File No: EX/162 (STD/1335)

April 2011

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Infineum 6399

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 Sustainability, Environment, Water, Population and Communities.

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 Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

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

This assessment report is for an extension of original assessment certificate for Infineum 6399. Based on the submission of new information by the extension notifier, some sections of the original assessment report for Infineum 6399 have been modified. These modifications have been made under the heading 'Extension Application' in the respective sections.

Infineum 6399

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Holder of the Original Assessment Certificate (No. 2992, STD/1335):
Infineum Australia Pty Ltd (ABN: 24 084 881 863)
Level 2/6 Riverside Quay
Southbank VIC 3006

Applicant for an Extension of the Original Assessment Certificate:
Caltex Australia Petroleum Pty Ltd (ABN: 17 000 032 128)
2 Solander Street
KURNELL, NSW 2231

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: chemical name, other names, CAS number, molecular and structural formula, molecular weight, spectra data, purity, details of use, import volumes, identity of recipients and methods of detection and determination.

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
The notified chemical has been approved for inclusion on the national inventories in the following countries: Korea, US, Canada, China and Japan. The notified chemical is currently undergoing notification in the Philippines.

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Infineum 6399 will be imported into Australia as a component of a commercial fuels additive package. It will not be imported in isolation. The trade name of the new product has not yet been finalised.

OTHER NAME(S)
Infineum 6399, WASA 6399

MOLECULAR WEIGHT
> 1000 Da

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

3. COMPOSITION

DEGREE OF PURITY > 90%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (>1% by weight) None

ADDITIVES/ADJUVANTS None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Cream coloured waxy/brittle solid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	51.8°C	Measured
Boiling Point	Not determined	Starts to decompose at 300°C
Density	948 kg/m^3	Measured
Vapour Pressure	5.85 × 10^{-4} Pa at 20°C	Measured
Water Solubility	< 1.0 µg/L at 20°C	Measured.
Hydrolysis as a Function of pH	Not Determined	Measurement unfeasible due to the low water solubility. Hydrolysis is unlikely given the extremely low water solubility, despite of the existence of hydrolysable function groups in the notified chemical.
Partition Coefficient (n-octanol/water)	log Pow > 6.5 at 40°C	Estimated.
Adsorption/Desorption	log K_{oc} = 6.62 at 40°C	Estimated.
Dissociation Constant	PK_a = ~ 4	Predicted based on the existence of dissociable functional groups in the notified chemical. Measurement not feasible due to the insolubility in water.
Particle Size	Not determined	Waxy solid
Flash Point	169°C at 101 kPa	Measured
Flammability	Not determined	Not expected to be flammable based on measured flash point.
Autoignition Temperature	Not determined	Not expected to autoignite under normal conditions of use.
Explosive Properties	Not expected to be explosive	The structural formula contains no explosophores.
Stability Testing	Thermally stable up to 297°C.	Measured

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

Reactivity
Starts to decompose at 300°C, but expected to be stable under normal conditions.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured within Australia. The notified chemical will be imported at a concentration of < 50 wt % in a new fuel additive package in bulk vessels or 205 L steel drums.
Extension Application
The notified chemical will be imported in diesel fuels containing the notified chemical at < 500 ppm.

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

Original application

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 20	< 20	< 50	< 50	< 50

Extension application

Year	1	2	3	4	5
Tonnes	325	325	325	325	325

PORT OF ENTRY
Melbourne, Sydney and Brisbane.

IDENTITY OF MANUFACTURER/RECIPIENTS
The notifier expects the notified chemical to be used by a number of formulators of diesel fuels.

TRANSPORTATION AND PACKAGING
The product containing the notified chemical will be brought into Australia in bulk vessels and 205 L drums. These containers will be transported by road for delivery to customer facilities or refineries. The finished diesel fuels containing the notified chemical at < 500 ppm will be distributed to service stations by road tankers. At the service stations, the diesel fuels will be transferred to underground tanks for storage.

USE
The notified chemical will be used as an additive in finished diesel fuels. The notified chemical will be imported at a concentration of < 50 wt % in a new fuel additive package. The notified chemical will be blended with diesel fuels and other additives within Australia to form finished fuels where it will be present at a concentration of < 500ppm.
Extension Application
The use of the notified chemical will be same as that for the original assessment certificate. However, no reformulation will take place in Australia.

OPERATION DESCRIPTION
Reformulation
At the reformulation sites the additive package containing the notified chemical will be blended with diesel fuel and other additives typically in batches of 10,000-100,000 litres, to form the finished diesel fuels. The transfer of the imported additive package containing the notified chemical into the blend tanks is automated apart from the connection and disconnection of the flexible transfer hoses. Blending of the additives will be conducted in a closed system and on completion of the blending the container, transfer hoses and pump are cleaned of additive by flushing with diesel fuel. *End use*
The finished fuels containing the notified chemical (at < 500 ppm) are then pumped out for distribution to service stations around Australia by road tanker. At the service stations, the diesel fuel will be transferred to underground tanks. When required, the diesel fuel would be pumped directly into automobile fuel tank.
Extension Application
The end use of the diesel fuels containing the notified chemical at < 500 ppm will be same as that for the original assessment certificate. However, no reformulation will take place in Australia.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

EXPOSURE DETAILS
Transport and dock workers will be exposed to the notified chemical only in the event of a spill due to an accident or leaking drum. *Reformulation*
During reformulation, dermal and ocular exposure to the additive package containing the notified chemical at a concentration of < 50 wt % is possible during the connection and disconnection of the vessels containing it to the transfer hoses. Inhalation exposure is likely to be negligible due to the low vapour pressure of the notified chemical and the automation of the reformulation process. Exposure is expected to be reduced by engineering controls such as flushing of the transfer hoses and containers with diesel and the use of PPE such as gloves, safety glasses, overalls and safety shoes.
End use
Worker exposure to the notified chemical at concentrations of < 500 ppm could occur during handling and

fueling of vehicles or equipment that are powered by diesel. The main route of exposure is expected to be dermal, although ocular exposure to splashes is possible. Maintenance on refinery plants and pipelines may also lead to worker exposure. Exposure during end use is expected to be minimized by the low (< 500 ppm) concentrations of the notified chemical in the finished diesel fuel and through good hygiene practices.

Extension Application

As only finished diesel containing the notified chemical at < 500 ppm concentration will be imported under the extension application, potential exposure will be limited to transportation, storage and end-use applications only.

6.1.2. Public exposure

The additive package containing the notified chemical at a concentration of < 50 wt % will not be sold to the public and hence exposure would only occur in the event of an accident during transportation. The public will be directly exposed (dermal and ocular) to the notified chemical when filling vehicles and equipment with finished diesel fuels containing it. Overall, direct exposure to the notified chemical in blended diesel is expected to be low due to the low concentration (< 500 ppm).

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	oral LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL > 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro Mammalian Chromosome Aberration Test.	non genotoxic
Genotoxicity – in vitro Mammalian Cell Gene Mutation Test.	non genotoxic
Reproduction/Developmental Toxicity Screening Test	NOAEL > 1000 mg/kg bw/day

Toxicokinetics, metabolism and distribution.

Based on the high molecular weight (> 1000 Da) and the lipophilicity of the notified chemical (water solubility < 1.0 µg/L at 20°C; log Pow > 6.5 at 40°C) dermal absorption is unlikely.

Acute toxicity.

The notified chemical is considered to be of low acute toxicity via the oral and dermal routes based on tests conducted in rats.

Irritation and Sensitisation.

Based on a test conducted on rabbits the notified chemical is considered to be non-irritating to the skin and slightly irritating to the eye. The notified chemical was found to be a non-sensitiser in a local lymph node assay in mice.

Repeated Dose Toxicity.

In a 28-day repeat dose oral toxicity study conducted on rats no test substance related effects were observed at all dose levels tested. Therefore, the No Observed (Adverse) Effect Level (NO(A)EL) was established as > 1000 mg/kg bw/day, based on the highest dose level examined.

Mutagenicity and Carcinogenicity.

The notified chemical was found to not be mutagenic using a bacterial reverse mutation test, and not clastogenic to human lymphocytes and mouse L5178Y TK+/- 3.7.2c cells *in vitro*.

Toxicity for reproduction.

In a reproduction toxicity study conducted on rats administered orally (gavage) no test substance related effects were observed at all dose levels tested. Therefore, the No Observed (Adverse) Effect Level (NO(A)EL) was established as > 1000 mg/kg bw/day, based on this being the highest dose tested.

Health hazard classification
Based on the available data the notified chemical is not classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
Occupational dermal and ocular exposure to the notified chemical for reformulation workers may occur during handling of the drums or bulk vessels, transfer of the additive package (≤ 50%) into the blend tank, cleaning and maintenance of the equipment. Dermal and ocular exposure may also occur in the end use where diesel (< 500 ppm notified chemical) or equipment powered by it is used.

Workers most at risk of irritancy will be reformulation workers handling the notified chemical at high concentrations i.e. in the imported additive. Given the engineering controls in place and proposed use of PPE including eye protection, the potential for exposure to the notified chemical should be minimised and therefore the risk to workers is not considered unacceptable.

Extension Application
As only finished diesel containing the notified chemical at < 500 ppm concentration will be imported under the extension application, exposure will be limited and therefore the risk to workers is not considered unacceptable.

6.3.2. Public health
The public will only be exposed to the notified chemical when it is present in diesel fuel (< 500 ppm). Due to the low concentration of the notified chemical in the diesel and the low hazard of the notified chemical the risk to the public is not considered unacceptable.

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 at a concentration of < 50 wt % in a new fuel additive package in bulk vessels or 205 L steel drums for further blending with diesel fuels and other additives to form finished fuels.
Fugitive emissions during transport and blending are considered to be negligible. Transfer to blending operation occurs in closed pipes and vessels, where the new chemical will be blended with diesel fuel and automatically pumped out for distribution by road tanker. If incidental spillage of the additive package occurs during normal blending procedures, it will be contained and soaked up with earth or sand before being transported off-site to an approved industrial facility for appropriate disposal to landfill. It would not be released to the environment.

Empty containers will be removed to a safe place to await appropriate recycling or disposal. Approximately 1% unused residues left inside "empty" containers will be either decomposed during the reconditioning of the containers forming water and oxides of carbon and nitrogen, or disposed of as consumer container residues.

RELEASE OF CHEMICAL FROM USE

The end use of the chemical will be as an additive in diesel fuel. There could be minor spills at petrol stations, which would mostly fall to the ground. Release of the new chemical to the atmosphere is unlikely to occur, particularly as the vapour pressure of the new chemical is negligible and its viscosity at ambient temperatures is high. In automotive engines, the notified chemical will be consumed together with the diesel fuel to generate primarily water and oxides of carbon and nitrogen.

RELEASE OF CHEMICAL FROM DISPOSAL
No significant release of the notified chemical from formulation to end use is expected. Any significant spill would most likely be disposed of to landfill.

7.1.2 Environmental fate

The notified chemical is considered to be readily biodegradable based on the study provided. The potential for bioaccumulation is expected to be low based on the modelling results. For the details of the environmental fate studies please refer to Appendix C.

Most of the notified chemical will share the fate of the diesel fuel containing it and be consumed in automotive engines. Residues in containers are not significant and are expected to be removed and treated properly, and most likely will be thermally decomposed to recover the calorific value at the container reconditioning facility. For the limited disposal to landfill, the notified chemical is expected to bind to organic matter based on the high estimated log Koc of 6.62, and undergo degradation processes via biotic and abiotic pathways. Either way, the notified chemical will be finally decomposed into small molecules of water and oxides of carbon and nitrogen.

7.1.3 Predicted Environmental Concentration (PEC)

The calculation of PEC is not conducted due to the minimal release estimated based on the reported use pattern.

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	LL50 > 100 mg/L WAF, nominal	Not toxic to fish up to the limit of the water solubility
Daphnia Toxicity	EL50 > 100 mg/L WAF, nominal	Not toxic to daphnia up to the limit of the water solubility
Algal Toxicity	EL50 > 100 mg/L WAF, nominal	Not toxic to algae up to the limit of the water solubility
Inhibition of Bacterial Respiration	IC50 > 1000 mg/L	Not toxic to sludge micro-organisms

The notified chemical is not considered toxic to the aquatic life up to the limit of its water solubility.

7.2.1 Predicted No-Effect Concentration

Calculation of PNEC for the notified chemical is not necessary given its property of no toxicity to aquatic life up to the limit of water solubility.

7.3. Environmental risk assessment

The Risk Quotient (RQ, PEC/PNEC) has not been determined given no significant release of the notified chemical to environment is expected from the reported use pattern. In case any release occurs to the environment, it readily biodegrades and therefore will not be a concern to the aquatic compartment.

Based on the above, the notified chemical is not expected to pose any unacceptable risk to the environment from the proposed application.

Extension Application

Considering that the notified chemical will share the fate of the diesel fuel and be consumed in automotive engines, the proposed increase in volume under the extension application is not expected to impact on the original environmental risk assessment.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available data the notified chemical is not classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)].

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.

Risk assessment relating to extension application
The proposed use and fate of the notified polymer will not change significantly under the proposed extension. The circumstances in the extension application are not expected to impact on the original human health and environmental risk assessment.

Recommendations

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following safe work practices to minimise occupational exposure when handling the notified chemical at high concentrations i.e. the imported additive:
  - Avoid eye contact

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure when handling the notified chemical at high concentrations i.e. the imported additive:
  - Protective eyewear

  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 or thermally decomposed during container reconditioning.

Emergency procedures

- Spills and/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 finished diesel fuels, or is likely to change significantly;
- the amount of chemical being introduced has increased from 325 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.

No additional secondary notification conditions are stipulated.

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.
Extension Application
The extension applicant has provided an MSDS of a product containing the notified chemical which was reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the extension applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 51.8ºC

Method	OECD TG 102 Melting Point/Melting Range.
Remarks	No significant protocol deviations. GLP compliant.
Test Facility	Wildlife International (2008a)

Boiling Point Not determined

Method	OECD TG 103 Boiling Point. US EPA OPPTS 830.7220, Boiling Point/Boiling Range
Remarks	The notified chemical starts to decompose at 300ºC. No significant protocol deviations. GLP compliant.
Test Facility	Wildlife International (2008b)

Density 948 kg/m^3

Method	OECD TG 109 Density of Liquids and Solids. US EPA OPPTS 830.7300, Density/Relative Density/Bulk Density
Remarks	Bulk density was measured using a Quantachrome Ultrapycnometer 1000 helium pycnometer. The temperature at which the test was conducted was not recorded. GLP compliant.
Test Facility	Wildlife International (2008c)

Vapour Pressure 5.85×10^{-4} Pa at 20ºC

Method	OECD TG 104 Vapour Pressure. US EPA OPPTS 830.7950 Vapour Pressure
Remarks	Spinning rotor gauge method used. Hexachlorobenzene was used as the reference substance, for which the tested vapour pressure was 1.35×10^{-3} Pa. This result is consistent with the literature value. The mean calculated vapour pressure for the new chemical at 20°C was 5.85×10^{-4} Pa, with a range of 4.96×10^{-4} to 7.61×10^{-4} Pa.
Test Facility	Wildlife International (2008d)

Water Solubility < 1 µg/L at 20ºC

Method	OECD TG 105 Water Solubility. US EPA OPPTS 830.7860.
Remarks	Column Elution Method. Samples were collected separately at water flow rate of 1.0 mL/min and 0.5 mL/min at 20˚C, and the concentrations were tested. For both cases the concentration was below the method limit of quantitation of 1 µg/L.
Test Facility	Wildlife International (2008e)

Partition Coefficient (n-octanol/water) log P_{OW} > 6.5

Method	OECD TG 117 Partition Coefficient (n-octanol/water). US EPA OPPTS 830.7570.
Remarks	HPLC Method. The column temperature was maintained at 40 ± 0.8˚C. The column dead time was determined to be 0.893 min by injecting thiourea. The notified chemical eluted as six peaks. All the six peaks eluted after the reference standard DDT that has the highest log P_{OW} of 6.5.
Test Facility	Wildlife International (2008f)

Adsorption/Desorption – screening test log K_{oc} = 6.62

Method	OECD TG 121 Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge Using High Performance Liquid Chromatography (HPLC).
Remarks	The column temperature was maintained at 40˚C. The column dead time was determined to be 1.836 min by injecting thiourea. The notified chemical eluted as five peaks. The corresponding log K_{OC} for the notified chemical ranged from unretained to an extrapolated value of 6.94. The weighted mean log K_{OC} of the notified chemical was calculated to be 6.62.
Test Facility	Wildlife International, Limited (2008g)

Flash Point 169ºC at 101 kPa

Method	US EPA OPPTS 830.6315 Flammability; D93-90 Flash Point by Pensky-Martens Closed Tester
Remarks	The flash point was determined using a PetroLab, Inc. Model PMA 2 flash point tester following the procedure presented in method D 93-90 of the American Society of Testing and Materials, Flash Point by Pensky-Martens Closed Tester. No significant protocol deviations. GLP compliant.
Test Facility	Wildlife International (2008h)

Stability Testing Thermally stable up to 297°C.

Method	OECD TG 113 Screening Test for Thermal Stability and Stability in Air. US EPA OPPTS 830.6316 Explodability
Remarks	Under the conditions specified, the thermal analysis determined one endothermic region with an average onset temperature of 36.1°C, consistent with phase transition (i.e., melting). An exothermic region prior to the boiling point of the test substance was also identified with an average onset temperature of 297°C, consistent with thermal decomposition. Therefore, the test substance was determined to be thermally stable below 297°C. No significant protocol deviations. GLP compliant.
Test Facility	Wildlife International (2008i)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Albino Crl : CD (SD) Female
Vehicle	Corn oil
Remarks - Method	No significant protocol deviations. GLP compliant.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	3 Female	2000	0
II	3 Female	2000	0

LD50	> 2000 mg/kg bw
Signs of Toxicity	There were no deaths. Clinical findings consisted of abnormal excretion (feces smaller than normal and/or decreased defecation). No other abnormal physical signs were noted during the study.
Effects in Organs	There were no remarkable necropsy findings.
Remarks - Results	Body weight gains were as expected.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	WIL (2008a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test.
Species/Strain	Rat/Sprague-Dawley CD (Crl : CD (SD) IGS BR)
Vehicle	Arachis oil was used to moisten the test substance.
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations. GLP compliant.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5 per sex	2000	0

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	There were no test substance-related dermal reactions.
Signs of Toxicity - Systemic	There were no deaths or test-substance related clinical signs. There were no signs of systemic toxicity.
Effects in Organs	No abnormalities were noted at necroscopy
Remarks - Results	Body weight gains were as expected.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	Safepharm (2008a)

B.3. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 2004/73/EC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 Male
Vehicle	Test substance administered as supplied
Observation Period	72 Hours
Type of Dressing	Semi-occlusive.
Remarks - Method	The pH of a 10% aqueous solution of the notified chemical was determined prior to the commencement of the study and found to be 8.3 immediately on preparation and 8.4 after 10 minutes. No significant protocol deviations. GLP compliant.
Remarks - Results	A single 4-hour, semi-occluded application of the test material to the intact skin of the three rabbits produced no evidence of skin irritation.
CONCLUSION	The notified chemical is non-irritating to the skin.
TEST FACILITY	Safepharm (2008b)

B.4. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Directive 2004/73/EC B.5 Acute Toxicity (Eye Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 (1 female and 2 male)
Observation Period	72 Hours
Remarks - Method	No significant protocol deviations. GLP compliant.

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	0.3	0.3	2	< 72 hours	0
Conjunctiva: chemosis	0.3	0	0	2	< 48 hours	0
Conjunctiva: discharge	0	0	0	2	< 24 hours	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	No corneal or iridial effects were noted. A single application of the test material to the non-irrigated eye of the 3 rabbits produced moderate conjunctival irritation 1 hour after treatment. Two treated eyes appeared normal at the 48 hour observation and the remaining treated eye appeared normal at the 72 hour observation.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	Safepharm (2008c)

B.5. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Notified chemical
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/Ca (CBA/CaOlaHsd) Female
Vehicle	Butanone
Remarks - Method	A preliminary screening test was conducted on 1 mouse with 50% w/w of the test substance in butanone. No signs of systemic toxicity were noted. Residual test material was noted post dose on day 2, with fur loss noted on day 3 and for the remainder of the test. α-Hexylcinnamaldehyde (85% in butanone) was used as the positive control. GLP compliant.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	735 (±169)	
10	1554 (±454)	2.12
25	1733 (±322)	2.36
50	2021 (±537)	2.75
Positive Control (% v/v)		
10		2.86
25		5.74

Remarks - Results	There were no deaths and no signs of systemic toxicity were noted in the test or control animals. Residual test material on the ears and fur loss was noted post dose on day 2 and for the remainder of the test in all animals treated with concentrations of 25 and 50% of the notified chemical. Body weight changes of the test animals were comparable to those seen in the control animals. A stimulation index of less than 3 was observed for all concentrations of the test material.
CONCLUSION	There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	Safepharm (2008d)

B.6. 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)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Corn oil

Remarks - Method	At the time of randomization male rats weighed only 153 to 185 g. One female animal in the control group did not have the histological processing performed on the adrenal glands. GLP compliant.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 per sex	0	0
low dose	5 per sex	100	0
mid dose	5 per sex	300	0
high dose	5 per sex	1000	0
control recovery	5 per sex	0	0
high dose recovery	5 per sex	1000	0

Mortality and Time to Death

There were no unscheduled deaths during the study.

Clinical Observations

Incidental findings of red/yellow material around the mouth, nose, eyes and anogenital regions, fur loss, scabbing and soft faeces were noted during the study. These isolated, incidental external changes were considered of no toxicological importance.

Behavioural Assessment – There were no significant treatment-related changes.

Functional Performance Tests – Mean rotarod performance for female animals in the high dose group (mean 48.1 with standard deviation 40.47) was significantly lower than the control animals (mean 120 with standard deviation 0) during the third week of the study. However the pretest measurements on the high dose group were of a similar value (mean 46.7 with standard deviation 46.0) and hence the result was considered of no toxicological importance. There were no other significant treatment-related changes observed in the functional performance tests.

Sensory Reactivity Assessments - There were no treatment-related changes in sensory reactivity.

Bodyweight – No significant changes in bodyweight were seen.

Food Consumption – There was a statistically significant increase in the food consumption for females in the high dose group in comparison to the controls. As this increase was not seen in the remainder of the study or for the high dose male group it was considered of no toxicological importance.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology – There was a statistically significant increase in the prothrombin time (PT) and in the large unstained cell parameter of the differential leukocyte count for male animals in the high dose recovery group. As the same increases were not seen for male animals in the high dose group the results were considered of no toxicological importance. There were no other significant test substance-related alterations in haematology and coagulation parameters.

Blood Chemistry – A statistically significant higher mean chloride level in male animals in the mid dose group was not considered of no toxicological importance as no dose response relationship was seen. Slightly higher mean potassium levels and lower mean sodium levels in the male high dose recovery group and higher mean albumin and triglycerides levels in the female high dose recovery group were considered of no toxicological importance due to the slight magnitude of the changes and because corresponding changes were not seen in the high dose groups. There were no other test substance-related alterations in serum chemistry parameters.

Urinalysis - Females in the high dose recovery group had an increase in urine volume together with a reduction in the specific gravity. The differences were considered of no toxicological importance due to there being no dose response relationship present and corresponding changes were not seen in the high dose groups. There were no other test substance-related alterations in urinalysis parameters.

Effects in Organs

Review of the gross necropsy observations revealed no observations that were considered to be associated with administration of the test substance.

Lower mean final bodyweights were measured in male animals in the low, mid and high dose groups. Higher mean adrenal gland and brain weights relative to final body weights were also seen in the male low and mid dose groups. The differences were considered of no toxicological importance due to there being no dose response relationship present. There were no other test substance-related alterations in final body weights or organ weights.

There were no test substance-related microscopic findings at the scheduled necropsies. All histologic changes were incidental findings or related to experimental manipulation other than the administration of the test substance.

Remarks – Results

Based on the results of this study, the notified chemical administered orally (gavage) to Crl:CD(SD) rats for 28 consecutive days did not result in toxicity in the evaluated parameters at dosage levels of 100, 300 or 1000 mg/kg/day.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as > 1000 mg/kg bw/day based on the absence of any test substance related effects at the highest dose tested.

TEST FACILITY WIL (2008b)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

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

Species/Strain *S. typhimurium*: TA1535, TA1537, TA98, TA100
E. coli: WP2uvrA$^-$

Metabolic Activation System Rat S9 fraction from phenobarbitone/β-napthoflavone induced rat liver

Concentration Range in Main Test
a) With metabolic activation: 50 – 5000 µg/plate
b) Without metabolic activation: 50 – 5000 µg/plate

Vehicle Tetrahydrofuran

Remarks - Method
No signs of toxicity were recorded in the preliminary test.
Although a particulate precipitate was observed at and above 500 µg/plate this did not prevent the scoring of revertant colonies.
No significant protocol deviations.
GLP compliant.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5000	> 5000	500	negative
Test 2		> 5000	500	negative
Present				
Test 1	> 5000	> 5000	500	negative
Test 2		> 5000	500	negative

Remarks - Results The test material was tested up to the maximum recommended dose level of 5000 µg/plate. 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.

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.

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

TEST FACILITY Safepharm (2008e)

B.8. 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
Cell Type/Cell Line	Lymphocyte
Metabolic Activation System	Rat S9 fraction from phenobarbitone/β-napthoflavone induced rat liver
Vehicle	Tetrahydrofuran
Remarks - Method	Due to the marginal toxicity of tetrahydrofuran to human lymphocytes when dosed at 1% by volume the maximum dose volume was reduced to 0.5% by volume. The selection of the maximum dose level for the main test was based on the pronounced precipitation seen in the preliminary test. GLP compliant.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 9.77, 19.53, 39.06*, 79.13*, 156.25*, 312.5*	4 hours	24 hours
Test 2	0*, 4.90, 9.77, 19.53*, 39.06*, 78.13*, 156.25*	24 hours	24 hours
Present			
Test 1	0*, 9.77, 19.53, 39.06*, 79.13*, 156.25*, 312.5*	4 hours	24 hours
Test 2	0*, 9.77, 19.53, 39.06*, 78.13*, 156.25*, 312.5*	4 hours	24 hours

*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	> 2500	> 312.5	79.13	Negative
Test 2		> 156.25	> 156.25	Negative
Present				
Test 1	> 2500	> 312.5	156.25	Negative
Test 2		> 312.5	156.25	Negative

Remarks - Results The positive and vehicle controls gave satisfactory responses, confirming the validity 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 human lymphocytes treated in vitro under the conditions of the test.

TEST FACILITY Safepharm (2008f)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
EC Directive 2000/32/EC B.17 Mutagenicity - In vitro Mammalian Cell Gene Mutation Test.

Species/Strain	Mouse
Cell Type/Cell Line	L5178Y TK+/- 3.7.2c
Metabolic Activation System	Rat S9 fraction from phenobarbitone/β-napthoflavone induced rat liver
Vehicle	Tetrahydrofuran
Remarks - Method	Due to the toxicity of tetrahydrofuran to L5178Y TK+/- 3.7.2c cells when dosed above 50 µL per 20 mL culture the maximum final concentration that could be tested was 1250 µg/mL. The selective agent used was 5-trifluorothymidine, the selection time was not recorded. GLP compliant.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	0, 78.13, 156.25, 312.5, 625, 937.5, 1250	4 hours	2 days	N/A
Test 2	0, 78.13, 156.25, 312.5, 625, 937.5, 1250	24 hours	2 days	N/A
Present				
Test 1	0, 78.13, 156.25, 312.5, 625, 937.5, 1250	4 hours	2 days	N/A
Test 2	0, 78.13, 156.25, 312.5, 625, 937.5, 1250	4 hours	2 days	N/A

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 1250	> 1250	78.13	Negative
Test 2	> 1250	> 1250	78.13	Negative
Present				
Test 1	> 1250	> 1250	78.13	Negative
Test 2		> 1250	78.13	Negative

Remarks - Results

The vehicle controls had acceptable mutant frequency values that were within the normal range for the L5178Y cell line at the TK +/- locus. The positive control materials induced marked increases in the mutant frequency indicating the satisfactory performance of the test and of the activity of the metabolising system.

The test material did not induce any toxicologically significant or dose-related increases in the mutant frequency at any dose level, either with or without metabolic activation, in either the first or the second experiment using a dose range where the maximum dose level was limited by difficulties in the test material formulation.

CONCLUSION The notified chemical was not clastogenic to mouse L5178Y TK+/- 3.7.2c cells treated in vitro under the conditions of the test.

TEST FACILITY Safepharm (2008g)

B.10. Developmental toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD 421 Reproduction/Developmental Toxicity Screening Test US EPA OPPTS Guidelines 870.3550
Species/Strain	Rat/Crl:CD(SD)
Route of Administration	Oral – gavage
Exposure Information	Exposure period - female: 39 – 52 days (14 days prior to pairing through to the day prior to euthanasia). Females with no evidence of mating or that failed to deliver were dosed through to the day prior to euthanasia for a total of 44-52 days. Exposure period - male: 28 days (14 days prior to pairing through to 1 day prior to scheduled euthanasia). Dose regimen: daily Post-exposure observation period: 0
Vehicle	Corn oil
Remarks - Method	No significant protocol deviations. GLP compliant.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
I	12 per sex	0	0
II	12 per sex	100	0
III	12 per sex	300	0
IV	12 per sex	1000	0

Mortality and Time to Death

All animals survived to the scheduled necropsy.

Effects on Dams

There were no test substance-related effects on mean body weights, body weight gains or food consumption for females during the pre-mating treatment period at any dosage levels. The mean bodyweight gain in the high dose group during lactation days 1 – 4 was significantly greater than in the control group. The differences in the body weight gain were considered of no toxicological importance due to the control group having an uncharacteristically low mean body weight gain and the lack of a concomitant increase in the food consumption at this stage. A significant increase in the food consumption of female animals in the high dose group was seen during gestation days 0 – 4, however there was no corresponding increase in the food consumption during this period and hence considered of no toxicological importance.

No test substance-related effects on reproductive performance or gestation length were seen in any of the test groups.

There were no test substance-related histopathologic effects on reproductive organs. No test substance-related macroscopic findings were noted at the scheduled necropsies for males or females at any dosage level.

There were no test substance related alterations in mean organ weights observed at any dosage level. Statistically significant differences in organ weight were observed including the higher mean left epididymis weight relative to brain weight in high dose males. However as the mean left epididymis absolute weight, the mean left epididymis weight relative to body weight and the mean right epididymis absolute and relative weights showed no difference in comparison to control it was considered of no toxicological importance. The mean pituitary gland weights (relative to brain and body weights) in the mid dose female group was also lower than in the control group. However as there was no dose response relationship present the finding was considered of no toxicological importance. There were no microscopic findings associated with either of the differences.

Effects on Foetus

The mean number of pups born, live litter size and the percentage of males at birth in all dose groups were similar to the control group values. Postnatal survival was unaffected by parental test substance administration.

Mean male and female pup body weights and body weight gains in all dose groups were unaffected by parental test substance administration.

Mandibular micrognathia was noted in one pup from the mid dose group, however as this condition was not seen in the high dose group it was considered of no toxicological importance.

Remarks - Results

In the absence of test substance-related effects on reproductive performance, gestation lengths and parturition, a dosage level of 1000 mg/kg/day (the highest dosage level tested) was considered to be the no-observed-adverse-effect level (NOAEL) for reproductive toxicity of the test substance when administered orally by gavage to Crl:CD(SD) rats. Based on the absence of test substance-related effects on parental survival, clinical condition, mean body weights, body weight changes, food consumption, organ weights or macroscopic changes at all dosage levels and microscopic changes at the 1000 mg/kg/day dosage level, the NOAEL for male and female systemic toxicity was considered to be 1000 mg/kg/day. The NOAEL for neonatal toxicity was also 1000 mg/kg/day based on the absence of effects on postnatal survival or pup body weights.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as > 1000 mg/kg bw/day based on the absence of any test substance related effects at the highest dose tested.

TEST FACILITY WIL (2008c)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 301B Ready Biodegradability: CO2 Evolution Test. Commission Directive, Annex V. 67/548/EEC C.4-C, Carbon Dioxide Evolution. ISO 10634: 1995 (E) Water Quality – Guideline for the preparation and Treatment of Poorly Water-soluble Organic Compounds for the subsequent Evaluation of their Biodegradability in An Aqueous Medium.
Inoculum	Activated sludge from Cambridge Wastewater Treatment Plant, Cambridge, Maryland
Exposure Period	28 days
Auxiliary Solvent	Test substance was dissolved in THF and applied to silica gel. Solvent was then completely removed prior to study. Test material was studied as solvent-free coating on silica gel powder. Polysorbate 85 was used as solubilizer in some of the test groups.
Analytical Monitoring	Measurement of CO_2 evolved throughout the study, dissolved organic carbon (DOC) of test chamber on Day 29.
Remarks - Method	The CO_2 produced from the degradation of organic carbon sources within the test chamber was trapped as K_2CO_3 in the KOH solution and the amount of inorganic carbon in the trapping solution was measured at various intervals during the study, using a Shimadzu Model TOC-V_{CSH} carbon analyzer. Sodium benzonate was used as the reference substance. The test contained a blank control group, reference group, treatment group, treatment toxicity control group, control group containing solubilizer, treatment group containing solubilizer, treatment toxicity control group containing solubilizer and a solubilizer toxicity control group. All groups were conducted in duplicates.

RESULTS

Test substance		*Sodium Benzonate*	
Day	*% Degradation**	*Day*	*% Degradation**
15	10	7	68
18	60	18	102.8

* Percentage of theoretical amount of carbon dioxide ($ThCO_2$).

Remarks - Results	The biodegradation of the reference reached 68% by day 7 of the test. The biodegradation of the notified chemical reached 102.5% of $ThCO_2$ at day 28, with an average of 10% by day 15 and 60% by day 18 of the study. The results indicate that the existence of solubilizer inhibited the biodegradation process for both the reference and the notified chemical. This may be attributed to the emulsifying effect of the solubilizer to the test substances which blocked the access of microbials to the test substances.
CONCLUSION	The notified chemical is considered ready biodegradable based on the test result.
TEST FACILITY	Wildlife International Limited (2008j)

C.1.2. Bioaccumulation

TEST SUBSTANCE	Notified Chemical
Remarks - Results	Test not conducted. The bioaccumulation potential of the notified chemical was evaluated based on related study data and in silico modelling. It was concluded that the material is not expected to be bioaccumulative. The ready biodegradability of the notified chemical also indicates a low potential for bioaccumulation.
CONCLUSION	The potential for bioaccumulation of the notified chemical is considered low.

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test - static test. US EPA OPPTS 850.1075: Fish Acute Toxicity Test, Fresh Water and Marine.
Species	fathead Minnow (*Pimephales promelas*)
Exposure Period	96 hours
Auxiliary Solvent	None.
Water Hardness	134 mg $CaCO_3$/L
Analytical Monitoring	HPLC with triple quadrupole mass spectrometry (LC/MS/MS) detection. The limit of quantitation (LOQ) was defined as 1.00 µg/L.
Remarks – Method	Three replicate test chambers were maintained at 22 ± 1 ˚C in the treatment group and in the control group, with 10 fish in each test chamber. Due to the low solubility of the notified chemical, test solutions were prepared as water accommodated fractions (WAFs) by directly mixing the notified chemical and water at a nominal loading rate of 100 mg/L. The solutions were filtrated through a 0.22 µm filter prior to distribution into the test chambers. The test solutions appeared clear and colourless following mixing and filtration.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
0		30	0	0	0	0	0
100		30	0	0	0	0	0

LL50	> 100 mg/ L WAF nominal at 96 hours.
NOEL	100 mg/L WAF nominal at 96 hours.
Remarks – Results	No mortality or overt signs of toxicity was observed in all the groups throughout the 96-hour test.
CONCLUSION	The notified chemical is not toxic to fathead minnow up to the limit of its water solubility.
TEST FACILITY	Wildlife International Limited (2009a).

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified Chemical

METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Cladoceran (Daphia magma). 48 hour static test US EPA OPPTS 850.1010 Acute Toxicity for Daphnia – Cladoceran (Daphia magma). 48 hour static test
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	137 mg $CaCO_3$/L
Analytical Monitoring	HPLC with triple quadrupole mass spectrometry (LC/MS/MS) detection. The limit of quantitation (LOQ) was defined as 1.00 µg/L.
Remarks - Method	Daphnids were exposed to the notified chemical of five loading rates (6.3, 13, 25, 50 and 100 mg/L) and a negative control (dilution water) in duplicates with 10 animals used for each replicate. Due to the low solubility of the notified chemical, test solutions were prepared as water accommodated fractions (WAFs) by directly mixing the notified chemical and water. The solutions were filtrated through a 0.22 µm filter prior to distribution into the test chambers. Test solutions appeared clear and colourless following mixing and filtration.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control		20	0	0
6.3		20	0	0
13		20	0	0
25		20	0	0
50		20	0	0
100		20	0	0

EL50	>100 mg/L at 24 hours > 100 mg/L at 48 hours
NOEL	100 mg/L at 48 hours
Remarks - Results	All the Daphnia in the negative control group and in all the treatment groups appeared normal through the test, with no mortality/immobility or overt signs of toxicity observed. One daphnia in the 13 mg/L treatment group was noted floating at the 24-hour observation, but was normal in appearance after gentle submersion. The no-mortality/immobility concentration and the NOEL were therefore determined to be 100mg/L.
CONCLUSION	The notified chemical is not toxic to *Daphnia magna* up to the limit of its water solubility.
TEST FACILITY	Wildlife International Limited (2009b).

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	Freshwater alga (*Pseudokirchneriella subcapitata*)
Exposure Period	72 hours
Concentration Range	Nominal: 6.3, 13, 25, 50 and 100mg/l Actual: < 0.001-0.00489 mg/L
Auxiliary Solvent	None
Water Hardness	N/A
Analytical Monitoring	HPLC with triple quadrupole mass spectrometry (LC/MS/MS) detection.

	The limit of quantitation (LOQ) was defined as 1.00 µg/L.
Remarks - Method	Algae were exposed to the notified chemical at five concentrations and a negative control (dilution water) in duplicates at 23 ± 2˚C and under continuous cool-white fluorescent lighting of 6,000 ± 20% intensity. Due to the low solubility of the notified chemical, test solutions were prepared as water accommodated fractions (WAFs) by directly mixing the notified chemical and algal medium. The mixtures were decanted followed by centrifuging 10 min at 9643 g to remove suspended material prior to distribution into the test chambers. Test solutions appeared clear and colourless following mixing and centrifugation.

RESULTS

Biomass		*Growth*	
E_bL50 mg/L at 72 h	*95 % confidence interval mg/L*	*E_rL50 mg/L at 72 h*	*95 % confidence interval mg/L*
> 100 (WAF, Nominal)	Not applicable Could not be calculated	> 100 (WAF, Nominal)	Not applicable Could not be calculated

Remarks - Results	There were no apparent treatment-related effects on any of the parameters measured for any of the concentration tested. Consequently, the 72-hour EL50 value based on cell density, area under the growth curve and growth rate was > 100mg/L and the 72-hour NOEL in this study was determined to be 100mg/L, the highest loading rate tested.
CONCLUSION	The notified chemical is not considered toxic to algae up to the limit of its water solubility.
TEST FACILITY	Wildlife International Limited (2009c).

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 67/548/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test.
Inoculum	Activated sludge collected from the Cambridge Wastewater Treatment Facility, Cambridge, Maryland
Exposure Period	3 hours
Concentration Range	Nominal: 10, 30, 100, 300 and 1000 mg/L
Remarks – Method	The respiration rates of the test solutions were measured using a YSI Model 50 B Dissolved Oxygen Meter.
RESULTS	Control, reference and treatment test mixtures were incubated at 20 ± 2˚C and aerated for three hours at a rate sufficient to provide aerobic conditions and maintain solids in suspension. 3,5-dichlorophenol was used for the reference control at levels of 3, 15 and 50 mg/L.
IC50	> 1000 mg/L
NOEC	24 mg/L
Remarks – Results	The respiration rates observed in the two controls were 36.0 and 32.7 mg O_2/L/hr, with a difference of approximately 10%. The EC50 value for the reference substance was 20.5 mg/L, with 95 percent confidence limits of 3 and 50, and was within the 5 to 30 mg/L range considered acceptable for the test. The EC50 and 95% confidence limits were calculated using binomial probability with nonlinear interpolation. Inhibitory effects upon respiration by the test substance at the concentrations evaluated in this study did not exhibit a concentration

	dependent dose response pattern. The dose-response curve starting below zero at 10 mg/L, and rising to a maximum around 100 mg/L, before dropping below zero again around 1000 mg/L. The observed percent inhibitions for the notified chemical ranged from – 4.8 – 34.2%. The EC50 value for the test substance is therefore considered to be greater than 1000 mg/L, the highest concentration tested.
CONCLUSION	The notified chemical is not considered to be toxic to sludge micro-organisms based on the test results.
TEST FACILITY	Wildlife International, Ltd (2008k)

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