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July 2010

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Oxetane, 3-ethyl-3-[[(2-ethylhexyl)oxy]methyl]-

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health and Ageing, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment, Water, Heritage and the Arts.

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

This Full Public Report is also available for viewing and downloading from the NICNAS website or available on request, free of charge, by contacting NICNAS. For requests and enquiries please contact the NICNAS Administration Coordinator at:

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

Director
NICNAS

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FULL PUBLIC REPORT

Oxetane, 3-ethyl-3-[[(2-ethylhexyl)oxy]methyl]-

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
DKSH Australia Pty Ltd (ABN 70 005 059 307)
14 - 17 Dansu Court, HALLAM VIC 3803

and

Gerber Scientific International (Aust) Pty Ltd (ABN 55 088 162 038)
9 Hamley Road, MOUNT KURING-GAI NSW 2080

NOTIFICATION CATEGORY
Standard: Chemical other than polymer

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: spectral data, purity, impurities, adjuvants/additives, use details and import volume.

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
UK (2003)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Aron Oxetane OXT-212

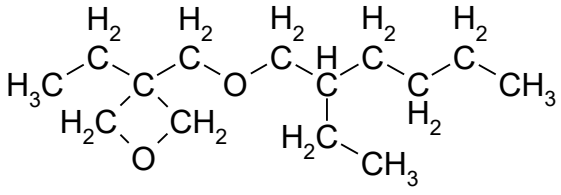
CAS NUMBER
298695-60-0

CHEMICAL NAME
Oxetane, 3-ethyl-3-[[(2-ethylhexyl)oxy]methyl]-

OTHER NAME(S)
EHOX

MOLECULAR FORMULA
$C_{14}H_{28}O_2$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
228 Da

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

3. COMPOSITION

DEGREE OF PURITY > 95%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Clear colourless liquid

Property	Value	Data Source/Justification
Freezing Point	< -20°C	Measured
Boiling Point	265°C at 101.64 kPa	Measured
Density	890 kg/m^3 at 20°C	Measured
Vapour Pressure	1.6 × 10^{-2} kPa at 25°C	Measured
Water Solubility	1.48 × 10^{-2} g/L at 20°C	Measured
Hydrolysis as a Function of pH	$t_{½}$ > 1 year	Measured
Partition Coefficient (n-octanol/water)	log Pow = 5.16 at 30°C	Measured
Adsorption/Desorption	log K_{oc} = 3.84 at 40°C	Measured
Dissociation Constant	Not determined	The notified chemical has no acidic or basic groups.
Particle Size	Not determined	Liquid
Flash Point	124°C at 101.33 kPa	Measured
Flammability	Not expected to be highly flammable.	Based on flash point.
Autoignition Temperature	194°C	Measured
Explosive Properties	Negative	There are no chemical groups that would infer explosive properties.
Surface Tension	59.1 mN/m at 21.0°C	Measured
Oxidising Properties	Negative	There are no chemical groups that would infer oxidising properties.

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

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported neat, in formulated ink preparations at < 70% and as a component of ink at < 20% in cartridges.

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

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

PORT OF ENTRY
Sydney and Fremantle

TRANSPORTATION AND PACKAGING
The ink cartridges and the concentrate or the formulated ink preparations containing the notified chemical (packaged in 20 L steel cans or 200 L steel drums) will be transported by road or rail to end user sites.

USE
Resin for ink and printing preparations in industrial settings.

OPERATION DESCRIPTION
The neat notified chemical will be blended with other ingredients at customer formulation sites, to make inks containing the notified chemical at < 70%. While the formulation process will vary with the product type and formulation site, it is expected that most sites will have closed, automated mixing and dosing equipment. The formulated inks will be transported to the printing facility for storage.

When used for printing, the ink formulations (containing at up to 70% of the notified chemical) will be transferred directly from the storage containers to the printing machine via automated lines. The printing machine will be fully automated and will be equipped with heat resource or UV lamps that cure the product immediately after coating.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Waterside & transport workers	6	2	10
Storage personnel at formulation facility	1	1	10
Formulation workers	2	8	100
Storage personnel at printing facility	1	8	100
Printer operators	2	8	100
Service technicians	5	1	20

EXPOSURE DETAILS
Workers involved in importation, transportation or storage are not expected to be exposed to the imported notified chemical except in the event of an accident where the packaging is breached.

At customer formulation facilities, exposure to the neat notified chemical is possible during handling of the import containers, cleaning and maintenance of the equipment. Skin and eye contact (due to splashing) are likely to be the main routes of exposure. Inhalation exposure is not likely due to the low volatility of the notified chemical, except where mists or aerosols are generated. The level of exposure would vary from site to site depending on the level of automation of the formulation process. Exposure to worker is likely to be minimised by use of industrial standard personal protective equipment (PPE).

The worst case dermal exposure is expected to be to workers directly handling the imported concentrate. NICNAS estimated it to be 0.1-1.0 mg/cm^2/day, based on EASE (the estimation and assessment of substance exposure) model (EASE) for the exposure scenario 'manual addition of liquids' (European Commission, 2003) and assuming the notified chemical is present at concentration of 100%. Assuming a surface area of 420 cm^2 (one hand) for a 70 kg worker and a 100% dermal absorption factor, systemic exposure is estimated to be 0.6-6.0 mg/kg bw/day. Exposure is likely to be minimised by use of industrial standard personal protective equipment (PPE).

Printer operators and service technicians will come in contact with the notified chemical (at < 70%) during certain processes, including printer maintenance, connecting and disconnecting bottles and handling the printed substrate once the ink has been fully cured. The most likely route of exposure will be dermal. Inhalation exposure is unlikely due to the low volatility of the notified chemical. Exposure will be limited by the expected use of local exhaust ventilation in areas of printing machines and workers wearing personal protection equipment, including impermeable gloves.

After application to substrate and cured, the ink containing the notified chemical will be reacted into a polymeric resin product and will not be bioavailable.

6.1.2. Public exposure

The ink product containing the notified chemical will only be used for industrial purposes. The general public is only expected to come into contact with the notified chemical after the ink or coating formulation is cured to the substrate. Once dried and cured, the notified chemical will be reacted into a polymeric resin product and will not be bioavailable. Therefore, public exposure is not expected.

6.2. Human health effects assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2500 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute inhalation toxicity (analogue)	LC50 > 4.78 mg/L/4 hour; low toxicity
Rabbit, skin irritation	irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation – adjuvant test	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOEL = 100 mg/kg bw/day
Mutagenicity – bacterial reverse mutation (2 tests)	non mutagenic
Genotoxicity – in vitro Mammalian Chromosome Aberration Test	non genotoxic

Toxicokinetics, metabolism and distribution

Given the high partition coefficient (log P_{ow} of 5.16), uptake into the stratum corneum is expected to be high. However, given the low water solubility the rate of transfer from the stratum corneum to the epidermis is expected to be slow and hence percutaneous absorption will be limited. Given the high lipophilicity and low water solubility, oral and respiratory absorption may occur through micellular solubilisation.

Acute toxicity

The notified chemical is of low acute toxicity *via* the oral, dermal and inhalation routes.

Irritation

The notifier has classified the notified chemical as corrosive (R34). However, the scores of the skin irritation study conducted on rabbits indicated the notified chemical to be an irritant. In addition the notified chemical was only slightly irritating to the eye providing further support that the notified chemical is likely to be a skin irritant and not corrosive.

Sensitisation

The notified chemical is not expected to have the potential to cause skin sensitisation based on a skin sensitisation test using the Magnusson & Kligman method.

Repeated dose toxicity

The No Observed Effect Level (NOEL) was established as 100 mg/kg bw/day in the repeat dose toxicity study, based on the histopathological changes in the liver and thyroid observed in males and females at 500 mg/kg bw/day.

Genotoxicity

The notified chemical tested was not mutagenic in two bacterial reverse mutation studies and not genotoxic in an

in vitro mammalian chromosome aberration test.

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

Xi; R38 Irritating to skin

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
Based on the toxicological data provided, the notified chemical is irritating to skin and slightly irritating to eyes. It is not a skin sensitiser and is not mutagenic, and is of low acute oral, dermal and inhalation toxicity. There is potential for systemic toxicity via the oral or inhalation route, however systemic toxicity via the dermal route is not expected as percutaneous absorption is expected to be negligible given the high lipophilicity of the notified chemical.

Dermal and ocular exposure to the notified chemical at high concentrations may occur during formulation (at up to 100%) and printing processes (at up to 70%). Given formulation will be conducted in enclosed systems and all workers are expected to wear PPE, exposure should be low and hence the risk of irritation from the notified chemical is considered low.

Exposure is mainly expected by the dermal route as oral and inhalation exposure is expected to be negligible given the low volatility of the notified chemical and that formulation is expected to occur in enclosed systems using local exhaust ventilation. The worst case exposure is likely to occur when workers handle the neat notified chemical as introduced and the maximum systemic exposure is estimated to be 6 mg/kg bw/day (without PPE) based on 100% dermal absorption. Given a NOEL of 100 mg/kg bw/day for the notified chemical the margin of exposure (MOE) is 17. A MOE of greater than 100 is considered to be acceptable to account for intra- and interspecies differences, hence the notified chemical may pose a risk to the health of workers. However, workers are expected to wear PPE during formulation of inks and therefore, the risk of systemic toxicity from repeated exposure to the notified chemical during formulation and end use is not considered unacceptable.

Overall the risk to the health of workers posed by use of the notified chemical under the occupational settings described is not considered unacceptable.

6.3.2. Public health
The public will only have dermal exposure to dried inks containing the notified chemical, from which it is not expected to be bioavailable. Therefore the risk to 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, a UV curable resin for inks and printing materials, will be imported into Australia as ready-to-use ink cartridges and as a raw material for blending. Accidental spills during transport or reformulation are expected to be collected with inert material for disposal. Residues of the notified chemical remaining in import containers and wastes generated from cleaning blending equipment are expected to be collected and disposed of by an authorised waste disposal company.

RELEASE OF CHEMICAL FROM USE
The notified chemical is a component of formulating and printing preparations which will be printed onto plastic sheet and plastic plate articles. The notified chemical is consumed during the curing process and is completely incorporated into the resin product. Waste generated during the printing and curing process is expected to be disposed of by an authorised waste disposal company.

Printing cartridges will be designed to prevent leakage and will not be open during use, transport, installation or replacement. Therefore, release of the ink formulation containing the notified chemical to the environment is not expected under normal conditions of use. If spillage does occur, the ink will be physically contained with adsorbent material and disposed of to landfill.

RELEASE OF CHEMICAL FROM DISPOSAL
Empty cartridges and printed articles at the end of their useful life will be disposed to landfill or recycled. During the recycling, the notified chemical may partition to the sludge from washing water due to its low solubility, and be sent to landfill. The notified chemical remaining on the cleaned recycled articles is then melted with the article, incorporated into the new plastic articles, and eventually be sent to landfill at the end of the articles' useful life.

7.1.2 Environmental fate

The majority of the notified chemical will be sent to landfill from direct disposal of ink cartridges, articles (including recycled articles) at the end of their useful life, and sludge generated from plastic recycling processes. In landfill, the notified chemical is expected to be immobile based on its adsorption coefficient (log Koc = 3.84) and low water solubility. It is expected not to be readily biodegradable, and in the water compartment it is expected to adsorb to sludge and sediment. Whilst the notified chemical has a moderately low molecular weight (MW = 228) and a high partition coefficient (log Pow = 5.16), it is not likely to bioaccumulate based on its low bioconcentration factor (BCF < 347). In addition, the notified chemical is not expected to be bioavailable as it is completely consumed during the printing and curing process, and is incorporated into the matrix of the resin. The notified chemical will slowly degrade to form water and oxides of carbon. For the details of the environmental fate studies, refer to Appendix C.

7.1.3 Predicted Environmental Concentration (PEC)

The PEC for the notified chemical has not been calculated as there is no expected significant release to the water environment based on its 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	LC50 (96 h) = 1.9 mg/L	Toxic to fish
Daphnia Toxicity	EC50 (48 h) = 1.6 mg/L	Toxic to aquatic invertebrates
Algal Toxicity	E_rC50 (72 h) = 2.0 mg/L	Toxic to algae
Inhibition of Bacterial Respiration	IC50 (3 h) >3200 mg/L	Not harmful to microbial respiration

Under the Globally Harmonised System of Classification and Labelling of Chemicals (United Nations, 2009) the notified chemical is toxic to fish, aquatic invertebrates and algae, but was found to be not harmful to microbial respiration.

7.2.1 Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) for the notified chemical has not been calculated as there is no expected significant release to the water environment based on its reported use pattern.

7.3. Environmental risk assessment

The notified chemical is unlikely to be released to the aquatic compartment in environmentally significant concentrations. Therefore, based on its reported use pattern the notified chemical is not expected to pose a risk to the environment.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

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

Xi; R38 Irritating to skin

and

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 2009) 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
Aquatic environment	Acute category 2 Chronic category 2	Toxic to aquatic life Toxic to aquatic life with long lasting effects

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 expected to pose a risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- Safe Work Australia should consider the following health hazard classification for the notified chemical:
  - Xi; R38 Irritating to skin

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - Conc ≥ 20%: R38

CONTROL MEASURES

Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical during formulation activities:
  - Automated and/or enclosed systems.
  - Local exhaust ventilation if aerosols or mists are generated.

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced:
  - Avoid skin and eye contact.

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical during formulation and handling of ink products:
  - 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.

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

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

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

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 resin for ink and printing preparations for industrial use, or is likely to change significantly;
  - the amount of chemical being introduced has increased from 1 tonne per annum, 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 the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Freezing Point < -20 ± 0.5ºC

Method EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks Using BS4633: Method for the Determination of Crystallising Point.
Test Facility Safepharm Laboratories Limited (2002a)

Boiling Point 265 ± 0.5ºC at 101.64 kPa

Method EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks By differential scanning calorimetry, using ASTM E537-86.
Test Facility Safepharm Laboratories Limited (2002a)

Density 890 kg/m^3 at 20 ± 0.5ºC

Method EC Directive 92/69/EEC A.3 Relative Density.
Remarks Using pycnometer.
Test Facility Safepharm Laboratories Limited (2002a)

Vapour Pressure 1.6×10^{-2} kPa at 25ºC

Method EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks Using an isoteniscope system with measurements being made at several temperatures and linear regression analysis used to calculate the vapour pressure at 25ºC.
Test Facility Safepharm Laboratories Limited (2002b)

Water Solubility 1.48×10^{-2} g/L at 20 ± 0.5ºC

Method EC Directive 92/69/EEC A.6 Water Solubility.
Remarks Flask Method. Three mixtures of test material and double distilled water were shaken at 30°C and allowed to stand for a period of not less than 24 hours before centrifugation. The concentration of test material in the sample solutions were determined by gas chromatography.
Test Facility Safepharm Laboratories Limited (2002a)

Hydrolysis as a Function of pH $t_{½}$ > 1 year

Method EC Directive 92/69/EEC C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$ years*
4	25°	> 1
7	25°	> 1
9	25°	> 1

Remarks Sample solutions at pH 4, 7 and 9 were maintained at 50.0 ± 0.5°C in accordance with the guidelines above. After 5 days the concentration of test substance was determined by gas chromatography. There was less than 10% hydrolysis after 5 days at 50°C in each sample, which is equivalent to a half life greater than 1 year at 25°C.
Test Facility Safepharm Laboratories Limited (2002a)

Partition Coefficient (n-octanol/water) log Pow = 5.16 at 30ºC

Method EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks HPLC Method. The partition coefficient was determined by interpolation from a calibration curve constructed from known standards (log Pow range 0.3-6.2) in accordance with the guidelines above. As the notified chemical was determined to have a log Pow of 5.16, it is expected that the notified chemical will partition from water to oil.

Test Facility Safepharm Laboratories Limited (2002a)

Adsorption/Desorption log K_{oc} = 3.84 at 40°C
– screening test

Method OECD TG 121 Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge Using High Performance Liquid Chromatography (HPLC).
Remarks HPLC Method. The adsorption coefficient was determined by interpolation from a calibration curve constructed from known standards (log Koc range 1.25-5.63) in accordance with the guidelines above. As the notified chemical was determined to have a log Koc of 3.84, it is expected to be immobile in soil.
Test Facility Safepharm Laboratories Limited (2002a)

Flash Point 124 ± 2°C at 101.33 kPa

Method EC Directive 92/69/EEC A.9 Flash Point.
Remarks Using a closed cup equilibrium method.
Test Facility Safepharm Laboratories Limited (2002b)

Autoignition Temperature 194 ± 5°C

Method EC Directive 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks It was determined by heating aliquots of the test substance in a flask and observing for any ignition.
Test Facility Safepharm Laboratories Limited (2002b)

Surface Tension 59.1 mN/m at 21.0 ± 0.5°C

Method ISO 304, EC Directive 92/69/EEC A.5 Surface Tension.
Remarks Concentration: 1.55×10^{-2} g/L using a ring method. The test substance is considered to be a surface-active material.
Test Facility Safepharm Laboratories Limited (2002a)

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/Sprague-Dawley CD
- Vehicle: None
- Remarks - Method: No deviations from the protocol.

RESULTS

Number and Sex of animals	*Dose (mg/kg bw)*	*Mortality*
3 F	2000	0
3 F	2000	0

- LD50: > 2500 mg/kg bw
- Signs of Toxicity: There were no signs of systemic toxicity.
- Effects in Organs: No abnormalities were noted at necropsy.
- Remarks - Results: All animals showed expected gains in bodyweight over the study period.

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

TEST FACILITY: Safepharm Laboratories Limited (2002c)

B.2. 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/Sprague-Dawley CD
- Vehicle: None
- Type of dressing: Semi-occlusive.
- Remarks - Method: No deviations from the protocol.

RESULTS

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

- LD50: > 2000 mg/kg bw
- Signs of Toxicity - Local: There were no signs of dermal irritation.
- Signs of Toxicity - Systemic: There were no signs of systemic toxicity.
- Effects in Organs: No abnormalities were noted at necropsy.
- Remarks - Results: All animals showed expected gains in bodyweight over the study period except for one female which showed bodyweight loss during the first week but expected gain in bodyweight over the second week of the study.

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

TEST FACILITY: Safepharm Laboratories Limited (2003a)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE	Analogue chemical (Oxetane, 3,3'-[oxybis(methylene)]bis[3-ethyl-, CAS No. 18934-00-4)
METHOD	OECD TG 403 Acute Inhalation Toxicity. EC Directive 92/69/EEC, 93/21/EEC B.2 Acute Toxicity (Inhalation).
Species/Strain	Rats/Sprague-Dawley Crl:CD (SD) IGS BR
Vehicle	None
Method of Exposure	Oro-nasal exposure.
Exposure Period	4 hours
Physical Form	liquid aerosol
Particle Size	3.21 μm (mean mass aerodynamic diameter) Geometric standard deviation (GSD) = 1.67 Predicted amount less than 4 μm = 66.6%
Remarks - Method	No deviations from the protocol.

RESULTS

Number and Sex of Animals	*Concentration <mg/L>*		*Mortality*
	Nominal	*Actual*	
5 per sex	17.3	4.78 ± 0.36	0

LC50	> 4.78 mg/L/4 hours
Signs of Toxicity	Signs of hunched posture and pilo-erection are commonly seen in animals for short periods on removal from the chamber following 4-hour inhalation studies. Wet fur is commonly recorded both during and for a short period after exposure. These observations are considered to be associated with the restraint procedure and, in isolation, are not indicative of toxicity. In addition to the observations considered to be due to the restraint procedure, the following abnormalities were detected: During exposure, on removal from the test chamber and one hour after exposure, increased respiratory rate was noted in all animals. One day after exposure all animals appeared normal and no further observation were recorded throughout the remainder of the recovery period. Normal bodyweight development was noted during the study. Any apparent differences between the male and female bodyweight gain are due to expected differences in growth rates between male and female animals and not due to toxicity of the test substance.
Effects in Organs	No macroscopic abnormalities were detected at necropsy.
Remarks - Results	

CONCLUSION	The analogue chemical is of low toxicity via inhalation.
TEST FACILITY	Safepharm Laboratories Limited (2003b)

B.4. 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 M
Vehicle	None
Observation Period	14 days
Type of Dressing	Semi-occlusive.

Remarks - Method No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	2	2.3	2	4[1]	14 days	1[1]
Oedema	1	1	1	1	< 9 days	0[2]

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.
[1]Thickning of the skin
[2]Desquamation (characterised by dryness and sloughing of the skin)

Remarks - Results

Clinical signs
There were no signs of toxicity or ill health in any rabbit during the observation period.

Dermal reactions
Severe erythema with very slight oedema was observed in one animal accompanied by necrotic patches and thickening of the skin. The necrotic patches peeled off on day 7. Well-defined erythema with very slight oedema was seen in the remaining two animals. In addition, desquamation (characterised by dryness and sloughing of the skin) was observed in all animals. Reactions had resolved in two animals by either day 9 or 11, however, very slight erythema was still evident in the remaining animal at study termination on day 14.

CONCLUSION The notified chemical is irritating to the skin.

TEST FACILITY Huntingdon Life Sciences Ltd (2000a)

B.5. 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 1 M, 2 F
Observation Period 72 hours
Remarks - Method No deviations from the protocol. In the absence of skin irritation data a Rabbit Enucleated Eye Test was performed prior to the *in vivo* study. The results indicated that the test substance was unlikely to cause severe ocular irritancy.

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	0.3	0.3	1	< 48 hours	0
Conjunctiva: chemosis	0	0	0	1 (1 hour)	< 24 hours	0
Conjunctiva: discharge	0	0	0	1 (1 hour)	< 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 No corneal or iridial effects were noted during the study.

Minimal conjunctival irritation was noted in all treated eyes at the 1-hour observation and in two treated eyes at the 24-hour observation.
One treated eye appeared normal at the 24-hour observation and the remaining two treated eyes appeared normal at the 48-hour observation.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY Safepharm Laboratories Limited (2002d)

B.6. Skin sensitisation

TEST SUBSTANCE Notified Chemical

METHOD OECD TG 406 Skin Sensitisation - <Magnusson & Kligman Method>.
EC Directive 96/54/EC B.6 Skin Sensitisation - <Magnusson & Kligman Method>.

Species/Strain Guinea pig/albino Dunkin-Harley

PRELIMINARY STUDY Maximum Non-irritating Concentration: 100%
intradermal: 0, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 25, 50, 75% (v/v) and 100%
topical: 25, 50, 75% (v/v) and 100%

MAIN STUDY

Number of Animals Test Group: 10 M Control Group: 5 M

INDUCTION PHASE Induction Concentration:
intradermal: 25% v/v
topical: 100%

Signs of Irritation *Intradermal injections*
Necrosis was recorded at sites receiving Freund's Complete Adjuvant in test and control animals
Sight irritation was seen in test animals at sites receiving the test substance (25% v/v) and slight irritation was observed in control animals receiving Alembicol D.

Topical application
Slight erythema was observed in four test animals following topical application with the test substance at 100%.
Slight erythema was seen in one control animal.

CHALLENGE PHASE

challenge topical: 50% (v/v) and 100%

Remarks - Method No significant protocol deviations. The positive control study was not done concurrently with the test of the notified chemical.

RESULTS The notified chemical was dissolved in Alembicol D (a product of coconut oil) as vehicle.

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: challenge*	
		24 h	*48 h*
Test Group	100%	0/10	0/10
	50%	0/10	0/10
Control Group	0%	0/10	0/10

Remarks - Results *Clinical signs*
No signs of ill health or toxicity were observed.

Bodyweight
All animals showed expected gains in bodyweight over the study period.

Positive control

	The positive control, hexyl cinnamic aldehyde (HCA) was tested to produce evidence of skin sensitisation thus confirming the sensitivity and reliability of the experimental technique.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Huntingdon Life Sciences Ltd (2000b)

B.7. Repeat dose toxicity

TEST SUBSTANCE	Notified Chemical
METHOD	Analogous to OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. Analogous to EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/Crj:CD (Sprague-Dawley)
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	The high dose level was set at 500 mg/kg bw/day based on the results of a 7-day repeated dose toxicity study in rats using dose levels of 0, 100, 500 and 1000 mg/kg bw/day. No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
control	6 per sex	0	0
low dose	6 per sex	20	0
mid dose	6 per sex	100	0
high dose	6 per sex	500	0
control recovery	6 per sex	0	0
high dose recovery	6 per sex	500	0

Mortality and Time to Death

No mortality was observed during the treatment phase.

Clinical Observations

Salivation was observed in the male and female rats in the 100 and 500 mg/kg bw/day group immediately after administration. The onset of salivation occurred on day 5 of administration for the males and on day 6 of administration for the females in the 500 mg/kg bw/day group, and the symptom continued throughout the administration period. In the 100 mg/kg bw/day group, salivation was observed in one male only on day 7 of administration and in one female only on day 6 of administration. The salivation was a transient change occurring immediately after administration but disappearing by the afternoon observation, and no changes (miosis, lacrimation, etc.) suggesting the toxic effects of the test substance on the autonomic nervous system were observed.

Some motor activity reductions were observed in 2 males and one female at 100 mg/kg bw/day and one male at 500 mg/kg bw/day. However, functional observation (detailed clinical observation), sensory reactivity to stimuli, grip strength, and motor activity revealed no remarkable changes at any observation times or in any test substance treatment group.

As a consequence, the study author considered salivation have been induced not by a toxic effect of the test substance but by the bitter taste (irritation) of the test substance, and is of be of no toxicological significance.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

None of the body weight, food consumption, urinalysis, haematological or biochemical examinations, or necropsy revealed any test substance-related changes in any test substance treatment group.

Effects in Organs

Organ weight measurement revealed a high value of relative liver weight in males in the 500 mg/kg bw/day group and high values of absolute and relative liver weights in females in the 500 mg/kg bw/day group at the end of administration period. In addition, a tendency toward a high value of absolute liver weight, which was not significant, was observed in males in the 500 mg/kg bw/day group.

At the end of recovery period, a high value of relative spleen weight was observed in males in the 500 mg/kg bw/day group. Since thus change was slight and was not observed at the end of the administration period, it was considered to be unrelated to administration of the test substance.

Histopathological examination revealed test substance-related changes were observed in the liver and thyroid. In the liver, slight hypertrophy of the centrilobular hepatocytes was observed in 3 females in the 500 mg/kg bw/day group. In the thyroid, slight hypertrophy of the follicular cells was observed in 3 males and 3 females in the 500 mg/kg bw/day group.

In addition, slight fatty degeneration of the periportal hepatocytes was observed in one male and 5 females in the control group, 5 females in the 100 mg/kg bw/day group, and 2 males and 2 females in the 500 mg/kg bw/day group. Since this change was also observed in the control group, it was considered to be related to the administration of corn oil as a vehicle, rather than to administration of the test substance. Massive necrosis of hypatocytes and slight fatty degeneration of periportal hepatocytes were observed in one female in the 20 mg/kg bw/day group. Since these changes were spontaneous changes in this rat and were not related to the dose levels, they were considered to be unrelated to administration of the test substance.

Remarks – Results

The above-mentioned changes had recovered by the end of the recovery period of 14 days, suggesting good reversibility.

CONCLUSION

The No Observed Effect Level (NOEL) was established as 100 mg/kg bw/day, based on the histopathological changes in the liver and thyroid observed in males and females at 500 mg/kg bw/day.

TEST FACILITY Panapharm Laboratories (PPL) Co., Ltd. (2001)

B.8. 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	S9 was prepared from the livers of phenobarbital/β-naphthoflavone induced male Sprague-Dawley rats.
Concentration Range in Main Test	a) With metabolic activation: 0, 50, 150, 500, 1500, 5000 µg/plate b) Without metabolic activation: 0, 50, 150, 500, 1500, 5000 µg/plate
Vehicle	Dimethyl sulphoxide
Remarks - Method	No deviations from the protocol.

RESULTS

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

Remarks - Results

The test substance caused no visible reduction in the growth of the bacterial background lawn at any dose level, although a significant decrease in revertant colony frequency was noted to TA100 at 5000 µg/plate. The test substance was therefore tested up to the maximum recommended dose level of 5000 µg/plate. No test substance precipitate was observed on the plates at any of the doses treated in either the presence or absence of S9-mix.

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

Results for the negative controls (spontaneous mutation rates) were considered to be acceptable.

All of the positive control chemicals (N-ethyl-N'-nitro-N-nitrosoguanidine , 9-Aminoacridine and 4-Nitroquinoline-1-oxide) 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 Laboratories Limited (2003c)

B.9. Genotoxicity – bacteria

TEST SUBSTANCE	Notified Chemical
METHOD	Analogous to OECD TG 471 Bacterial Reverse Mutation Test. Analogous to EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Pre incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 was prepared from the livers of phenobarbital/β-naphthoflavone induced male Sprague-Dawley rats.
Concentration Range in Main Test	a) With metabolic activation: 0, 2.4, 4.9, 10, 20, 39, 78 µg/plate (for *S. typhimurium)* and 0, 313, 625, 1250, 2500, 5000 µg/plate (for *E. coli*) b) Without metabolic activation: 0, 0.61, 1.2, 2.4, 4.9, 10, 20 µg/plate (for *S. typhimurium)* and 0, 313, 625, 1250, 2500, 5000 µg/plate (for *E. coli*)
Vehicle	Dimethyl sulphoxide
Remarks - Method	No deviations from the protocol. Only *S. typhimurium* was tested in both main tests.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	≥ 20			
Test 1		≥ 10	> 5000	negative
Test 2		≥ 10	> 20	negative
Present	≥ 78			
Test 1		≥ 39	> 5000	negative
Test 2		≥ 39	> 78	negative

Remarks - Results	In both the dose-finding test and the main tests, neither increase in the number of revertant colonies more than twice as many as that of the negative control in any strains and at any doses of base-pair substitution type or frame-shift type, with or without metabolic activation, nor dose-related response was observed. The growth inhibition was observed at ≥ 10 µg/plate in the absence of metabolic activation and at ≥ 39 µg/plate in the presence of metabolic activation, and the precipitate of the test substance on the plates was not observed with or without metabolic activation. The revertant colonies of the positive controls showed increase more than twice that of the negative controls, confirming the validity of the study.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	BML Inc. (1999)

B.10. Genotoxicity – in vitro

TEST SUBSTANCE	Notified Chemical
METHOD	Analogous to OECD TG 473 In vitro Mammalian Chromosome Aberration Test. Analogous to EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.
Cell Type/Cell Line	The CHL/IU cell line derived from the lung of a female Chinese hamster.
Metabolic Activation System	S9 was prepared from the livers of phenobarbital/β-naphthoflavone induced male Sprague-Dawley rats.
Vehicle	Acetone
Remarks - Method	No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0, 12.5*, 25*, 50*, 75*, 100, 125	6	24
Test 2	0, 3.1*, 6.3*, 12.5*, 25*, 50*, 75*	24	24
Present			
Test 1	0, 25*, 50*, 100*, 150*, 200*, 250*	6	24

*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	78.2 (calculated)			
Test 1		> 50	> 125	negative
Test 2	39.1 (calculated)	> 50	> 75	negative
Present	156.2 (calculated)			
Test 1		> 200	> 250	negative

Remarks - Results	The incidences of structural chromosomal aberration or numerical aberration were less than 5% in all exposure groups. The incidences of structural aberrations in the positive control groups (mitomycin C and benzo(a)pyrene) were significantly higher than those in negative control groups (acetone). No statistical method was used for data analysis.
CONCLUSION	The notified chemical was not clastogenic to the CHL/IU cell line treated in vitro under the conditions of the test.
TEST FACILITY	Japan Oilstuff Inspectors' Corporation (2001)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I).
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Shimadzu TOC-5000 total organic carbon (TOC) analyser for dissolved organic carbon (DOC) and TOC, and gas chromatography/flame ionisation detector (GC/FID) for residual substance concentration.
Remarks - Method	The oxygen uptake of the test substance in inoculated medium was measured over 28 days in a darkened enclosed respirometer, conducted in accordance with the guidelines above. A reference control (aniline) was run in parallel. Biodegradation is expressed as the percentage oxygen uptake, corrected for the blank, of the theoretical uptake (ThOD). Test conditions were: 25 ± 1°C, pH 7.1-8.0.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	0	7	54
14	0	14	61
21	0	21	61
28	0	28	61

Remarks - Results	Based on biological oxygen demand (BOD), the test substance was not readily biodegradable under the conditions of the test. A decrease in test substance concentration, as determined by GC/FID, was attributed to adsorption of the test substance to the test vessel. The test is considered valid if the percentage degradation of aniline is > 65% after 14 days. The percentage degradation of aniline reached 61% after 14 days, narrowly missing the pass level, where upon degradation plateaued. Although the test is not considered valid, it appears the notified chemical is not readily biodegradable, especially given there was no degradation over 28 days.
CONCLUSION	The notified chemical is expected to be not readily biodegradable.
TEST FACILITY	Mitsubishi Chemical Safety Institute Ltd. (2000)

C.1.2. Bioaccumulation

TEST SUBSTANCE	Notified chemical
METHOD	Testing methods for New Chemical Substances (1974, amended 1998) as required by the Chemical Substances Control Law of Japan.
Species	Carp (*Cyprinus carpio*)
Exposure Period	Exposure: 60 days Depuration: 0 days
Auxiliary Solvent	2-methoxyethanol and HCO-40
Concentration Range	Nominal: 0.04 mg/L and 0.004 mg/L Actual: ~0.0348 mg/L and ~0.00355 mg/L

Analytical Monitoring	Gas chromatography/flame ionisation detector (GC/FID) for the determination of the test substance concentration.
Remarks - Method	The bioaccumulation test system was set up in triplicate. Two groups of carp were exposed to the test substance at different concentrations levels, in addition to a control. During the exposure period, the concentrations of the test substance in water and fish were measured periodically. There was no depuration period. Test conditions were: 23.2°C - 24.9°C, pH 6.9-7.2, 5.0 – 7.8 mg O_2/L, 67 mg $CaCO_3$/L.

RESULTS

Bioconcentration Factor	BCF < 347
CT50	Not determined
Remarks - Results	The concentrations of the test substance in the fish were calculated to be 4.36-12.78 µg/g at high concentration and 0.353-1.089 µg/g at low concentration, resulting in BCF ranges of 125-347 and 99-308 respectively. The fat content of the fish ranged from 6.0% (n = 3, 5.0-7.1%) at the beginning of the test to 4.6% (n=3, 4.3-5.0%) at the end of the test. There was no significant matter that may have affected the reliability of the test results during the exposure period of 60 days and, as there were no dead fish or abnormal conditions during this period, the test is considered valid. No steady state was reached within 60 days, and the steady state bioconcentration factor (BCF_{ss}) was not calculated accordingly.

CONCLUSION	The notified chemical does not have the potential for bioaccumulation.
TEST FACILITY	Mitsubishi Chemical Safety Institute Ltd. (2001)

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	Rainbow trout (*Onchorynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	Gas chromatography/flame ionisation detector (GC/FID) was used for the determination of the test substance concentration.
Remarks – Method	The test was conducted according to the guidelines above at nominal test substance concentrations of 0.44, 0.79, 1.4, 2.5, and 4.4 mg/L in the final definitive test. Due to the poor solubility of the test substance, a modification of the standard method for the preparation of aqueous media was undertaken. A saturated solution of the test substance was prepared by stirring an excess of test substance in dechlorinated tap water for a period of 24 hours and then the undissolved material was removed by filtration through a pre-conditioned filter. Test conditions were: 13.1-14.6°C, pH 7.5-8.0, 9.4-10.2 mg O_2/L, 16 h/8 h light/dark cycle. The median lethal concentration was taken as the geometric mean of the highest concentration showing no mortality and the lowest concentration showing 100% mortality.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*

0	0	10	0	0	0	0	0
0.44	0.49	10	0	0	0	0	0
0.79	0.93	10	0	0	0	0	0
1.4	1.6	10	0	0	0	0	0
2.5	2.8	10	0	10	10	10	10
4.4	5.0	10	10*	10	10	10	10

*After approximately 2.5 hours all the fish were observed to be moribund. These fish were killed and classed as mortalities.

LC50	1.9 mg/L at 96 hours (95% CI: 1.4 mg/L and 2.5 mg/L).
NOEC	0.44 mg/L at 96 hours.
Remarks – Results	The No Observed Effect Concentration (NOEC) is based on zero mortalities and the absence of any sub-lethal effects at this concentration. Sub-lethal effects were observed at test concentrations of 0.79, 1.4 and 2.5 mg/L, and included increased pigmentation, loss of equilibrium, swimming at the bottom of the test vessel, and the presence of moribund fish. There was no mortality in the control vessel, thus validating the test.

CONCLUSION The notified chemical is toxic to fish.

TEST FACILITY SafePharm Laboratories Limited (2003d)

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	250 mg $CaCO_3$/L
Analytical Monitoring	Gas chromatography/flame ionisation detector (GC/FID) was used for the determination of the test substance concentration.
Remarks - Method	The test was conducted according to the guidelines above at nominal test substance concentrations of 0.14, 0.25, 0.44, 0.79, 1.4, 2.5, 4.4 and 7.9 mg/L in the final definitive test. Due to the poor solubility of the test substance, a modification of the standard method for the preparation of aqueous media was undertaken. A saturated solution of the test substance was prepared by stirring an excess of test substance in dechlorinated tap water for a period of 24 hours and then the undissolved material was removed by filtration through a preconditioned filter. Test conditions were: 20.9-21°C, pH 7.9, 8.1-8.5 mg O_2/L, 16 h/8 h light dark cycle. Statistical values were calculated by the maximum-likelihood probit method (ToxCalc).

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	< LOQ	2 × 10	0	0
0.14	0.146	2 × 10	0	0
0.25	0.261	2 × 10	0	0
0.44	0.422	2 × 10	0	0
0.79	0.918	2 × 10	0	3
1.4	1.65	2 × 10	1	7
2.5	2.89	2 × 10	7	14

4.4	5.63	2 × 10	17	20
7.9	8.66	2 × 10	20	20

LC50	1.6 mg/L at 48 hours (95% CI: 1.4-2.0 mg/L)
NOEC	0.44 mg/L at 48 hours
Remarks - Results	There was no mortality observed in the control, thus validating the test.

CONCLUSION The notified chemical is toxic to aquatic invertebrates.

TEST FACILITY SafePharm Laboratories Limited (2003e)

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	*Scenedesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 0.88-14 mg/L Actual: 0.439-6.695 mg/L
Auxiliary Solvent	None
Water Hardness	0.15 mmol Ca^{2+} & Mg^{2+}
Analytical Monitoring	Gas chromatography/flame ionisation detector (GC/FID) was used for the determination of the test substance concentration. Cell concentrations were determined by a Coulter Multisizer Particle Counter.
Remarks - Method	After a range finding test, the definitive test was conducted according to the guidelines above at nominal test substance concentrations of 0.88, 1.75, 3.5, 7.0 and 14 mg/L in triplicate. Due to the poor solubility of the test substance, a modification of the standard method for the preparation of aqueous media was undertaken. A saturated solution of the test substance was prepared by stirring an excess of test substance in dechlorinated tap water for a period of 24 hours and then the undissolved material was removed by filtration through a preconditioned filter. Test conditions: 24 ± 1°C, pH 7.5-8.3, continuous illumination. Statistical values were determined by Bartlett's test for homogeneity of variance and Dunnett's multiple comparison procedure (SAS).

RESULTS

Biomass		*Growth*	
E_bC_{50} mg/L at 72 h	*NOEC mg/L*	*E_rC_{50} mg/L at 72 h*	*NOEC mg/L*
1.2	0.88	2.0	0.88

Remarks - Results	Based on mean measured test concentrations the E_bC50 and E_rC50 at 72 hours were 0.58 mg/L and 1.0 mg/L (95% CI: 0.89-1.1 mg/L), and the NOEC was 0.439 mg/L. However, a decline in the measured test concentration was considered to be due to adsorption of the test substance to algal cells. Cell density in the control increased 80-fold, thus validating the test.

CONCLUSION The notified chemical is toxic to algae.

TEST FACILITY SafePharm Laboratories Limited (2003f)

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 sewage sludge microorganisms
Exposure Period	3 hours
Concentration Range	Nominal: 10-3200 mg/L Actual: Not reported
Remarks – Method	After a range finding test, the definitive test was conducted according to the guidelines above at nominal test substance concentrations of 10, 32, 100, 320, 1000 and 3200 mg/L with the addition of a synthetic sewage of a respiratory substrate. A blank control and reference (3,5-dichlrorphenol) control were run in parallel. The rate of respiration was determined after 3 h contact time and compared to the results from the control and reference material. Test conditions: 21°C, pH 7.6-7.9, 100 mg $CacO_3$/L.
RESULTS	
IC50	> 3200 mg/L
NOEC	10 mg/L
Remarks – Results	At all test concentrations tested, oily globules of test material were visible on the surface, and dispersed throughout the test media at 0 h, 30 min and 3 h contact time. The reference compound IC50 (3 h) was < 32 mg/L, and the variation in the respiration rates of the control at 3 h was ± 1%, thus validating the test.
CONCLUSION	The notified chemical is not harmful to microbial respiration
TEST FACILITY	SafePharm Laboratories Limited (2003g)

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