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September 2010

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Oxetane, 3,3'-[oxybis(methylene)]bis[3-ethyl-

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

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

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

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Director
NICNAS

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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
USA (2006), Japan (2003), EU (2001) and UK (2003)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Aron Oxetane OXT-221

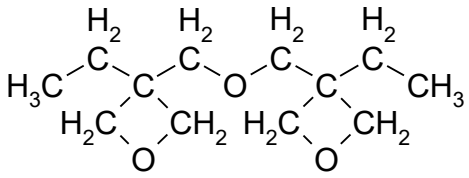
CAS NUMBER
18934-00-4

CHEMICAL NAME
Oxetane, 3,3'-[oxybis(methylene)]bis[3-ethyl-

OTHER NAME(S)
DOX

MOLECULAR FORMULA
$C_{12}H_{22}O_3$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
214 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 liquid

Property	Value	Data Source/Justification
Freezing Point	< -20°C	Measured
Boiling Point	~291°C at 101.05 – 102.40 kPa (with minor decomposition)	Measured
Density	996 kg/m^3 at 20°C	Measured
Vapour Pressure	4.24 × 10^{-2} kPa at 25°C	Calculated
Water Solubility	34.6 g/L at 20°C	Measured
Hydrolysis as a Function of pH	$t_{½}$ > 1 year, pH 4-9	Measured
Partition Coefficient (n-octanol/water)	log Pow = 2.34 at 40°C	Measured
Adsorption/Desorption	log K_{oc} = 1.68 at 40°C	Measured
Dissociation Constant	Not determined	The notified chemical does not contain dissociable functionality
Particle Size	Not determined	Liquid
Flash Point	136°C at 101.18 kPa	Measured
Flammability	Not expected to be highly flammable.	Based on flash point.
Autoignition Temperature	200°C	Measured
Explosive Properties	Negative	There are no chemical groups that would infer explosive properties.
Surface Tension	58.1 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 and as a component of ink preparations and in printer cartridges (at < 70%).

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤ 1	≤ 5	≤ 5	≤ 5	≤ 10

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 preparations, which will be printed onto plastic sheet and plastic plate articles.

OPERATION DESCRIPTION
The concentrate containing the notified chemical at 100% 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 < 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 is 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 concentrate containing the notified chemical at 100% is possible during handling of the import containers, cleaning and maintenance of the equipment. Skin, inhalation and eye contact (due to splashing) are likely to be the main routes of exposure. The level of exposure would vary from site to site depending on the level of automation of the formulation process. Exposure 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 without PPE. NICNAS estimated dermal exposure without PPE 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 without PPE. 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. Oral exposure is not expected. 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, the ink containing the notified chemical is cured into an inert plastic plate or sheet and exposure is not expected.

6.1.2. Public exposure

The ink product containing the notified chemical will only be used for industrial purposes. The public will not be exposed to the neat substance or the concentrated product except in the event of accidental spillage during road transportation. 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 and is not bioavailable. Therefore, public exposure is not expected.

6.2. Human health effects assessment

The results from toxicological investigations conducted on the notified chemical or structurally similar 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 300-500 mg/kg bw; harmful
Rat, acute dermal toxicity (analogue)	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute inhalation toxicity	LC50 > 4.78 mg/L/4 hour; low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation – adjuvant test	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days	NOEL = 40 mg/kg bw/day (M), 200 mg/kg bw/day (F)
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosome aberration test	genotoxic
Genotoxicity – in vivo mammalian erythrocyte micronucleus test	non genotoxic

Toxicokinetics, metabolism and distribution

Given a log Pow at 2.34 (at 40ºC) and low molecular weight, there is potential for absorption through dermal, oral and respiration routes. Bioaccumulation is not likely given the high water solubility of the notified chemical.

Acute toxicity

The notified chemical is of low acute toxicity *via* the dermal (based on studies conducted on the analogue Oxetane, 3-ethyl-3-[[(2-ethylhexyl)oxy]methyl]- CAS No. 298695-60-0) and inhalation routes but harmful *via* the oral route.

Irritation

The notified chemical is slightly irritating to the skin and eyes.

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

A high level of liver weight and hypertrophy of the centrilobular hepatocytes were observed in males and females at 1000 mg/kg bw/day during repeated administration of the test substance to rats for 28 days. In addition, a high value of kidney weight, which did not accompany the histopathological changes, was observed in males at 1000 mg/kg bw/day, and a high value of urine specific gravity was observed in males in the 200 and 1000 mg/kg bw/day groups. These changes were reversible at the end of the recovery period of 14 days.
The No Observed Effect Level (NOEL) of 40 mg/kg bw/day for males and 200 mg/kg bw/day for females were established under the conditions of this study.

Genotoxicity

The notified chemical was not mutagenic in a bacterial reverse mutation study but was found to be genotoxic in an *in vitro* mammalian chromosome aberration test. However, the notified chemical was not genotoxic in an *in*

vivo mammalian erythrocyte micronucleus 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:

Xn; R22 Harmful if swallowed

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
Based on the toxicological data provided, the notified chemical is slightly irritating to skin and eyes and is harmful *via* the oral route. It is not a skin sensitiser and is not mutagenic, and is of low acute dermal and inhalation toxicity.

Dermal and ocular exposure to the notified chemical at high concentrations may occur during formulation (at up to 100%) and when using printing ink (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 ink 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 based on 100% dermal absorption. Using the NOEL of 40 mg/kg bw/day for the notified chemical the margin of exposure (MOE) is calculated as 7. 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 is used without PPE during formulation of inks. 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 use of inks 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 on substrates, 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. The notified chemical is a volatile compound and a fraction of the imported quantity of this chemical will partition to air during ink reformulation. Formulations containing the notified chemical, however, are non-volatile. 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 recycling, the notified chemical is expected to be melted with the article for incorporation into new plastic articles and will eventually be sent to landfill at the end of the articles' useful life.

7.1.2 Environmental fate

The notified chemical will be sent to landfill from direct disposal of ink cartridges and articles incorporating the notified chemical (including recycled articles) at the end of their useful life. In landfill, the notified chemical residues from cartridges may leach, due to the notified chemical's ready water solubility and low soil adsorption coefficient. It is not biodegradable, but the notified chemical is not expected to bioaccumulate based on its ready water solubility, low partition coefficient and bioconcentration factor (BCF < 32).

The notified chemical is a volatile compound and a fraction of the imported quantity of this chemical will partition to air during ink reformulation. The half-life of the notified chemical in air was calculated to be 5.2 h, based on reactions with hydroxyl radicals over a 12 hour day, and reaction with ozone is not expected (AOPWIN, v1.92; EPISuite, US EPA 2009). The notified chemical is therefore not expected to persist in the air compartment.

The majority of the notified chemical will be consumed during printing and will be irreversibly incorporated into the resin matrix. Notified chemical in the cured resin on plastic articles is not expected to be mobile or be bioavailable. The notified chemical is likely to 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 predicted environmental concentration (PEC) for the notified chemical has not been calculated as it is a UV curable ink which will be irreversibly incorporated into resin matrices and is unlikely to be released to the aquatic compartment in environmentally significant concentrations.

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) = 76 mg/L	Harmful to fish
Daphnia Toxicity	EC50 (48 h) >100 mg/L	Not harmful to aquatic invertebrates
Algal Toxicity	E_rC50 (72 h) >100 mg/L	Not harmful to algae
Inhibition of Bacterial Respiration	IC50 (3 h) = 2800 mg/L	Does not inhibit respiration of waste water microorganisms

Under the Globally Harmonised System of Classification and Labelling of Chemicals (United Nations, 2009) the notified chemical is harmful to fish, but is not harmful to aquatic invertebrates and algae. The notified chemical is not readily biodegradable and, based on its acute toxicity to fish, it is classified as harmful to aquatic life with long lasting effects.

7.2.1 Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) for the notified chemical has not been calculated as it is a UV curable ink which will be irreversibly incorporated into resin matrices and is unlikely to be released to the aquatic compartment in environmentally significant concentrations.

7.3. Environmental risk assessment

The notified chemical is a UV curable ink which will be irreversibly incorporated into resin matrices and 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:

Xn; R22 Harmful if swallowed

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***
Acute toxicity	4	Harmful if swallowed
Aquatic environment	Acute category 3 Chronic category 3	Harmful to aquatic life Harmful 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:
  - Xn; R22 Harmful if swallowed

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - Conc ≥ 25%: R22

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.

- 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 repeatedly during formulation and handling of ink preparations:
  - 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 chemical 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 ink preparations for industrial use, or is likely to change significantly;
  - the amount of chemical being introduced has increased from 10 tonne per year, 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 ~291ºC at 101.05 – 102.40 kPa (with minor decomposition)

Method EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks By differential scanning calorimetry, using ASTM E537-86. Some decomposition was observed prior to boiling at ~200 ºC, this response was greater under air, indicating this was probably due to oxidation.
Test Facility Safepharm Laboratories Limited (2002a)

Density 996 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 4.24×10^{-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 (2003a)

Water Solubility 34.6 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, pH 4-9

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 = 2.34 at 40ºC

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 0.9-3.6) in

accordance with the guidelines above.
Test Facility Safepharm Laboratories Limited (2002a)

Adsorption/Desorption – screening test log K_{oc} = 1.68 at 40°C

Method EC Directive 200/59/EC C.19 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 K_{oc} range 1.25-5.63) in accordance with the guidelines above. As the notified chemical was determined to have a log K_{oc} of 1.68, it is expected to be mobile in soil.
Test Facility Safepharm Laboratories Limited (2002a)

Flash Point 136 ± 2ºC at 101.18 kPa

Method EC Directive 92/69/EEC A.9 Flash Point.
Remarks Using a closed cup equilibrium method.
Test Facility Safepharm Laboratories Limited (2003a)

Autoignition Temperature 200 ± 5ºC

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 (2003a)

Surface Tension 58.1 mN/m at 21.0 ± 0.5ºC

Method ISO 304, EC Directive 92/69/EEC A.5 Surface Tension.
Remarks Concentration: 1.09 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
- Species/Strain: Rat/Sprague-Dawley CD
- Vehicle: Arachis oil BP for 300 mg/kg bw dose group. Test substance used undiluted for 2000 mg/kg bw dose group.
- Remarks - Method: No deviations from the protocol.

RESULTS

Group	*Number and Sex of animals*	*Dose (mg/kg bw)*	*Mortality*
1	3 F	2000	1
2	3 F	2000	3
3	3 F	300	0
4	3 F	300	0

- LD50: 300-500 mg/kg bw
- Signs of Toxicity: Signs of systemic toxicity noted in animals treated at dose level of 2000 mg/kg bw during the day of dosing were decreased respiratory rate, laboured and/or noisy respiration, occasional body tremors and coma. Hunched posture was noted in one animal four hours and one day after dosing. One animal appeared normal throughout the study.
  There were no signs of systemic toxicity noted in animals treated at a dose level of 300 mg/kg bw.
- Effects in Organs: Abnormalities noted at necropsy of animals that died during the study were dark liver, pale liquid present in the stomach and sloughing of the non-glandular region of the stomach. No abnormalities were noted at necropsy of animals that survived to the end of the study.
- Remarks - Results: Four animals out of six treated at 2000 mg/kg bw were found dead during the day of dosing and there were no deaths noted at 300 mg/kg bw.
  The surviving animals showed expected gains in bodyweight over the study period.

CONCLUSION: The notified chemical is harmful via the oral route.

TEST FACILITY: Safepharm Laboratories Limited (2002b)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Analogue Chemical (Oxetane, 3-ethyl-3-[[(2-ethylhexyl)oxy]methyl]- CAS No. 298695-60-0)

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 analogue chemical is of low toxicity via the dermal route.

TEST FACILITY Safepharm Laboratories Limited (2003b)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE Notified Chemical

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	Mean mass aerodynamic diameter (MMAD) = 3.21 μm 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 notified chemical is of low toxicity via inhalation.

TEST FACILITY Safepharm Laboratories Limited (2003c)

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	1 M, 2 F
Vehicle	None
Observation Period	9 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	0.7	0.7	1	2[1]	< 9 days	0
Oedema	0.7	0	0	2	< 6 days	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.
[1]Thickening 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
Very slight to well-defined erythema with or without slight oedema was seen in all animals, resolving completely by either day 7 or 9. In addition, thickening of the skin was observed in one animal.

CONCLUSION: The notified chemical is slightly 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	7 days
Remarks - Method	No deviations from the protocol.

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	2	1.7	1.3	2	< 7 days	0
Conjunctiva: chemosis	1.3	1	0.3	2	< 7 days	0
Conjunctiva: discharge	1.3	1	1	2	< 7 days	0
Corneal opacity	1	0.7	0	1	< 7 days	0
Iridial inflammation	0.7	0.3	0	1	< 72 hours	0

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

Remarks - Results	Scattered or diffuse corneal opacity was noted in two treated eyes at the 24 and 48-hour observations and persisted in one treated eye at the 72-hour observation. Iridial inflammation was noted in all treated eyes 1 hour after the treatment, in two treated eyes at the 24-hour observation and persisted in one treated eye at the 48-hour observation. Conjunctival irritation was noted in all treated eyes one and 24 hours after treatment. Conjunctival irritation was noted in two treated eyes with minimal conjunctival irritation in one treated eye at the 48-hour observation. Conjunctival irritation was noted in one treated eye with minimal conjunctival redness in two treated eyes at the 72-hour observation. All treated eyes appeared normal at the 7-day observation.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	Safepharm Laboratories Limited (2002c)

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: 10% 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 (10% v/v in Alembicol D) and slight irritation was observed in control animals receiving Alembicol D. *Topical application* Slight erythema was observed in seven test animals following topical application with the test substance as supplied in Alembicol D. Slight erythema was seen in one control animal.
CHALLENGE PHASE	
challenge	topical: 37.5% (v/v) and 75%
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
Negative/Vehicle Control Group	0%	0/5	0/5

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

OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.

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 1000 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, which showed clear effects in males and females. The middle- and low-dose levels were set at 200 and 40 mg/kg bw/day, respectively, as 5-fold dose decrement.

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	40	0
mid dose	6 per sex	200	0
high dose	6 per sex	1000	0
control recovery	6 per sex	0	0
high dose recovery	6 per sex	1000	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 200 and 1000 mg/kg bw/day group immediately after administration. The onset of salivation occurred on day 1 of administration for the males and females in the 1000 mg/kg bw/day group and on day 10 of administration for the males in the 200 mg/kg bw/day group, and the symptom continued throughout the administration period. The salivation was a transient change occurring immediately after administration but disappearing by the afternoon observation. In addition, trauma was observed in one female in the control group on day 21 of administration, and it continued throughout the

administration period.
No abnormal change was observed throughout the recovery period.
There was no difference between the control group and any test substance group in the function observation, sensory reactivity and grip strength of the foreleg or hind leg to stimuli before the start of administration, or during the administration or recovery period.
In week 4 of administration, a high value of motor activity in males in the 40 mg/kg bw/day group was observed at 50-60 minute-intervals after the start of the measurement,. Since this was not related to the dose levels, it was considered to be unrelated to administration of the test substance.
There was no difference between the control group and any test substance group in the motor activity in the recovery period.
The body weight changes of all test substance groups were almost the same as those of control group throughout the administration and recovery periods.
A high value of food consumption was observed in males in the 1000 mg/kg bw/day group on day 28 of administration. Since it was slight and had no toxicological significance, it was considered to be unrelated to administration of the test substance.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
For urinalysis, in week 4 of administration, a high value of specific gravity was observed in the 200 and 1000 mg/kg bw/day groups. There were no remarkable changes in any test substance groups in the recovery period.

For hematological examination, a low value of monocytes in females in the 40 mg/kg bw/day group was observed at the end of administration period. Since the change was slight and was not related to the dose levels, it was considered to be unrelated to administration of the test substance. At the end of the recovery period, a high value of erythrocytes and a low value of MCHC (mean corpuscular hemoglobin concentration) were observed at the end of the administration period, they were considered to be unrelated to administration of the test substance.

For biochemical examination, at the end of the administration period, high values of γ-GTP and BUN and low values of glucose and chloride were observed in males in the 1000 mg/kg bw/day group. In addition, a high value of sodium was observed in females in the 1000 mg/kg bw/day group, but this was slight and was considered to be unrelated to administration of the test substance.
At the end of recovery period, a low value of ALP and high values of total cholesterol and phospholipids were observed in males in the 1000 mg/kg bw/day group. Low values of A/G ratio and ALP and high values of total cholesterol and phospholipids were observed in females in the 1000 mg/kg bw/day group. Since these changes were not observed at the end of the administration period, they were considered to be unrelated to administration of the test substance.

Effects in Organs
At necropsy, no test substance-related abnormal findings were observed at the end of administration period.
At the end of administration period, small testis and epididymis were observed in one male in the 1000 mg/kg bw/day group. Since these changes occurred unilaterally, and no similar changes were observed in any other male in the 1000 mg/kg bw/day group, they were considered to be unrelated to administration of the test substance. Light yellow nodules of the epididymis (unilateral) in one male in the control group and trauma of the skin were observed in one female in the control group. These changes were considered to be unrelated to administration of the test substance because they occurred in the control group.
No abnormal findings were observed at the end of recovery period.

A high value of relative liver weight in males and females in the 1000 mg/kg bw/day group and high value of relative kidney weight in males in the 1000 mg/kg bw/day group were observed at the end of administration period.
In addition, high values of relative brain and adrenal weights were observed in females in the 1000 mg/kg bw/day group. Since these changes were slight and within the normal ranges, they were considered to be unrelated to administration of the test substance.
At the end of the recovery period, a high value of relative liver weight was observed in males and females in the 1000 mg/kg bw/day group, and high values of absolute and relative kidney weights were observed in males at 1000 mg/kg bw/day.
In addition, high values of absolute and relative adrenal weights were observed in males at 1000 mg/kg bw/day. Since these changes were not observed at the end of administration period, they were considered to be unrelated to administration of the test substance.

Test substance-related changes were observed in the liver. At the end of administration, slight hypertrophy of the centrilobular hepatocytes was observed in one male and 3 females in the 1000 mg/kg bw/day group. This change was not observed at the end of the recovery period. There were no histopathological changes in the kidneys related to the weight changes.
In addition, atrophy of the seminiferous tubule of the testis and decreased sperm in the lumen of the epididymis were observed in one male in the 1000 mg/kg bw/day group. These changes were spontaneous changes in this strain of rat and occurred unilaterally in this study. Moreover, no abnormal changes in the testes were observed in any other males at 1000 mg/kg bw/day. They were therefore considered to be unrelated to administration of the test substance. Fatty degeneration of the periportal hepatocytes, subcapsular granuloma of the liver, the appearance of the foam cells in the lungs, cyst of kidney, and spermatic granuloma of the epididymis were observed in the control, 200 and 1000 mg/kg bw/day groups. Since their incidences and degrees were not related to the dose levels, these changes were considered to be unrelated to administration of the test substance.

Remarks – Results
A high level of liver weight and hypertrophy of the centrilobular hepatocytes were observed in males and females at 1000 mg/kg bw/day during repeated administration of the test substance. In addition, a high value of kidney weight, which did not accompany the histopathological changes, was observed in males in the 1000 mg/kg bw/day group, and a high value of urine specific gravity was observed in males at 200 and 1000 mg/kg bw/day.
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 40 mg/kg bw/day for males and 200 mg/kg bw/day for females under the conditions of this study.

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

B.8. Genotoxicity – bacteria

TEST SUBSTANCE	Notified Chemical
METHOD	Analogous to OECD TG 471 Bacterial Reverse Mutation Test. 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, 156, 313, 625, 1250, 2500, 5000 µg/plate b) Without metabolic activation: 0, 156, 313, 625, 1250, 2500, 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	> 1250	> 1250	> 5000	negative
Present				
Test	> 1250	> 1250	> 5000	negative

Remarks - Results

In both the dose-finding test and main test, there was 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 observed.

The revertant colonies of the positive controls showed an increase more than twice that of the negative controls, 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

BML Inc. (1999)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE

Notified Chemical

METHOD

Analogous to OECD TG 473 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	0, 250*, 500*, 1000*, 1500*, 2000*, 2500	6	24
Present			
Test	0, 250*, 500*, 1000*, 1500*, 2000*, 2500	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				
Test	1251.8 (calculated)	> 1500	> 2500	positive
Present				
Test	1251.5 (calculated)	> 1500	> 2500	positive

Remarks - Results

In the cell growth inhibition test, precipitated test substance was observed in the medium before incubation, but was not observed after incubation.

In the pulse treatment chromosomal aberration test, the incidence of structural chromosomal aberration was 37.0% at 2000 µg/mL without metabolic activation and 30.0% at 2000 µg/mL with metabolic activation.

The incidences of numerical aberration were less than 5% in all treatment groups both with and without metabolic activation. The incidences of structural aberrations in the positive control groups (mitomycin C and benzo(a)pyrene) were significantly higher than the ones in the negative control groups (acetone).

CONCLUSION The notified chemical was clastogenic to the CHL/IU cell line treated in vitro under the conditions of the test.

TEST FACILITY Japan Oilstuff Inspectors' Corporation (2002)

B.10. Genotoxicity – in vivo

TEST SUBSTANCE Notified chemical

METHOD OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
EC Directive 2000/32/EC B.12 Mutagenicity - Mammalian Erythrocyte Micronucleus Test.

Species/Strain Mouse/ albino Crl:CD-1(ICR)BR
Route of Administration Oral – gavage
Vehicle Arachis oil
Remarks - Method In the range-finding toxicity test, the test substance showed no marked difference in its toxicity to male or female mice. It was therefore considered acceptable to use males only for the micronucleus test.
In the range-finding toxicity test, doses of 1500 mg/kg and above produced premature deaths and clinical signs of toxicity. The maximum tolerated dose (MTD) of the test substance was selected as 1000 mg/kg bw.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	7 + 7 M	0	24 & 48
II (low dose)	7 M	250	24
III (mid dose)	7 M	500	24
IV (high dose)	7 + 7 M	1000	24 & 48
V (positive control, CP)	5 M	50	24

CP=cyclophosphamide.

RESULTS

Doses Producing Toxicity There were no premature deaths seen in any of the dose groups. Clinical signs were not observed in animals dosed with the test substance.

Genotoxic Effects No statistically significant decreases in the PCE/NCE (polychromatic erythrocytes/normochromatic erythrocytes) ratio were observed in the 24 or 48-hour test substance dose groups where compared to their concurrent control groups. However, in both of the 1000 mg/kg test substance dose groups marked reduction in PCE/NCE ratios were observed and this was taken to indicate that systematic absorption and exposure to the target tissue had occurred.

There were no statistically significant increases in the frequency of micronucleated PCEs in any of the test substance dose groups when compared to their concurrent vehicle control groups.

The positive control group showed a marked increase in the incidence of micronucleated polychromatic erythrocytes hence confirming the sensitivity of the system to the known mutagenic activity of cyclophosphamide under the conditions of the test.

Remarks - Results	The test substance was found not to produce a significant increase in the frequency of micronuclei in polychromatic erythrocytes of mice under the conditions of the test.
CONCLUSION	The notified chemical was not clastogenic under the conditions of this in vivo micronucleus test in the mouse.
TEST FACILITY	Safepharm Laboratories Limited (2004)

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 6.4-7.9.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	0	7	65
14	0	14	70
21	0	21	68
28	0	28	66

Remarks - Results	The pass level (60% of ThOD) was not reached by the test substance within a ten day window, or over the test period, thus it is not considered to be readily biodegradable. The percentage degradation of the reference substance (aniline) surpassed the pass level by day 7, thereby validating the test.
CONCLUSION	The notified chemical is 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: 28 days Depuration: 0 days
Auxiliary Solvent	None
Concentration Range	Nominal: 0.2 mg/L and 0.02 mg/L Actual: ~0.191 mg/L and ~0.0179 mg/L
Analytical Monitoring	Gas chromatography/flame ionisation detector (GC/FID) for the determination of the test substance concentration.
Remarks - Method	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. The bioconcentration factor (BCF) was determined by comparing the concentration of the test substance in the fish to the mean concentration

of test substance in the test water. Test conditions were: 23.2°C - 24.9°C, pH 7.1-7.2, 7.7-8.4 mg O_2/L, 61 mg $CaCO_3$/L.

RESULTS

Bioconcentration Factor	BCF < 32
CT50	Not determined
Remarks - Results	The concentrations of the test substance in the fish at the end of the test were determined to be ≤ 0.62 µg/g at high concentration and ≤ 0.57 µg/g at low concentration, resulting in BCF values of ≤ 4 and ≤ 32 respectively. The fat content of the fish ranged from 7.7% (n = 3, 6.8-8.5%) at the beginning of the test to 8.0% (n=3, 7.8-8.1%) 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 28 days and, as there were no dead fish or abnormal conditions during this period, the test is considered reliable.

CONCLUSION The notified chemical has a low potential to bioaccumulate in fish

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; Limit of quantitation (LOQ) was determined to be 3.1 mg/L.
Remarks – Method	After a range-finding test, a definitive test at nominal concentrations 5.6, 10, 18, 32, 56 and 100 mg/L was conducted according to the guidelines above. The fish, 10 per test solution, were observed for mortality and sublethal responses every 24 hours. Test conditions were: 14.0°C, pH 7.6-8.33, and 8.5-9.9 mg O_2/L. Statistical values were determined by Spear-Kaber method (ToxCalc software, 1999).

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual**		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	< LOQ	10	0	0	0	0	0
5.6	4.5	10	0	0	0	0	0
10	8.2	10	0	0	0	0	0
18	16	10	0	0	0	0	0
32	29	10	0	0	0	0	0
56	51	10	0	0	0	0	0
100	97	10	0	0	0	0	8

*Mean measured concentration

LC50	76 mg/L at 96 hours (95% CI: 67-87 mg/L).
NOEC	16 mg/L at 96 hours.
Remarks – Results	Sublethal effects of exposure, swimming at the bottom of the test vessel

and/or increased pigmentation, were observed at test concentrations of 32 mg/L and above.
The pH of the control group varied between 7.6 and 8.3. Although the test guidelines state that the pH should not vary by more than one pH unit, this variation is not considered to affect the validity or integrity of the test as no adverse reactions were observed in the control group.
Actual measured test concentrations ranged from 61-110% of nominal, and sampling, analytical variation and/or volatility of the test sample may account for the various loss of the test substance. On the majority of occasions no significant loss of test substance was shown over each 24-hour renewal period, however results of less than 80% of nominal were observed. Therefore, the results were calculated on the mean measured test concentrations to give a worst case analysis.
There was no mortality in the control group after 96 hours of exposure, thereby validating the test.

CONCLUSION The notified chemical is harmful 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 – 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; Limit of quantitation (LOQ) was determined to be 3.1 mg/L.
Remarks - Method	After a range finding test, a limit test at a nominal concentration of 100 mg/L was conducted according to the guidelines above. Four replicates each had 10 daphnia added. The daphnia were observed for immobilisation every 24 hours over the course of the test. Test conditions were: 21.0°C, 16 h/8 h light dark cycle, 8.5-8.7 mg O_2/L, pH 7.9-8.1.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	< LOQ	2 × 10	0	0
100	79.7-97.6	4 × 10	0	0

EC50	> 100 mg/L at 48 hours
NOEC	100 mg/L at 48 hours
Remarks - Results	All test solutions appeared clear and colourless throughout the test. After 48 hours of exposure, there was no immobility observed in the test concentrations or control, thereby validating the test.

CONCLUSION The notified chemical is not harmful 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	*Pseudokirchneriella subcapitata* (formerly known as *Scenedesmus subspicatus*)
Exposure Period	72 hours
Concentration Range	Nominal: 100 mg/L Actual: 88-101 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, a limit test at a concentration of 100 mg/L was conducted according to the guidelines above. Test conditions: 24 ± 1°C, pH 7.2–7.6, continuous illumination. Statistical values were determined by a Students Test incorporating Bartlett's test for homogeneity of variance (SAS software, 1996).

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*
> 100	100	> 100	100

Remarks - Results	There were no abnormalities detected in any of the control or test cultures when inspected microscopically at 72 hours. The test was considered valid according to current OECD TG 201 guidelines.
CONCLUSION	The notified chemical is not harmful to algae.
TEST FACILITY	SafePharm Laboratories Limited (2002d)

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
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-dichlorophenol) 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 7.9–8.2, 100 mg $CaCO_3$/L.
RESULTS	
IC50	2800 mg/L at 3 hours
NOEC	320 mg/L
Remarks – Results	There was a relatively large increase in the respiration rate observed in the test vessels. It was reported to be possibly due to a hormetic response

	of the activated sewage sludge microorganisms to the test substance, i.e. the stimulation of biological activity due to the presence of test substance at concentrations below its toxic level. The 3 h IC50 of the reference compound (11 mg/L) was found to be within the expected normal range of 5 to 30 mg/l, and the variation in the respiration rates of the control at 3 h was ± 1%, thereby validating the test.
CONCLUSION	The notified chemical does not inhibit respiration of waste water microorganisms
TEST FACILITY	SafePharm Laboratories Limited (2002e)

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