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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Sclareolate

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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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Firmenich Limited (ABN 86 002 964 794)
73 Kenneth Road Balgowlah NSW 2093

NOTIFICATION CATEGORY
Standard: Chemical other than polymer.

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication:
Chemical Name, Other Names, CAS Number, Molecular Formula, Structural Formula, Molecular Weight, Spectral Data, Method of Detection and Determination, Identity and Weight Percent of Impurities, additives and Adjuvants

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)
LVC/576 and LVCR/24

NOTIFICATION IN OTHER COUNTRIES
USA (2002), EU (2004), Switzerland (2007), Japan (2007), Canada (2007), South Korea (2006), Philippines (2006)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Sclareolate

MOLECULAR WEIGHT
< 500 Da

ANALYTICAL DATA
Reference UV, IR, ^{1}H AND ^{13}C NMR, MS and GC spectra were provided.

3. COMPOSITION

DEGREE OF PURITY 99%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Colourless liquid

Property	Value	Data Source/Justification
Freezing Point	< -50°C	Measured
Boiling Point	219°C at 101.3 kPa	Measured
Density	905 kg/m^3 at 20°C	Measured
Vapour Pressure	6.41 × 10^{-2} kPa at 25°C	Measured
Water Solubility	1.01 g/L at 22°C	Measured
Hydrolysis as a Function of pH	Stable (half-life > 1 year) at pH 4 and 7, half-life 67.9 days at pH 9	Measured
Partition Coefficient (n-octanol/water)	log P_{ow} = 3.4 at 25°C	Measured
Adsorption/Desorption	log K_{oc} = 1.7 at 30°C	Measured
Dissociation Constant	Not determined	The notified chemical has no acidic or basic groups.
Surface tension	48.3 mN/m	Measured
Particle Size	Not determined	Liquid
Flash Point	87.5°C at 101.3 kPa	Measured
Flammability Limits	Not determined	Not expected to form flammable mixtures in air
Autoignition Temperature	> 400°C	Measured
Explosive Properties	negative	Estimated

DISCUSSION OF PROPERTIES

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

Reactivity

The notified chemical is stable at normal conditions but could be hydrolysed in presence of strong acid or strong base

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL OVER NEXT 5 YEARS

The notified chemical will not be manufactured in Australia. It will be introduced as a small component (maximum 10%) of fragrance preparations.

MAXIMUM INTRODUCTION VOLUME OF NOTIFIED CHEMICAL OVER NEXT 5 YEARS

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

PORT OF ENTRY

Sydney, by wharf or airport

IDENTITY OF RECIPIENTS

After import, the fragrance preparations containing the notified chemical will be stored at the notifier's site prior to distribution to customers for reformulation into a wide variety of cosmetics, toiletries and household products.

TRANSPORTATION AND PACKAGING

The fragrance preparations containing the notified chemical will be transported by road from the wharf or airport of entry to the Firmenich Ltd warehouse for storage before Firmenich Ltd will forward them directly to the clients, typically by road, when needed. These fragrance preparations will be imported and distributed in tightly closed lacquered drums, typically of 180 kg size, but also of 100, 50, 25 10 or 5 kg size. Final consumer products will be transported to retail stores for distribution, and will be sold in a variety of small package sizes, typical of consumer-sized containers.

USE

The notified chemical will be used as a fragrance ingredient in a variety of cosmetic and household products. The concentration of the notified chemical will be a maximum of 2% in fine perfumes, and a maximum of 0.06% in other cosmetic products and household products.

OPERATION DESCRIPTION

The fragrance preparations containing the notified chemical will be used in the reformulate of other cosmetics, and household cleaning products and detergents. The process will involve a blending operation which mainly will be automated and occur in a fully enclosed environment, followed by automatic filling in containers of various sizes.

The final consumer products will be distributed to retail outlets, displayed and sold to the public.

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)*
Transport workers	4	1 hour/day	2 days/year
Mixer	5	4 hours/day	2 days/year
Drum handling	5	4 hours/day	2 days/year
Drum cleaning	8	4 hours/day	2 days/year
Maintenance	5	4 hours/day	2 days/year
Quality control	1	0.5 hour/day	1 day/year
Packaging	10	4 hours/day	2 days/year

EXPOSURE DETAILS

Transport and distribution workers are not expected to be exposed to the notified chemical except in an unlikely event of an accident and breakage of the packaging of the consumer products containing up to 10% of the notified chemical. Accidental exposure of transport and distribution workers is also unlikely in the case of import and distribution of raw material. In case of such accidental exposure, main routes of exposure would be dermal and ocular. However, the likelihood of such an accidental exposure is minimal.

In case of import of raw material for reformulation into consumer products, dermal and ocular exposure of workers involved in reformulation may occur during the manual transfer of the notified chemical (< 10%) from the drums and pails in to the mixing vessel. However, this exposure could be minimised by the use of PPE for skin and eye protection by the workers. Therefore, no significant exposure is likely for these workers except in the case of an accident.

Packers could also have dermal and ocular exposure to the notified chemical up to 10%. However exposure is likely to be minimised through the automation of the process and the use of safety glasses and gloves.

Overall, the exposure of workers to the notified chemical is expected to be low.

6.1.2. Public exposure

End-use products are designed to be sold to consumers. The general public will be repeatedly exposed to low-levels of the notified chemical via a number of different consumer products (a maximum of 2% in fine perfumes, and a maximum of 0.06% in other cosmetic products and household products).

Chronic dermal exposure

The worst-case long-term dermal exposure to the notified chemical can be estimated by assuming that the notified chemical is present in the maximum amount in all cosmetic products used by the consumer, and that there is 100% dermal absorption. The estimated systemic dose from skin surface residue to fragrances due to use of a number of cosmetic products is estimated as 2.547 mg/kg bw/day (Cadby, 2002). Therefore based on a concentration of 2% notified chemical in the fragrance compound the long-term dermal exposure to the notified chemical is estimated as 0.051 mg/kg bw/day.

Public exposure from transport, storage, reformulation or disposal is considered to be negligible.

6.2. Human health effects assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	oral LD50 > 2000 mg/kg bw, low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw, low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation – adjuvant test.	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL = 150 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro Mammalian Chromosome Aberration Test	non genotoxic
Genotoxicity – in vitro Mammalian Cell Gene Mutation Test	non genotoxic

Toxicokinetics, metabolism and distribution

Given the low molecular weight of the notified chemical and its log P_{ow} of 3.4, it is likely to be significantly absorbed following oral and dermal exposure. The octanol-water partition coefficient suggests that distribution of the notified chemical is unlikely to be bioaccumulate. Hydrolysis is likely to minimise the amount of the notified chemical available for distribution. Metabolism is likely to be extensive.

Acute toxicity

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

Irritation

Based on the studies provided, the notified chemical is considered to be slightly irritating to eyes and skin.

Sensitisation

There was no evidence of skin sensitisation to the notified chemical in an adjuvant Magnusson and Kligman test using guinea pig.

Repeated Dose Oral Toxicity

The No Observed Effect Level (NOEL) was established as 150 mg/kg bw/day for females and 15 mg/kg bw/day for males in the study, based on treatment-related increased liver weights at 1000 mg/kg bw/day in females and at 150 mg/kg bw/day in males.

The No Observed Adverse Effect Level (NOAEL) was established as 150 mg/kg bw/day for both sexes in the study, based on changes detected at 150 mg/kg bw/day were confined to a marginal but dose-related increase in liver weight (relative to bodyweight) with no histopathological correlates.

Genotoxicity

The notified chemical tested was not mutagenic in a bacterial reverse mutation study and not genotoxic in an *in*

vitro mammalian chromosome aberration test or an *in vitro* mammalian cell gene mutation test.

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

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
Dermal and possibly ocular exposure to the notified chemical could occur during the transfer of the fragrance mixture to the blending vessel. The level of exposure would vary from site to site depending on the level of automation of the formulation process. The estimated dermal exposure is 42 mg/day, based on EASE model using reasonable worst case defaults (without PPE) for the exposure scenario 'manual addition of liquids' (European Commission, 2003) and assuming the notified chemical is present at concentration of 10%. Therefore, for a 70 kg worker using a 100% dermal absorption factor, systemic exposure is estimated to be 0.6 mg/kg bw/day.

Based on a NOAEL of 150 mg/kg bw/day derived from a 28-day repeat dose oral toxicity study, the margin of exposure (MOE) for the transfer of the fragrance mixture to the blending vessel is 250. MOE greater than or equal to 100 accounting for intra- and inter-species differences are considered acceptable.

Overall, the use of the notified chemical is not expected to pose an unacceptable risk to the workers.

6.3.2. Public health
The public may come into contact with the notified chemical (< 2%) through the use of a range of cosmetic and consumer products.

Systemic Toxicity
The worst -case long-term dermal exposure to the notified chemical is estimated as 0.051 mg/kg bw/day. A dermal NOAEL was not determined, however a NOAEL of 150 mg/kg bw/day was established in a 28-day oral study in the rat. The use of this NOAEL results in a margin of exposure (MOE) of 2900. MOE greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. The MOE is based on conservative assumptions (e.g. 100% dermal absorption) and therefore likely to overestimate the risk.

The risk to the public of systemic effects after the use of cosmetic products is considered to be minimal based on the known systemic toxicity of the notified chemical and the estimated MOE.

Local Toxicity
The public will be exposed to the chemical at a maximum concentration of 2%. At this concentration the notified chemical is unlikely to be a skin irritant and therefore the risk of local irritancy effects is considered to be low.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will not be manufactured in Australia. Releases during formulation may amount to 0.2% of the imported quantity, as spills and container residues. Formulation equipment will be washed with water, with the aqueous washings reused.

RELEASE OF CHEMICAL FROM USE
While there will be some releases of this moderately volatile fragrance chemical to the atmosphere, the dominant release will be to sewer, when cosmetic and toiletries products are washed from the skin, and when cleaning agents are used.

RELEASE OF CHEMICAL FROM DISPOSAL
Formulation wastes would be disposed of to landfill or by incineration, while residues in empty consumer product containers are likely to be disposed of to landfill with the containers, or to sewer when containers are rinsed by consumers before recycling.

7.1.2 Environmental fate

The notified chemical did not satisfy criteria for ready biodegradability, but the extent of biodegradation (49%) in the test (see Appendix C1 for details) indicates that biodegradation can be expected to occur in the environment, and to some extent during sewage treatment. If released to the atmosphere, the notified chemical is expected to degrade through reaction with hydroxyl radicals ($t_{½}$ = 11.3 hours, EPIWIN). Most of the notified chemical is expected to remain in the aquatic phase, because of its water solubility, and may be released from sewage treatment plants to receiving waters, where it will disperse and biodegrade. Some partitioning to sludge may be expected as the notified chemical is surface active. A small proportion may be applied to land when effluent is used for irrigation or sewage sludge containing residues of the notified chemical is used as soil amendment, and would be expected to biodegrade. Similarly, residues disposed of to landfill are expected to slowly biodegrade.

7.1.3 Predicted Environmental Concentration (PEC)

The PEC in water can be estimated as tabulated below based on the worst case assumption that all of the chemical will be discharged to sewer, and subsequently released to receiving waters.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	1000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	2.74	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	21.374	million
Removal within STP	0%	
Daily effluent production:	4,275	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.6	µg/L
PEC - Ocean:	0.06	µg/L

Based on model predictions of 3% partitioning to sludge, partitioning to biosolids in STPs Australia-wide may result in an average biosolids concentration of 0.194 mg/kg (dry wt). Biosolids are applied to agricultural soils, with an assumed average rate of 10 t/ha/year. Assuming a soil bulk density of 1300 kg/m^3 and a soil-mixing zone of 10 cm, the concentration of the notified chemical may approximate 0.001 mg/kg in applied soil. This assumes that degradation of the notified chemical occurs in the soil within 1 year from application. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated biosolids application, the concentration of notified chemical in the applied soil in 5 and 10 years may approximate 0.005 mg/kg and 0.01 mg/kg, respectively.

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1300 kg/m^3). Using these assumptions, irrigation with a hypothetical worst case concentration of 0.6 µg/L may potentially result in a soil concentration of approximately 4.6 x 10^{-3} mg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 2.3 x 10^{-2} mg/kg and 4.6 x 10^{-2} mg/kg, respectively.

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 C2.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity (96 hours)	LC50 = 13 mg/L	Harmful
Daphnia Toxicity (48 hours)	EC50 = 20 mg/L	Harmful
Algal Toxicity (72 hours)	EC50 > 100 mg/L	Not harmful
Inhibition of Bacterial Respiration	IC50 > 100 mg/L	Not harmful

The results of the studies indicate that the notified chemical is harmful to fish and daphnids, but not harmful to algae and bacteria.

7.2.1 Predicted No-Effect Concentration

The PNEC for the aquatic compartment can be calculated by dividing the most sensitive test result by 100, as data are available for three trophic levels.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
Acute fish toxicity	13	mg/L
Assessment Factor	100	
Mitigation Factor	1.00	
PNEC:	130	µg/L

7.3. Environmental risk assessment

The PEC/PNEC ratios for the aquatic environment are tabulated below.

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.6	130	< 0.005
Q - Ocean	0.06	130	< 0.0005

The notified chemical is not considered to pose a risk to the aquatic environment as the PNEC exceeds the PECs by factors of more than 100.

While the PEC/PNEC ratio cannot be determined for the terrestrial environment, as there are no terrestrial toxicity data, the notified chemical is not considered to pose a risk to the terrestrial environment as the PEC in soil is well below 1 mg/kg, even when estimated under the unrealistic assumption that residues will accumulate from annual applications over a decade. The notified chemical is not expected to persist in sludge amended soils or in landfill.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

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

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

	Hazard category	***Hazard statement***
Acute hazards to the aquatic environment	3	Harmful to aquatic life

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 PEC/PNEC ratio and the reported use pattern, the notified chemical is not considered to pose a risk to the environment.

Recommendations

CONTROL MEASURES
Occupational Health and Safety

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

  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 containment, collection and subsequent safe disposal.

Regulatory Obligations

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

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

(1) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from a maximum of 2% in fine perfumes, and a maximum of 0.06% in other cosmetic products and household products, 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 < -50ºC

Method OECD TG 102 Melting Point/Melting Range.
EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks The freezing point is less than -50°C (limit of the method) and probably less than -56°C under the conditions of the test.
Test Facility SEPC (2000a)

Boiling Point 219 ± 1 ºC at 101.325 kPa

Method OECD TG 103 Boiling Point.
EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks The Siwoloboff modified method was used.
Test Facility RCC (2001)

Density 905 kg/m^3 at 20.0 ± 0.5ºC

Method EC Directive 92/69/EEC A.3 Relative Density.
Remarks The pycnometer method was used.
Test Facility SafePharm Laboratories (2002a)

Vapour Pressure 6.41×10^{-2} kPa at 25ºC

Method EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks The vapour pressure at 25ºC was extrapolated from measurements made at temperatures > 190ºC with an isoteniscope.
Test Facility SafePharm Laboratories (2002b)

Water Solubility 1.01 ± 0.05 g/L at 22 ± 2ºC

Method OECD TG 105 Water Solubility.
EC Directive 92/69/EEC A.6 Water Solubility.
Remarks The water solubility was determined by the flask method with quantitation by means of HPLC (UV). The test report contained some unexplained anomalies. However, the result of this test is consistent with the observation that a 500 mg/L stock solution could be prepared for the fish toxicity test, and with EPIWIN modelling.
Test Facility CIT (2000a)

Hydrolysis as a Function of pH

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

pH	*T (°C)*	$t_{½}$
4	25	> 1 year
7	25	> 1 year
9	25	67.9 days

Remarks The test was conducted at 50ºC in filtered sterile buffer solution with 1% acetonitrile co-solvent. Hydrolysis reached about 10% after 5 days at pH 4, indicating a half-life greater than, but close to, 1 year at 25ºC. Hydrolysis at pH 7 reached about 4%. Hydrolysis reached about 50% at pH 9. Further testing was conducted at 60ºC and 70ºC in order to determine the rate constant and half-life.
Test Facility SafePharm Laboratories (2002a)

Partition Coefficient (n-octanol/water) log P_{ow} = 3.4 at 25ºC

Method OECD TG 117 Partition Coefficient (n-octanol/water).
EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks HPLC Method. The result appears a little high, given the water solubility, and should be treated with caution. Partitioning by the flask method was briefly investigated while studying the surface tension. Significant emulsification was observed in the organic layer, confirming the surface activity of the notified chemical. The partition coefficient cannot be reliably determined for surface active chemicals, which tend to partition to phase boundaries rather than between phases.
Test Facility CIT (2000b)

Adsorption/Desorption – screening test log K_{oc} = 1.73 at 25°C

Method OECD TG 121 Adsorption - Desorption Using the HPLC Screening Method.
Remarks The test should be treated with caution given the limitations of the HPLC method when testing surface active substances. The authors consider the method to be valid, noting the surface activity but also the low likelihood that the test substance would interact or react with the stationary phase in any way other than partitioning, based on inspection of the chemical structure.
Test Facility SafePharm Laboratories (2002a)

Flash Point 87.5 ± 0.5ºC at 101.3 kPa

Method EC Directive 92/69/EEC A.9 Flash Point.
Remarks The closed cup equilibrium method was used.
Test Facility SEPC (2000b)

Autoignition Temperature > 400ºC

Method EC Directive 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks By heating aliquots of the test substance in a flask up to 400ºC and observing for any ignition. Grey fumes were emitted at 100ºC and above.
Test Facility SafePharm Laboratories (2001a)

Explosive Properties Not explosive

Method EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks There are no chemical groups that would imply explosive properties and the oxygen balance has been calculated at –237.3, therefore the result has been predicted negative.
Test Facility SafePharm Laboratories (2001a)

Surface Tension 48.3 mN/m at 21ºC

Method OECD TG 115 Surface Tension of Aqueous Solutions.
EC Directive 92/69/EEC A.5 Surface Tension.
Remarks The nominal test concentration was about 1 g/L. Substances with a surface tension below 60 mN/m are considered surface active.
Test Facility SafePharm Laboratories (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.
EC Directive 92/69/EEC B.1tris Acute Oral Toxicity – Acute Toxic Class Method.

Species/Strain	Rat/Sprague-Dawley
Vehicle	Corn oil
Remarks - Method	Minor deviations of the protocol were not considered to have compromised the validity or integrity of the study.

RESULTS

Number and Sex of Animals	*Dose (mg/kg bw)*	*Mortality*
3 M	200	0
3 per sex	2000	0

LD50	> 2000 mg/kg bw
Signs of Toxicity	No clinical signs and no mortality were observed in the animals given 200 or 2000 mg/kg.
Effects in Organs	At necropsy, no apparent abnormalities were observed in any animal.
Remarks - Results	The body weight gain of the animals was not affected by treatment with the test substance.

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

TEST FACILITY: CIT (2000c)

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 (Crl: CD® (SD) IGS BR)
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 sign 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 dermal route.

TEST FACILITY: SafePharm Laboratories (2001b)

B.3. 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	Until reversibility of cutaneous reactions.
Type of Dressing	Semi-occlusive.
Remarks - Method	The minor deviation (the relative humidity recorded in the animal room was sometimes outside of the target ranges specified in the protocol) of the protocol was not considered to have compromised the validity or integrity of the study.

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	1.3	0.3	0.7	2	72 hours	0
Oedema	0	0	0	0	-	0

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

Remarks - Results	A very slight or well-defined erythema was noted in all animals from day 1 up to day 2 (one animal), 3 (one animal) or 4. No other cutaneous reactions were observed during the study.
CONCLUSION	The notified chemical is slightly irritating to the skin.
TEST FACILITY	CIT (2000d)

B.4. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 M
Observation Period	Until reversibility of ocular reactions.
Remarks - Method	The minor deviation (the relative humidity recorded in the animal room was sometimes outside of the target ranges specified in the protocol) of the protocol was not considered to have compromised the validity or integrity of the study.

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	1.3	0.7	0.3	2	96 hours	0
Conjunctiva: chemosis	0	0	0	0	-	0
Conjunctiva: discharge	0	0	0	0	-	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	Very slight or slight conjunctival reactions (very slight chemosis (one animal) and very slight or slight redness of the conjunctivae (all three animals)) were observed from day 1; these reactions persisted up to day 2 (one animal), 3 (one animal) or 4. No other ocular reactions were noted during the study.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	CIT (2000e)

B.5. Skin sensitisation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 406 Skin Sensitisation - <Magnusson and Kligman>. EC Directive 96/54/EC B.6 Skin Sensitisation - <Magnusson and Kligman>.
Species/Strain	Guinea pig/Harley Crl: (HA) BR
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: 10% (w/w) in corn oil topical: 100% (w/w) in corn oil
MAIN STUDY	
Number of Animals	Test Group: 10 per sex Control Group: 5 per sex
INDUCTION PHASE	Induction Concentration: intradermal: 10% (w/w) in corn oil topical: 100%
Signs of Irritation	On day 2, 24 hours after the intradermal injections and on day 10, after removal of the dressing of the cutaneous application, signs of irritation were observed at the treatment site in the animals of the control and treated groups.
CHALLENGE PHASE	
challenge	topical: 100%

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after challenge:*	
		24 h	*48 h*
Test Group	100%	0/20	0/20
Control Group	100%	0/10	0/10

Remarks - Results	Only a dryness of the skin was observed at the 48-hour reading, in 2/10 animals of the control group and in 1/20 animals of the treated group. No other cutaneous reactions were noted.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	CIT (2000f)

B.6. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/Sprague-Dawley CD (Crl: CD® (SD) IGS BR)
Route of Administration	Oral – gavage

Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: not applicable
Vehicle	Arachis oil BP
Remarks - Method	No deviations from the protocol.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 per sex	0	0
low dose	5 per sex	15	0
mid dose	5 per sex	150	0
high dose	5 per sex	1000	0

Mortality and Time to Death

There were no deaths during the study.

Clinical Observations

Increased salivation prior to and up to 10 minutes after dosing was detected in animals of either sex treated with 1000 mg/kg bw/day from day 3 persisting throughout the study period. Incidents of associated stained fur were also observed during the first two weeks of treatment. One female of this dose group showed hunched posture five hours after doing on day 12 only. Isolated signs of increased salvation were detected at 150 mg/kg bw/day but no abnormalities were detected at 15 mg/kg bw/day. Such observations are often reported following oral administration of unpalatable or slightly irritant test substance formulation and are considered not to be indicative of systemic toxicity. No treatment-related effects were detected at 150 mg/kg bw/day.

At any dose level, no treatment-related effects were detected for behaviour assessment, functional performance test, sensory reactivity assessment, no adverse effect on bodyweight development was detected, no adverse effect on dietary intake or food efficiency was detected and no intergroup differences were detected for water consumption.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Animals of either sex treated with 1000 mg/kg bw/day showed decreased erythrocyte count and mean corpuscular haemoglobin concentration and an elevation in mean corpuscular volume. A reduced haemoglobin concentration was also detected for females from this treatment group. No treatment-related effects were detected for animals of either sex at 150 or 15 mg/kg bw/day.

Animal of either sex treated with 1000 mg/kg bw/day showed a statistically significant reduction in plasma cholesterol compared with control animals. No such effects were detected for animals of either sex at 150 or 15 mg/kg bw/day.

Effects in Organs

A statistically significant increase in liver weight, both absolute and relative to terminal weight, was detected for animals of either sex treated with 1000 mg/kg bw/day whilst relative and absolute epididymides weight were reduced for males from this group. A slight, but statistically significant increase in liver weight was also detected for males but not females treated with 150 mg/kg bw/day. No toxicologically significant changes were detected at 15 mg/kg bw/day.

At necropsy, macroscopic abnormalities were confined to one 1000 mg/kg bw/day male which showed an enlarged liver. Macroscopic examination of tissue sections revealed treatment-related liver and thyroid changes:

Liver: Centrilobular hepatocyte enlargement was observed in relation to treatment for animals of either sex treated with 1000 mg/kg bw/day, but probably not at any other dose level. This condition is occasionally encountered in untreated animals.

Thyroid: A small increase in the incidence of follicular cell hypertrophy was probably associated with treatment at the 1000 mg/kg bw/day dose level for male animals.

Remarks – Results

CONCLUSION
The No Observed Effect Level (NOEL) was established by the study author as 150 mg/kg bw/day for females and 15 mg/kg bw/day for males in the study, based on treatment-related increased liver weights at 1000 mg/kg bw/day in females and at 150 mg/kg bw/day in males.

The No Observed Adverse Effect Level (NOAEL) was established by NICNAS as 150 mg/kg bw/day for both sexes in the study, based on changes detected at 150 mg/kg bw/day were confined to a marginal but dose-related increase in liver weight (relative to bodyweight) with no histopathological correlates.

TEST FACILITY SafePharm Laboratories (2002c)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

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

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA$^-$
Metabolic Activation System	S9 was prepared from the livers of male Sprague-Dawley rats that had each orally received three consecutive daily doses of phenobarbital/β-naphthoflavone (80/100 mg per kg per day) prior to S9 preparation on Day 4.
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	> 5000	5000	5000	positive only in *Salmonella* strains
Present	> 5000	> 5000	5000	Negativein all *Salmonella* and *E. coli* strains

Remarks - Results
The test substance caused a weak by slightly intermittent toxic response to the *Salmonella* strains, predominantly in the absence of S9, at 5000 µg/plate. In the range-finding test, there was no visible reduction in the growth of the bacterial background lawn, however several strains exhibited substantial decreases in revertant colony frequency at the maximum recommended dose level. In the main test, toxicity was exhibited a slight weakening of the bacterial background lawns of all the *Salmonella* strains dose in the absence of S9. In the presence of S9, decreases in revertant colony frequency were noted to TA1535 and TA 1537. These results were not indicative of toxicity sufficiently severe enough to prevent the test substance being tested up to the maximum recommended dose level of 5000 µg/plate. No test material precipitate was observed to the naked eye, however an oily precipitate was noted under an inverted microscope at 5000 µg/plate.
No significant increases in the frequency of revetant colonies were recorded for any of the bacterial strains, at any dose level either with or without metabolic activation.

All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacteria strains.

CONCLUSION The notified chemical was considered to be non-mutagenic to bacteria under the conditions of the test.

TEST FACILITY SafePharm Laboratories (2006)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

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

Cell Type/Cell Line Human lymphocytes

Metabolic Activation System S9 was prepared from the livers of male Sprague-Dawley rats that had each received orally three consecutive daily doses of phenobarbital (80 mg/kg) and β-naphthoflavone (100 mg/kg) prior to S9 preparation on the fourth day.

Vehicle Dimethyl sulphoxide

Remarks - Method No deviations from the protocol.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 63.13, 126.25, 252.5*, 505*, 1010*, 2020, MMC 0.4*	4 hours	20 hours
Test 2	0*, 126.25, 252.5*, 505*, 1010*, 1515, 2020, MMC 0.2*	24 hours	24 hours
Present			
Test 1	0*, 63.13, 126.25, 252.5*, 505*, 1010*, 2020, CP 10*	4 hours	20 hours
Test 2	0*, 126.25, 252.5*, 505*, 1010*, 2020, CP 10*	4 hours	20 hours

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	≥ 252.5		≥ 1010	
Test 1		> 2020	≥ 1010	negative
Test 2		> 2020	2020	negative
Present	> 2020		≥ 1010	
Test 1		> 2020	≥ 1010	negative
Test 2		> 2020	2020	negative

Remarks - Results All vehicles (solvent) controls gave frequencies of cells with aberrations within the range expected for normal human lymphocytes.
All the positive control treatments gave statistically significant increases in the frequency of cells with aberrations indicating the satisfactory performance of the test and of activity of the metabolising system.
The test substance did not induce any statistically significant increases in the frequency of cells with aberrations, in either of two separate experiments.

CONCLUSION The notified chemical was considered to be non-clastogenic to human lymphocytes in vitro under the conditions of the test.

TEST FACILITY SafePharm Laboratories (2002d)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 476 In vitro Mammalian Cell Gene Mutation Test. EC Directive 2000/32/EC B.17 Mutagenicity - In vitro Mammalian Cell Gene Mutation Test.
Cell Type/Cell Line	L5178Y TK +/- 3.7.2c mouse lymphoma cells (heterozygous at the thymidine kinase locus)
Metabolic Activation System	PB/βNF S9 was prepared from the livers of male Sprague-Dawley rats that had each received orally three consecutive daily doses of phenobarbital/β-naphthoflavone (80/100 mg per kg per day) prior to S9 preparation on the fourth day. 20% S9-mix was prepared by mixing S9, NADP (5 mM), G6P (5mM), KCl (33mM) and $MgCl_2$ (8mM) in R0. The final concentration of S9 was 2% throughout the study.
Vehicle	Dimethyl sulphoxide
Remarks - Method	No deviations from the protocol.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*
Absent		
Test 1	0, 23.44, 46.88, 93.75, 187.5, 375, 500, 750, 1000	4 hours
Test 2	0, 31.25, 62.5, 125, 187.5, 250, 375, 500, 750	24 hours
Present		
Test 1	0, 46.88, 93.75, 187.5, 375, 500, 750, 1000, 1500	4 hours
Test 2	0, 187.5, 250, 375, 500, 750, 1000, 1250, 1500	4 hours

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	≥ 505.75			
Test 1		> 1000	≥ 750	negative
Test 2		> 750	> 750	negative
Present	≥ 505.75			
Test 1		> 1500	≥ 750	negative
Test 2		> 1500	> 1500	negative

Remarks - Results	The maximum dose level used was limited by test substance induced toxicity. A greasy/oily precipitate of test substance was observed at and above 750 µg/mL in test 1. The vehicle (solvent) controls had mutant frequency values that were considered acceptable for the purpose pf this study. The positive control materials induced marked increases in the mutant frequency indicating the satisfactory performance of the test and of the activity of the metabolising system. The test substance did not induce any statistically significant or dose-related increases in the mutant frequency at any dose level, either with or without metabolic activation, in either the first or the second test including dose levels where optimum or near optimum levels of toxicity were achieved.
CONCLUSION	The notified chemical was considered to be non-mutagenic to L5178Y cells under the conditions of the test.
TEST FACILITY	SafePharm Laboratories (2007)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test.
Inoculum	Solids filtered from secondary effluent obtained from water treatment plant (Evreux, France) receiving mainly domestic effluent.
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Not conducted
Remarks - Method	Biodegradation was determined by measurement of biological oxygen demand

RESULTS

Test substance		*Sodium acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
3	31.75	3	54.81
10	41.30	10	76.92
28	49.37	28	80.13

Remarks - Results	The test substance was not inhibitory to microbial respiration based on the results (64.5% of theoretical oxygen demand after 28 days) from the toxicity control containing test and reference substance.
CONCLUSION	Not readily biodegradable, but having the potential for rapid biodegradation.
TEST FACILITY	CIT (2000g)

C.1.2. Bioaccumulation

Bioaccumulation was not tested, but a BCF value of about 81 was estimated using EPIWIN. This suggests a low potential for bioaccumulation. The results from the biodegradability test, and the presence of functionality that is likely to be metabolised in fish, indicate that the notified chemical is unlikely to bioaccumulate in fish.

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 (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	Gas chromatography with flame ionisation detection (LOQ 0.082 mg/L)
Remarks – Method	The test was conducted in sealed vessels with no aeration and no headspace, because of the suspected volatility of the test substance. Test media were renewed at 24 hour intervals. The LC50 values at 48, 72 and 96 hours were calculated using the geometric mean of the concentrations producing 0% and 100% mortality at these times. The value at 24 hours was calculated using the trimmed Spearman-Karber method.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*6 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	< LOQ	10	0	0	0	0	0
10	11.8/8.05	10	0	0	0	0	0
18	21.7/15.6	10	0	9	10	10	10
32	38.0/30.7	10	0	10	10	10	10
56	68.1/58.6	10	10	10	10	10	10
100	117/100	10	10	10	10	10	10

LC50	14 mg/L (95%CI: 13-16 mg/L) at 24 hours 13 mg/L at 48 hours. 13 mg/L at 72 hours. 13 mg/L at 96 hours.
NOEC	10 mg/L at 96 hours.
Remarks – Results	There was no evidence of instability, insolubility or adherence to glass in stability samples. Exposure concentrations declined between renewals, as revealed by the measured data tabulated above which were taken at 0 and 24 hours. Analysis of the 10 mg/L solution at 96 hours recovered 91% of the nominal concentration. Comparison of test and stability vessels suggests that the test substance was taken up by or sorbed to the fish. Results are expressed as nominal concentrations. Fish lost equilibrium and became moribund before death.

CONCLUSION The notified chemical is harmful to fish.

TEST FACILITY SafePharm Laboratories (2002e)

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 with flame ionisation detection (LOQ 0.082 mg/L)
Remarks - Method	The test was conducted in sealed vessels with no aeration and no headspace, because of the suspected volatility of the test substance. The 48 hour EC50 and associated confidence intervals were calculated using the trimmed Spearman-Karber method.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	< LOQ	20	0	0
1.0	0.866/0.770	20	0	0
1.8	1.61/1.48	20	0	0
3.2	3.00/2.78	20	0	0
5.6	5.14/4.48	20	0	0
10	9.52/8.60	20	0	0
18	17.4/16.3	20	0	7
32	30.9/27.7	20	0	20
56	55.1/47.3	20	3	20
100	98.9/93.3	20	7	20

LC50	> 100 mg/L at 24 hours 20 mg/L (95%CI: 17-22 mg/L) at 48 hours
NOEC	10 mg/L at 48 hours
Remarks - Results	There was no evidence of instability, insolubility or adherence to glass in stability samples. Exposure concentrations declined during the test, as revealed by the measured data tabulated above which were taken at 0 and 48 hours. Comparison of test and stability vessels suggests that the test substance sorbed to the daphnids. Results are expressed as nominal concentrations.
CONCLUSION	The notified chemical is harmful to daphnids.
TEST FACILITY	SafePharm Laboratories (2002f)

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: 100 mg/L Actual: 85 mg/L (97 mg/L at 0 hours, 74 mg/L at 72 hours)
Auxiliary Solvent	None
Water Hardness	Not stated. Typical algal culture medium containing various salts and chelators, including 4.4 mg/L calcium chloride dihydrate.
Analytical Monitoring	Gas chromatography with flame ionisation detection (LOQ 0.082 mg/L)
Remarks - Method	A limit test only was conducted. Cell concentrations in controls increased by a factor of 21, satisfying the validity criterion of a 16 fold increase.

RESULTS

Biomass		*Growth*	
EC50 *mg/L at 72 h*	*NOEC* *mg/L*	*EC50* *mg/L at 72 h*	*NOEC* *mg/L*
> 100	100	> 100	100

Remarks - Results	There was no evidence of instability, insolubility or adherence to glass in stability samples. Exposure concentrations declined during the test, as revealed by the measured data taken at 0 and 72 hours. Comparison of test and stability vessels suggests that the test substance sorbed to the algal cells, though some volatilisation may have occurred when samples were taken at 24 hour intervals for enumeration. Some hydrolysis is also likely to have occurred, given the high final pH, and the difference in final pH between test and control vessels (10.0 and 10.3, respectively). Results are expressed as nominal concentrations. Corresponding mean measured concentrations are 85 mg/L. Growth and biomass increased relative to controls, by 12% and 28% respectively. The pH increased during the test, consistent with good algal growth. The absence of carbon dioxide exchange with the atmosphere, due to the use of sealed vessels, probably contributed to the high final pH (10.3 in controls, 10.0 in test vessels).
CONCLUSION	The notified chemical is not harmful to green algae.
TEST FACILITY	SafePharm Laboratories (2002g)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Solids filtered from secondary effluent obtained from water treatment plant (Evreux, France) receiving mainly domestic effluent.
Exposure Period	3 hours
Concentration Range	Nominal: 1, 3.16, 10, 31.6, 100 mg/L
Remarks – Method	3,5-Dichlorophenol was used as reference substance.
RESULTS	
IC50	> 100 mg/L
NOEC	100 mg/L
Remarks – Results	Oxygen consumption was only measured at 100 mg/L, as respiration was equivalent to (within 15% of) the control.
CONCLUSION	The notified chemical is not harmful to waste water treatment microorganisms.
TEST FACILITY	CIT (2000h)

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