File No: STD/1413

July 2012

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Chemical in Leucophor S and Leucophor SAC

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

For the purposes of subsection 78(1) of the Act, this Public Report may be inspected at our NICNAS office by appointment only at Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

This 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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SUMMARY

The following details will be published in the NICNAS *Chemical Gazette:*

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS SUBSTANCE	INTRODUCTION VOLUME	USE
STD/1413	Chemcolour Industries Australia Pty Limited	Chemical in Leucophor S and Leucophor SAC	No	≤ 20 tonnes per annum	A component in paper making

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

Human health risk assessment
Under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

When used in the proposed manner, the notified chemical is not considered to pose an unreasonable risk to public health.

Environmental risk assessment
On the basis of the PEC/PNEC ratio and the assessed use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

CONTROL MEASURES
Occupational Health and Safety

- Employees should use standard engineering controls, work practices and personal protective equipment to reduce worker exposure during use of the notified chemical.

  Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

- If the notified chemical or products containing it are introduced in powder form local exhaust ventilation should be used during handling.

- 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 disposal.

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if
- there is a change in the location of use;
- the notified chemical is imported in pure form.

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component in paper making, or is likely to change significantly;
- the amount of chemical being introduced has increased from 20 tonnes 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.

Material Safety Data Sheet
The MSDS of a product containing 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.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Chemcolour Industries Australia Pty Limited (ABN 70 125 602 271)
Monash Business park
20-22 Gardiner Road
NOTTING HILL VIC 3168

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: chemical name, CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, impurities and additives/adjuvants.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: vapour pressure, flash point, flammability, autoignition temperature, explosive properties, oxidising properties, acute dermal toxicity, skin irritation, eye irritation and skin sensitisation.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
Commercial Evaluation Permit (2010), CEC/789.

NOTIFICATION IN OTHER COUNTRIES
United Kingdom (2000)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Leucophor S (product containing 18.5% notified chemical)
Leucophor SAC (product containing 18.5% notified chemical)

MOLECULAR WEIGHT
> 1,000 Da

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

3. COMPOSITION

DEGREE OF PURITY > 60%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Yellow solid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	> 360 °C	Measured
Boiling Point	> 360 °C at 101.3 kPa	Measured
Density	1,890 kg/m^3 at 21°C	Measured
Vapour Pressure	4.6 × 10^{-7} kPa at 25°C	Based on data for analogue 1
Water Solubility	> 499 g/L at 20°C	Measured
Hydrolysis as a Function of pH	t ½ > 1 year at 25°C at pH 4, 7 and 9	Measured
Partition Coefficient (n-octanol/water)	log Kow < -2.04 at 23°C	Measured
Surface Tension	64.9 mN/m at 24.0°C	Measured
Adsorption/Desorption	log K_{oc} = 3.75 at 23.0°C	Measured
Dissociation Constant	Not determined	The notified chemical is a salt and is expected to be dissociated in the environment.
Particle Size	Not determined	Imported in an aqueous solution
Flash Point	Not determined	Imported in an aqueous solution
Flammability	Not expected to be highly flammable	Based on data for analogue 1
Autoignition Temperature	390°C	Based on data for analogue 1
Explosive Properties	Not expected to be explosive	Based on data for analogue 1
Oxidising Properties	Not expected to be oxidising	Based on data for analogue 1

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

Reactivity
Stable under normal conditions of use.

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 not be manufactured in Australia. The notified chemical will be imported as a component (18.5%) of an optical brightener product for paper making.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	10	10	15	20	20

PORT OF ENTRY
Sydney and Melbourne

IDENTITY OF MANUFACTURER/RECIPIENTS
The notified chemical will be imported by Chemcolour Industries Australia Pty Limited and the end user will be Australian Paper Pty Ltd.

TRANSPORTATION AND PACKAGING
The products containing the notified chemical will be imported in 200 L drums or 1000 L IBCs and will be transported throughout Australia by road.

USE
The notified chemical will be used as an optical brightener for use in paper making.

OPERATION DESCRIPTION
The notified chemical will not be manufactured or reformulated within Australia, but will be imported in aqueous optical brightening products, at a concentration of 18.5%, for use in paper making.

After import the products containing the notified chemical (18.5%) will be transported to the notifier's warehouse for storage prior to distribution to the end user's paper manufacturing facility. At the paper manufacturing facility the products containing the notified chemical (18.5%) will be pumped from the imported containers through permanently installed pipework into the paper making machinery. The imported containers will be held in a bunded area during transfer of the product to the paper making machinery. The paper making process is expected to be a closed system where the imported product containing the notified chemical will be mixed with other components before being applied to the paper as a surface coating.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport workers	1	5
Warehouse staff – importer and end user	2	5
Process workers – paper manufacture	1	40
Laboratory technicians	1	100
Container recyclers	1	5

EXPOSURE DETAILS
Dermal and ocular exposure of workers to the notified chemical at a concentration of 18.5% may occur during connection/disconnection of hoses, or cleaning/maintenance of equipment. Such exposure is only likely to

occur accidentally and workers are expected to wear personal protective equipment (PPE) such as coveralls, gloves and safety glasses to minimise exposure levels. Following application to the paper, the notified chemical is expected to be fixed into the cellulose matrix and not be available for exposure.

6.1.2. Public Exposure

The notified chemical will not be used by the public. The public may come into contact with finished paper products containing the notified chemical, however, the notified chemical is expected to be fixed into the cellulose matrix of the paper, with minimal leaching anticipated during use. Thus the notified chemical is not expected to be available for exposure.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical and analogous chemicals 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 > 2,000 mg/kg bw; low toxicity
Rat, acute dermal toxicity*	LD50 > 2,000 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 – 90 days.	NOAEL = 50 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro Mammalian Cell Gene Mutation Test	non genotoxic

* Studies conducted on analogue 1.

Toxicokinetics, metabolism and distribution.

Gastrointestinal (GI) and dermal absorption is expected to be limited by the high molecular weight (> 1000 Da) and high water solubility (> 499 g/L at 20°C) of the notified chemical.

Acute toxicity.

The notified chemical is considered to be of low acute toxicity via the oral route based on a test conducted in rats. Analogue 1 was considered to be of low acute toxicity via the dermal route based on a test conducted in rats. Based on the read across data the notified chemical is also expected to be of low acute toxicity via the dermal route.

Irritation and Sensitisation.

Based on tests conducted in rabbits analogue 1 is considered to be slightly irritating to the skin and eye. Analogue 1 was also found to be a non-sensitiser in a guinea pig maximisation test. Based on the read across data the notified chemical is expected to be a slight dermal and eye irritant, and not a skin sensitizer.

Repeated Dose Toxicity.

A 90 day oral study in 20 rats with the notified chemical gave a NOAEL of 50 mg/kg bw/day. At the only higher dose tested (750/500 mg/kg bw/day) 3/4 rats died.

Mutagenicity.

The notified chemical was found to not be mutagenic using a bacterial reverse mutation test, and is not clastogenic to L5178Y TK +/- cells in vitro.

Reproductive effects

The US EPA previously held concerns for the potential developmental/reproductive toxicity of a group of chemicals that includes the notified chemical; however, based on newer toxicological data, they now consider the group to be of lower concern. Reproductive or developmental studies are not available for the notified chemical. In the 90-day repeat dose oral study in rats on the notified chemical, significant adverse effects were seen at the highest dose only (500/750 mg/kg bw/day) on several body systems and organs, including the male reproductive system. It is not known whether the effects on the male rat reproductive system are specific to the notified chemical or, as proposed by the study authors, are related to the general severe toxicity of the chemical at this dosage. The notifier has provided summaries of three reproductive / developmental studies carried out on

an analogue chemical, which indicated low concern for that chemical. Based on the available data, the reproductive/developmental toxicity of the notified chemical cannot be ruled out.

Health hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The available toxicological information indicates that the notified chemical has low acute oral and dermal toxicities in rats with LD50's > 2000 mg/kg bw. It is expected to be a slight dermal and eye irritant, and not a skin sensitizer. For subchronic toxicity a NOAEL of 50 mg/kg bw/day was established. The notified chemical was not mutagenic in a bacterial reverse mutation test, and is not clastogenic to L5178Y TK +/- cells in vitro.

There is potential for incidental occupational exposure to the notified chemical (18.5%) during connecting/disconnecting hoses and cleaning/maintenance of equipment, mainly via the dermal and ocular routes. Such exposure is expected to be reduced through the use of PPE (coveralls, gloves and safety glasses) and the closed nature of the equipment used.

Given the available test data on the notified chemical, an analogous chemical and the expected low exposure levels in the presence of PPE, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

6.3.2. Public Health
The notified chemical is expected to be bound to the cellulose matrix of the paper. Thus, based on the expected minimal exposure, the risk to the public presented by the notified chemical is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will be imported as a product for use as a brightening agent in the manufacture of paper. No significant release of the notified chemical to the environment is expected from transport and storage processes.

RELEASE OF CHEMICAL FROM USE
At the industrial customer's site, the product containing the notified chemical will be pumped into a storage tank from where it will be dosed to the paper whitening machine. The notified chemical will be used during the white paper manufacturing process as coating to the surface of paper. It is assumed that at least 90% of the notified chemical will adsorb onto the paper surface during the application processes. The remainder may be released into waste water produced by the manufacturing process. The notifier indicates that the customer has on-site wastewater treatment facilities. The notified chemical would only be released from the paper mill in the backwater/effluent discharge after treatment. The treatment process will be completely enclosed and water will not be released from the site until the treatment process has finished.

RELEASE OF CHEMICAL FROM DISPOSAL
Empty IBCs containing residual notified chemical will enter the second-hand IBC market for reuse through a professional IBC recycling company. It is expected that residues in the import containers would be washed out on-site at the paper mill and the rinsates treated in the on-site water treatment facility. Alternatively, aqueous rinsates from used IBCs would be washed out at the recycling company facility and the residues discharged to sewer after appropriate on-site water treatment. The losses of notified chemical in container residues are estimated to be <1% per annum. It is assumed that 50% of the paper to which the notified chemical is applied will end up in landfill and the remainder will undergo paper recycling processes.

7.1.2. Environmental Fate

The notified chemical reached a biodegradation rate of 23% after 28 days in ready biodegradation study which indicates it is partially biodegradable aerobically but not readily biodegradable. The notified chemical is stable to hydrolysis in the environmental pH range of 4–9 and abiotic degradation mediated by hydrolytic mechanisms is therefore expected to be slow. Stability tests conducted during the course of ecotoxicity testing indicate that the notified chemical undergoes primary degradation on continued exposure to light.

The notified chemical is therefore not expected to be fully removed from the effluents of waste water treatment plants. Although the notified chemical has a high water solubility, some of notified chemical discharged in treated effluent is expected to partition to sludge and sediment, based on the high measured adsorption/desorption coefficient (log K_{oc} = 3.75). The rate of abiotic degradation may be slow based on the apparent hydrolytic stability of the chemical and it may persist in the water column. Although potentially persistent in the water compartment, the notified chemical is unlikely to bioconcentrate in aquatic organisms based on the low measured log Kow and the relatively large molecular dimensions of the chemical.

During recycling processes, waste paper is repulped using a variety of chemical agents, which, amongst other things, enhance detachment of inks and coatings from the fibres. The measured adsorption/desorption coefficient indicates that the notified chemical has the potential to strongly adsorb to soil and sediment in the sludge fraction. Therefore, only a low proportion of the notified chemical is expected to remain in the effluent water from both on-site waste water treatment plants and municipal water treatment plants to which treated effluent from paper recycling facilities may be discharged. Sludge (containing the notified chemical) generated during the recycling process is expected to be sent to landfill for disposal.

In either landfill or water, the notified chemical will ultimately decompose to water, oxides of carbon, nitrogen, and sulfur, and inorganic salts.

7.1.3. Predicted Environmental Concentration (PEC)

At the paper mill, waste water from the manufacturing process will undergo on-site secondary treatment prior to discharge into a river with an estimated daily flow rate of 130 ML. For a worst case release scenario it is assumed that 10% of the annual import quantity of the notified chemical (2000 kg) is not retained in the paper manufactured on-site and is released in the water discharged into the on-site secondary water treatment plant. It is further assumed that none of the chemical is removed by adsorption or biodegradation prior to release to the river and that releases occur on 260 days each year. Based on this scenario, the maximum daily discharge rate of notified chemical is 7.69 kg/day which could result in concentrations of up to 59.2 µg/L notified chemical (= 7.69 kg day^{-1} / 130 ML) in the river receiving effluent from the paper mill.

Up to 50% of the imported quantity of notified chemical that is applied to paper at the paper mill (9000 kg (= 0.5 × 0.9 × 20,000 kg)) could potentially enter paper recycling in Australia. This equates to 45% of the total imported volume. The typical concentrations of the notified chemical in surface waters resulting from paper recycling can be calculated using a worst case continental model in which it is assumed that none of the notified chemical entering waste water treatment plants (either on-site or municipal) is removed from the effluents.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	20,000	kg/year
Proportion expected to be released to sewer	45%	
Annual quantity of chemical released to sewer	9,000	kg/year
Days per year where release occurs	260	days/year
Daily chemical release:	34.62	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	0%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	7.65	µg/L
PEC - Ocean:	0.765	µg/L

As the notified chemical is a water soluble, highly sorptive substance, the SimpleTreat model was not used to estimate the removal of the notified chemical within STPs. This is because SimpleTreat utilises the n-

octanol/water partition coefficient (Kow), rather than the soil adsorption coefficient (K_{oc}) of the chemical, to estimate removal of the notified chemical from the water column due to sorption to sludge. Hence the use of SimpleTreat may lead to an overestimation of the aquatic exposure concentration of water soluble, highly sorptive chemicals (European Commission, 2003). The predicted environmental concentrations (PECs) calculated above are therefore upper limits since a high proportion of the notified chemical is expected to sorb to sludge in STPs. Furthermore, the notified chemical has a potential for biodegradation, however the low rate of degradation cannot be accounted for by the SimpleTreat model, in this case.

Based on the above calculations, the maximum PEC for the notified chemical in surface water is 66.85 µg/L (= 59.2 + 7.65) for river water and 6.685 µg/L (= 59.2/10 + 0.765) for ocean waters receiving combined effluents from paper recycling and paper manufacture.

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 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 7.654 µg/L may potentially result in a soil concentration of approximately 51.03 µg/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 255 µg/kg and 510 µg/kg, respectively. These are maximum values as the notified chemical is expected to sorb to sludge and biodegrade.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Acute Studies		
Fish Toxicity (96 h; study 1)	LC50 > 100 mg/L	Not Harmful
Fish Toxicity (96 h; study 2)	LC50 > 100 mg/L	Not Harmful
Daphnia Toxicity (48 h)	EC50 > 100 mg/L	Not Harmful
Algal Toxicity (72 h)	E_rC50 > 39 mg/L	Potentially harmful
Inhibition of Bacterial Respiration (3 h)	IC50 > 1000 mg/L	Not inhibitory to bacterial respiration
Earthworm Toxicity (14 d)	LC50 > 1000 mg/kg	Very slightly toxic
Chronic Studies		
Daphnia Toxicity (21 d)	NOEC = 3.2 mg/L	Not harmful with long lasting effects
Algal Toxicity (72 h)	NOEC = 39 mg/L	Not harmful with long lasting effects

Under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009) the notified chemical is considered to be not acutely harmful to fish and aquatic invertebrates and not chronically harmful to aquatic invertebrates or algae. In the absence of a defined endpoint for the algal 72 hr E_rC50, it is not appropriate to utilise this algal endpoint to determine the formal GHS Hazard Classifications for the notified chemical. Therefore based on the acute toxicity to fish and daphnia and the chronic toxicity to daphnia and algae, the notified chemical is formally not classified for acute or long-term hazard under the GHS.

7.2.1. Predicted No-Effect Concentration

As there were both acute and chronic endpoints available for the notified chemical, the Predicted No-Effect Concentration (PNEC) was calculated with both the lower limit for the acute endpoints and lowest chronic endpoint. The lowest resultant PNEC was used in the assessment. The lowest PNEC was derived from the chronic endpoints, and an assessment factor of 50 was used as chronic toxicity endpoints are available for the effects of the notified chemical on aquatic species from two trophic levels.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
NOEC (daphnia, 21d)	3.2	mg/L
Assessment Factor	50	
PNEC:	64	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	66.85	64	1.04
Q - Ocean	6.685	64	0.104

The risk quotient (Q = PEC/PNEC) for aquatic exposure is calculated to be very close to 1 based on the above calculated PEC and PNEC values. The calculated risk quotient is an upper limit since it is likely that a substantial amount of notified chemical will sorb to sludge during paper recycling processes and in sewage treatment plants where it is expected to biodegrade. As the risk quotient is anticipated to be < 1 due to sorption to sludge and biodegradation, the notified chemical is therefore not expected to pose an unreasonable risk to the aquatic environment from its assessed use pattern.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point > 360 ºC

Method EC Council Regulation No 96/69/EEC A.1 Melting/Freezing Temperature.
ASTM E537-86
Remarks Measured using differential scanning calorimetry.
No significant protocol deviations.
Test Facility Safepharm (2000a)

Density 1,890 kg/m^3 at 21.0 ºC

Method EC Council Regulation No 92/69/EEC A.3 Relative Density.
Remarks Testing was carried out using a Quantachrome MVP-2 gas comparison pycnometer.
No significant protocol deviations.
Test Facility Safepharm (2000a)

Water Solubility > 499 g/L at 20 ± 0.5°C

Method 92/69/EEC A.6 Water Solubility.
Remarks Flask method. Test substance (~12 g) was added to three separate flasks each with approximately 12 g of double distilled water. The flasks were shaken at approximately 30°C, and after standing for 20°C for a period of not less than 24 hours, the contents were filtered and concentration of test substance was determined by HPLC.
Test Facility Safepharm (2000a)

Hydrolysis as a Function of pH $t_{½}$ > 1 year at 25°C at pH 4, 7 and 9

Method 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 were prepared in stoppered glass flasks at nominal concentration of 1 g/L in three buffer solutions (pH 4, 7 and 9). The solutions were shielded from light whilst maintained at the test temperature of 50.0 ± 0.5°C for a period of 5 days. Aliquots of sample solutions were taken from flasks at various times and the pH and concentration of test substance was measured. Less than 10% hydrolysis was observed after 5 days at 50°C at pH 4, 7 and 9 and therefore the estimated half-life at 25°C is > 1 year.
Test Facility Safepharm (2000a)

Partition Coefficient (n-octanol/water) log Kow < -2.04 at 23 ± 0.5ºC

Method 92/69/EEC A.8 Partition Coefficient.
Remarks Shake Flask Method. Test material (2.5069 g) was added to 500 mL n-octanol saturated water to prepare a stock solution. The pH was adjusted to 7.1 with HCl. Six partitions were performed by shaking stock solution with water saturated n-octanol. The shaking was performed by inversion of flasks over a 5 min period. After separation, aliquots of both phases were analysed for test substance concentration by HPLC. The test should be carried out on the unionised form of the test substance, however this was not possible as the test substance since the test substance has functions with pKas outside of the working pH range of the test.
Test Facility Safepharm (2000a)

Surface Tension 64.9 mN/m at 24.0 ºC

Method EC Council Regulation No 92/69/EEC A.5 Surface Tension.

Remarks Concentration: 1.01 g/L
Test Facility Safepharm (2000a)

Adsorption/Desorption – screening test $\log K_{oc} = 3.75$ at $23.0 \pm 3.0^{o}C$

Method OECD TG 106 Adsorption - Desorption Using a Batch Equilibrium Method.

Soil Number/Type	*Organic Carbon Content (%)*	*pH*	*K_{oc}*
1/Typical brown earth Wick series	0.6	4.8	7.43×10^3
2/Typical brown earth Bearsted series	1.8	5.5	6.34×10^3
3/Typical brown earth Wick series	0.6	7.3	2.98×10^3

Remarks A screening level batch equilibrium adsorption/desorption study was conducted on three soil types. All samples were protected from light to avoid photodegradation.

Adsorption test
Replicate soil samples were prepared by adding ~0.1 g (soil 1) or ~5 g (soils 2 and 3) to a solution of test substance containing $CaCl_2$ (0.016M). For each soil a control was also prepared and a soil-less control was also run in parallel. After 16 hours of agitation the samples were centrifuged and an aqueous aliquot was taken for analysis by HPLC. The log K_{oc} was 3.97, 3.80 and 3.47 for the soils 1, 2 and 3 respectively. The mean of the log K_{oc} values for the three soils was reported. The notified chemical was demonstrated to be either slightly mobile or immobile in the soils tested.

Desorption test
For each soil sample, the supernatant removed during the adsorption step was replaced with an equivalent volume (20.0 mL) of 0.01M $CaCl_2$. The samples were re-equilibrated by shaking for the same time in the adsorption step. After equilibration the samples were centrifuged to separate phases and an aliquot was taken for analysis by HPLC. The desorption step was repeated using a further volume (20.0 mL) of 0.01M $CaCl_2$ solution. The mean test material desorbed was 26.8%, 1.74% and 8.51% (%w/w) for soils 1, 2 and 3 respectively. Significant desorption of the test substance from soils was observed under the conditions of the test.

Test Facility Safepharm (2000b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Sprague-Dawley
Vehicle	Water
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	3 per sex	2,000	0/6

LD50	> 2,000 mg/kg bw
Signs of Toxicity	There were no deaths. No signs of systemic toxicity were noted.
Effects in Organs	No abnormalities were noted at necroscopy
Remarks - Results	Body weight gains were as expected.

CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Safepharm (1999a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Analogue 1 (78.5% purity)
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test. EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal) – Limit Test.
Species/Strain	Rat/Sprague-Dawley
Vehicle	Test substance administered as supplied.
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5 per sex	2,000	0/10

LD50	> 2,000 mg/kg bw
Signs of Toxicity - Local	There were no test substance related dermal reactions.
Signs of Toxicity - Systemic	There were no deaths or test-substance related clinical signs. There were no signs of systemic toxicity.
Effects in Organs	No abnormalities were noted at necroscopy
Remarks - Results	Body weight gains were as expected for male animals. Three female animals showed a temporary initial weight loss followed by weight gain to the expected level in the second week.

CONCLUSION	Analogue 1 is of low toxicity via the dermal route.
TEST FACILITY	Safepharm (1994a)

B.3. Irritation – skin

TEST SUBSTANCE	Analogue 1 (78.5% purity)
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Council Regulation No 92/69/EEC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	Test substance administered as supplied.
Observation Period	72 hours
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	0	0	1	< 24 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 single 4-hour, semi-occluded application of the test material to 3 rabbits produced slight erythema at the 1 hour observation in 2 animals. All treated skin sites appeared normal at the 24-hour observation. No corrosive effects were noted.
CONCLUSION	Analogue 1 is slightly irritating to the skin.
TEST FACILITY	Safepharm (1994b)

B.4. Irritation – eye

TEST SUBSTANCE	Analogue 1 (78.5% purity)
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Council Regulation No 92/69/EEC B.5 Acute Toxicity (Eye Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Observation Period	72 Hours
Remarks - Method	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			
Conjunctiva: redness	0.7	0.7	0.3	2	< 72 Hours	0
Conjunctiva: chemosis	0.3	0.3	0	2	< 48 Hours	0
Conjunctiva: discharge	0	0	0	2	< 24 Hours	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	1	< 24 Hours	0

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

Remarks - Results	A single application of the test material to the non-irrigated eye of three rabbits produced mild conjunctival irritation, which had resolved in all animals by the 72 h observation.
CONCLUSION	Analogue 1 is slightly irritating to the eye.

TEST FACILITY Safepharm (1994c)

B.5. Skin sensitisation

TEST SUBSTANCE Analogue 1 (78.5% purity)

METHOD OECD TG 406 Skin Sensitisation – Guinea Pig Maximisation Test.
EC Directive 92/69/EEC B.6 Skin Sensitisation – Guinea Pig Maximisation Test.

Species/Strain Guinea pig/Dunkin-Hartley

PRELIMINARY STUDY Maximum Non-irritating Concentration:
intradermal: < 1% in water
topical: < 10% in water

MAIN STUDY

Number of Animals Test Group: 10 Control Group: 5

INDUCTION PHASE Induction Concentration:
intradermal: 10% in water
topical: 75% in water

Signs of Irritation Following intradermal induction very slight erythema was noted in all test group animals at the 24 and 48-hour observations. No signs of irritation were noted in the control animals. Following topical induction very slight erythema was noted in all test group sites at the 1-hour observation and one test animal at the 24-hour observation. Very slight erythema was also noted in one control group animal at the 1-hour observation.

CHALLENGE PHASE

1st challenge topical: 50% and 25% in water

Remarks - Method No significant protocol deviations

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: 1st challenge*	
		24 h	*48 h*
Test Group	25%	0/10	0/10
	50%	0/10	0/10
Control Group	25%	0/5	0/5
	50%	0/5	0/5

Remarks - Results There were no skin reactions noted at any of the challenge sites in both the test and control animals. Body weight gains were comparable between the test and control groups.

CONCLUSION There was no evidence of reactions indicative of skin sensitisation to analogue 1 under the conditions of the test.

TEST FACILITY Safepharm (1994d)

B.6. Repeat dose toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents.

Species/Strain Rat/Sprague-Dawley Crl:CD (SD) IGS BR

Route of Administration Oral – gavage

Exposure Information Total exposure days: 90 days
Dose regimen: 7 days per week
Post-exposure observation period: none

Vehicle Water

Remarks - Method The high dose level was reduced from 750 mg/kg bw/day to 500 mg/kg

bw/day on day 56 due to the level of mortalities.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10 per sex	0	0/20
low dose	10 per sex	5	0/20
mid dose	10 per sex	50	0/20
high dose	10 per sex	750/500	7/20

Mortality and Time to Death

Seven animals in the high dose group died during the course of the study. Three female animals died during the administration of the 750 mg/kg bw/day dose on days 28, 42 and 53. After the dose was reduced to 500 mg/kg bw/day a further two female animals died on days 58 and 74 and two male animals were killed *in extremis* on days 85 and 89.

Clinical Observations

Animals in the high dose group showed a hunched posture, pilo-erection, pallor of the extremities and a tiptoe gait. There were no treatment related clinical signs noted during the study for animals in the mid and low dose groups. Reduced bodyweight gain was recorded in high dose males and during the first three weeks of the study for high dose female animals. Bodyweight gains for the mid and low dose groups were comparable to the control animals. There was reduced food consumption in the high dose group throughout the study but no changes were seen in the mid and low dose groups. There were no differences between the groups in relation to water consumption and there were no adverse ophthalmologic findings.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

High dose group animals showed signs of microcytic hypochromic anaemia, with statistically significant reductions in haemoglobin, haematocrit and erythrocyte counts and statistically significant increases in the mean cell haemoglobin. Male animals in the high dose group also showed a statistically significant reduction in clotting times and statistically significant increases in the mean cell haemoglobin concentration and leucocyte count, these effects were observed in female animals in the high dose group but did not reach statistical significance.

Animals in the high dose group showed statistically significant reductions in albumin concentration. Male animals in the high dose group also showed a statistically significant reduction in total protein and statistically significant increases in the alanine aminotransferase, aspartate aminotransferase and phosphorus. Female animals in the high dose group also showed a statistically significant increase in sodium concentration.

No adverse effects were seen in the mid and low dose groups.

Effects in Organs

Liver and kidney weights relative to the terminal body weight showed statistically significant reductions in high dose animals of either sex. Further statistically significant reductions in the high dose group were attributed by the study authors to the reduced body weight development. Relative brain and heart weights in both sexes in the high dose group showed a statistically significant increase and a statistically significant reduction in the relative adrenal and epididymis weight in males in the high dose was also observed. Female animals in the low dose group had reduced absolute ovary weights and in the mid dose group reduced absolute spleen and relative liver weights in the absence of supporting evidence the findings were considered to be of no toxicological significance.

Animals that died before the end of the study had pale, red or small adrenals, dark intestines/stomach contents, raised limiting ridge of the stomach, pale or mottled appearance of the liver and kidneys and lung discolouration with dark foci present with isolated cases of pale pancreas/uterus and small testes/seminal vesicles. High dose animals killed at the end of the study displayed the following symptoms pale kidneys, liver, gastro-intestinal tract, pituitary gland, small darkened adrenals, small testes seminal vesicles and ovaries. No adverse macroscopic findings were noted in the organs of animals in the mid and low dose groups.

In high dose animals hepatotoxic effects consisting of generalised hepatocyte enlargement, single cell hepatocyte necrosis, karyomegaly and variation in nuclear size, increased mitotic activity, bile duct

proliferation, eosinophillic inclusions, accumulations of pigment, periportal hepatocyte lipid vacuolation, mulifocal hepatocyte necrosis and periportal hepatocyte degeneration/necrosis were observed. Male animals in the high dose had statistically significant increases in the pigment accumulation in the spleen, vacuolation of groups of interstitial cells in the myocardium, trabecular bone formation in the femur, adipose infiltration of the bone marrow, bilateral testicular atrophy, a reduction of epididymal spermatozoal content, prostrate secretory content and seminal vesicles secretory content. In the high dose group an oedematous appearance of the exocrine pancreas, cortical atrophy of adrenals, tubular basophillia and degeneration in the kidneys, accumulations of eosinophillic pigment in the kidneys, accumulations of alveolar macrophages in the lungs, vacuolated histocytes in the lymph nodes and lymphoid atrophy in the thymus was observed in both sexes. The ovaries of some female animals in the high dose group also contained no developing follicles.

Remarks – Results

In the high dose group a wide range of adverse effects including death were seen in both sexes. In the low and mid dose groups female animals had reduced absolute ovary weights, reduced absolute spleen and relative liver weights however, in the absence of other related changes the findings were considered to be of no toxicological significance. Therefore, based on the adverse effects seen in the high dose group the No Observed (Adverse) Effect Level (NO(A)EL) was established as 50 mg/kg bw/day in this study.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 50 mg/kg bw/day in this study, based on adverse effects including mortality seen at the higher dose.

TEST FACILITY Safepharm (2004a)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Directive 92/69/EEC 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 fraction from phenobarbitone/β-napthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 50 – 5,000 µg/plate b) Without metabolic activation: 50 – 5,000 µg/plate
Vehicle	Water
Remarks - Method	No significant protocol deviations.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5,000	> 5,000	> 5,000	negative
Test 2		> 5,000	> 5,000	negative
Present				
Test 1	> 5,000	> 5,000	> 5,000	negative
Test 2		> 5,000	> 5,000	negative

Remarks - Results

The test material was tested up to the maximum recommended dose level of 5,000 µg/plate. No toxicologically significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation.

All the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the

activity of the S9-mix and the sensitivity of the bacterial strains.

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

TEST FACILITY Safepharm (1999b)

B.8. 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
Metabolic Activation System Unknown
Vehicle Unknown
Remarks - Method There was a page missing from the test report that covered the preparation of the metabolic activation system and the test and control samples.
The osmolality of the test material increased to levels greater than 50 mOsm at dose levels above 4,000 µg/mL and hence the maximum concentration used in the main study was limited to 4,000 µg/mL.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	0*, 500*, 1000*, 2000*, 3000*, 3500*, 4000*	3 hours	2 days	10-14 days
Test 2	0*, 1000*, 2000*, 2500*, 3000*, 3500*, 4000*	24 hours	2 days	10-14 days
Present				
Test 1	0*, 500*, 1000*, 2000*, 3000*, 3500*, 4000*	3 hours	2 days	10-14 days
Test 2	0*, 1000*, 2000*, 2500*, 3000*, 3500*, 4000*	3 hours	2 days	10-14 days

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 3,380	> 4,000	> 4,000	negative
Test 2		> 4,000	> 4,000	negative
Present				
Test 1	> 3,380	> 4,000	> 4,000	negative
Test 2		> 4,000	> 4,000	negative

Remarks - Results The positive and vehicle controls gave satisfactory responses, confirming the validity of the test system.
The test material did not induce any statistically significant increases in the mutant frequency at the TK +/- locus in L5178Y cells.

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

TEST FACILITY Safepharm (2003)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE: Notified chemical (purity > 60%)

METHOD: OECD TG 301 D Ready Biodegradability: Closed Bottle Test.

Inoculum	Sewage treatment microorganisms from domestic sewage treatment plant
Exposure Period	28 days
Auxiliary Solvent	None reported
Analytical Monitoring	O_2 concentration
Remarks - Method	The test was conducted in accordance with the guideline above. All preparations were carried out under non-actinic laboratory safety light to minimise photodegradation of the test substance. The ThOD was not calculated as the identity of all components of the test substance was not known. The COD of the test material was determined instead to calculate the biodegradation of test substance and was reported to be 0.64 mg O_2/ mg.

RESULTS

Test substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
3	8	3	8
9	9	9	45
15	12	15	62
28	23	28	71

Remarks - Results: All validity criteria were satisfied. Degradation of the reference compound, sodium benzoate, exceeded the pass level of 60% degradation after 14 days. In the toxicity test, there was more than 25% degradation after 14 days, hence the test substance is not inhibitory to the inoculum at the tested concentration.

CONCLUSION: The notified chemical is not readily biodegradable

TEST FACILITY: Safepharm (1999c)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish (study 1)

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 203 Fish, Acute Toxicity Test – Semi-static

Species	*Oncorhynchus mykiss* (Rainbow trout)
Exposure Period	96 hours
Auxiliary Solvent	None reported
Water Hardness	~ 100 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	Following a range finding test, a limit test was conducted in duplicate at nominal concentration of 100 mg/L under semi-static conditions with daily renewal according to the guidelines above. Test conditions: 14.0°C, pH 7.7–7.8, 16-h light to 8-h darkness photoperiod, dissolved oxygen concentration 9.7–9.8 mg/L. A low intensity non-actinic light source was used to prevent photo-degradation of the test substance.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	< LOQ	10	0	0	0	0	0
100	102 – 107	20	0	0	0	0	0

LOQ (limit of quantification) = 1.5 mg/L

LC50	> 100 mg/L at 96 hours (based on nominal concentrations)
NOEC	100 mg/L at 96 hours (based on nominal concentrations)
Remarks – Results	All validity criteria for the test were satisfied and no significant deviations to protocol were reported. There were no sub-lethal effects or mortalities observed in the test organisms.

CONCLUSION The notified chemical is not harmful to fish

TEST FACILITY Safepharm (1999d)

C.2.2. Acute toxicity to fish (study 2)

TEST SUBSTANCE Notified chemical (purity > 60%)

METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-static
Species	*Oncorhynchus mykiss* (Rainbow trout)
Exposure Period	96 hours
Auxiliary Solvent	None reported
Water Hardness	~ 100 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	Following a range finding test, a limit test was conducted in duplicate at nominal concentration of 100 mg/L under semi-static conditions with daily renewal according to the guidelines above. Test conditions: 14.0°C, pH 7.4–7.5, 16-h light to 8-h darkness photoperiod, dissolved oxygen concentration 9.9–10.1 mg/L. Test vessels were shielded from light throughout the test for the study duration.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	< LOD	10	0	0	0	0	0
100	99*	20	0	0	0	0	0

LOD (Limit of Detection) = 0.576 mg/L
*Measured at 96 hours

LC50	> 100 mg/L at 96 h (based on nominal concentrations)
NOEC	100 mg/L at 96 (based on nominal concentrations)
Remarks – Results	All validity criteria for the test were satisfied and no significant deviations to protocol were reported. There were no mortalities or any adverse reactions to exposure observed in the test organisms.

CONCLUSION The notified chemical is not harmful to fish

TEST FACILITY Safepharm (1995)

C.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD	OECD TG 202 *Daphnia sp.* Acute Immobilisation Test – Static
Species	*Daphnia magna*
Exposure Period	48 hours

Auxiliary Solvent	None reported
Water Hardness	~250 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	Following a range finding test, a limit test was conducted at nominal concentrations 100 mg/L under static conditions according to the guidelines above. Test solutions were prepared under low intensity non-actinic light source to prevent photo-degradation of the test substance. Test conditions were: 21.0°C, pH 8.0 – 8.2, 8.0 – 8.3 mg/L dissolved O_2.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	< LOQ	20	0	0
100	101.5*	40	0	0

LOQ (limit of quantitation) = 1.5 mg/L
*Mean of 4 replicates

EC50	> 100 mg/L at 48 hours (based on nominal concentrations)
NOEC	100 mg/L at 48 hours (based on nominal concentrations)
Remarks - Results	All validity criteria for the test were satisfied and no significant deviations to protocol were reported. There was no immobilisation observed in the test organisms.

CONCLUSION The notified chemical is not harmful to aquatic invertebrates

TEST FACILITY Safepharm (1999e)

C.2.4. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 211 *Daphnia magna,* Reproduction Test – Flow-through

Species	*Daphnia magna*
Exposure Period	21 days
Auxiliary Solvent	Not reported
Water Hardness	242 – 260 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	Based on the information from the Acute Toxicity test to *Daphnia magna* (reference: Safepharm 1999e) a definitive test was conducted at nominal concentrations of 1.0, 3.2, 10, 32 and 100 mg/L under flow-through conditions according to the guidelines above. Test solutions were renewed continuously under at a rate of approximately 30 mL/min. Test conditions: temperature maintained at approximately 21°C, dissolved oxygen %ASV 93-97%, pH 7.9 – 8.0. The EC50 (immobilisation) value at 21 days was calculated by the trimmed Spearman-Karber method. The EC50 (reproduction) value at 21 days was calculated by the maximum-likelihood probit method.

Day 21				
Nominal concentration (mg/L)	*Measured concentration (mg/L)*	*Percentage of parental generation surviving*	*Mean number of offspring produced per female – (standard deviation)*	*Mean body length / mm (standard deviation)*
Control	< LOQ	100	109.6 (12.9)	4.5 (0.4)
1.0	0.892	100	109.2 (9.1)	4.6 (0.3)
3.2	3.50	100	110.8 (18.9)	4.6 (0.3)
10	10.7	100	72.8 (11.7*)	4.5 (0.3)
32	34.9	10	16	**

100	Not reported	0	0	**

*Significant difference **Not measured due to adult mortality
LOQ = 0.11 mg/L

EC50 (reproduction)	16 mg/L at 21 days (based on nominal concentrations) (95% CI 9.8 – 26 mg/L)
NOEC (reproduction)	3.2 mg/L at 21 days (based on nominal concentrations)
EC50 (immobilisation)	20 mg/L at 21 days (based on nominal concentrations) (95% CI 16 – 25 mg/L)
NOEC (immobilisation)	3.2 mg/L at 21 days (based on nominal concentrations)
Remarks - Results	No significant deviations to protocol were reported and all validity criteria for the test were satisfied.

Due to the nature of the test it was not possible to shield the test vessels from light. However precautionary measures were taken to prevent photo-degradation of test material including the use of a non-actinic light source during preparation of stock solutions and the dynamic, continuous flow testing conditions. Analysis of the test solutions sampled throughout the test show measured test concentrations were 62% – 142% of nominal values. However the majority of test concentrations were within 80% – 120% of nominal values and hence it was considered justified to based the results on nominal concentrations only.

There was a significant effect on size and colour of the parental generation daphnids at test concentration 100 mg/L. The organisms at this concentration were markedly smaller and paler in colour than the control animals from Days 5 – 11, when all were eliminated due to toxic effects of the test material. A significant effect on size and colour of the daphnids at the 32 mg/L concentration was observed from Day 14 to termination of the test on Day 21 where 50 – 100% were markedly paler in colour than the control animals. The parent daphnids in the remaining test concentrations were observed to be the same size and colour as the control animals.

After 21 days there were no statistically significant differences between the control and the 1.0, and 3.2 mg/L test groups in terms of the number of live young produced per adult. The 10 mg/L test group showed a significant difference from the control and remaining test groups after 21 days in terms of producing fewer of live young per adult. The 32 mg/L group was not included in the statistical analysis due to significant mortalities in the parental generation. The 100 mg/L group was not included in statistical analysis as exposure to the test material eliminated all daphnids prior to Day 21 of the test.

There were no statistically significant differences ($P \geq 0.05$) between the control and the 1.0, 3.2 and 10 mg/L test groups in terms of length of the daphnids after 21 days exposure to the test material. The 32 mg/L group and 100 mg/L group were not included in the statistical analyses as per the reasons given in the above paragraph.

CONCLUSION	The notified chemical is not harmful to aquatic invertebrates with long lasting effects
TEST FACILITY	Safepharm (2004b)

C.2.5. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test

Species	*Scenedesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 100 mg/L Actual: 102 mg/L (0 h), 9.72 mg/L (72 h)
Auxiliary Solvent	Not reported
Water Hardness	0.15 mmol Ca^{2+} and Mg^{2+}
Analytical Monitoring	HPLC
Remarks - Method	Following a range finding test, a limit test was conducted in triplicate in accordance with the guidelines above. No significant deviations to protocol were reported. Test conditions: 24 ± 1°C, pH 7.4 – 9.2, continuous illumination (~7000 lux). The growth endpoints were estimated by applying a Students t-test to determine if there was a statistically significant difference (P ≥ 0.05) between the area under the growth curves for the control and 100 mg/L test group.

RESULTS

Biomass		*Growth*	
E_yC_{50} mg/L at 72 h	*NOEC mg/L at 72 h*	*E_rC50 mg/L at 72 h*	*NOEC mg/L at 72 h*
> 39	Not reported	> 39	39

Remarks - Results	The test substance measured concentrations at the beginning of the test were near nominal (102%) but decreased to 10% of nominal at 72 hours. This was considered to be due to photo-degradation of the test substance based on a stability study carried out on the test substance (under light the test substance decreased to 6% of its initial concentration after 72 hrs). Hence time weighted mean measured test concentrations were used to determine the EC50 and NOEC. The test results should be treated with caution as the toxicity can be considered to be due to the test substance and degradants of the test substance. The notified chemical is considered potentially harmful to algae as based on the lower threshold for E_rC50 reported, it is possible that the E_rC50 < 100 mg/L.

CONCLUSION The notified chemical is potentially harmful to algae

TEST FACILITY Safepharm (1999f)

C.2.6. Acute toxicity to earthworms

TEST SUBSTANCE Notified chemical

METHOD OECD TG 207 Earthworm, Acute Toxicity Tests

Species	*Eisenia foetida* (earthworm)
Exposure Period	14 days
Analytical Monitoring	None reported
Remarks – Method	Following a preliminary range-finding study, earthworms were exposed to the test substance according to guidelines above. Test conditions were: temperature 19.9 – 22.1°C, pH 6.1 – 6.3, soil moisture content (as % of dry weight) 25 – 30%. The LC50 for the test substance was estimated by inspection of mortality data. Statistical analysis of the earthworm weight data was performed using Bartlett's test for homogeneity of variance and a Students t-test. The results from a positive control study using chloroacetamide were reported.

RESULTS

Concentration mg/kg		*Cumulative mortality / total earthworms*		*Mean earthworm weight (g)*	
Nominal	*Actual*	*Day 7*	*Day 14*	*Day 0*	*Day 14*

Control	Not reported	1*/40	1*/40	0.373	0.298
1000	Not reported	0/60	1*/60	0.407	0.257

*Considered to be due to natural causes and/or handling stress

LC50	> 1000 mg/kg at 14 days (based on nominal test concentration)
NOEC	1000 mg/kg at 14 days (based on nominal test concentration)
Remarks – Results	The validity criterion for the test was satisfied (mortality in the controls < 10% at the end test) and no significant deviations to protocol were reported. The LC50 for the positive control study was 29 mg/kg after 14 day, which was within the normal range for the reference material. The worms in the 1000 mg/kg test group were shown to be significantly larger ($P < 0.05$) than those in the control group on Day 0. However given that no significant differences ($P \geq 0.05$) were found in terms of weight between the control and the 1000 mg/kg test group on Day 14, this difference was considered not to have affected the overall outcome of the test.
CONCLUSION	The notified chemical is not very toxic to earthworms (Mensink et al., 1995)
TEST FACILITY	Safepharm (2004c)

C.2.7. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sewage sludge
Exposure Period	3 hours
Concentration Range	Nominal: 1000 mg/L Actual: Not measured
Remarks – Method	Following a range finding test, a limit test was conducted in triplicate in accordance with the guidelines above. No significant deviations to protocol were reported. A blank control and reference (3,5-dichlorophenol) control were run in parallel. Test conditions were: 21°C, pH 7.5–7.7, normal laboratory lighting.
RESULTS	
IC50	> 1000 mg/L at 3 hours
NOEC	1000 mg/L at 3 hours
Remarks – Results	The validation criteria for the control respiration rates and reference material, (3,5-dichlorophenol) EC_{50} were satisfied.
CONCLUSION	Not inhibitory to bacterial respiration
TEST FACILITY	Safepharm (1999g)

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