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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Anthraquinine dye in PictureMate Photo Cartridge T5852

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act) and *Industrial Chemicals (Notification and Assessment) Regulations 1990*. 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 and Heritage.

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

Director
NICNAS

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FULL PUBLIC REPORT

E-300

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Epson Australia Pty Ltd (ABN 91 002 625 783)
3 Talavera Road
North Ryde NSW 2113

NOTIFICATION CATEGORY
Limited-small volume: Chemical other than polymer, (1 tonne or less per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: Identity of chemical (Chemical name, CAS No., Molecular and Structural formulae, Molecular weight, Spectral data), Composition (Purity, impurities), Import volume.

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None.

NOTIFICATION IN OTHER COUNTRIES
A VIIA Notification in the EU (UK) and a VIIA Notification in Switzerland.

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
E-300
Anthraquinine dye in PictureMate Photo Cartridge T5852

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

3. COMPOSITION

DEGREE OF PURITY
> 80%

4. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20ºC and 101.3 kPa

Property	Value	Data Source/Justification
Melting Point/Freezing Point	288ºC	Measured
Boiling Point	>331ºC at 101.3 kPa	Measured
Density	1590 kg/m^3 at 22.1ºC	Measured
Vapour Pressure	<2.7 x 10^{-8} kPa at 25ºC.	Measured
Water Solubility	In the range 49.9 to 51.9% (w/w) at 20°C.	Measured
Surface Tension	72.4 mN/m at 22.2ºC	Measured

Hydrolysis as a Function of pH	>365 d at pH 4, 7 and 9 at 25°C	Measured
Partition Coefficient (n-octanol/water)	log P_{OW} <-6.41at 20°C	Measured
Adsorption/Desorption	log K_{OC} <1.25 at 30°C	Measured
Dissociation Constant	pKa = 4.2 and 0.7 for the two major dissociable functional groups	Estimated
Particle Size	Inhalable fraction (<100 µm): 27.9% Respirable fraction (<10 µm): 4.66%	Measured
Flash Point	Not determined	Solid (high M.P. and low vapour pressure)
Flammability	Not highly flammable	Measured
Autoignition Temperature	> 400°C	Measured
Explosive Properties	Not predicted to be explosive	Estimated
Oxidising Properties	Not predicted to be oxidising	Estimated

Discussion of Observed Effects

For full details of the physical-chemical properties tests please refer to Appendix A.

Reactivity

Based on the chemical structure and experience in use the test material is predicted to be stable under normal environmental conditions.

Dangerous Goods classification

Based on the available physico-chemical properties the notified chemical is not classified as a Dangerous Good according to the Australian Dangerous Goods Code (FORS, 1998).

5. INTRODUCTION AND USE INFORMATION

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

In sealed inkjet cartridges (5 – 100 mL) filled and sealed prior to being imported to Australia.

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

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

PORT OF ENTRY

Sydney.

IDENTITY OF MANUFACTURER/RECIPIENTS

Inkjet cartridge distributors.

TRANSPORTATION AND PACKAGING

The notified chemical with be imported ready packed in 5 – 100 mL sealed inkjet cartridges in plastic shrink wrap.

USE

The notified chemical is used in water-soluble ink for use in ink-jet printers with plain paper. This notified chemical is at < 10% in the ink.

OPERATION DESCRIPTION

No reformulation or repackaging of the product occurs in Australia. The product is delivered to the end-user as it is imported into Australia. The sealed ink cartridges will be handled by service technicians or office workers replacing the spent cartridges in the printer.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure assessment

6.1.1. Occupational exposure

Number and Category of Workers

Category of Worker	*Number*	*Exposure Duration*	*Exposure Frequency*
Importation/Waterside workers	10	4 hours per day	70 days per year
Storage and transport	100	6 hours per day	240 days per year
Office worker/Service technician	10000	<0.1/0.5 hours per day	20/100 days per year

Exposure Details

The notified chemical is contained in sealed cartridges at a concentration of <10% in the ink.

Office workers, customer service engineers and the public will replace spent ink cartridges. Replacement of printing cartridges involves removal of the old printing cartridge from the printing machine and directly loading the new cartridge. These workers may have dermal contact with very small quantities of the notified chemical if they touch the print heads while replacing the cartridges, or by handling before the ink is adequately dried or if printing to a non-absorbent substrate occurs as an error. After the ink is dry the notified chemical is bound to the paper and is not expected to be readily bioavailable. Dermal and possible ocular exposure could occur when handling faulty or ruptured cartridges.

6.1.2. Public exposure

Limited exposure as described above may occur while changing the ink cartridges, however this will be relatively infrequent and should only result in very limited exposure to small quantities of the notified chemical.

After the ink is dry the notified chemical is bound to the paper and is not expected to be readily bioavailable. The public may be dermally exposed to the notified chemical, while handling printed paper or other substrates, where the ink is only partially dried.

6.2. Human health effects assessment

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	low toxicity, LD50 estimated >2000 mg/kg bw active ingredient
Rat, acute dermal toxicity	low toxicity, LD50 >2000 mg/kg bw active ingredient
Rabbit, skin irritation	not irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL = 25 mg/kg bw/day
Genotoxicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro chromosomal aberrations	non genotoxic

The notified chemical was of low acute toxicity via the oral and dermal routes in rats. It was not irritant to rabbit skin and was a slight eye irritant in rabbits. It was not a skin sensitiser in a mouse local lymph node assay and was not mutagenic in bacteria or clastogenic in Chinese Hamster lung cells in vitro. Subchronic toxicity was evident in a 28-day repeat dose oral toxicity study in rats with degenerative effects on the kidneys at and above a dose of 25 mg/kg bw/day. Changes in the lymph nodes in animals treated at and above a dose of 150 mg/kg bw/day were also noted and inflammatory gastric changes and changes to the spleen were noted in animals treated at the top dose (1000 mg/kg bw/day).

The notified chemical is not expected to be acutely toxic via the oral or dermal routes. It is noteworthy that red kidneys were noted after acute oral administration but not after dermal application. It would be predicted from the high molecular weight of the notified chemical and the low log P_{ow} that absorption across biological membranes should not occur. It would appear that absorption occurs from the g.i. tract but not via the skin. Therefore, metabolism in the gut may be responsible rather than the discolouration of the kidneys being due to absorption of lower molecular weight coloured impurities. This discolouration was also seen in the oral repeat dose study in rats.

The notified chemical is not classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999). Although a NOAEL of 25 mg/kg bw/day was indicated in the 28-day oral repeated dose study the nature of the adverse effects were not sufficient to warrant a classification of R48. The notifier also stated that the notified chemical did not require the harmful classification with R48/22 by the UK Competent Authority.

6.3. Human health risk characterisation

6.3.1. Occupational health and safety

As the notified chemical is introduced in sealed ink jet cartridges, ingestion is an unlikely route of exposure. Exposure via the dermal route may occur intermittently from replacement of cartridges in a printer, handling substrates on which ink has yet to dry or during printer maintenance. It can be stated with some certainty that current ink jet printers and cartridges are designed such that ink spillage in the printer is unlikely to avoid compromising printer function. Therefore, exposure of maintenance workers should not occur or should be minimal. As the notified chemical is at < 10% in the ink and it is designed to be used on plain paper, droplet size is reduced to allow very fast drying. Thus, exposure to wet ink on plain paper or other absorbent substrates (eg inkjet printable CDs) is unlikely and in any case exposure to the notified chemical is already low. Thus, the only likely exposure scenario is when accidental printing occurs on non-absorbent substrates by error. If it assumed that 1 mg of notified chemical is printed on an A4 sized substrate, the following calculation may be done:

Area of contact with finger ends (four fingers on one hand) = 8 cm^2
A4 sized paper = ~600 cm^2
% Removal = (8/600) × 0.5 × 100 = <1% (assuming 50% of the ink dries on the paper)
∴ Exposure to fingertips per event = <1% of 1 mg = <u><0.01 mg per event</u>.

For extensive contact with wet ink on paper or other substrate (i.e. >10 events per day) the daily exposure, assuming no washing between events, a 70 kg person and 10% absorption (as a worst case), would be:

Daily exposure = (<0.001 (mg/event)×10) ÷ 70 = <u>~1.4 x 10^{-4} mg/kg bw/day</u>.

Based on a NOAEL of 25 mg/kg bw/day, derived from a 28-day rat oral study the margin of exposure (MOE) is significantly greater than 100. MOEs greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. Therefore, the risk of systemic effects to is considered acceptable.

Exposure during replacement of cartridges could occur but the cartridges require extreme care so as not to compromise the quality of the output of the printer. Nevertheless, some exposure to ink may occur as the ink outlet needs to be manually uncovered to allow ink flow to the print head by removal of adhesive tape. Such exposure is difficult to quantify but should be less than exposure via non-absorbent substrates as previously calculated. Therefore risk of systemic

effects is likely to be low.

Although the notified chemical is slightly irritating to the eye the risk of eye irritation during the end use by office workers and customer service engineers is expected to be low because of the concentration of the notified chemical in the printing ink (<10%) and limited potential for ocular exposure.

6.3.2. Public health

The inkjet cartridges containing the notified chemical will also be sold to the public with the same risk as calculated for office workers in section 6.3.1. Therefore, the risk of adverse health effects is low. Once the ink is dried on the substrate to which it is applied the notified chemical will not be bioavailable as it will be in a solid matrix and there is therefore no risk of systemic effects.

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 in sealed ink cartridges. There will be no release to the environment due to reformulation or repackaging.

RELEASE OF CHEMICAL FROM USE

The ink cartridges are designed to prevent leakage and will not be opened during transport, use, installation or replacement. Therefore, release of ink containing the notified chemical to the environment is not expected under normal conditions. These ink cartridges will be changed by office workers, customer service engineers and the public. However, if leakage or spillage does occur, the ink will be contained with absorbent material, which will presumably be disposed of via landfill from normal office garbage along with the empty cartridges.

The sealed ink cartridges are contained within the printer until they are removed for disposal. The minimal amount of residual ink left in the empty cartridges is expected to be disposed of to landfill.

Most of the notified chemical will be dried on to printer paper, which will be disposed of to landfill, recycled or incinerated. Recycling of treated paper may result in the release of a proportion of the notified chemical to the aquatic compartment. Waste paper is repulped using a variety of chemical treatments, which result in fibre separation and ink detachment from the fibres. The wastes are expected to go to trade waste sewers. Due to the low percentage of notified chemical in the ink and the widespread use, release to the aquatic compartment will be highly diffused. The notified chemical adsorbed to sludge during the recycling process will be disposed of to landfill.

RELEASE OF CHEMICAL FROM DISPOSAL

No special precautions are required. Empty cartridges containing the ink preparation are expected to be disposed of by landfill.

7.1.2 Environmental fate

A single Ready Biodegradability test was conducted on the notified chemical, which attained 9% degradation after 28 days, and therefore, cannot be considered as readily biodegradable under the strict terms and conditions of OECD Guideline No 301D. For the details of the environmental fate study please refer to Appendix C.

7.1.3 Predicted Environmental Concentration (PEC)

Manufacture, reformulation and packaging into end-use containers occurs overseas, and release is not expected. After use, printed paper may be disposed of by incineration, to landfill or be recycled. Notified chemical disposed of to landfill, may be mobile, however the low proposed annual import volume, and diffuse release throughout Australia will mitigate any potential exposure while the notified chemical slowly degrades.

In Australia, approximately 50% of printed paper is recycled. The following Predicted Environmental Concentration calculation assumes this 50% recycling, and as a worst case scenario assumes no recovery within STPs.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1,000	kg/year
Proportion expected to be released to sewer	50%	
Annual quantity of chemical released to sewer	500	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	1.37	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	20.496	million
Removal within STP	0%	
Daily effluent production:	4,099	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.33	µg/L
PEC - Ocean:	0.03	µg/L

7.2. Environmental effects assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	EC50 >100 mg/L	Not harmful.
Daphnia Toxicity	EC50 >100 mg/L	Not harmful.
Algal Toxicity	E_bC50 >100 mg/L	Not harmful.
	E_rC50 >100 mg/L	
Inhibition of Bacterial Respiration	EC50 >1000 mg/L	Not harmful.

The notified chemical was found not to be harmful to any of the test species exposed during ecotoxicity testing.

7.2.1 Predicted No-Effect Concentration

Aquatic ecotoxicity data were provided for three trophic levels, without any statistically significant toxicity being observed up to the maximum concentrations tested. The following Predicted No-Effect Concentration has been calculated using an assessment factor of 100.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50	>100	mg/L
Assessment Factor	100	
PNEC	>1	mg/L

7.3. Environmental risk assessment

Based on the above PEC and PNEC values, the following Risk Quotient has been calculated.

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River:	0.33	>1000	<0.00033
Q - Ocean:	0.03	>1000	<0.00003

This indicates that the proposed import volume and use pattern is not expected to pose an unacceptable risk to the aquatic environment.

8. CONCLUSIONS – SUMMARY OF RISK ASSESSMENT FOR THE ENVIRONMENT AND HUMAN HEALTH

8.1. Hazard classification

Based on the available data the notified chemical is not classified as hazardous under the NOHSC *Approved Criteria for Classifying Hazardous Substances*.

and

As a comparison only, the classification of 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***
Health, Environment	Not Classified	

8.2. Environmental risk assessment

On the basis of the PEC/PNEC ratio, the chemical is not considered to pose a risk to the environment based on its reported use pattern.

8.3. Human health risk assessment

8.3.1. Occupational health and safety

Under the conditions of the occupational settings described, the risk to workers is considered to be acceptable.

8.3.2. Public health

When used in the proposed manner the risk to the public is considered to be acceptable.

9. MATERIAL SAFETY DATA SHEET

The MSDS for ink containing the notified chemical provided by the notifier was reviewed by NICNAS and is published here as a matter of public record. The accuracy of the information on the MSDS remains the responsibility of the applicant. The MSDS was found to be in accordance with the NOHSC *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 2003).

10. RECOMMENDATIONS

CONTROL MEASURES

Occupational Health and Safety

- 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 NOHSC *Approved Criteria for Classifying Hazardous Substances*, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Environment

Disposal

- The notified chemical should be disposed of by incineration or to landfill.

Emergency procedures

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

11. REGULATORY OBLIGATIONS

This risk assessment is based on the information available at the time of notification. If the circumstances under which the notified chemical was assessed change a reassessment may be needed. Under 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 whether or not the notified chemical has been listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director of Chemicals Notification and Assessment 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 as a component of printer inks has changed, or is likely to change significantly;
- the amount of chemical being introduced (< 1 tonne) has increased, or is likely to increase, significantly;
- if 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.

12 . BIBLIOGRAPHY

FORS (Federal Office of Road Safety) (1998) Australian Code for the Transport of Dangerous Goods by Road and Rail (ADG code), 6th Edition, Canberra, Australian Government Publishing Service.

Kurume Laboratory (2005), Biodegradation Study of E-300 by Microorganisms, study number: 14407. Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan (unpublished report submitted by notifier).

NOHSC (1994) National Code of Practice for the Labelling of Workplace Substances [NOHSC:2012(1994)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

NOHSC (2003) National Code of Practice for the Preparation of Material Safety Data Sheets, 2nd edition [NOHSC:2011(2003)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

NOHSC (2004) Approved Criteria for Classifying Hazardous Substances, 3rd edition [NOHSC:1008(2004)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

SafePharm Laboratories Ltd (2005a) JDP Magenta E-300: Determination of General Phyico-chemical Properties. SPL Project No: 1307/390, Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK (unpublished report submitted by notifier).

Safepharm Laboratories Ltd (2005b) JDP Magenta E-300: Acute Oral Toxicity in the Rat – Acute Toxic Class Method, SPL project number: 1307/392. Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK (unpublished report submitted by notifier).

Safepharm Laboratories Ltd (2005c) JDP Magenta E-300: Acute Dermal Toxicity in the Rat, SPL project number: 1307/421. Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK (unpublished report submitted by notifier).

Safepharm Laboratories Ltd (2005d) JDP Magenta E-300: Acute Dermal Irritation in the Rabbit, SPL project number: 1307/393. Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK (unpublished report submitted by notifier).

Safepharm Laboratories Ltd (2005e) JDP Magenta E-300: Acute Eye Irritation in the Rabbit, SPL project number: 1307/382. Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK (unpublished report submitted by notifier).

Safepharm Laboratories Ltd (2005f) JDP Magenta E-300: Local Lymph Node Assay in the Mouse, SPL project number: 1307/383. Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK (unpublished report submitted by notifier).

Safepharm Laboratories Ltd (2005g) JDP Magenta E-300: Reverse Mutation Assay "Ames Test" Using *Salmonella typhimurium* and *Escherichia coli*, SPL project number: 1307/384. Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK (unpublished report submitted by notifier).

SafePharm Laboratories Ltd (2005h) JDP Magenta E-300: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*). SPL Project No: 1307/396, 14 October 2005, Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK (unpublished report submitted by notifier).

SafePharm Laboratories Ltd (2005i) JDP Magenta E-300: Acute Toxicity to *Daphnia magna*. SPL Project No: 1307/397, 13 October 2005, Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK (unpublished report submitted by notifier).

SafePharm Laboratories Ltd (2005j) JDP Magenta E-300: Algal Inhibition Test. SPL Project No: 1307/398, 13 October 2005, Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK (unpublished report submitted by notifier).

SafePharm Laboratories Ltd (2005k) JDP Magenta E-300: Assessment of the Inhibitory Effect on the Respiration of Activated Sewage Sludge. SPL Project No: 1307/400, 08 September 2005, Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK (unpublished report submitted by notifier).

SafePharm Laboratories Ltd (2006a) JDP Magenta E-300: Determination of Hazardous Phyico-chemical Properties. SPL Project No: 1307/391, Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK (unpublished report submitted by notifier).

Safepharm Laboratories Ltd (2006b) JDP Magenta E-300: Twenty-Eight Day Repeat Dose Oral (Gavage) Toxicity Study in the Rat, SPL project number: 1307/394. Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK (unpublished report submitted by notifier).

Safepharm Laboratories Ltd (2006c) JDP Magenta E-300: Chromosome Aberration Test in CHL Cells *In-Vitro*, SPL project number: 1307/395. Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK (unpublished report submitted by notifier).

United Nations (2003) Globally Harmonised System of Classification and Labelling of Chemicals (GHS). United Nations Economic Commission for Europe (UN/ECE), New York and Geneva.

Appendix A: Physico-Chemical Properties

Melting Point/Freezing Point 288ºC

METHOD EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks Method: Differential Scanning Calorimetry.
TEST FACILITY Safepharm Laboratories Ltd (2005a)

Boiling Point >331ºC at 101.3 kPa

METHOD EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks Method: Differential Scanning Calorimetry. The test material was found to boil and/or decompose from 331°C. A calculated value of boiling temperature gave a result of >1486°C (based on a smaller simplified structure of the test material due to software issues).
TEST FACILITY Safepharm Laboratories Ltd (2005a)

Density 1590 kg/m^3 at 22.1ºC

METHOD EC Directive 92/69/EEC A.3 Relative Density.
Remarks Method: Gas comparison pycnometer.
TEST FACILITY Safepharm Laboratories Ltd (2005a)

Vapour Pressure <2.7 x 10^{-8} kPa at 25ºC

METHOD EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks Method: Vapour pressure balance. Vapour pressure estimated by extrapolation from data point chosen (241°C) to give highest vapour pressure.
TEST FACILITY Safepharm Laboratories Ltd (2006a)

Water Solubility In the range 49.9 to 51.9% w/w at 20°C.

METHOD EC Directive 92/69/EEC A.6 Water Solubility.
Remarks The standard A6 Flask Method was not applicable to this test material due to the high indeterminable saturation levels produced. It was therefore not possible to prepare samples at five times the saturation level as recommended in the guideline. No analysis could be performed due to the high solubility producing unfilterable mixtures and thus water solubility was estimated based on visual inspection.
TEST FACILITY Safepharm Laboratories Ltd (2005a)

Surface Tension 72.4 mN/m at 22.2ºC

METHOD EC Directive 92/69/EEC A.5 Surface Tension.
Remarks Concentration: 0.999 g/L. Ring method using an interfacial tension balance. The test material is not considered to be surface active.
TEST FACILITY Safepharm Laboratories Ltd (2005a)

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 (ºC)*	*$t_{½}$ days*
4	25	>365
7	25	>365
9	25	>365

Remarks Analysis by HPLC.
TEST FACILITY Safepharm Laboratories Ltd (2005a)

Partition Coefficient (n-octanol/water) log Pow at 20ºC is <-6.41

METHOD EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks Analytical Method: HPLC. No significant deviations from the test protocol (Shake Flask Method) were reported.

It is evident from the information obtained in the hydrolysis test and data relating to the pH of the test material in water that negligible hydrolysis of the sample solutions occurred during the course of the test.
TEST FACILITY Safepharm Laboratories Ltd (2005a)

Adsorption/Desorption – screening test log K_{oc} is <1.25 at 30°C.

METHOD EC Directive 2001/59/EC C.19 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography
Remarks Test was performed using the HPLC screening method at pH 7. The notified chemical eluted before the standard solution of acetanilide, indicating it is highly mobile in soil or sediment.

The low adsorption properties of the test material containing salted anionic functional groups determined by the HPLC estimation method were consistent with the extremely high water solubility and low partition coefficient characteristics. Although the determined value is believed to accurately assess the affinity of the test material for the organic carbon content of soils and sewage sludge, the method guideline specifically requiring the analysis of substances in an ionised form if present within the environmentally relevant pH range of 5.5 to 7.5; the mobility of the test material in soil and sewage sludge may also be influenced by additional interactions other than partitioning not addressed by the test method, due to the anionic nature of the test material.
TEST FACILITY Safepharm Laboratories Ltd (2005a)

Dissociation Constant Not Determined

Remarks There are two main dissociating functional groups and pK_a's are typically approximately 0.7 and 4.2 from literature references. Therefore, at environmental pH (4-9), the material is always ionised i.e. fully dissociated.

Particle Size

METHOD OECD TG 110 Particle Size Distribution/Fibre Length and Diameter Distributions.

Range (µm)	*Mass (%)*
< 100	27.9
< 10.2	4.66
< 5.4	0.952

Remarks Method: Cascade impactor and sieve.
TEST FACILITY Safepharm Laboratories Ltd (2005a)

Flash Point Not determined.

Remarks Chemical is a solid with a high melting point and low vapour pressure.

Flammability Not highly flammable.

METHOD EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks Failed to ignite in a preliminary test.
TEST FACILITY Safepharm Laboratories Ltd (2006a)

Autoignition Temperature > 400°C

METHOD	92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks	Did not ignite in an oven up to 400°C.
TEST FACILITY	Safepharm Laboratories Ltd (2006a)

Explosive Properties Not predicted to be explosive

METHOD	EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks	Predicted negative on the basis of structure.
TEST FACILITY	Safepharm Laboratories Ltd (2006a)

Oxidising Properties Not predicted to have oxidising properties.

METHOD	EC Directive 92/69/EEC A.17 Oxidising Properties (Solids).
Remarks	Predicted negative on the basis of structure.
TEST FACILITY	Safepharm Laboratories Ltd (2006a)

Appendix B: Toxicological Investigations

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method - Limit Test EC Directive 2004/73/EC B.1tris Acute Oral Toxicity – Acute Toxic Class Method- Limit Test.
Species/Strain	Rat/Sprague-Dawley CD
Vehicle	Distilled water
Remarks – Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	3/female	2099	0
II	3/female	2099	0

LD50	> 2099 mg/kg bw (equivalent to 2000 mg/kg bw active ingredient)
Signs of Toxicity	There were no signs of systemic toxicity. All animals showed expected gains in bodyweight over the study period.
Effects in Organs	Red kidneys were noted at necropsy of all animals.
Remarks – Results	None.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Safepharm Laboratories Ltd (2005b)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal) – Limit Test.
Species/Strain	Rat/Sprague-Dawley CD
Vehicle	The test material was moistened with distilled water.
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	2099	0/10

LD50	>2099 mg/kg bw (equivalent to 2000 mg/kg bw active ingredient)
Signs of Toxicity - Local	Crust formation was noted at the treated skin sites of two males and four females three to six days after treatment. Red-coloured staining, which prevented evaluation of erythema, was noted at all treated skin sites up to three days after treatment. No other signs of dermal irritation were noted.
Signs of Toxicity - Systemic	There were no signs of systemic toxicity. All animals showed expected gains in bodyweight over the study period.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks – Results	None.

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

TEST FACILITY Safepharm Laboratories Ltd (2005c)

B.3. Acute toxicity – inhalation

Data not provided.

B.4. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Directive 2004/73/EC B.4 Acute Toxicity (Skin Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	The test material was moistened with distilled water.
Observation Period	3 days.
Type of Dressing	Semi-occlusive.
Remarks – Method	No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0	0	0	0	N/A	0
Oedema	0	0	0	0	N/A	0

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

Remarks – Results Pink-coloured staining was noted at all treated skin sites throughout the study. This did not affect evaluation of skin reactions.

CONCLUSION The notified chemical is not irritating to skin.

TEST FACILITY Safepharm Laboratories Ltd (2005d)

B.5. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 2004/73/EC B.5 Acute Toxicity (Eye Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Observation Period	7 days.
Remarks – Method	No significant protocol deviations. A Rabbit Enucleated Eye Test (REET) was performed prior to the *in vivo* test.

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	0.67	0.33	1	2	< 7 days	0

Conjunctiva: chemosis	0	0	0	1	< 24 hours	0
Conjunctiva: discharge	0.33	0.33	0.67	2	< 72 hours	0
Corneal opacity	0	0	0	0	N/A	0
Iridial inflammation	0	0	0	0	N/A	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 moderate conjunctival irritation. A small area of petechial haemorrhage on the nictitating membrane was also noted in one treated eye. No ocular effects were noted in one treated eye at the 48-hour observation, in one other treated eye at the 72-hour observation or in the remaining treated eye at the 7-day observation.

Purple coloured staining of the cornea and conjunctival membranes was noted in all treated eyes during the study. This staining was not evident at the 7 day observation. The staining did not affect evaluation of ocular effects.

CONCLUSION

The notified chemical is slightly irritating to the eye.

TEST FACILITY

Safepharm Laboratories Ltd (2005e)

B.6. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE

Notified chemical

METHOD

OECD 429 Skin Sensitisation: Local Lymph Node Assay
EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay).

Species/Strain

Mouse / CBA/Ca

Vehicle

1% pluronic in distilled water.

Remarks – Method

No significant protocol deviations. The test substance concentration and choice of vehicle were selected based on the solubility observed in a vehicle determination study.

RESULTS

Concentration	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
5% (w/w)	843.30	2.24
10% (w/w)	554.20	1.47
25% (w/w)	622.90	1.65
Positive Control		
5% (w/v) in 70% ethanol in distilled water		2.6
10% (w/v) in 70% ethanol in distilled water		8.4
25% (w/v) in 70% ethanol in distilled water		12.9

Remarks – Results

There were no signs of systemic toxicity.

CONCLUSION

There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY

Safepharm Laboratories Ltd (2005f)

B.7. 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). US EPA Health Effects Test Guidelines. OPPTS 870.3050. Repeated Dose 28-day Oral Toxicity Study in Rodents. The Japanese Ministry of Economic Trade and Industry, Ministry of Health Labour and Welfare, and Ministry of the Environment Guidelines of 21 November 1973 for a twenty-eight day repeat dose oral toxicity study as required by the Law Concerning the Evaluation of Chemical Substances and Regulation of their Manufacture etc. (Chemical Substance Control Law) 1973 Ministry of International Trade and Industry amended 2004.
Species/Strain	Rat/Sprague-Dawley CD
Route of Administration	Oral – gavage.
Exposure Information	Total exposure days: 28 days; Dose regimen: 7 days per week; Post-exposure observation period: 14 days.
Vehicle	Distilled water.
Remarks – Method	There were no significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose* mg/kg bw/day*	*Mortality*
I (control)	5/sex	0	0/10
II (low dose)	5/sex	25	0/10
III (mid dose)	5/sex	150	0/10
IV (high dose)	5/sex	1000	0/10
V (control recovery)	5/sex	0	0/10
VI (high dose recovery)	5/sex	1000	0/10

*incorporating a correction factor for 93% purity

Mortality and Time to Death

There were no unscheduled deaths during the study.

Clinical Observations

No toxicologically significant clinical signs were detected.

Behavioural Assessments: There were no treatment related changes in the behavioural parameters measured.

Functional Performance Tests: There were no treatment-related changes in sensory reactivity.

Bodyweight: High dose females showed a statistically significant reduction in bodyweight gain at Week 2. No such effects were detected in low or mid dose animals or in recovery animals during the 14 days without treatment.

Food Consumption: No treatment related effects were detected.

Water Consumption: Daily visual inspection of water bottles revealed no intergroup differences.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology: No treatment-related effects were detected.

Blood Chemistry: Statistically significant increases in plasma levels of urea, albumin, aspartate

aminotransferase, bilirubin and creatinine together with elevation in the albumin/globulin ratio and statistically significant decreases in plasma levels of triglycerides and alanine aminotransferase were evident in the high dose males at the end of the treatment period. Mid dose males also showed a statistically significantand high dose females increase in aspartate aminotransferase. High dose females showed a decrease in plasma alanine aminotransferase. Significant differences from controls for these parameters were not evident at the end of the recovery period.

No treatment-related changes were detected in low dose animals when compared to controls.

Urinalysis: An increase in urine volume (but not dose related) was noted in all treatment groups. This effect was not observed at the end of the recovery period. Pink coloured urine was noted in all treatment groups (except mid dose males) including at the end of the recovery period. Due to the colour of the urine the urinalytical parameters pH, protein, glucose, ketones, urobilinogen, bilirubin and blood could not be measured in four out of five high dose males and all high dose females at the end of the treatment period. Blood (leucocytes or erythrocytes) was detected at the end of the treatment period in one mid dose male and in the urine of the one high dose male that could be measured and at the end of the recovery period in all treated males and three out of five treated females. There were no differences at the end of the recovery period compared with controls for the other parameters that could not be measured at the end of the treatment period.

Effects in Organs

Organ Weights: High dose animals showed an increase in kidney weight at the end of both the treatment period and the recovery period. This was accompanied in the males by increase in spleen weight (also observed at both the end of the treatment and recovery periods).

Necropsy: High dose animals showed pink coloured contents in the gastrointestinal tract. Pink discolouration was evident in a number of tissues at *post mortem* examination. Two high dose males and females showed dark discolouration of the spleen. In addition, two males at this dose level showed an enlarged spleen.

Histopathology: The oral administration of the notified chemical to rats by gavage for a period of 28-days at a maximum dose level of 1000 mg/kg bw/day resulted in treatment-related morphological changes in the spleen, kidneys, lymph nodes and stomach.

The following treatment related changes were observed:

SPLEEN: Higher grades of severity of extramedullary haemopoiesis were seen in relation to treatment for high dose males but not at any other treatment level. The condition was observed to have regressed among recovery high dose animals following an additional fourteen days without treatment.

KIDNEY: Tubular vacuolation was observed for high dose animals, and for female rats at low and mid doses. Accumulations of red pigment were observed in the proximal tubular epithelium high dose animals. This was associated in many instances with tubular degeneration and/or tubular basophilia. There were indications of partial regression of pigment accumulation and tubular changes, with the exception of epithelial vacuolation, among recovery group animals following completion of the fourteen day recovery period.

LYMPH NODES: Vacuolated histiocytes were observed in the cervical and mesenteric lymph nodes as an effect of treatment for high dose animals, and probably also at the mid dose although the incidence of rats affected was not especially convincing at this treatment level. There was no indication of regression of the condition in either locality in lymph nodes from recovery high dose animals.

STOMACH: Agglomeration of secretion, superficial mucosal basophilia, and/or vacuolation of mucosal cells adjacent to the limiting ridge, were observed in relation to treatment for high dose animals. Two female rats dosed at 150 mg/kg bw/day were also affected by agglomeration of secretion and vacuolation. With the exception of vacuolation of mucosal cells adjacent to the limiting ridge, which appeared to be marginally worse for recovery high dose female rats, all conditions were observed to have regressed following completion of the fourteen day recovery period.

Remarks – Results

The changes in clinical chemistry parameters (in high and mid dose animals) and possible urinalysis changes in the high dose animals together with the increased kidney weights and tubular degeneration suggested an

effect on kidney function in high dose animals extending to mid dose females. Minimal microscopic effects in low dose females were not thought to be an adverse effect. The irritant nature of the test substance was indicated by inflammatory gastric changes.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 25 mg/kg bw/day in this study based on the changes within the renal tubular epithelium. However R48 classification was not warranted as the vacoulation was minimal to the extent that normal cell integrity was evident.

TEST FACILITY Safepharm Laboratories Ltd (2006b)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OEC D 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 and TA100
E. coli: WP2 *uvr*A

Metabolic Activation System S9 fraction from phenobarbitone/ß-naphthoflavone-induced rat liver.

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

Vehicle Sterile distilled 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*
Present				
Test 1	>5000 µg/plate	>5000 µg/plate	>5000 µg/plate	None
Test 2		>5000 µg/plate	>5000 µg/plate	None
Absent				
Test 1	>5000 µg/plate	>5000 µg/plate	>5000 µg/plate	None
Test 2		>5000 µg/plate	>5000 µg/plate	None

Remarks – Results The notified chemical did not induce a 2-fold or more increase in the number of revertant colonies compared to the negative control, either with or without metabolic activation.

The vehicle control plates gave counts of revertant colonies within the normal range. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with and without metabolic activation. Thus, the sensitivity of the assay and the efficacy of the S9-mix were validated.

The test material caused no visible reduction in the growth of the bacterial background lawn at any dose level. The test material was, therefore, tested up to the maximum recommended dose level of 5000 µg/plate. A pink colour, which became increasingly darker with increasing test material concentrations, was noted from 50 µg/plate, this did not prevent the scoring of revertant colonies.

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

TEST FACILITY Safepharm Laboratories Ltd (2005g)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical.

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

Cell Type/Cell Line: Chinese hamster lung cells
Metabolic Activation System: S9 fraction from phenobarbitone/ß-naphthoflavone-induced rat liver.
Vehicle: Minimal Essential Media (MEM)
Remarks – Method: No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
*Present***			
Test 1	0*, 156.25, 312.5, 625, 1250*, 2500*, 5000*	6 hours	24 hours
Test 2	0*, 156.25, 312.5, 625, 1250*, 2500*, 5000*	6 hours	24 hours
Absent			
Test 1	0*, 156.25, 312.5, 625, 1250*, 2500*, 5000*	6 hours	24 hours
Test 2	0*, 78.13, 156.25*, 312.5*, 625*, 937.5, 1250	24 hours	24 hours

*Cultures selected for metaphase analysis. ** S9 at 5% in Test 1 and 2% in Test 2

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in PreliminaryTest	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Present				
Test 1	>5000	Ca. 5000	>5000	Negative
Test 2		>5000	>5000	Negative
Absent				
Test 1	>5000	>5000	>5000	Negative
Test 2		625	>5000	Negative

Remarks – Results: The test material did not induce any statistically significant increases in the number of cells carrying structural chromosome aberrations or in the numbers of polyploid cells either in the absence or presence of metabolic activation.

The vehicle control had frequencies of cells with aberrations within the range expected for the CHL cell line. All of the positive control materials induced highly significant increases in the frequency of cells with aberrations indicating the satisfactory performance of the test and of the activity of the metabolising system.

CONCLUSION The notified chemical was not clastogenic to Chinese Hamster lung cells treated in vitro under the conditions of the test.

TEST FACILITY Safepharm Laboratories Ltd (2006c)

Appendix C: Environmental Fate and Ecotoxicological Investigations

ENVIRONMENTAL FATE

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical.
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I).
Inoculum	Activated sewage sludge.
Exposure Period	28 days.
Auxiliary Solvent	None.
Analytical Monitoring	The change in BOD of the test solutions was measured by autoreading using a data sampler, confirmed by HPLC.
Remarks – Method	No significant protocol deviations. Test concentration of the notified chemical was 100 mg/L.

RESULTS

Test substance		*Aniline*	
Day	*% degradation (BOD)*	*Day*	*% degradation*
7	0	7	71
14	1.7	14	76
21	7.3	21	77
28	9	28	76

Remarks – Results	All relevant OECD criteria were met. Aniline attained 76% degradation after 14 days thereby confirming the suitability of the inoculum and test conditions. A parallel analysis by HPLC indicated an average of 1% biodegradation of the test item, and a further parallel analysis of DOC indicated an average of 0% biodegradation.
CONCLUSION	The notified chemical cannot be classed as ready biodegradable.
TEST FACILITY	Kurume Laboratory (2005)

C.1.2. Bioaccumulation

CONCLUSION	The notified chemical has high water solubility and a low octanol/water partition coefficient. As such it has a low degree of lipophilicity and low potential to cross biological membranes.

ECOTOXICOLOGICAL INVESTIGATIONS

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical.
METHOD	
	EC Directive 92/69/EEC C.1 Acute Toxicity for Fish – semi-static.
Species	Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period	96 h
Auxiliary Solvent	None.
Water Hardness	Ca. 100 mg $CaCO_3$/L
Analytical Monitoring	
Remarks – Method	No significant protocol deviations.
RESULTS	Based on the results of the range-finding test, a "limit test" was conducted at a concentration of 100 mg/L to confirm that at the maximum concentration given in the OECD/EEC Test Guidelines, no mortalities or sub-lethal effects of exposure were observed. An amount of test material (4.00 g) was dissolved in dechlorinated tap water and the volume adjusted to 1 L to give a 4.0 g/L stock solution. The entire volume was further diluted in a final volume of 20 L and stirred using a flat bladed mixer for approximately 1 minute to give the 100 mg/L test concentration.

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	0	7	0	0	0	0	0
100	95.8	7	0	0	0	0	0

LC50	>100 mg/L at 96 hours.
NOEC	100 mg/L at 96 hours.
Remarks – Results	The control was observed to be a clear, colourless solution throughout the duration of the test. The 100 mg/L test preparation was observed to be a magenta coloured solution. Analysis of the test preparations at 0, 24 and 96 hours showed measured test concentrations to range from 92.4%to 98.9% of nominal and so the results are based on nominal test concentrations only.
CONCLUSION	The notified chemical is not harmful to Rainbow trout.
TEST FACILITY	Safepharm laboratories Ltd (2005h)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical.
METHOD	EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia – static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None.
Water Hardness	Ca. 250 mg $CaCO_3$/L
Analytical Monitoring	HPLC analysis of test concentrations.
Remarks – Method	Based on the results of the range-finding test, a "limit test" was conducted at a concentration of 100 mg/L to confirm that at the maximum concentration given in the OECD/EEC Test Guidelines, no immobilisation or adverse reactions were observed. An amount of test material (100 mg) was dissolved in dechlorinated tap water and the volume adjusted to 1 L to give the 100 mg/L test concentration. No significant protocol deviations were reported.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	0	10	0	0
100	92.5	20	0	0

LC50	>100 mg/L at 48 hours
NOEC	100 mg/L at 48 hours
Remarks – Results	No immobilisation was observed at the test concentration of 100 mg/L. It was considered unnecessary and unrealistic to test at concentrations in excess of 100 mg/L. The control test medium was observed to be a clear colourless solution and the 100 mg/L test medium was observed to be a clear magenta solution throughout the test. Analysis of the test preparations at 0 and 48 hours showed measured test concentrations to range from 85% to 99% of nominal value and so the results are based on nominal test concentrations only.
CONCLUSION	The notified chemical is not harmful to *Daphnia magna*.
TEST FACILITY	Safepharm laboratories Ltd (2005i)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical.
METHOD	EC Directive 92/69/EEC C.3 Algal Inhibition Test.
Species	*Scenedesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 100 mg/L Actual: 93-105% of Nominal
Auxiliary Solvent	None.
Analytical Monitoring	HPLC
Remarks – Method	Based on the result of the range-finding test a "limit-test" was conducted at a concentration of 100 mg/L to confirm that at the maximum concentration given in the OECD/EEC Test Guidelines no effect on algal growth was observed. An amount of test material (100 mg) was dissolved in culture medium and the volume adjusted to 500 mL to give a 200 mg/L stock solution. An aliquot (250 mL) of this stock solution was mixed with algal suspension (250 mL) to give the required test concentration of 100 mg/L. A Student's t-test incorporating Bartlett's test for homogeneity of variance was carried out on the area under the growth curve data at 72 h for the control and the 100 mg/L test concentration to determine any statistically significant differences between the test and control groups.

RESULTS

Biomass		*Growth*	
EbC_{50} *mg/L at 72 h*	*NOEC* *mg/L*	*ErC_{50}* *mg/L at 0-72 h*	*NOEC* *mg/L*
>100	100	>100	100

Remarks – Results	Analysis of the test preparations at 0 and 72 hours showed measured test concentrations to range from 93% to 105% of nominal and so the results are based on nominal test concentrations only. The actual mean-measured concentration was found to be 99 mg/L. There were no statistically significant differences ($P \geq 0.05$) in either biomass or growth rate between the control and 100 mg/L test group and therefore, the NOEC was 100 mg/L. The cell concentration of the control cultures increased by a factor of 111 after 72 hours, which was in line with the OECD Guideline that states the enhancement must be at least by a factor of 16 after 72 hours.
CONCLUSION	The notified chemical is not harmful to *Scenedesmus subspicatus.*
TEST FACILITY	Safepharm laboratories Ltd (2005j)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical.
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test US EPA Draft Ecological Effects Test Guidelines OPPTS 850.6500.
Inoculum	Activated sewage sludge.
Exposure Period	3 hours
Concentration Range	Nominal: 1000 mg/L
Remarks – Method	Based on the results of a range-finding test, a "limit test" was conducted at a concentration of 1000 mg/L (three replicates) to confirm that at this concentration no effect on respiration of the activated sewage sludge was observed. An amount of test material (2000 mg) was dissolved in water and the volume adjusted to 1000 mL to give a 2000 mg/L stock solution. An aliquot (250 mL) of this stock solution was dispersed with synthetic sewage (16 mL), activated sewage sludge (200 mL) and water, to final volume of 500 mL, to give the required concentration of 1000 mg/L. Analysis of the concentration, homogeneity and stability of the test material in the test preparations was not appropriate according to the Test Guidelines. For the purpose of the test a reference material, 3,5-dichlorophenol was used as a toxic reference material.
RESULTS	
IC50	>1000 mg/L
NOEC	1000 mg/L
Remarks – Results	The test validation criteria were satisfied. Observations made throughout the test period showed that at the test concentration of 1000 mg/L no undissolved test material was visible. Validation criteria were satisfied for the test.
CONCLUSION	The notified chemical is not harmful to activated sludge micro-organisms.
TEST FACILITY	SafePharm Laboratories Ltd (2005k)