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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

CIM-08

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

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

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

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

Director
NICNAS

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Canon Australia Pty Ltd (ABN 66 005 002 951)
1 Thomas Holt Drive
NORTH RYDE NSW 2113

NOTIFICATION CATEGORY
Standard: Chemical other than polymer.

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication:
Chemical name, Other names, CAS number, Molecular formula, Structural formula, Molecular weight, Spectral data, Purity, Non-hazardous impurities, Use details and Import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows:
Flash point, Reactivity and Acute inhalation toxicity.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
LVC/746

NOTIFICATION IN OTHER COUNTRIES
USA (2007)
UK (2007)
Switzerland (2008)
Japan (2008)
Korea (2008)
Philippines (2008)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
CIM-08
C-Y9
Yellow C-Y9 Liq
Yellow C-Y9

MOLECULAR WEIGHT
>1000 Da

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

3. COMPOSITION

DEGREE OF PURITY > 75%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS None

ADDITIVES/ADJUVANTS None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Yellow powder

Property	Value	Data Source/Justification
Melting Point	Decomposed without melting from 350°C.	Measured
Boiling Point	Not determined	Not applicable as decomposes prior to melting.
Density	1600 kg/m^3 at 20°C	Measured
Vapour Pressure	< 1.8 x 10^{-8} kPa at 25°C	Estimated
Water Solubility	320 – 339 g/L at 20 ± 0.5°C	Measured
Hydrolysis as a Function of pH	$t_{1/2}$ > 1 year at pH 4, 7 and 9, 25°C	Measured
Partition Coefficient (n-octanol/water)	log P_{OW} = -3.92 at 23 ± 1.0°C	Measured
Surface Tension	72.3 mN/m at 21°C	Measured
Adsorption/Desorption	log K_{oc} < 1.25 at 30°C	Measured
Dissociation Constant	pKa = 4.64	Measured
Particle Size	Inhalable fraction (< 100 μm): 34.3% Respirable fraction (< 10.0 μm): 3.18%	Measured
Flash Point	Not determined	Not applicable as low volatility solid.
Flammability	Not highly flammable	Measured
Autoignition Temperature	> 400°C	Measured
Explosive Properties	Not explosive	Measured
Oxidising Properties	Not oxidising	Predicted based on structure.

DISCUSSION OF PROPERTIES

The notified chemical is highly water soluble, hydrolytically stable, not surface active, has a low vapour pressure and is expected to be ionic throughout the environmental pH range 4-9. Based on the measured log Koc, the notified chemical is not likely to absorb to soil or sludge consistent with its hydrophilic nature.

A significant proportion (34%) of the powdered notified chemical is inspirable and could be inhaled into the upper respiratory tract. However, only a small fraction (<4%) was of small enough particle sizes to reach the lower respiratory tract (<10 μm).

Details of tests on physical and chemical properties are provided in Appendix A.

Reactivity

The notified chemical is predicted to be stable under normal conditions of use.

5. INTRODUCTION AND USE INFORMATION

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

The notified chemical will be imported only as a component of ink (< 7%), which has already been incorporated into cartridges.

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

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

PORT OF ENTRY

Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS

Offices and office equipment retailers nationwide.

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a component of ready-to-use (5 ml and 900 ml) plastic inkjet cartridges, which will be packed in plastic bags inside cardboard boxes. The cartridges will be transported by road from the wharf to the notifier's warehouse prior to distribution to offices and office equipment retailers nationwide.

USE
The notified chemical is used as a component (< 7%) of inkjet printer ink.

OPERATION DESCRIPTION
No reformulation or repackaging of the notified chemical will occur in Australia. The products containing the notified chemical will be delivered to the end-user in the same form in which they are imported. The cartridges will be installed or replaced into the inkjet printer by office workers, service technicians or consumers.

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	50	< 8 hours per day	10-50 days per year
Storage and transport	15	< 8 hours per day	10-50 days per year
Office worker/consumer	2,000,000	10 seconds per day	2 days per year
Service Technicians	100	1 hour per day	170 days per year

EXPOSURE DETAILS
Exposure to the notified chemical during the importation, transport and storage of the printer cartridges is not expected, except in the unlikely event of an accident where the cartridge and its packaging may be breached.

Both office workers and service technicians may be exposed (dermal or ocular) to the notified chemical in inks (< 7% concentration) while changing printer cartridges, and service technicians may additionally be exposed during printer maintenance. Dermal exposure to small quantities of the notified chemical may occur if the print heads are touched while replacing the cartridges. In addition, dermal and possibly ocular exposure could occur when handling faulty or ruptured cartridges. Exposure during handling and cleaning or printer components is likely to be limited to the fingertips. Therefore, the exposure of these workers is expected to be minimal and infrequent.

Dermal exposure of workers may also occur when handling printed media before the ink is adequately dried, especially when printing on non-absorbent materials. Dermal exposure of office workers to the notified chemical from dried inks on printed paper is expected to be minimal, as the dye will be largely bound to the paper within the matrix of the dried ink.

6.1.2. Public exposure
The exposure of the public to the notified chemical in inkjet printer inks is expected to be identical, or of a lesser extent, than that experienced by office workers using the same ink.

6.2. Human health effects assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation	non-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 > 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation (Ames)	non mutagenic
Mutagenicity – bacterial reverse mutation (incorporating modified Ames test for azo dyes)	non-mutagenic
Genotoxicity – in vitro mammalian chromosome aberration test (Chinese hamster CHL/IU)	non genotoxic

Acute toxicity
The notified chemical was found to be of low acute oral and dermal toxicity in studies conducted on rats (LD50 > 2,000 mg/kg bw). The NOEL in a 28-day oral repeat dose study in rats was 150 mg/kg bw/day on the basis of the treatment related changes observed in the stomach at 300 and 1000 mg/kg bw/day. However, as these changes showed regression following the 14-day recovery period, the effects were generally regarded as not being adverse. Hence, the NOAEL is considered as > 1000 mg/kg bw/day.

Irritation and Sensitisation
The notified chemical was found to be slightly irritating to the eye, though not severe enough to warrant hazard classification, and non-irritating to the skin.

The notified chemical was not a skin sensitiser when tested up to a concentration of 25% in a mouse local lymph node assay. Therefore, the notified chemical is unlikely to cause skin sensitisation in exposed humans.

Mutagenicity and Carcinogenicity
Azo dyes are a concern for their potential induction of mutagenicity and carcinogenicity. The azo linkage is the most labile portion of an azo dye molecule, and it is readily enzymatically metabolised in mammals, including man (SCCNFP, 2002). Liver azo reductase enzymes reductively cleave the molecule into component amines. Some metabolism may also occur in the cells of the bladder wall, and during percutaneous absorption. Anaerobic intestinal bacteria are also capable of reductive cleavage of the azo linkage.

The aromatic amines that arise from the azo reduction of azo dyes are thought to be activated through their *N*-oxidation by cytochrome P450 isozymes (SCCNFP, 2002). These *N*-hydroxylarylamines may be further glucuronated (activated) or acetylated (inactivated), which may influence their mutagenicity (Bartsch, 1981). Under acidic pH, they form reactive nitrenium ions that can alkylate bases in DNA, particularly the nucleophilic centres in guanine. This mechanism is thought to contribute to the carcinogenicity of many azo dyes.

The notified chemical is not expected to be reductively cleaved to release any of the restricted aromatic amines specified in either the Appendix to EC Directive 76/769/EEC (EC, 2004) or the annexes of EU SCCNFP/0495/01 (SCCNFP, 2002). However, the notified chemical can be broken by azo reduction into a number of arylamine species, although these are unlikely to be mutagenic.

In addition, azo dyes are renowned for their content of impurities, particularly for the presence of component arylamines (SCCNFP, 2002; Øllgaard *et al* 1998). Such impurities are thought to contribute to their carcinogenicity, as these species may be more readily absorbed, and activated as carcinogens. The HPLC trace provided by the notifier indicates that the sample of the notified chemical contains a number of impurities. The impurities have been identified to be mainly isomers of the notified chemical. As such, these impurities are unlikely to contribute to carcinogenicity of the notified chemical. However, there are a small percentage of 4 unidentified impurities that may be free amine species. Free amines may exhibit a higher risk of toxicity as they are likely to be more readily absorbed across the skin.

The notifier supplied test results showing that the notified chemical was found to be not mutagenic in bacteria (under the conditions of the Ames test used), and did not induce chromosomal aberrations in mammalian cells *in vitro*. Furthermore, the notifier also supplied test results from a non-GLP study showing that the notified chemical was found to be not mutagenic using the modified Ames test for azo dyes (Prival and Mitchell, 1982). This modified test is thought to yield a greater detection of mutagenic azo dyes as it is recognised that the standard procedure is not sufficiently sensitive for these chemicals, likely due to their complex metabolism *in vivo* (Brown and DeVito, 1993, referenced in Øllgaard *et al* 1998). The Prival and Mitchell modified Ames test utilises a reductive pre-incubation step (during which the azo dye is reduced to amine species) before the test is carried out.

Overall, on the basis of weight of evidence the notified chemical is not expected to be genotoxic.

6.3. Human health risk characterisation

6.3.1. Occupational health and safety

Dermal and ocular exposure of workers to the notified chemical should occur infrequently and be of small amounts, given the containment of the notified chemical within ink cartridges.

The level of repeat dermal exposure for service technicians and office workers handling sealed cartridges of printing inks containing the notified chemical at < 7% is not expected to be significant compared to the NOEL of 150 mg/kg bw/day established in the 28 day rat study.

The notified chemical has the potential to be irritating to the eye based on an eye irritation study in rabbits. However, ocular exposure is not expected under normal circumstances, unless the ink residues containing the notified chemical are deposited on the fingers and then rubbed into the eyes. In addition the irritation potential is reduced due to the concentration of the notified chemical (< 7%) in the inks.

Overall, the risk presented by the notified chemical to the health and safety of workers is not expected to be unacceptable.

6.3.2. Public health

The exposure and hazard of the notified chemical to the members of the public during the use of inkjet printers are expected to be identical or similar to that experienced by office workers. Therefore, the risk of the notified chemical to the health of the public is considered to be low.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported into Australia as a component of inkjet printer ink in ready-to-use cartridges. Release of the ink solution to the environment from manufacturing and reformulation of the notified chemical is not expected in Australia.

RELEASE OF CHEMICAL FROM USE

Environmental release of the substance is possible during paper recycling and from the disposal of used print cartridges. Around 5% of the ink will remain in “empty” cartridges. The ink remaining in the ink cartridges during the recycling process will not be reused.

RELEASE OF CHEMICAL FROM DISPOSAL

The notifier collects the used cartridges by setting up the collection boxes in general merchandising stores and post offices, etc. The collected cartridges will be sent to the subcontractor to be recycled as raw materials, for example, a plastic material to be used to make plastic goods. The notifier does not collect and re-use the used cartridges as new cartridges by refilling the ink. The other cartridges which are not collected will be disposed of to landfill. The residual ink separated from the used cartridges will be disposed of under the regulation of Australia and be most likely sent to landfill.

The notified chemical which is imparted on printed paper will share the fate of the paper substrate, which may be disposed of to landfill or recycled. Approximately 50% of the printed paper may undergo recycling process and a significant proportion of the notified chemical associated to the paper is expected to end up with the aquatic environment due to its solubility and low log K_{OC}.

7.1.2 Environmental fate

The notified chemical is not considered to be bioconcentrating and not readily biodegradable based on the environmental fate study reports. For the details of the studies please refer to Appendix C.

In landfill, the notified chemical is expected to leach given the high water solubility and low log K_{OC}. Therefore, most of the notified chemical is predicted to end up with the aquatic environment followed with slow degradation process via biotic and abiotic pathways forming small molecules of water, salts and oxides of carbon, nitrogen and sulphur.

7.1.3 Predicted Environmental Concentration (PEC)

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	1000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	2.74	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	21.161	million
Removal within STP	0%	
Daily effluent production:	4,232	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.65	µg/L
PEC - Ocean:	0.06	µg/L

The calculation of PEC has been conducted assuming all the notified chemical is released to the water environment which is for the worst case scenario.

7.2. Environmental effects assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	LC50 > 100 mg/L	Not toxic to fish
Daphnia Toxicity	EC50 > 100mg/L	Not toxic to daphnids
Algal Toxicity	E_rC50 > 320 mg/L	Not toxic to algae
Inhibition of Bacterial Respiration	IC50 >1000 mg/L	Not toxic to microorganisms

The notified chemical is not toxic to the aquatic ecosystem based on the study results.

7.2.1 Predicted No-Effect Concentration

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50 (Fish).	> 100	mg/L
Assessment Factor	10	
Mitigation Factor	1	
PNEC:	> 10,000	µg/L

The PNEC has been calculated using the LC50 endpoint for fish and an assessment factor of 10, as more than three studies have been provided.

7.3. Environmental risk assessment

Risk Assessment	*PEC µg/L*	*PNEC µg/L*	*Q*
Q - River:	0.65	> 10,000	« 0.01
Q - Ocean:	0.06	> 10,000	« 0.01

The risk quotient (Q) value has been calculated to be much less than 0.01 for both river and ocean water, based on the worst case scenario of assuming all the notified chemical is released to the aquatic environment. Therefore, the notified chemical is not expected to pose an unacceptable risk to the aquatic ecosystem from the intended application in Australia.

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1300 kg/m^3). Using these assumptions, irrigation with a concentration of 0.65 µg/L may potentially result in a soil concentration of approximately 5.0 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 25 µg/kg and 50 µg/kg, respectively.

Based on the dispersed low level release and low toxicity, the notified chemical is not expected to cause harmful effects in the environment.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

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

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

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

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

Recommendations

CONTROL MEASURES
Occupational Health and Safety

- No specific engineering controls, work practices or personal protective equipment are required for the safe use of the notified chemical itself, however, these should be selected on the basis of all ingredients in the formulation.

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

- A copy of the MSDS should be easily accessible to employees.

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

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component of inkjet printer ink, or is likely to change significantly;
- the amount of chemical being introduced has increased from one tonne per annum, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

No additional secondary notification conditions are stipulated.

Material Safety Data Sheet
The MSDS of products 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.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point Decomposed without melting from 350ºC.

Method OECD TG 102 Melting Point/Melting Range.
Differential Scanning Calorimetry
Remarks Similar thermographic profiles were obtained using air and nitrogen atmospheres indicative that the observed decomposition is most likely thermal and not oxidative.
Test Facility SafePharm Laboratories (2007a)

Density 1600 kg/m^3 at 20ºC

Method OECD TG 109 Density of Liquids and Solids.
Gas comparison pycnometer
Test Facility SafePharm Laboratories (2007b)

Vapour Pressure < 1.8 x 10^{-8} kPa at 25ºC

Method OECD TG 104 Vapour Pressure.
Remarks No statistical analyses were performed because the balance readings were too low and variable for a line of best fit to have any meaning. Instead it was considered more appropriate to impose a regression slope on a chosen data point to provide an estimate of the maximum value for the vapour pressure at 25ºC.
Test Facility SafePharm Laboratories (2007c)

Water Solubility 320 – 339 g/L at 20 ± 0.5ºC

Method OECD TG 105 Water Solubility.
EC Directive 92/69/EEC A.6 Water Solubility.
Remarks The determination was carried out by visual assessment using a flask method. The solution pH was determined to be about 7.5.
A preliminary test was conducted and a solubility range of 20.0 – 30.7% was determined.
A further definitive test result gave a water solubility of 32.0 – 33.9% at 20.0 ± 0.5˚C.
It was not possible to prepare samples at five times saturation level as specified in the test guideline due to the extremely high water solubility of the notified chemical.
Test Facility SafePharm Laboratories (2007a)

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

Method OECD TG 111 Hydrolysis as a Function of pH.
EC Directive 92/69/EEC C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$ <hours>*
4	50±0.5	> 120
7	50±0.5	> 120
9	50±0.5	> 120

Remarks Duplicate tests were conducted by keeping the test solutions of the notified chemical shielded from light 5 days at 50˚C and pH 4, 7, and 9. The nominal concentration of the solutions for test was 250 mg/L.
Less than 10% hydrolysis was detected at pH 4 and 7 after 120 hours, which is equivalent to a half-life greater than 1 year at 25˚C. About 16% hydrolysis was detected at pH 9 after 120 hours. However, a further timepoint at 172 hours indicated that the test material had not hydrolysed as assumed from the 120 hour timepoint. It was deemed that the 120 hour sample result was erroneous.
Test Facility SafePharm Laboratories (2007b)

Partition Coefficient (n-octanol/water) log P_{OW} = -3.92 at 23 ± 1.0 ºC

Method OECD TG 107 Partition Coefficient (n-octanol/water): Shake Flask Method.
EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks HPLC Method/Flask Method.
A preliminary estimate of the partition coefficient was made based on the approximate solubilities of the test material in n-octanol and water.
The definitive test was conducted in duplicate at the n-octanol/water volume ratios of 5:1, 10:1 and 20:1.
The notified chemical contains strong acid salts and therefore the pKa values lies outside the environmental relevant range for the determination of partition coefficient. Therefore, the test was performed at pH 7 as recommended for chemicals containing salts.
Test Facility SafePharm Laboratories (2007a)

Surface Tension 72.3 mN/m at 21°C

Method EC Directive 92/69/EEC A.5 Surface Tension.
Ring based method.
Remarks Concentration: 0.983 g/l (corrected for purity).
Test Facility SafePharm Laboratories (2008a)

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

Method OECD 121 Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography
EC Directive 2001/59/EC C.19 Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography
Remarks The test was conducted at 30°C with a nominal concentration of 100 mg/L in reverse osmosis water. The dead time was determined by measuring the retention time of formamide. It is impossible to carry out the test in the unionised form for the notified chemical, due to the existence of strong acid salts. Therefore, testing was carried out at approximately neutral pH range of 6.35 – 7.11.
The log K_{OC} was determined to be < 1.25 since the retention time (1.785) was less than that of acetanilide (4.127, log K_{OC} = 1.25) which was the lowest among the reference materials.
The determined low value is consistent with the high water solubility and low partition coefficient of the notified chemical, and is believed to accurately assess the affinity of the chemical for the organic carbon content of soils and sewage sludge.
Test Facility SafePharm Laboratories (2007b)

Dissociation Constant pKa = 4.64

Method OECD TG 112 Dissociation Constants in Water.
Remarks Test was conducted in triplicate at a nominal concentration of 10.6 g/L.
Test Facility SafePharm Laboratories (2007b)

Particle Size

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

Range (μm)	*Mass (%)*
< 100	34.3
< 10.0	3.18
< 5.5	0.505

Remarks Particle size distribution was determined using a Marple Miller Cascade Impactor. Too few particles were of a size less than 10 μm to allow accurate assessment of mass median aerodynamic diameter.
Test Facility SafePharm Laboratories (2007b)

Flammability Not highly flammable

Method	EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks	Failed to ignite in preliminary test. Moisture content determined to be 3.079% by drying to constant weight at 105 °C.
Test Facility	SafePharm Laboratories (2007d)

Autoignition Temperature > 400°C

Method	EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Test Facility	SafePharm Laboratories (2007c)

Explosive Properties Not explosive

Method	EC Directive 92/69/EEC A.14 Explosive Properties. Three tests conducted: 1. BAM fall hammer test 2. BAM friction test 3. Koenen steel tube test
Remarks	Tests were conducted on dried material.
Test Facility	SafePharm Laboratories (2007c)

Oxidizing Properties Not oxidising

Method	EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Remarks	Based on the chemical structure the result for the oxidising properties has been predicted negative.
Test Facility	SafePharm Laboratories (2007c)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE Notified chemical

METHOD OECD TG 420 Acute Oral Toxicity – Fixed Dose Method.
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*
1	1F	2000	0
2	4F	2000	0

LD50 > 2000 mg/kg bw
Signs of Toxicity No treatment related clinical effects observed.
Effects in Organs No treatment related effects observed.
Remarks - Results No deaths occurred during the 14-day observation period.

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

TEST FACILITY SafePharm Laboratories (2007e)

B.2. Acute toxicity – dermal

TEST SUBSTANCE Notified chemical

METHOD OECD TG 402 Acute Dermal Toxicity.
Species/Strain Rat/Sprague-Dawley CD
Vehicle Arachis oil BP
Type of dressing Semi-occlusive.
Remarks - Method No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5M	2000	0
2	5F	2000	0

LD50 > 2000 mg/kg bw
Signs of Toxicity - Local No treatment related effects observed.
Signs of Toxicity - Systemic No treatment related effects observed.
Effects in Organs No treatment related effects observed.
Remarks - Results No deaths occurred. No signs of dermal irritation observed. All animals showed expected gains in bodyweight over the study period.

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

TEST FACILITY SafePharm Laboratories (2008b)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 3
Vehicle Distilled water
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	0	0	0
Oedema	0	0	0	0	0	0

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

Remarks - Results Yellow-coloured staining was noted at all treated skin sites throughout the study. This did not affect evaluation of skin reactions.
No evidence of skin irritation was noted during the study.

CONCLUSION The notified chemical is non-irritating to the skin.

TEST FACILITY SafePharm Laboratories (2007f)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
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	1	1	0.7	2	48 hrs	0
Conjunctiva: chemosis	0.3	0.3	0.3	1	24 hrs	0
Conjunctiva: discharge	0.3	0.3	0.3	2 (1 hr)	24 hrs	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

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

Remarks - Results Yellow-coloured staining of the fur was noted around all treated eyes throughout the study. No corneal or iridial effects were noted.
Moderate conjunctival irritation was noted in all treated eyes one hour after treatment with minimal to moderate conjunctival irritation noted in all treated eyes at the 24-hour observation and minimal conjunctival irritation noted in all treated eyes at the 48-hour observation.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY SafePharm Laboratories (2007g)

B.5. Repeat dose toxicity

TEST SUBSTANCE Notified chemical

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

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	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose* mg/kg bw/day*	*Mortality*
control	5F/5M	0	0
low dose	5F/5M	25	0
mid dose I	5F/5M	150	0
mid dose II	5F/5M	300	0
high dose	5F/5M	1000	0
control recovery	5F/5M	0	0
high dose recovery	5F/5M	1000	0

* Corrected for purity of notified chemical.

Mortality and Time to Death

No mortalities occurred at any dose level.

Clinical Observations

No toxicologically significant signs of toxicity were detected throughout the treatment period or during the recovery period.

An isolated incident of generalised red/brown fur staining was evident in one non-recovery female treated with 1000 mg/kg bw/day. Observations of this nature are often reported following oral administration of an unpalatable or slightly irritant test material and, in isolation, are considered not to be indicative of systemic toxicity.

Yellow fur staining was evident in four non-recovery 1000 mg/kg bw/day females on Day 15 and in one male treated with 300 mg/kg bw/day between Days 12 and 14. Such observations are often reported following excretion of a coloured test material or metabolite and, following normal grooming behaviour, dispersal of the colouration over the external fur surface, and are of no toxicological significance.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There were no toxicologically significant changes in blood chemical, urinalytical and haematological parameters.

Effects in Organs

There were no toxicologically significant changes in organ weights. The only significant toxicological effects observed were in the stomach:

Agglomeration of secretion, superficial mucosal basophilia and hyperplasia of mucous cells were observed in the gastric mucosa immediately adjacent to the limiting ridge among animals of either sex treated with 1000 mg/kg bw/day. Acanthosis and hyperkeratosis of the limiting ridge were also seen among both sexes at this dose level. There was also an effect of treatment on animals at the 300 mg/kg bw/day dose level with two males and one female being similarly affected to a greater or lesser extent. Agglomeration of secretion was observed for one female rat treated with 25 mg/kg bw/day. However, it is stated in the report that this

condition is also seen occasionally among control animals as a spontaneous change. There was, however, no evidence of an effect at either of the remaining treatment levels. These gastric changes regressed following an additional 14 days without treatment.

Remarks – Results

Treatment related effects were observed in animals of either sex at dose levels of 1000 and 300 mg/kg bw/day. These included microscopic changes identified in the stomach as agglomeration of secretion, mucous cell hyperplasia, mucosal basophilia/atrophy and acanthosis and hyperkeratosis of the limiting ridge. No treatment related effects were observed in the 150 and 25 mg/kg bw/day dose groups. The No Observed Effect Level (NOEL) was therefore considered to be 150 mg/kg bw/day.

The gastric changes observed showed regression following the 14-day recovery period and therefore the effects were generally regarded as not being adverse. For these reasons the histopathological changes were considered not to represent an adverse health effect and the No Observed Adverse Effect Level (NOAEL) was therefore considered to be > 1000 mg/kg bw/day.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as > 1000 mg/kg bw/day in this study, based on no adverse effects observed at the highest dose level used in this study.

TEST FACILITY SafePharm Laboratories (2008c)

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

TEST SUBSTANCE Notified chemical

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay

Species/Strain Mouse/Female CBA/Ca (CBA/CaBkl)

Vehicle 1% pluronic L92 in distilled water

Remarks - Method No significant protocol deviations

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	612.72	n/a
5	454.76	0.74
10	687.31	1.12
25	863.95	1.41
Positive Control		
1	Not reported	1.39
10	Not reported	11.33
20	Not reported	19.34

Remarks - Results There were no mortalities during the study and no signs of systemic toxicity. Bodyweight changes of the test animals were comparable to those observed in the corresponding control group animals over the same period. Orange coloured staining on the ears was noted post-dose on Days 1 and 2 in animals treated with the highest dose.

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

TEST FACILITY SafePharm Laboratories (2007h)

B.7. Genotoxicity – bacteria (1)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA$^-$
Metabolic Activation System	S9 fraction from phenobarbitone/β-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	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*
Absent	> 5000			
Test 1		> 5000	> 5000	negative
Present	> 5000			
Test 1		> 5000	> 5000	negative

Remarks - Results

The test substance caused no visible reduction in the growth of the bacterial background lawn at any dose level. The test substance was, therefore, tested up to the maximum recommended dose level of 5000 µg/plate. A yellow colour was noted from 50 µg/plate but this did not prevent the scoring of revertant colonies. No test material precipitate was observed on the plates at any of the doses tested in either the presence or absence of metabolic activation.

No toxicologically significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test substance, either with or without metabolic activation. A small, statistically significant increase in revertant colony frequency was observed for tester strain TA1537 without activation at 5000 µg/plate in the main test only. This increase was considered to be of no biological relevance because there was no evidence of a dose-response relationship or reproducibility. Furthermore, the revertant counts at 5000 µg/plate were within the in-house historical control range for the tester strain. No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test substance, either with or without metabolic activation.

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

TEST FACILITY SafePharm Laboratories (2007i)

B.8. Genotoxicity – bacteria (2)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Pre-incubation procedure. The method incorporated the Prival and Mitchell modification for azo dyes (Prival and Mitchell, 1982). Test 1 – Standard Ames Test Test 2 – Azo modification method

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA$^-$
Metabolic Activation System	Test 1: Rat liver S9-mix. Test 2: uninduced Hamster liver S9-mix.
Concentration Range in Main Test	a) With metabolic activation: 19.5-5000 µg/plate b) Without metabolic activation: 19.5-5000 µg/plate
Vehicle	Distilled water
Remarks - Method	Non-GLP study. Bacterial strains TA98 and TA100 were only used for azo modification method (Test 2).

RESULTS

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

Remarks - Results	No increase in the number of revertants was observed under all conditions tested in both the standard Ames test and modified Prival and Mitchell method for azo dyes.

CONCLUSION The notified chemical was not mutagenic to bacteria under the conditions of the standard Ames test and modified Prival and Mitchell method for azo dyes.

TEST FACILITY Canon (2007)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.

Species/Strain	Chinese Hamster
Cell Type/Cell Line	CHL/IU
Metabolic Activation System	S9 fraction from phenobarbitone/β-naphthoflavone-induced rat liver.
Vehicle	Eagle's Minimal Essential Medium (MEM)
Remarks - Method	No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 156.25, 312.5, 625, 1250*, 2500*, 5000*	6 hrs	24 hrs
Test 2	0*, 19.5, 39, 78.1*, 156.25*, 312.5*, 625*	24 hrs	24 hrs
Present			
Test 1	0*, 156.25, 312.5, 625, 1250*, 2500*, 5000*	6 hrs	24 hrs
Test 2	0*, 156.25, 312.5, 625, 1250*, 2500*, 5000*	6 hrs	24 hrs

*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	1250[1], > 5000[2]			
Test 1		> 5000	> 5000	negative
Test 2		> 625	> 625	negative

Present	> 5000			
Test 1		> 5000	> 5000	negative
Test 2		> 5000	> 5000	negative

[1]For 24 hr continuous exposure
[2]For 6 hr exposure

Remarks - Results	Cytotoxicity was only observed in the 24-hour continuous exposure group in the preliminary test at a dose of 1250 µg/mL. The test substance did not induce any statistically significant increases in the frequency of cells with aberrations or numbers of polyploid cells in any of the exposure groups. All of the positive controls 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 (2007j)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I).
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Measurement of biochemical oxygen demand (BOD) with a closed system oxygen measuring apparatus; Determination of dissolved organic carbon (DOC) by a total organic carbon analysis (TOC); Determination of test item by HPLC.
Remarks - Method	On-site sludge sampling was carried out at 10 locations in Japan by collecting return sludge, surface water and surface soil that were in contact with atmosphere. The test was conducted in triplicate at 25±1°C at a concentration of 100 mg/L for the notified chemical and 30 mg/L for activated sludge. Aniline was used as a reference item to confirm that the sludge was sufficiently active. Control tests conducted included a blank control of culture medium only, a control test of the notified chemical without sludge and a control with the reference chemical.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	0.3	7	63
14	0.0	14	73
21	-1.0	21	75
28	0.3	28	76

* The percentages of degradation were calculated by BOD.

Remarks - Results	All the validity criteria of the test were met. The percentage of biodegradation at 28 days was 0% by BOD, 3% by DOC and 1% by HPLC. The notified chemical is not considered readily biodegradable based on the test result.
CONCLUSION	The notified chemical is not considered readily biodegradable.
TEST FACILITY	CERI (2007)

C.1.2. Bioaccumulation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 305 Bioconcentration: Flow-through Fish Test.
Species	*Cyprinus carpio*
Exposure Period	Exposure: 28 days Depuration: No
Auxiliary Solvent	None
Concentration Range	Nominal: 0.189, 1.89 mg/L Actual: 0.179, 1.82 mg/L
Analytical Monitoring	Liquid chromatography-mass spectrometry (LC-MS) for determination of

	the concentrations of the notified chemical in water and fish.
Remarks - Method	The test was conducted at two different concentrations, 1.89 mg/L and 0.189 mg/L at a flow rate of 2.0 mL/min for stock solution and 800 mL/min for dilution water, with 1155 L/day of test water being supplied. A control test was also carried out at a flow rate of 800 mL/min with 1152 L/day of water being supplied. 28 fish was used for each test treatment and 12 fish was used for the control test. All the tests were controlled at 24.3 ± 0.3°C and a pH range of 8.0 – 8.2.

RESULTS

Bioconcentration Factor	BFC ≤ 1.1, 11 for nominal concentration of 1.89 and 0.189 mg/L, respectively.
CT50	
Remarks - Results	No depuration has been conducted after the exposure of the fish to the test chemical. This is considered fine given the detected concentrations of the notified chemical in all test fish at the last three successive analyses were not more than the minimum determination limit. Since all the BCFs were ≤ 1.1 for the 1.89 mg/L test and ≤ 11 for the 0.189 mg/L test, it was evaluated that a steady-state was reached after 28 days and the notified chemical is not considered to be bioconcentrating.

CONCLUSION	The notified chemical is not considered to be bioconcentrating based on the test results.
TEST FACILITY	CERI (2008)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test -semi-static. EC Directive 92/69/EEC C.1 Acute Toxicity for Fish- semi-static.
Species	*Oncorhynchus mykiss* (Rainbow Trout)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	Approximately 140 mg $CaCO_3$/L
Analytical Monitoring	HPLC for determination of concentrations of the notified chemical.
Remarks – Method	The water used for both the range-finding and definitive tests was laboratory tap water after being dechlorinated by passage through an activated carbon filter (Pure Series 500) and partly softened, and adjusted to required temperature. Following a preliminary range-finding test, a definitive test was conducted in duplicate at a nominal concentration of 100 mg/L, a temperature of 14°C and a pH range of 7.8 - 8.1, each with 10 fish being used. A control test was conducted under identical conditions except without the chemical.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*3h*	*24h*	*48h*	*72h*	*96h*
Control	< limit of quantitation	10	0	0	0	0	0
$100R_1$*1	99(0hr), 103(24hr), 99(96hr)	10	0	0	0	0	0
$100R_2$*1	106(0hr), 105(24hr), 99(96hr)	10	0	0	0	0	0

*1: R_1 and R_2 = Replicates 1 and 2

LC50	>100 mg/L at 96 hours.
NOEC	100 mg/L at 96 hours.

Remarks – Results	The test medium was yellow clear solution and renewed on a daily base. Verification of test concentrations throughout the test period shows that the notified chemical is stable in the test medium. The recovery of solutions was 94% of the nominal concentration. Neither mortality nor sub-lethal effects of the exposure was observed for a period of 96 hours.
CONCLUSION	The notified chemical is not toxic to *Oncorhynchus mykiss* (Rainbow Trout).
TEST FACILITY	SafePharm Laboratories (2007k)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - static. EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	approximately 250 mg $CaCO_3$/L
Analytical Monitoring	Visual observation, HPLC
Remarks - Method	No significant protocol deviations. Following a preliminary range-finding test, 4 replicates of 5 daphnids were exposed to a test concentration of 100 mg/L of the notified chemical for 48 hours at 21 – 22°C. A positive control test was conducted using potassium dichromate as the reference material at concentrations of 0.32, 0.56, 1.0, 1.8 and 3.2 mg/L for 48 hours at 21 – 22°C.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	<limit of quantitation>	20	0	0
100	99-103	20	0	0

EC50	>100 mg/L (nominal) at 48 hours
NOEC	100 mg/L (nominal) at 48 hours
Remarks - Results	The 48-Hour EC50 for the reference material to *Daphnia magna* based on nominal concentrations was 0.75 mg/L with 95% confidence limits of 0.56 – 1.0 mg/L. Verification of test concentration at 0 and 48 hours shows that the notified chemical is stable in the test medium. The recovery of solutions was 99 – 101% of the nominal concentration. No immobilisation was reported throughout the 48 hours test at 100 mg/L.
CONCLUSION	The notified chemical is not toxic to *Daphnia magna*.
TEST FACILITY	SafePharm Laboratories (2007l)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical at
METHOD	OECD TG 201 Alga, Growth Inhibition Test. EC Directive 92/69/EEC C.3 Algal Inhibition Test.
Species	*Desmodesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 3.2, 10, 32, 100 and 320 mg/L Actual: 3.58, 10.1, 32.7, 100 and 318 mg/L
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	HPLC for determination of the test material concentration. A Coulter® Multisizer Particle Counter for the analysis of algal cell concentration.
Remarks - Method	Following a preliminary range-finding test, parallel experiments A and B were conducted in triplicates by exposing algae to the notified chemical for 72 hours at 25 – 26°C under constant illumination and shaking. In experiment A, algae were directly exposed to test material solution and covered by glass Petri dishes above the test vessels containing culture medium. In experiment B, algae were exposed to culture medium alone and covered by glass Petri dishes above the test vessels containing the test material solutions. A positive control test was conducted using potassium dichromate as the reference material at concentrations of 0.0625, 0.125, 0.25, 0.5 and 1.0 mg/L

RESULTS

Biomass		*Growth*	
E_bC50	*NOEC*	*E_rC50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L at 0-72 h*	*mg/L*
A 130	32	>320	32
B 81	10	>320	32

Remarks - Results	All test validation criteria were met. Verification of test concentrations at 0 hour and 72 hours show that the notified chemical is stable under the test conditions. The positive control test gives an E_rC50 (0-72h) of 0.58 mg/L (95% CI 0.47 - 0.71 mg/L), and an E_bC50 (0-72h) of 0.20 mg/L (95% CI 0.17 - 0.24 mg/L). The significant differences between experiments A and B indicate that the effects of the notified chemical on algae were not only due to a reduction in light intensity, but also due to the intrinsic toxic properties of the notified chemical. The results from experiment A should be used for toxic classification.
CONCLUSION	The notified chemical is not toxic to *Desmodesmus subspicatus* based on the test result E_rC50.
TEST FACILITY	SafePharm Laboratories (2007m)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test
Inoculum	A mixed population of activated sewage sludge micro-organisms from the aeration stage of the Severn Trent Water Plc sewage treatment plant at UK

	which treats predominantly domestic sewage
Exposure Period	3 hours
Concentration Range	Nominal: 1000 mg/L
Remarks – Method	The water used in the test was laboratory tap water dechlorinated by passage through an activated carbon filter (Purite Series 500) and partly softened (Elga Nimbus 1248D Duplex water softener). The water had a total hardness of approximately 140 mg/L as $CaCO_3$ and was adjusted to required temperature before use. For preparation of the test solution, the notified chemical was dissolved directly in water. A range finding test was conducted by exposing sewage sludge to a series of concentrations of 1.0, 10, 100 and 1,000 mg/L of the notified chemical. On the basis of the range finding test a definitive test was conducted at approximately 21˚C in triplicates by exposing sewage sludge to 1,000 mg/L of the notified chemical. The chemical 3,5-dichlorophenol was used as reference material at 3.2 and 32 mg/L in the control test.

RESULTS

IC50	>1000 mg/L
NOEC	1000 mg/L
Remarks – Results	The refernce material gives a 3-Hour EC50 value of 12 mg/L. No significant effect on respiration was observed at both the rang-finding and the definitive tests. The notified chemical is not considered to be inhibitory to sewage sludge microorganisms.

CONCLUSION	The notified chemical is not toxic to sewage sludge microorganisms.
TEST FACILITY	SafePharm Laboratories (2008d)

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