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**NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME
(NICNAS)**

FULL PUBLIC REPORT

CIM-03

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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FULL PUBLIC REPORT**CIM-03****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 (more than 1 tonne per year).

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; Identity of toxic or hazardous impurities; % Weight of Hazardous and Non-hazardous 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)

Low volume category permit (2007)

NOTIFICATION IN OTHER COUNTRIES

USA

UK

Switzerland

Japan

Korea

China

Philippines

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)

CIM-03

ANALYTICAL DATA

Reference NMR, IR, HPLC, GC, LCMS and UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY > 80%

ADDITIVES/ADJUVANTS None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Red solid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	> 360°C	Measured
Boiling Point	Not determined	Not applicable as notified chemical is a high melting point solid
Density	1510 kg/m ³ at 20 ± 1.0°C	Measured
Vapour Pressure	< 2.1 x 10 ⁻⁸ kPa at 25°C	Estimated
Water Solubility	40.1 – 45.2% w/w at 20.0 ± 0.5°C	Measured
Hydrolysis as a Function of pH	t _{1/2} > 1 year at pH 4, 7 and 9 at 25°C	Measured
Partition Coefficient (n-octanol/water)	log Pow < -2.40 at 23.5 ± 0.5°C	Measured
Adsorption/Desorption	log K _{oc} = < 1.25 at 40°C	Measured
Dissociation Constant	Not determined	Notified chemical is a salt of a very strong acid and as a consequence will remain dissociated throughout the environmental pH range of 4-9.
Particle Size	Inhalable fraction (<100 µm): 24% Respirable fraction (<10.2 µm): 3.7%	Measured
Flash Point	Not determined	Not applicable as notified chemical is a low volatility solid.
Flammability	Not highly flammable	Measured
Autoignition Temperature	290°C	Measured
Explosive Properties	Not explosive	Measured

DISCUSSION OF PROPERTIES

For full details of tests on physical and chemical properties, please refer to 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, which has already been incorporated into cartridges (< 2.5% concentration).

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

Year	1	2	3	4	5
Tonnes	< 0.1	< 0.1	< 0.5	< 0.5	< 0.5

PORT OF ENTRY

Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS

The ink-jet printing systems will be potentially supplied to offices nationwide.

TRANSPORTATION AND PACKAGING

The notified chemical will be imported as a component of ready-to-use (16 ml and 150 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 warehouse, where they will be expected to be stored in an undercover area on a pallet rack..

USE

The notified chemical will be used as a dye component of imported inkjet printer inks (< 2.5%).

The inks will be used by office workers and the public for routine but varied colour printing operations in home and small office scenarios. Sealed ink cartridges containing the notified chemical will be used as necessary to replace spent cartridges in inkjet printers.

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

<i>Category of Worker</i>	<i>Number</i>	<i>Exposure Duration (hours/day)</i>	<i>Exposure Frequency (days/year)</i>
Importation/Waterside workers	50	< 8 hours per day	10-50 days per year
Storage and transport	100	< 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 (< 2.5% 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.

The extent of dermal exposure of workers to wet ink on paper or other non-absorbent substrate has been estimated by the notifier. One kilogram of pure dye would be expected to produce several million sheets of A4 coloured text or graphics. Under worst-case conditions, each piece of A4 paper could be assumed to incorporate a maximum of 1 mg of notified chemical. Based on a 50% transfer on contact when handling printed paper or other substrate (assuming partially dry ink), and the relative contact area of fingertips and paper size:

Area of contact with finger ends (four fingers on one hand) = 8 cm²

A4 sized paper = ~600 cm²

% Removal = $(8/600) \times 0.5 \times 100 = < 1\%$

∴ Exposure to fingertips per event = < 1% of 1 mg = < 0.01 mg per event.

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 conservative estimate of 100% absorption, would be:

Daily exposure = $(< 0.01 \text{ (mg/event)} \times 10) \div 70 = \underline{\sim 0.0014 \text{ mg/kg bw/day}}$.

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.

<i>Endpoint</i>	<i>Result and Assessment Conclusion</i>
Rat, acute oral toxicity	LD50 = 2500 mg/kg bw, low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw, low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly/moderately irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOEL = 25 mg/kg bw/day
Genotoxicity – bacterial reverse mutation (Ames)	non mutagenic
Genotoxicity –Modified Ames for azo compounds	non-mutagenic
Genotoxicity – in vitro mammalian chromosome aberration test	non genotoxic

Acute toxicity

The notified chemical was of low acute oral and dermal toxicity in rats (LD50 > 2,000 mg/kg bw). The NOEL in a 28-day oral repeat dose study in rats was 25 mg/kg bw/day on the basis of the treatment related changes observed in the kidneys and/or stomach at 150 and 400 mg/kg bw/day.

Irritation and Sensitisation

The notified chemical was found to be moderately irritating to the eye, though not severe enough to warrant hazard classification, and a slight irritant 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. Relatively few azo dyes have been demonstrated to be skin sensitisers (Øllgaard *et al* 1998). 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 isomers of the notified chemical. As such, the impurities are unlikely to contribute to carcinogenicity of the notified chemical.

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 showing that the notified chemical was found to be not mutagenic using the modified Ames test for azo dyes (Prival and Mitchell, 1982). 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. 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).

Classification

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

6.3. Human health risk characterisation

6.3.1. Occupational health and safety

Dermal and 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 notified chemical, present in inks at concentrations < 2.5%, is not likely to be toxic at the highest levels of probable exposure (worst-case exposure estimate of ~0.0014 mg/kg bw/day, compared with NOEL of 25 mg/kg bw/day), although it may cause slight eye and skin irritation.

Given that exposure of workers to the notified chemical is expected to be low, the OHS risk is considered acceptable.

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 assessed to be low. The unlikely but potential public exposure through accidents during importation, transportation or storage is assessed as negligible.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

Printer ink will be imported in ready-to-use cartridges (containing < 2.5% notified chemical). No release is expected as manufacturing and reformulation of the ink containing the notified chemical will not take place in Australia. Environmental release of the notified chemical is unlikely during importation, storage and transportation, and spillage during a transport accident is the most likely reason for environmental release. Individual container capacity, container and packaging specifications would limit the extent of release.

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 of use. In the unlikely case of spills arising during installation and replacement, it is expected that the ink containing the notified chemical will be contained and collected with absorbent material and be subsequently disposed of to landfill. Cartridges are contained within the printer until the contents are used then they are removed and sent to a recycling and disposal centre or directly to landfill.

Most of the notified chemical (> 98%) will be bound to the printed 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 waste is expected to go to trade waste sewers. Approximately 50% of the ink printed on paper will enter paper recycling of which a proportion of the ink is expected to be recovered during recycling. While most may partition to water, due to the low percentage of the notified chemical in these inks and the widespread use, release to the aquatic compartment from any given recycling plant will still be low based on worst case assumptions. Any notified chemical absorbed to sludge during the recycling process will be disposed of to landfill.

RELEASE OF CHEMICAL FROM DISPOSAL

The majority of the annual import volume of the notified chemical will ultimately be disposed of as normal office/domestic waste that will end up in either landfill or be incinerated. Some waste paper printed with the ink may be disposed of directly to landfill with the notified chemical bound to the paper, while some will enter the paper recycling process. Used cartridges may be sent to recycling and disposal centres or directly to landfill. The cartridges may be broken down into component parts for recycling. Residual ink (< 2% of the notified chemical) left in the empty cartridges will be separated from the cartridges and incinerated during the recycling of the cartridges.

The notified chemical that is incinerated is expected to thermally decompose to form predominantly simple organic compounds and various salts. Similarly, the notified chemical that is disposed of to landfill should eventually degrade to form predominantly simple organic compounds and various salts.

7.1.2 Environmental fate

One test relating to biodegradability was presented. The result indicate that the notified chemical is poorly biodegradable. For the details of the environmental fate studies please refer to Appendix C.

7.1.3 Predicted Environmental Concentration (PEC)

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	500	kg/year
Proportion expected to be released to sewer	50%	
Annual quantity of chemical released to sewer	250	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	0.68	kg/day
Water use	200	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:	0.17	µg/L
PEC - Ocean:	0.017	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.

<i>Endpoint</i>	<i>Result</i>	<i>Assessment Conclusion</i>
Fish Toxicity	LC50 2.3 mg/L	Toxic
Daphnia Toxicity	EC50 51 mg/L	Harmful
Algal Toxicity	EC50 28 mg/L	Harmful
Inhibition of Bacterial Respiration	EC50 > 3200 mg/L	Not harmful
<i>Lemna minor</i> (duckweed) toxicity	EC50 > 100 mg/L	Not harmful

7.2.1 Predicted No-Effect Concentration

Aquatic ecotoxicity data were provided for three trophic levels, with fish demonstrating the highest level of sensitivity to the notified chemical. 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 (fish)		2.3	mg/L
Assessment Factor		100.0	
Mitigation Factor		1.00	
PNEC:		23	µg/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	0.17	23	0.007
Q - Ocean	0.017	23	0.0007

This indicates that the current import volume and use pattern is not expected to pose an unacceptable risk to the aquatic 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)].

and

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

	<i>Hazard category</i>	<i>Hazard statement</i>
Health		
Acute oral toxicity	5	May be harmful if swallowed
Environment		
Acute	2	Toxic to aquatic life
Chronic	2	Toxic to aquatic life with long lasting effects

Human health risk assessment

Under the conditions of the occupational settings described and based on the use pattern, the notified chemical is not considered to pose an unacceptable risk to the health of workers and the public.

Environmental risk assessment

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

Recommendations**CONTROL MEASURES****Occupational Health and Safety**

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the printer cartridges containing the notified chemical:
 - Avoid contact with eyes and skin
- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical:
 - Service personnel should wear cotton or disposable gloves when removing spent printer cartridges containing the notified chemical and during routine maintenance and repairs.
- 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.

Public Health

- The following measures should be taken by the suppliers of the printer cartridges to minimise public exposure to the notified chemical:
 - Printer cartridges containing the notified chemical should be labelled with the following safety direction: Avoid skin and eye contact with ink.

Environment**Disposal**

- The notified chemical should be disposed of by landfill.

Emergency procedures

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

Regulatory Obligations*Secondary Notification*

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

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

- 1) Under Section 64(1) of the Act; if
- the notified chemical is imported in any fashion other than within an inkjet ink cartridge.

or

- (2) Under Section 64(2) of the Act; if
- the function or use of the chemical intended as a component ($< 2.5\%$) in inkjet printer inks, has changed from, or is likely to change significantly;
 - the amount of chemical being introduced has increased from half a tonne per annum, 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.

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/Freezing Point > 360 °C

Method EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Differential scanning calorimetry
Remarks The notified chemical was not observed to melt between 25 and 360 °C.
Test Facility SafePharm Laboratories Ltd (2005a)

Density 1510 kg/m³ at 20 ± 1.0 °C

Method EC Directive 92/69/EEC A.3 Relative Density.
Gas comparison pycnometer
Test Facility SafePharm Laboratories Ltd (2005a)

Vapour Pressure < 2.1 x 10⁻⁸ kPa at 25°C

Method EC Directive 92/69/EEC A.4 Vapour Pressure, vapour pressure balance.
Remarks The given value is the highest extrapolated estimate, based on readings at 241 °C.
Test Facility SafePharm Laboratories Ltd (2006a)

Water Solubility 40.1 – 45.2% w/w at 20.0 ± 0.5 °C

Method Based on EC Directive 92/69/EEC A.6 Water Solubility.
Remarks Flask Method. Samples of the notified chemical could not be prepared at five times the saturation level, as recommended in the test guideline, as the saturation point was unable to be determined. No analysis could be performed due to the high solubility producing unfilterable mixtures. Therefore, the water solubility was estimated based on visual inspection.
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.

<i>pH</i>	<i>T (°C)</i>	<i>t</i> _½ <hours or days>
4	25	>365
7	25	>365
9	25	>365

Remarks The preliminary test showed less than 10% hydrolysis (by HPLC) after 5 days at 50°C in buffers of pH 4, 7 and 9, which is estimated to be equivalent to a half-life of >1 year at 25°C at any pH.

Test Facility SafePharm Laboratories Ltd (2005a)

Partition Coefficient (n-octanol/water) log Pow <-2.40 at 23.5 ± 0.5°C

Method EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks Shake Flask Method with HPLC analysis.
Test Facility SafePharm Laboratories Ltd (2005a)

Adsorption/Desorptionlog K_{oc} < 1.25 at 40°C

Method	OECD TG 121 Estimation of the Adsorption Coefficient (K_{oc}) on Soil and on Sewage Sludge using HPLC.
Remarks	<p>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. As the test material eluted prior to the first calibration standard, a limit value has been reported for the adsorption coefficient, that of less than the valid calibration range.</p> <p>The low adsorption properties of the test material containing acidic 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 requires 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.</p> <p>The alternative use of computer-based estimation programs and/or Quantitative Structure Activity Relationships (QSAR's) for materials of this nature are considered invalid as estimates are typically derived from the partition coefficient value. Therefore, once more the possible secondary interaction originating from the anionic charges present on the test material is not addressed.</p>
Test Facility	SafePharm Laboratories Ltd (2005a)

Particle Size

Method	OECD TG 110 Particle Size Distribution/Fibre Length and Diameter Distributions. Sieve method and cascade impactor method								
<table border="1"> <thead> <tr> <th>Range (μm)</th><th>Mass (%)</th></tr> </thead> <tbody> <tr> <td>Proportion of test material having an inhalable particle size less than 100 μm</td><td>24.0</td></tr> <tr> <td>Proportion of test material having a thoracic particle size less than 10.2 μm</td><td>3.7</td></tr> <tr> <td>Proportion of test material having a respirable particle size less than 5.4 μm</td><td>0.8</td></tr> </tbody> </table>		Range (μm)	Mass (%)	Proportion of test material having an inhalable particle size less than 100 μm	24.0	Proportion of test material having a thoracic particle size less than 10.2 μm	3.7	Proportion of test material having a respirable particle size less than 5.4 μm	0.8
Range (μm)	Mass (%)								
Proportion of test material having an inhalable particle size less than 100 μm	24.0								
Proportion of test material having a thoracic particle size less than 10.2 μm	3.7								
Proportion of test material having a respirable particle size less than 5.4 μm	0.8								
Remarks	Too few particles were of a size < 10.2 μm to allow accurate assessment of mass median aerodynamic diameter.								
Test Facility	SafePharm Laboratories Ltd (2005a)								

Flammability

The notified chemical was determined to be not highly flammable.

Method	EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks	The notified chemical failed to ignite during the preliminary screening test.
Test Facility	SafePharm Laboratories Ltd (2006a)

Autoignition Temperature

290°C

Method	92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Test Facility	SafePharm Laboratories Ltd (2006a)

Explosive Properties

The notified chemical was determined not to have explosive properties

Method EC Directive 92/69/EEC A.14 Explosive Properties.
Test Facility SafePharm Laboratories Ltd (2006a)

Oxidizing Properties

Predicted to be negative

Method EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Remarks The notified chemical does not contain any chemical groups that would imply oxidising properties
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. EC Directive 2004/73/EEC B.1tris Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/ Sprague Dawley CD (CrI: CD (SD) IGS BR)
Vehicle	Distilled water
Remarks - Method	No significant protocol deviations. Corrections were made for the purity of the test material.

RESULTS

<i>Group</i>	<i>Number and Sex of Animals</i>	<i>Dose mg/kg bw</i>	<i>Mortality</i>
1	3F	300	0
2	3F	300	1
3	3F	2000	1
4	3F	2000	1

LD50 2500 mg/kg bw

Signs of Toxicity One animal treated with 300 mg/kg was found dead approximately 3 minutes after dosing.
Two animals treated at 2000 mg/kg were found dead 1 day after dosing.
Red staining of the urine and faeces were noted in surviving animals (300 mg/kg) four hours to three days after dosing. Animals appeared normal two or four days after dosing.
Hunched posture, diarrhoea stained red and noisy respiration were noted in animals treated at a dose level of 2000 mg/kg. Red staining of faeces, fur and urine were also noted. Surviving animals appeared normal seven, nine, or eleven days after dosing.

Effects in Organs At necropsy, the animal that died after dosing with 300 mg/kg had red material in the stomach. Animals that died during the study that were dosed with 2000 mg/kg had abnormally red lungs, dark liver, dark kidneys, stomach stained red with red liquid or material present and small and large intestines stained red.
No abnormalities were observed at necropsy in animals killed at the end of the study.

Remarks - Results

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 (88% purity)
METHOD	OECD TG 402 Acute Dermal Toxicity. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/Sprague-Dawley CD (CrI: CD(SD) IGS BR)
Vehicle	Moistened with distilled water
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations.

Corrections were made for the purity of the test material.

RESULTS

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

LD50 >2000 mg/kg bw
 Signs of Toxicity - Local None
 Signs of Toxicity - Systemic None
 Effects in Organs None
 Remarks - Results Red coloured staining was noted at the treatment sites of all animals one to four days after dosing. This prevented evaluation of erythema.

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

TEST FACILITY SafePharm Laboratories Ltd (2006b)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
 EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).
 Species/Strain Rabbit/New Zealand White
 Number of Animals 3
 Vehicle Distilled water
 Observation Period 7 days
 Type of Dressing Semi-occlusive.
 Remarks - Method One of the three animals was treated initially, where the test substance was applied to three sites for time periods of 3 minutes, 1 hour and 4 hours prior to its removal and evaluation of skin reactions. The other two animals were treated for 4 hours only.

RESULTS

<i>Lesion</i>	<i>Mean Score* Animal No.</i>			<i>Maximum Value</i>	<i>Maximum Duration of Any Effect</i>	<i>Maximum Value at End of Observation Period</i>
	1	2	3			
<i>Erythema/Eschar</i>	0	1	1	1	<7 days	0
<i>Oedema</i>	0	0.7	0.7	1	<72 hours	0

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

Remarks - Results No skin irritation was observed after 3 minute or the 1 hour exposure period on the single animal tested.

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

TEST FACILITY SafePharm Laboratories Ltd (2005c)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
 EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Observation Period	72 hours for 2 animals, 7 days for one animal
Remarks - Method	A single rabbit was treated initially and an assessment of the initial pain reaction was made. After consideration of the ocular responses produced in the first treated animal, two additional animals were treated. In order to minimise pain on application of the test material, one drop of local anaesthetic (amethocaine hydrochloride 0.5%) was instilled into both eyes of the final animal 1-2 minutes prior to treatment. One treated eye was observed on day 7 to assess the reversibility of the ocular effect.

RESULTS

Lesion	Mean Score* Animal No.			Maximum Value	Maximum Duration of Any Effect	Maximum Value at End of Observation Period
	1	2	3			
<i>Conjunctiva: redness</i>	1	1	1.7	2	<7 days	1
<i>Conjunctiva: chemosis</i>	1	0.7	1.7	2	<7 days	1
<i>Conjunctiva: discharge</i>	1	0.7	1.7	2	<7 days	1
<i>Corneal opacity</i>	0.7	0	0.3	1	<72 hr	0
<i>Iridial inflammation</i>	0.7	0	0.7	1	<72 hr	0

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

Remarks - Results	<p>Red coloured staining of the fur was noted around all treated eyes during the study.</p> <p>Red coloured staining of the cornea was noted in one treated eye at the 24 and 48 hour observation. The staining did not affect evaluation of corneal effects.</p> <p>An area of haemorrhage over the nictitating membrane was noted in one treated eye at the 24, 48 and 72 hour observations.</p> <p>Two treated eyes appeared normal at the 72 hour observation and the remaining treated eye appeared normal at the 7 day observation.</p> <p>The notified chemical is classified as a moderate irritant according to a modified Kay and Calandra classification system.</p>
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CONCLUSION	The notified chemical is slightly irritating/non-irritating to the eye.
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TEST FACILITY	SafePharm Laboratories Ltd (2005d)
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B.5. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical (88% purity)
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/Sprague-Dawley Crl:CD (SD) IGS BR
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Distilled water
Remarks - Method	No significant protocol deviations. A 14-day repeated dose range finding study was performed at 500 and 1000 mg/kg bw/day, using 3 male and 3 female animals in each group. The test method was similar to the main study, and was used to select the doses for the main study.

RESULTS

<i>Group</i>	<i>Number and Sex of Animals</i>	<i>Dose mg/kg bw/day</i>	<i>Mortality</i>
control	5M, 5F	0	0
low dose	5M, 5F	25	0
mid dose	5M, 5F	150	0
high dose	5M, 5F	400	0
control recovery	5M, 5F	0	0
high dose recovery	5M, 5F	400	0

Mortality and Time to Death

No mortality was observed during the treatment or recovery phases.

Clinical Observations

Isolated incidents of increased salivation, respiratory pattern changes, red/brown staining around the snout and diarrhoea were evident in animals of either sex treated with 400 mg/kg/day throughout the treatment period. Fur staining was detected in animals of either sex treated with 400 and 150 mg/kg/day and in males treated with 25 mg/kg/day from day 2 onwards. The staining remained in some animals of both sexes in the recovery group.

There were some changes in body weight gain, however, these were considered to be incidental. No significant changes in body weight, functional observations, food or water consumption were observed.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Blood chemistry

Some statistically significant changes were observed at high and medium dose (decrease in chloride levels, decrease in alkaline phosphatase, increase in cholesterol, increase in inorganic phosphorus, and changes in sodium concentrations). However, such changes were considered to be of no toxicological significance as individual values were within normal ranges for rats of the strain and age used.

Haematology

There were no changes in haematological parameters that were considered to be of toxicological significance.

Urinalysis

Animals of both sexes treated with 400 mg/kg/day showed pink urine, an effect that was also observed in males treated with 150 mg/kg/day. This was considered to be due to the coloured nature of the test material, rather than an indication of toxicity.

Effects in Organs

Organ weights

Recovery 400 mg/kg/day females showed a reduction in absolute and relative thymus weight following 14 days without treatment. The absence of similar effects detected in non-recovery animals at the end of the dosing period indicates that such changes are likely to be of no toxicological significance.

Necropsy

Animals of both sexes treated with 400 mg/kg/day, as well as a few animals treated at lower doses, showed pink contents/staining of the gastro-intestinal tract. In addition, males showed pink discolouration of the testes. Such observations were considered to be of no toxicological significance.

Histopathology

Kidney: Hypertrophy of distal tubules and collecting ducts was observed in relation to treatment for animals of either sex treated with 400 mg/kg/day but not at other treatment levels. The condition regressed in recovery animals treated with the same dose following 14 days without treatment. The effect was considered to be treatment related.

Stomach: Agglomeration of secretion was observed in the gastric mucosa of animals of either sex treated with 400 mg/kg/day, with a smaller incidence among animals treated with 150 mg/kg/day. The condition was observed to regress after the 14 day recovery period. This was considered to be a treatment related effect.

A number of other effects were observed in the heart, liver, spleen, kidney, lung and bone marrow of some of the treated animals. However, effects of similar severity and in a similar number of control animals were also

observed. In addition, many such effects are considered to be common in laboratory rats. As such, the effects were considered not to be of toxicological significance. No effects were found in other organs examined.

Remarks – Results

Treatment related effects were observed in the kidneys and stomach of animals treated with 400 mg/kg/day, and in the stomach of animals treated with 150 mg/kg/day. Adverse changes were observed to have regressed following cessation of the treatment.

CONCLUSION

The No Observed Effect Level (NOEL) was established as 25 mg/kg bw/day in this study, based on hypertrophy of distal tubules and collecting ducts in the kidneys, and agglomeration of secretion in the stomach.

TEST FACILITY SafePharm Laboratories Ltd (2006c)

B.6. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.
Plate incorporation procedure
Species/Strain *S. typhimurium*: TA1535, TA1537, TA98, TA100
E. coli: WP2uvrA
Metabolic Activation System S9 fraction from phenobarbitone/β-naphthoflavone induced livers of male Sprague-Dawley rats.
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.
As the notified chemical is an azo compound, the OECD test method strongly recommends the use of a modified Ames test with a reducing pre-incubation step to improve sensitivity (eg Prival and Mitchell, 1982). However, such a modification was not used in this test.

RESULTS

Metabolic Activation	Test Substance Concentration (µg/plate) Resulting in:			
	Cytotoxicity in Preliminary Test	Cytotoxicity in Main Test	Precipitation	Mutagenic Effect
<i>Absent</i>				
Test 1	>5000 µg/plate	>5000 µg/plate	>5000 µg/plate	Negative
Test 2		>500 µg/plate	>5000 µg/plate	Negative
<i>Present</i>				
Test 1	>5000 µg/plate	>1500 µg/plate	>5000 µg/plate	Negative
Test 2		1500 µg/plate	>5000 µg/plate	Negative

Remarks - Results A pink colour was observed at ≥50 µg/plate, however, this did not prevent the scoring of revertant colonies. The test substance did not cause a marked increase in the number of revertants per plate of any of the tester strains either in the presence or absence of metabolic activation. Positive controls confirmed the sensitivity of the test system. Negative controls were within historical limits.

As a reductive pre-incubation step was not used in this study, the result (non-mutagenic) is indicative only of the conditions of this particular Ames test. Many carcinogenic Azo dyes test negative in Ames tests without the use of a modified test (SCCNFP, 2002).

CONCLUSION The notified chemical was not mutagenic to bacteria under the conditions of the test. However, this negative result is considered inconclusive, as a modified test (eg Prival modification) was not used.

TEST FACILITY SafePharm Laboratories Ltd (2005e)

B.7. Genotoxicity – bacteria (Prival and Mitchell modification for Azo dyes)

TEST SUBSTANCE Notified chemical

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

Species/Strain *S. typhimurium*:
TA1535, TA1537, TA102, TA98, TA100

Metabolic Activation System 30% liver S9 in modified co-factors

Concentration Range in Main Test With metabolic activation: 50 - 5000 µg/plate.

Vehicle Sterile distilled water

Remarks – Method The method incorporated the Prival and Mitchell modification for azo dyes (Prival and Mitchell, 1982).

RESULTS

Metabolic Activation	Test Substance Concentration (µg/plate) Resulting in:			
	Cytotoxicity in Preliminary Test	Cytotoxicity in Main Test	Precipitation	Mutagenic Effect
<i>Present</i>				
Test 1	>5000 µg/plate	>5000 µg/plate	>5000 µg/plate	Negative
Test 2	>5000 µg/plate	>5000 µg/plate	>5000 µg/plate	Negative

Remarks - Results The vehicle control plates gave counts of revertant colonies generally within the normal range. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies. Thus, the sensitivity of the assay and the efficacy of the induced rat liver 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 red/pink colour was noted from 50 µg/plate; this observation did not prevent the scoring of revertant colonies. No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material.

CONCLUSION The test material was considered to be non-mutagenic under the conditions of the test.

TEST FACILITY SafePharm Laboratories Limited (2007)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical (88% purity)

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

Species/Strain Chinese Hamster Lung (CHL) cells

Metabolic Activation System Phenobarbitone/β-naphthoflavone-induced rat liver S9 microsome mix

Vehicle Eagle's Minimal Essential Medium (MEM)

Remarks - Method

Corrections were made for the purity of the test material.
No significant protocol deviations

<i>Metabolic Activation</i>	<i>Test Substance Concentration (µg/mL)</i>	<i>Exposure Period</i>	<i>Harvest Time</i>
<i>Absent</i>			
Test 1	0*, 39.06, 78.13, 156.25, 234.38*, 312.5*, 468.75*	6 hr	24 hr
Test 2	0*, 9.77, 19.53, 39.06*, 58.6*, 78.13*, 156.25	24 hr	24 hr
<i>Present</i>			
Test 1 (S9 at 5% final conc)	0*, 78.13, 156.25*, 312.5*, 468.75, 625*, 937.5	6 hr	24 hr
Test 2 (S9 at 2% final conc)	0*, 78.13, 156.25*, 312.5*, 468.75*, 625, 937.5	6 hr	24 hr

RESULTS

<i>Metabolic Activation</i>	<i>Test Substance Concentration (µg/mL) Resulting in:</i>			
	<i>Cytotoxicity in Preliminary Test</i>	<i>Cytotoxicity in Main Test</i>	<i>Precipitation</i>	<i>Genotoxic Effect</i>
<i>Absent</i>				
Test 1	> 312.5	> 468.75	>5000	Negative
Test 2	> 312.5	> 156.25	>5000	Negative
<i>Present</i>				
Test 1	> 625	> 625	>5000	Negative
Test 2	-	625	>5000	Negative

Remarks - Results

Some low-level structural chromosomal aberrations, at higher levels than in the negative controls, were observed. However, none of these apparent increases over control levels was found to be statistically significant, and in most cases was not dose-dependent.

CONCLUSION

The notified chemical was not clastogenic to CHL cells treated in vitro under the conditions of the test.

TEST FACILITY

SafePharm Laboratories Ltd (2006d)

B.9. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE

Notified chemical (86.4% purity)

METHOD

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

Species/Strain

Mouse/ CBA strain, female

Vehicle

Dimethyl formamide

Remarks - Method

A preliminary screening study on two animals treated with 10% and 25% w/w notified chemical, was performed to determine its toxicity/irritancy potential. The mice were treated with test substance on the dorsal surface of each ear daily for three days. A 25% test substance concentration was considered the highest concentration that could be prepared homogeneously to a visible acceptable level.

In the main test, the doses used were 5, 10, or 25% w/w notified chemical. Following sacrifice of the mice, their lymph cells were extracted and pooled individually for each animal.

A laboratory historical positive control was used.

RESULTS

<i>Concentration (% w/w)</i>	<i>Proliferative response (DPM/lymph node)</i>	<i>Stimulation Index (Test/Control Ratio)</i>
<i>Test Substance</i>		
0 (vehicle control)	211 ± 38	1.0
5	287 ± 26	1.4 ± 0.2
10	377 ± 86	1.8 ± 0.3
25	562 ± 106	2.7 ± 0.3
<i>α-Hexylcinnamaldehyde (Positive Control)</i>		
Vehicle (Acetone:Olive oil (4:1))	262 ± 31	1.0
5	551 ± 173	2.1 ± 0.7
10	952 ± 226	3.6 ± 0.6
25	1969 ± 236	7.5 ± 0.4

Remarks - Results

In the preliminary study, no deaths or signs of systemic toxicity were observed during the study. Staining by the test substance prevented scoring for erythema. Based on the results of the preliminary study, the dose levels for the main study were selected.

During the main study, no deaths or signs of systemic toxicity were noted in the animals. Although a dose related increase in the stimulation index was observed, the stimulation index was < 3 at the highest dose tested.

CONCLUSION

There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical under the test conditions at concentrations up to 25%.

TEST FACILITY

NOTOX (2006)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test.
Inoculum	Sewage Treatment Micro-organisms
Exposure Period	28 d
Auxiliary Solvent	Nil
Analytical Monitoring	Dissolved Oxygen
Remarks - Method	An amount of test material (114 mg) was dissolved in culture medium with the aid of ultrasonification for approximately 5 minutes and the volume adjusted to 100 mL to give a 1000 mg/L stock solution. An aliquot (15 mL) of this stock solution was dispersed in a final volume of 6 L of inoculated culture medium to give a test concentration of 2.5 mg/L. For the purposes of the test, a standard material, sodium benzoate, was used.

RESULTS

<i>Test substance</i>		<i>Sodium benzoate</i>	
<i>Day</i>	<i>% Degradation</i>	<i>Day</i>	<i>% Degradation</i>
3	2	3	51
7	5	7	55
14	4	14	62
21	12	21	61
28	12	28	61

Remarks - Results The toxicity control attained 27% degradation after 14 days and 30% degradation after 28 days, therefore confirming that the test material was not toxic to the sewage treatment micro-organisms used in the study. The standard material, sodium benzoate, attained 62% degradation after 14 days and 61% degradation after 28 days thereby confirming the suitability of the test method and culture conditions.

CONCLUSION The test material cannot be considered to be readily biodegradable under the strict terms and conditions of OECD Guideline 301D.

TEST FACILITY SafePharm Laboratories Ltd (2005f)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test–semi static. EC Directive 92/69/EEC C.1 Acute Toxicity for Fish – semi static
Species	Rainbow trout (<i>Oncorhynchus mykiss</i>) [juvenile]
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	100 mg CaCO ₃ /L
Analytical Monitoring	Chemical analysis at 0, 24, 48, 72 and 96 hours
Remarks – Method	Following a preliminary range-finding test, fish were exposed, in groups of ten, to an aqueous solution of the test material over a range of concentrations for a period of 96 hours at a temperature of approximately

14°C under semi-static test conditions.

An amount of test material (1136 g) was dissolved in dechlorinated tap water and the volume adjusted to give a 1000 mg ai/L stock solution. Aliquots were each separately dispersed in a final volume of 20 L to give the test concentrations.

The concentration and stability of the test material in the test preparations were verified by chemical analysis at 0 hours (fresh media), 24 and 96 (old media) hours.

The LC50 value and associated confidence limits were calculated by the trimmed Spearman-Kärber method using the ToxCalc computer software package. When only one partial response is shown, the trimmed Spearman-Kärber method is appropriate.

RESULTS

Concentration mg/L		Number of Fish	Mortality				
Nominal	Actual		1 h	24 h	48 h	72 h	96 h
Control		10	0	0	0	0	0
1.0		10	0	0	0	0	0
1.8		10	1	1	1	1	1
3.2		10	10	10	10	10	10
5.6		10	10	10	10	10	10
10		10	10	10	10	10	10

LC50 2.3 mg/L at 96 hours. 95% CI = 2.0 – 2.5 mg/L

NOEC (or LOEC) 1.0 mg/L at 96 hours.

Remarks – Results Throughout the duration of the test the test preparations were observed to be red solutions increasing in colouration as concentration increased. Analysis of the test preparations at 0, 24 and 96 hours showed measured test concentrations to range from 89% to 103% of nominal and so it was considered justifiable to calculate the LC50 values in terms of the nominal test concentration only. No sub-lethal effects were observed.

CONCLUSION

The notified chemical was found to be toxic to rainbow trout.

TEST FACILITY

SafePharm Laboratories Ltd (2005g)

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.

Species *Daphnia magna*

Exposure Period 48 hours

Auxiliary Solvent None

Water Hardness 250 mg CaCO₃/L

Analytical Monitoring HPLC

Remarks - Method An amount of test material (568 mg) was dissolved in reconstituted water and the volume adjusted to give a 1000 mg ai/L stock solution, from which the test concentrations were derived.

RESULTS

Concentration mg/L		Number of <i>D. magna</i>	Number Immobilised	
Nominal	Actual		24 h	48 h
1.8	1.60 - 1.43	10	0	0
3.2	2.90 - 2.78	10	0	0
5.6	5.10 - 4.76	10	0	0
10	9.32 - 9.01	10	0	0
18	17.7 - 17.2	10	0	0
32	32.3 - 31.1	10	0	4
56	55.8 - 56.1	10	0	9
100	98.4 - 97.4	10	0	20
180	180 - 175	10	0	20

*Actual values found at 0 h and 48 h respectively.

LC50 51 mg/L at 48 hours (95% confidence level of 43 – 60 mg/L)
 NOEC (or LOEC) 18 mg/L at 48 hours
 Remarks - Results No effects were observed at test concentrations of less than 18 mg/L.

Analytical monitoring at 0 and 48 hours showed measured test concentrations to range from 85% to 101% of nominal and so the results are based on the nominal test concentrations only. The 1.8 mg ai/L test sample at 48 hours showed a measured test concentration of 79%, which was below the 80% limit allowed by the test guidelines. However, as this test concentration was below the NOEC it was considered that this result did not affect the outcome or integrity of the study.

The control was observed to be a clear, colourless solution throughout the duration of the test. The 1.8 to 180 mg ai/L test concentrations were observed to be clear, pink/red solutions, increasing in colouration with increasing concentration, throughout the duration of the test.

CONCLUSION The notified chemical is harmful to *Daphnia magna*.

TEST FACILITY SafePharm Laboratories Ltd (2005h)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test.
 EC Directive 92/69/EEC C.3 Algal Inhibition Test.

Species *Scenedesmus subspicatus*

Exposure Period 72 hours

Concentration Range Nominal: 1.0, 3.2, 10, 32, and 100 mg/L

Actual: 87-103% of Nominal

Auxiliary Solvent Nil

Analytical Monitoring HPLC

Remarks - Method A preliminary range-finding test was conducted following the modified algal test method for coloured test substances. The results obtained indicated that despite the use of a reduced test volume and increased light intensity significant inhibition of growth was observed. Therefore, it was considered appropriate to conduct the test following the methods described above and further refined for coloured test substances, to differentiate between a reduced growth of algae due to a true toxic effect of the chemical or due to an indirect effect, a reduction in growth by light absorption of the coloured test substance (Memmert *et al* 1994).

Following preliminary range-finding tests, *Scenedesmus subspicatus* was

exposed to an aqueous solution of the test material for 72 hours under constant illumination and stirred continuously via magnetic stirrer at a temperature of $24 \pm 1^\circ\text{C}$. The test was conducted using two experimental methods performed in parallel.

Experiment A

The algae were exposed to test material concentrations of 3.2, 10, 32, 100 and 320 mg/L. Glass Petri dishes above the test vessels contained the culture medium alone. Therefore, inhibition of algal growth in these test vessels was due to a combination of both the toxic effects of the test material and reduction in light intensity.

Experiment B

The glass Petri dishes above the test vessels contained the test material solutions at concentrations of 3.2, 10, 32, 100 and 320 mg/L. The test vessels contained algal cells in culture medium alone. Therefore inhibition of algal growth was due to a reduction in light intensity alone.

The difference between the inhibition values obtained in Experiment A and B can be interpreted as the true toxic effect of the test material on the algal cells.

Pre-culture gave an algal suspension in log phase growth characterised by a cell density of 1.77×10^6 cells per mL. This suspension was diluted to a cell density of 2.00×10^4 cells per mL prior to use.

One way analysis of variance incorporating Bartlett's test for homogeneity of variance and Dunnett's multiple comparison procedure for comparing several treatments with a control was carried out on the area under the growth curve data for Experiments A and B at 72 h for the control and all test concentrations to determine any statistically significant differences between the test and control groups.

RESULTS

Experiment A

<i>Biomass</i>		<i>Growth</i>	
<i>E_bC₅₀</i> <i>mg/L at 72 h</i>	<i>NOEC</i> <i>mg/L</i>	<i>E_rC₅₀</i> <i>mg/L at 72 h</i>	<i>NOEC</i> <i>mg/L</i>
28 (95% CI: 22 - 35)	3.2	83 (95% CI: 66 - 100)	3.2

Experiment B

<i>Biomass</i>		<i>Growth</i>	
<i>E_bC₅₀</i> <i>mg/L at 72 h</i>	<i>NOEC</i> <i>mg/L</i>	<i>E_rC₅₀</i> <i>mg/L at 72 h</i>	<i>NOEC</i> <i>mg/L</i>
26 (95% CI: 21 - 32)	3.2	50*	3.2

*It was not possible to calculate 95% confidence limits for the $E_{rC_{50}}$ value as the data generated did not fit the models available for the calculation of confidence limits.

Remarks - Results

Given that greater inhibition of growth was observed in Experiment B it was considered that the effect of the test material on algal growth was probably due to a reduction in light intensity alone, not the intrinsic toxic properties of the test material

Analysis of the test preparation at 0 and 72 hours showed measured test concentrations to range from 97% to 107% of nominal and so it was

considered justifiable to estimate the EC50 values in terms of the nominal test concentrations only.

CONCLUSION	The notified chemical is at worst harmful to <i>Scenedesmus subspicatus</i> .
TEST FACILITY	SafePharm Laboratories Ltd (2005i)

C.2.4. *Lemna* growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	Draft OECD TG <i>Lemna</i> , Growth Inhibition Test (April 2004)
Species	<i>Lemna minor</i>
Exposure Period	7 days
Concentration Range	Nominal: 100 mg/L Actual: 88.8-101% of nominal
Auxiliary Solvent	Nil
Analytical Monitoring	HPLC
Remarks - Method	Following a preliminary range-finding test, <i>Lemna minor</i> was exposed to an aqueous solution of the test material at a concentration of 100 mg/L for a period of 7 days, under constant illumination at a temperature of 24±2°C. The test solutions were renewed on days 3 and 5. The number of fronds in each control and treatment group was recorded on days 0, 3, 5, and 7, along with observations on plant development. An amount of test material (227 mg) was dissolved in culture medium and the volume adjusted to 2 litre to give a 100 mg/L test solution. This method of preparation was repeated in order to provide the required test concentrations for the media renewal on days 3 and 5. Statistical analysis of the yield data was carried out for the control and all test concentrations using one way analysis of variance incorporating Bartlett's test for homogeneity of variance and Dunnett's multiple comparison procedure for comparing several treatments with a control
RESULTS	
Remarks - Results	Analysis of the freshly prepared test concentrations on Day 0 and the old or expired test concentrations on Days 3, 5 and 7 showed measured test concentrations to be near nominal and hence the results are based on nominal test concentrations only.
CONCLUSION	The notified chemical was not found to be harmful to <i>Lemna minor</i> .
TEST FACILITY	SafePharm Laboratories Ltd (2005j)

C.2.5. 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: 10, 32, 100, 320, 1000 and 3200 mg/L
Remarks – Method	Following preliminary range-finding tests, activated sewage sludge was exposed to an aqueous solution of the test material at a range of concentrations for a period of 3 h at 21°C with the addition of a synthetic sewage as a respiratory substrate. The rate of respiration was determined

after 30 minutes and 3 hours contact time and compared to data for the control and a reference material, 3,5-dichlorophenol.

An amount of test material (7273 mg) was dissolved in water and the volume adjusted to 1000 mL to give a 6400 mg/L stock solution from which serial dilutions were made. Analysis of the concentration, homogeneity and stability of the test material in the test preparations was not required by the Test Guidelines. For the purpose of the test a reference material, 3,5-dichlorophenol was used.

RESULTS

IC50

>3200 mg/L

NOEC

32 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)

BIBLIOGRAPHY

- Bartsch H (1981) Metabolic activation of aromatic amines and azo dyes. *IARC Sci Publ.* (40):13-30.
- Brown MA and DeVito SC (1993). Predicting azo dye toxicity, *Critical Reviews in Environmental Science and Technology*, 23(3): 249-324.
- 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.
- 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.
- Notox B.V (2006), Assessment of Contact Hypersensitivity to FSM-002M in the Mouse (Local Lymph Node Assay), Notox Project 456108.
- Øllgaard H, Frost L, Galster J and Hansen OC (1998). Survey of azo-colorants in Denmark: Consumption, use, health and environmental aspects. Danish Technological Institute, Environment, Danish Environmental Protection Agency.
- Prival MJ and Mitchell VD (1982) Analysis of a method for testing azo dyes for mutagenic activity in *Salmonella typhimurium* in the presence of flavin mononucleotide and hamster liver S9. *Mutat Res.* 97(2): 103-16.
- SafePharm Laboratories Limited (2005a) [Notified chemical]: Determination of General Physico-Chemical Properties, SPL project number: 2125/001. SafePharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, UK (unpublished report submitted by notifier).
- SafePharm Laboratories Limited (2005b) [Notified chemical]: Acute Oral Toxicity in the Rat – Acute Toxic Class Method, SPL project number: 2125/003. SafePharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, UK (unpublished report submitted by notifier).
- SafePharm Laboratories Limited (2005c) [Notified chemical]: Acute Dermal Irritation in the Rabbit, SPL project number: 2125/004. SafePharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, UK (unpublished report submitted by notifier).
- SafePharm Laboratories Limited (2005d) [Notified chemical]: Acute Eye Irritation in the Rabbit, SPL project number: 2125/005. SafePharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, UK (unpublished report submitted by notifier).
- SafePharm Laboratories Limited (2005e) [Notified chemical]: Reverse Mutation Assay “Ames test” using *Salmonella Typhimurium* and *Escherichia Coli*, SPL project number: 2125/0009. SafePharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, UK (unpublished report submitted by notifier).
- SafePharm Laboratories Limited (2005f) [Notified chemical]: Assessment of Ready Biodegradability; Closed Bottle Test, SPL project number: 2125/013. SafePharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, UK (unpublished report submitted by notifier).
- SafePharm Laboratories Limited (2005g) [Notified chemical]: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*), SPL project number: 2125/010. SafePharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, UK (unpublished report submitted by notifier).
- SafePharm Laboratories Limited (2005h) [Notified chemical]: Acute Toxicity to *Daphnia Magna*, SPL project number: 2125/011. SafePharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, UK (unpublished report submitted by notifier).
- SafePharm Laboratories Limited (2005i) [Notified chemical]: Inhibition of Algal Growth Caused by Coloured Test Substances, SPL project number: 2125/060. SafePharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, UK (unpublished report submitted by notifier).

- SafePharm Laboratories Limited (2005j) [Notified chemical]: *Lemna* Growth Inhibition Test, SPL project number: 2125/059. Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, UK (unpublished report submitted by notifier).
- SafePharm Laboratories Limited (2005k) [Notified chemical]: Assessment of the Inhibitory Effect on the Respiration of Activated Sewage Sludge, SPL project number: 2125/014. Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, UK (unpublished report submitted by notifier).
- SafePharm Laboratories Limited (2006a) [Notified chemical]: Determination of Hazardous Physico-Chemical Properties, SPL project number: 2125/0002 (r). Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, UK (unpublished report submitted by notifier).
- SafePharm Laboratories Limited (2006b) [Notified chemical]: Acute Dermal Toxicity (Limit Test) in the Rat, SPL project number: 2125/0061. Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, UK (unpublished report submitted by notifier).
- SafePharm Laboratories Limited (2006c) [Notified chemical]: Twenty-eight Day Repeated Dose Oral (Gavage) Toxicity Study in the Rat, SPL project number: 2125/0007. Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, UK (unpublished report submitted by notifier).
- SafePharm Laboratories Limited (2006d) [Notified chemical]: Chromosome Aberration Test in CHL cells *in vitro*, SPL project number: 2125/008. Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, UK (unpublished report submitted by notifier).
- SafePharm Laboratories Limited (2007) [Notified chemical]: Reverse Mutation Assay “Ames test” using *Salmonella Typhimurium* Prival and Mitchell Modification for Azo Compounds, SPL project number: 2125/0069. Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, UK (unpublished report submitted by notifier).
- SCCNFP (2002) The Safety Review Of The Use Of Certain Azo-Dyes In Cosmetic Products: Opinion Of The Scientific Committee On Cosmetic Products And Non-Food Products Intended For Consumers.. SCCNFP/0495/01 (prepared in the context of Directive 76/768/EEC).
- 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.