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May 2008

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

CIM-04

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:

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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; CAS number; Molecular formula; Structural formula; Molecular weight; Spectral data; Purity; % Weight of non-hazardous ingredients; Import volume; % Weight of additives/adjuvants

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
UK (2006)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
CIM-04

ANALYTICAL DATA
Reference NMR, IR, HPLC, UV/vis, and mass spectra were provided.

3. COMPOSITION

DEGREE OF PURITY > 80%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS None

ADDITIVES/ADJUVANTS None

4. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa Dark yellow powder

Property	Value	Data Source/Justification
Melting Point/Freezing Point	Could not be determined	Measured
Boiling Point	Could not be determined	Measured
Density	1560 kg/m^3 at 20.4 ± 0.5°C	Measured
Vapour Pressure	2.3 x 10^{-8} kPa at 25°C	Measured
Water Solubility	309-328 g/L at 20°C	Measured

Hydrolysis as a Function of pH	pH 4 ~1 yr at 25°C pH 7 > 1 yr at 25°C pH 9 > 1 yr at 25°C	Measured
Partition Coefficient (n-octanol/water)	log P_{OW} < -2.79 at 20ºC	Measured
Surface Tension	71.5 mN/m at 21.4 ± 0.4ºC	Measured
Adsorption/Desorption	log K_{OC} < 1.25 at 40°C	Measured
Dissociation Constant	pKa = -1.98±0.50 – 4.49±0.10	Modelled
Particle Size	Inhalable fraction (< 100 μm): 97% Respirable fraction (< 10 μm): ~9.9%	Measured
Flash Point	Not determined	Not applicable, notified chemical is a solid
Flammability Limits	Not highly flammable	Measured
Autoignition Temperature	296ºC	Measured
Explosive Properties	Not explosive	Measured
Oxidising Properties	Not oxidising	Predicted

Discussion of Observed Effects

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

Reactivity

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

Dangerous Goods classification

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

5. INTRODUCTION AND USE INFORMATION

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

The notified chemical will be imported only as a component of ink 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	< 0.1	< 0.5	< 0.5	< 1.0	< 1.0

PORT OF ENTRY

Sydney Airport and Sydney Harbour

IDENTITY OF MANUFACTURER/RECIPIENTS

The ink cartridges will be stored at the notifier's warehouse prior to distribution to offices nationwide and office equipment retailers.

TRANSPORTATION AND PACKAGING

The notified chemical will be imported as a component of ready-to-use sealed inkjet cartridges of 16-150 mL volumes. The cartridges will be transported by road in Australia.

USE

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

OPERATION DESCRIPTION

No processing such as reformation, repackaging, filling or refilling of the cartridges containing the notified chemical, or any other handling of the notified chemical is carried out in Australia. Sealed ink cartridges containing the notified chemical will be handled by service technicians, office workers or the public, who will replace spent cartridges in the printers as necessary. Office workers and public will use printers for varied printing work.

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	50	< 8 hours/day	10-50 days/year
Storage and Transport	15	< 8 hours/day	10-50 days/year
Office worker/ consumer	2,000,000	< 0.1 hrs /day	2 days/year
Service Technicians	100	1 hours/day	170days/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 ink 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.

Dermal exposure of workers may also occur when handling printed media before the ink is adequately dried, especially when printing on non-absorbent materials. One kilogram of pure dye would be expected to print several million A4 paper sheets of coloured text or graphics. Under worst-case conditions, each piece of A4 paper could be assumed to incorporate 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 cm^2
A4 sized paper = ~600 cm^2
% Removal = (8/600) × 0.5 × 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 (mg/event)×10) ÷ 70 = ~0.0014 mg/kg bw/day.

It is not possible to estimate the level of exposure that is expected for the smaller arylamine species that may be derived from the notified chemical.

After printed inks are dry, the notified chemical will be bound to the paper or other media, and is not expected to be readily available.

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	oral LD50 > 2000 mg/kg bw, low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw, low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of skin sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOEL 400 mg/kg bw/day

Genotoxicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosome aberration test	non clastogenic

Toxicokinetics, metabolism and distribution:
Many azo dyes are unlikely to be absorbed through the skin because of their size and polarity (Øllgaard *et al* 1998). Smaller species may undergo percutaneous absorption, dependent on their properties. There was no evidence from the toxicological investigations to suggest that the notified chemical was absorbed following dermal exposure. However, bacterial skin microflora have been speculated to be able to break down azo dyes into smaller species through azo reduction, which may be more readily absorbed (SCCNFP, 2002).

Ultimately, the metabolites of azo dyes are excreted in the urine and bile (Brown & DeVito, 1993, referenced in Øllgaard *et al*, 1998).

General 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 400 mg/kg bw/day on the basis of the treatment related changes observed in the stomach at the higher dose level of 750 mg/kg bw/day.

In addition, the notified chemical was found to be a slight irritant, when administered in high concentrations to the skin or eye.

The notified chemical was not a skin sensitiser, as shown 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:
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 used contains low levels (0.2%) of one impurity. The identity of the contaminant is unknown, but it may be an aromatic amine.

The notifier supplied test results showing that the notified chemical was found to be not mutagenic in bacteria (using both the standard and the Prival-Mitchell (Prival MJ and Mitchell VD 1982) modified test), and it did not induce chromosomal aberrations in mammalian cells *in vitro*. However, while these results are suggestive, they do not rule out the notified chemical as a possible carcinogen. In general, the degree of correlation between mutagenicity study results and the carcinogenicity of azo dyes as a class is poor, likely due to their complex metabolism *in vivo* (Brown and DeVito, 1993, referenced in Øllgaard *et al* 1998).

Based on the currently available data, the notified chemical cannot be classified as a hazardous substance in accordance with 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 < 10%, 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 400 mg/kg bw/day), although it may cause slight skin and eye irritation.

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

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

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 open 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. Installation and replacement will be contained with absorbent and disposed of in landfill.

Cartridges are contained within the printer until the contents are used. They are then removed and sent to a recycling and disposal centre. The cartridges will then be broken down into component parts for recycling. Residual ink (< 2% of the notified chemical) left in empty cartridges will be separated from the cartridge and incinerated during the recycling of the cartridges.

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 chemical absorbed to sludge during recycling process will be disposed of to landfill.

RELEASE OF CHEMICAL FROM DISPOSAL

The total import volume of the notified chemical will ultimately be disposed 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. Some will enter the paper recycling process. Used cartridges may be sent to recycling and disposal centres. In this scenario, the cartridges will be broken down into component parts for recycling. Residual ink (< 2% of the notified chemical) left in the empty cartridges would 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 and nitrogen based compounds and various salts. Similarly, the notified chemical that is disposed of to landfill should eventually degrade.

7.1.2 Environmental fate

A single Ready Biodegradability test was conducted on the notified chemical, which attained 12% degradation after 28 days, and therefore, cannot be considered as readily biodegradable under the strict terms and conditions of OECD Guideline No 301D. For the details of the environmental fate study please refer to Appendix C.

7.1.3 Predicted Environmental Concentration (PEC)

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

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

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1,000	kg/year
Proportion expected to be released to sewer	50%	
Annual quantity of chemical released to sewer	500	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	1.37	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	20.496	million
Removal within STP	0%	
Daily effluent production:	4,099	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.33	µg/L
PEC - Ocean:	0.03	µg/L

7.2. Environmental effects assessment

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

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

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

7.2.1 Predicted No-Effect Concentration

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

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

7.3. Environmental risk assessment

Based on the above PEC and PNEC values, the following Risk Quotients have been calculated.

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River:	0.33	> 1	< 0.33
Q - Ocean:	0.03	> 1	< 0.03

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

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

8.1. Hazard classification

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

8.2. Environmental risk assessment

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

8.3. Human health risk assessment

8.3.1. Occupational health and safety

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

8.3.2. Public health

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

9. MATERIAL SAFETY DATA SHEET

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

10. RECOMMENDATIONS

CONTROL MEASURES
Occupational Health and Safety

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

- 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 NOHSC *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

- Products 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 incineration or to landfill.

Emergency procedures

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

11. REGULATORY OBLIGATIONS

This risk assessment is based on the information available at the time of notification. 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 regardless of whether the notified chemical has been listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director 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 importation volume exceeds one tonne per annum notified chemical; or
  - 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 (< 7%) in inkjet

printer inks, has changed, or is likely to change significantly;
- the amount of chemical being introduced has increased, 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.

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Appendix A: Physico-Chemical Properties

Melting Point/Freezing Point Could not be determined

METHOD EC Directive 92/69/EEC A.1 Melting/Freezing Temperature
Differential scanning calorimetry
Remarks The notified chemical decomposed at 359.65 ºC at 102.09 kPa. As such, the melting point could not be determined.
TEST FACILITY SafePharm Laboratories Ltd (2005a)

Boiling Point Could not be determined

METHOD EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks No value for boiling temperature was determined as the notified chemical decomposed prior to melting,
TEST FACILITY SafePharm Laboratories Ltd (2005a)

Density 1560 kg/m^3 at 20.4 ± 0.5ºC

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

Vapour Pressure < 2.3 x 10^{-8} kPa at 25ºC

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 243°C.
TEST FACILITY SafePharm Laboratories Ltd (2005b)

Water Solubility 309-328 g/L at 20ºC

METHOD EC Directive 92/69/EEC A.6 Water Solubility.
Remarks Flask Method. Various amounts of the test substance were added to flasks, followed by double-distilled water. After shaking for 72 h at 30°C, the samples were equilibrated at 20.0 ±0.5°C for approximately 24 h. High indeterminable saturation levels were produced, 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.

pH	*T (°C)*	*$t_{½}$ <hours or days>*
4	25	~1 yr
7	25	> 1 yr
9	40	> 1 yr

Remarks Aliquots of the sample solutions were taken from the flasks at various times and the pH of each sample was recorded. The concentration of the sample solution was determined by HPLC. Although there was an apparent decrease in concentration at pH 4 over the course of the test, this was considered relatively insignificant and probably not hydrolysis. Only approximately 13% of initial concentration was lost over 120 hours at 50°C and this was not indicative of a pseudo-first order reaction. Therefore, it was considered appropriate to conclude that the half-life at pH 4 at 50°C was equivalent to approximately 1 year at 25°C.
TEST FACILITY SafePharm Laboratories Ltd (2005a)

Partition Coefficient (n-octanol/water) log P_{OW} < -2.79 at 20ºC

METHOD EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks Shake Flask Method. A stock solution was prepared by diluting test material (0.5343 g) to 500 mL with n-octanol saturated water. This was adjusted to pH 7 using 0.1 M hydrochloric acid. Six partitions were performed. In each test, the combined volume of both phases occupied not less than 90% of the total volume of the test vessel. Analysis of concentrations was determined by HPLC.
TEST FACILITY SafePharm Laboratories Ltd (2005a)

Surface Tension 71.5 mN/m at 21.4 ± 0.4ºC

METHOD EC Directive 92/69/EEC A.5 Surface Tension.
Remarks The determination was carried out using a White Electrical Institute interfacial tension balance and a procedure based on the ISO 304 ring method. An aliquot (0.1145 g) of test material was diluted to 100 mL with glass double-distilled water. After shaking by hand for 1 minute, the sample solution was transferred to the measuring vessel. The surface tension result was not corrected using the Harkins-Jordan correction table, as the correction is not applicable to the apparatus used. Once calibrated, the balance and ring assembly used in this test give a direct reading for surface tension that is within the required accuracy (±0.5 mN/m); this is as a result of the reduced ring dimensions.

The test material is considered not to be a surface-active material.
TEST FACILITY SafePharm Laboratories Ltd (2005a)

Adsorption/Desorption log K_{OC} < 1.25 at 40°C

METHOD EC Directive 2001/59/EC Method C19 – HPLC Screening Method
Remarks 0.1016 g of test material was diluted to 100 mL with methanol with subsequent HPLC analysis compared against 12 reference substances. The test material eluted prior to the first reference substance, Acetanilide.
TEST FACILITY SafePharm Laboratories Ltd (2005a)

Dissociation Constant pKa = -1.98±0.50 – 4.49±0.10

METHOD Estimation using ACS/I-Lab Web Service (ACD/pKa 8.03) Software.
Remarks Testing was not carried out since the test material had numerous overlapping dissociation constants. Also, some dissociation constants were outside the range of the prescribed test methods.
TEST FACILITY SafePharm Laboratories Ltd (2005a)

Particle Size

METHOD OECD TG 110 Particle Size Distribution/Fibre Length and Diameter Distributions. Sieve and cascade impactor method

Range (μm)	*Mass (%)*
Proportion of test material having an inhalable particle size less than 100 μm	99.7
Proportion of test material having a thoracic particle size less than 10.2 μm	9.9
Proportion of test material having a respirable particle size less than 5.4 μm	2.6

Remarks Too few particles were of a size < 10.2μm to allow accurate assessment of mass median aerodynamic diameter.
TEST FACILITY SafePharm Laboratories Ltd (2005a)

Flash Point Not determined (< 296ºC)

METHOD EC Directive 92/69/EEC A.9 Flash Point.
Remarks Not applicable as notified chemical is a solid.

Flammability Limits 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 (2005b)

Autoignition Temperature 296ºC

METHOD 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
TEST FACILITY SafePharm Laboratories Ltd (2005b)

Explosive Properties The notified chemical does not have explosive properties

METHOD EC Directive 92/69/EEC A.14 Explosive Properties.
TEST FACILITY SafePharm Laboratories Ltd (2005b)

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 (2005b)

Appendix B: Toxicological Investigations

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical (87.4%)
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method. EC Directive 2004/73/EC B.1tris Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Sprague Dawley CD (Crl: CD (SD) IGS BR)
Vehicle	Distilled Water
Remarks - Method	No significant protocol deviations. Corrections were made for the purity of the test material.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	3F	2000	0
II	3F	2000	0

LD50	> 2000 mg/kg bw
Signs of Toxicity	Hunched posture was noted in five animals during the day of dosing, and in two animals one day after dosing, with lethargy noted in one animal two and four hours after dosing. One animal appeared normal throughout the study and the remaining animals appeared normal one or two days after dosing.
Effects in Organs	No abnormalities were noted at necropsy. All animals showed expected bodyweight gains during the first week, but this was significantly reduced in 5 out of 6 animals during the second week.
Remarks - Results	None
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	SafePharm Laboratories Ltd (2006a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity (Limit test). EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/Sprague-Dawley CD strain
Vehicle	Dried arachis oil BP
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations. The test material was moistened with dried arachis oil BP prior to application.

RESULTS

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

LD50	> 2000 mg/kg bw

Signs of Toxicity - Local	Orange coloured staining was noted at all treatment sites one day after treatment, which did not affect the evaluation of skin reactions. There were no signs of dermal irritation.
Signs of Toxicity - Systemic	There were no deaths or test substance related clinical signs.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks - Results	None

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

TEST FACILITY SafePharm Laboratories Ltd (2006b)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical (87.4%)

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

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 (2 males, 1 female)
Vehicle	Distilled water
Observation Period	72 hr
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations. Prior to application, 0.5g of test material was moistened with 0.5mL of distilled water. Test material was removed from the application site following exposure using cotton wool soaked in 74% industrial methylated spirits.

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	0.3	0.3	0.3	1	47 hr	0
Oedema	0	0	0	0	0 hr	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 treatment sites throughout the study, which did not affect the observation of skin reactions. Very slight erythema was observed at all treated skin sites at the 24 hour observation. No evidence of skin irritation was noted from the 48 hour observation onward.

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY SafePharm Laboratories Ltd (2006c)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical

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

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 males
Observation Period	72 hours
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.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0	0.3	0.3	1	48 h	0
Conjunctiva: chemosis	0	0	0	1	24 h	0
Conjunctiva: discharge	0	0	0	1	24 h	0
Corneal opacity	0	0	0	0	0 h	0
Iridial inflammation	0	0	0	1	24 h	0

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

Remarks - Results: Yellow-coloured staining of fur was noted around all treated eyes throughout the study. Iridial inflammation, slight chemosis and slight discharge were noted in two treated eyes one hour after treatment. Minimal conjunctival redness was noted in all treated eyes one hour after treatment and in two treated eyes at the 24 hour observation.

CONCLUSION: The notified chemical is slightly irritating to the eye.

TEST FACILITY: SafePharm Laboratories Ltd (2006d)

B.5. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE: Notified chemical (87.4%)

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/Ca (CBA/CaBkl) female

Vehicle: Propylene glycol

Remarks - Method: A preliminary screening study on a single mouse, treated with 10% w/w notified chemical in propylene glycol, was performed to determine its toxicity/irritancy potential. This mouse was treated on the dorsal surface of each ear daily for three days.
In the main test, the doses used were 2.5, 5, or 10% w/w notified chemical in propylene glycol. Following sacrifice of the mice, their lymph cells were extracted and pooled together for each experimental group.

A laboratory historical positive control was used (recent relative to the study date).

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	443.13	N/A
2.5	684.94	1.55
5	649.71	1.47
10	585.22	1.32
α-Hexylcinnamaldehyde (positive control)		
5	Unknown	2.64
10	Unknown	8.36

25	Unknown	12.94

Remarks - Results	In the preliminary study, no deaths or signs of systemic toxicity were observed in the animal during the study. Yellow staining of the fur and ears was noted one hour post-dosing on all three days. 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.
CONCLUSION	There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical under the study conditions.
TEST FACILITY	SafePharm Laboratories Ltd (2006e)

B.6. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical (87.4%)
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	Dried Arachis oil BP
Remarks - Method	No significant protocol deviations. A 14-day repeated dose range finding study was performed at 500 and 1000 mg/kg bw/day. The test method was similar to the main study.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
I (control)	5M, 5F	0	0
II (low dose)	5M, 5F	25	0
III (mid dose 1)	5M, 5F	150	0
IV (mid dose 2)	5M, 5F	400	0
V (high dose)	5M, 5F	750	0
VI (control recovery)	5M, 5F	0	0
VII (high dose recovery)	5M, 5F	750	0

Preliminary 14-Day Range Finding Test

During the preliminary 14-day range finder test, animals of either sex treated with 1000 and 500 mg/kg/day showed isolated incidences of increased salivation and fur staining by the test material. One male treated with 1000 mg/kg/day showed staining around the mouth on one occasion.

At necropsy, following the preliminary test, all animals treated with 1000 mg/kg/day showed a raised limiting ridge of the stomach. One male treated with 1000 mg/kg/day showed yellow coloured contents in the stomach. In addition, one female at this dose level showed one enlarged kidney and one small kidney. This was considered likely to be a congenital abnormality unrelated to treatment. With dose levels of 500 mg/kg/day, two males and one female showed yellow coloured contents in the stomach. No other treatment related effects were found.

Mortality and Time to Death

No mortality was observed during the treatment or recovery phases.

Clinical Observations

There were no significant clinical observations in the animals, except for yellow/orange fur or mouth staining in some treatment groups.

There were some changes in body weight gain, however, these were considered to be incidental.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Blood chemistry

Decreased levels of glucose and albumin were observed in males treated with 750 mg/kg/day. Decreased levels of albumin were also noted for males treated with 400 mg/kg/day. An increase in albumin was noted in females treated with 750 mg/kg/day. These observations were considered not to be of toxicological importance, as the values were within normal historical ranges.

Increased levels of alanine aminotransferase were observed in females at all treatment doses. This was considered not to be of toxicological significance because individual values were within normal historical ranges for rats of the age and strain, the change was not dose related, and there was no corroborative evidence of impaired liver function.

Females treated with 150 mg/kg/day showed an increase in plasma calcium levels. This was not considered to be of toxicological importance given that no dose related response was observed, and that all other electrolyte levels were comparable to controls across all dose groups.

Haematology

An increase (not dose related) in the number of white blood cells, namely for lymphocytes, was observed in females treated with 750, 400, 150 mg/kg/day. Decreased numbers of white blood cells and lymphocytes were observed for males treated with 750 mg/kg/day following a 14 day treatment-free period. These values were within normal historical ranges and were considered to be of uncertain toxicological importance.

An increase in hematocrit and haemoglobin, and a decrease in the number of platelets were observed in males treated with 750 mg/kg/day following a 14 day treatment-free period. In the absence of similar findings in non-recovery 750 mg/kg/day males at the end of the treatment period, the findings were considered to be of no toxicological importance.

Urinalysis

All differences in pH and the presence of ketones, glucose, haemoglobin, and reducing substances were considered to be a result of normal variation for rats of the age and strain used.

Effects in Organs

Organ weights

Females at all treatment levels showed an increase in uterus weights, both absolute and relative to terminal bodyweight. This was considered to be of no toxicological significance, given that it was not a dose related effect, and that there was no correlation with histopathological findings.

Necropsy

One male treated with 750 mg/kg/day showed yellow discolouration of the testes, epididymides and skin (hind limb). Yellow contents of the stomach were also observed. These observations are not considered to be indicative of toxicity, rather it was considered to be the result of excretion of the coloured notified chemical or its metabolites.

Histopathology

Stomach: Acanthosis and/or hyperkeratosis of the limiting ridge were observed in rats of either sex treated with 750 mg/kg/day. Agglomeration of secretion, mucosal basophlia and mucous cell hyperplasia were also seen in response to treatment at this dose level. All conditions were observed to have regressed among the recovery 750 mg/kg/day animals following 14 days without treatment. These treatment related changes are likely to be the result of direct contact with the notified chemical. No associated erosion or ulceration was observed. There were also isolated incidences of minimal agglomeration of secretion observed at the 400 mg/kg/day level, though these were considered to be of no toxicological significance.

Bone marrow – Femur: Adipose infiltration of the marrow was observed in males treated with 750 mg/kg/day and the corresponding recovery dose group.

For females there was a slight elevation in the incidence of higher grades of adipose infiltration for all treatment groups but no evidence of a dose response.

Bone marrow – Sternum: There was evidence in several treatment groups of adipose infiltration indicative of mild marrow hyperplasia. The toxicological significance of these observations is doubtful given the absence of a uniform dose response, and the fact that adipose infiltration of the bone marrow is a variable condition even among control animals.

A number of effects were observed in the heart, liver, spleen, kidney, thyroid, lung and uterus 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 stomach in animals treated with 750 mg/kg/day of the notified chemical. These changes were considered to be the result of direct contact with the notified chemical. No associated erosion or ulceration was observed and the changes regressed following cessation of the treatment.

CONCLUSION
The No Observed Effect Level (NOEL) was established as 400 mg/kg bw/day in this study, based on microscopic changes in the stomach of the animals identified as acanthosis and/or hyperkeratosis of the limiting ridge, and to a lesser extent agglomeration of secretion, mucosal basophilia and mucous cell hyperplasia.

TEST FACILITY SafePharm Laboratories Ltd (2006f)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

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

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA$^-$
Metabolic Activation System	S9 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	Acetone
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*	*Mutageni Effect*
Absent				
Test 1	> 5000 µg/plate	> 5000 µg/plate	> 5000 µg/plate	Negative
Test 2	-	> 5000 µg/plate	> 5000 µg/plate	Negative
Present				
Test 1	> 5000 µg/plate	> 5000 µg/plate	> 5000 µg/plate	Negative
Test 2	-	> 5000 µg/plate	> 5000 µg/plate	Negative

Remarks - Results	A yellow 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.
TEST FACILITY	SafePharm Laboratories Ltd (2005c)

B.8. 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. Pre incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA102, TA98, TA100
Metabolic Activation System	30% uninduced hamster liver S9, in modified co-factors (c.f. 10% in standard)
Concentration Range in Main Test	With metabolic activation: 50-5,000 µg/plate
Vehicle	Acetone
Remarks - Method	The method incorporated the Prival and Mitchell modification for azo dyes (Prival MJ and Mitchell VD 1982).

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Mutageni Effect*
Present				
Test 1	> 5,000	> 5,000	≥ 5000	None
Test 2	> 5,000	> 5,000	≥ 5000	None

Remarks - Results

The vehicle control plates gave counts of revertant colonies within the normal range. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies. 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 green colour and particulate precipitate was noted from 5000 µg/plate; these observations 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.

The Prival-Mitchell modification positive control, Congo Red, used in the

test induced marked increases in the frequency of TA98 and TA100 revertant colonies, with metabolic activation only. Thus, the sensitivity of the assay and the efficacy of the uninduced hamster liver S9-mix was validated.

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

TEST FACILITY SafePharm Laboratories Ltd (2007)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical (87.4%)

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Cell Type/Cell Line 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 No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 312.5, 625, 1250*, 2500*, 3750*, 5000*	6 hr	24 hr
Test 2	0*, 10, 20, 40, 80*, 120*, 160*	24 hr	24 hr
Present			
Test 1 (S9 at 5% final conc)	0*, 312.5, 625*, 1250*, 2500*, 3750*, 5000	6 hr	24 hr
Test 2 (S9 at 2% final conc)	0*, 625, 1250, 2500*, 3125*, 3750*, 5000*	6 hr	24 hr

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	~5000	~5000	> 5000	Negative
Test 2	156.25	> 160	> 5000	Negative
Present				
Test 1	2500	3750	> 5000	Negative
Test 2	-	3125	> 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.

The notified chemical did not induce any statistically significant increases in the frequency of cells with aberrations in Test 1 with the presence and absence of metabolic activation. In Test 2 in the presence and absence of metabolic activation, the notified chemical did not induce statistically significant increases in the frequency of cells with aberrations. However, it should be noted that marked increases (5%) in the number of gaps were seen at 80 and 120 μg/mL in Test 2 (absence of metabolic activation). This response was considered to have no toxicological significance, as it is likely to have been the result of the poor quality of the slides, which may have resulted in artefactual gap-type aberrations. The notified

	chemical did not induce a statistically significant increase in the numbers of polyploid cells at any dose level in any of the exposure groups.
CONCLUSION	The notified chemical was not clastogenic to CHL cells treated in vitro under the conditions of the test.
TEST FACILITY	SafePharm Laboratories Ltd (2006g)

Appendix C: Environmental Fate and Ecotoxicological Investigations

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 (30 mL) of this stock solution was dispersed in a final volume of 6 L of inoculated culture medium to give a test concentration of 5.0 mg/L. For the purposes of the test, a standard material, sodium benzoate, was used.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
3	7	3	59
7	13	7	73
14	13	14	80
21	12	21	81
28	12	28	82

Remarks - Results	Variation in degradation rates on different sampling days was considered to be due to variation in respiration rates between control and test vessels. The toxicity control attained 26% degradation after 14 days and 27% 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 80% degradation after 14 days and 82% 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 (2006h)

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*
Exposure Period	96 h
Auxiliary Solvent	Nil
Water Hardness	40 mg $CaCO_3$/L
Analytical Monitoring	HPLC analysis of test concentrations.
Remarks – Method	Preliminary solubility work carried out showed that the test material

solubility increased with a decrease in the hardness of the test medium. Therefore, as the test material was shown to be soluble over 24 h at a hardness of 40 mg/L as $CaCO_3$, the range-finding and definitive tests were conducted using dechlorinated tap water with a water hardness of 40 mg/L as $CaCO_3$.

Based on the results of the range-finding test, a "limit test" was conducted at a concentration of 100 mg/L to confirm that at the maximum concentration given in the OECD/EEC Test Guidelines, no mortalities or sub-lethal effects of exposure were observed.

An amount of test material (2288 mg) was dissolved in dechlorinated tap water and the volume adjusted to 1 L to give a 2000 mg/L stock solution. The entire volume was further diluted in a final volume of 20 L and stirred using a flat bladed mixer for approximately 1 minute to give the 100 mg/L test concentration.

RESULTS

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

LC50	> 100 mg/L at 96 hours.
NOEC (or LOEC)	100 mg/L at 96 hours.
Remarks – Results	The control was observed to be a clear, colourless solution throughout the duration of the test. The 100 mg/L test preparation was observed to be a clear, yellow solution at 0 hours and after each media renewal. The old test media at 24 h was observed to be a clear, yellow solution, however, the old test media at 48, 72 and 96 h was observed to be a very slightly cloudy, yellow solution. This was considered to be due to a small amount of the test material precipitating out of solution in the test diluent. However, this precipitate was only very slight and was homogenously dispersed throughout the diluent as indicated by the results of chemical analysis, which showed all measured test concentrations to be near nominal values. Therefore, give that no mortalities or sub-lethal effects of exposure were observed at 100 mg/L due to a physical effect of the precipitate, it was considered not to have affected the outcome or validity of the test. Analysis of the test preparation at 0, 24 and 96 h showed measured test concentrations to be near nominal and so it was considered justifiable to estimate the LC50 values in terms of the nominal test concentrations only.
CONCLUSION	The notified chemical is not harmful to *Oncorhynchus mykiss*.
TEST FACILITY	SafePharm Laboratories Ltd (2005d)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - semi-static. EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia – semi-static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Nil

Water Hardness	40 mg $CaCO_3$/L
Analytical Monitoring	HPLC analysis of test concentrations.
Remarks - Method	Preliminary solubility work carried out showed that the test material solubility increased with a decrease in the hardness of the test medium. Therefore, as the test material was shown to be soluble over 24 h at a hardness of 40 mg/L as $CaCO_3$, the range-finding and definitive tests were conducted using dechlorinated tap water with a water hardness of 40 mg/L as $CaCO_3$. Dechlorinated tap water was used as opposed to reconstituted water as it was not possible to amend the hardness of reconstituted water to 40 mg/L without significantly altering the medium in a way that would likely kill the test organisms. Based on the results of the range-finding test, a "limit test" was conducted at a concentration of 100 mg/L to confirm that at the maximum concentration given in the OECD/EEC Test Guidelines, no immobilisation or adverse reactions were observed. An amount of test material (229 mg) was dissolved in dechlorinated tap water and the volume adjusted to 2 L to give the 100 mg/L test concentration.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h [acute]* *14 d [chronic]*	*48 h [acute]* *21 d [chronic]*
100		20	0	0

LC50	> 100 mg/L at 24 hours > 100 mg/L at 48 hours
NOEC	100 mg/L at 48 hours
Remarks - Results	No immobilisation was observed at the test concentration of 100 mg/L. It was considered unnecessary and unrealistic to test at concentrations in excess of 100 mg/L. The control test media was observed to be a clear colourless solution and the 100 mg/L test media was observed to be a yellow coloured solution throughout the duration of the test. Analysis of the test preparation at 0, 24 and 48 h showed measured test concentrations to be near nominal and so it was considered justifiable to estimate the LC50 values in terms of the nominal test concentrations only.

CONCLUSION The notified chemical is not harmful to *Daphnia magna*.

TEST FACILITY SafePharm Laboratories Ltd (2005e)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

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

Species	*Scenedesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 100 mg/L Actual: 103-108% of Nominal
Auxiliary Solvent	Nil
Analytical Monitoring	HPLC
Remarks - Method	Based on the result of the range-finding test a "limit-test" was conducted

at a concentration of 100 mg/L to confirm that at the maximum concentration given in the OECD/EEC Test Guidelines no effect on algal growth was observed.

An amount of test material (114 mg) was dissolved in culture medium and the volume adjusted to 500 mL to give a 200 mg/L stock solution. An aliquot (250 mL) of this stock solution was mixed with algal suspension (250 mL) to give the required test concentration of 100 mg/L.

Pre-culture gave an algal suspension in log phase growth characterised by a cell density of 2.81 x 10^6 cells per mL. This suspension was diluted to a cell density of 2.20 x 10^4 cells per mL prior to use.

A Student's t-test incorporating Bartlett's test for homogeneity of variance was carried out on the area under the growth curve data at 72 h for the control and the 100 mg/L test concentration to determine any statistically significant differences between the test and control groups.

RESULTS

Biomass		*Growth*	
E_bC50 mg/L at 72 h	*NOEC mg/L*	*E_rC50 mg/L at 72 h*	*NOEC mg/L*
> 100	100	> 100	100

Remarks - Results

Analysis of the test preparation at 0 and 72 hours showed measured test concentrations to range from 103% to 108% of nominal and so it was considered justifiable to estimate the EC50 values in terms of the nominal test concentrations only. It was considered unnecessary and unrealistic to test at concentrations in excess of 100 mg/L.

There were no statistically significant differences (P≥0.05) between the control and 100 mg/L test group and therefore, the NOEC was 100 mg/L.

The cell concentration of the control cultures increased by a factor of 26 after 72 hours, which was in line with the OECD Guideline that states the enhancement must be at least by a factor of 16 after 72 hours.

CONCLUSION The notified chemical is not harmful to *Scenedesmus subspicatus.*

TEST FACILITY SafePharm Laboratories Ltd (2005f)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 209 Activated Sludge, Respiration Inhibition Test.
EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test
US EPA Draft Ecological Effects Test Guidelines OPPTS 850.6500.

Inoculum Activated sewage sludge.
Exposure Period 3 hours
Concentration Range Nominal: 1000 mg/L
Remarks – Method Based on the results of a range-finding test, a "limit test" was conducted at a concentration of 1000 mg/L (three replicates) to confirm that at this concentration no effect on respiration of the activated sewage sludge was observed.

An amount of test material (1144 mg) was dissolved in water and the

	volume adjusted to 500 mL to give a 2000 mg/L stock solution. An aliquot (250 mL) of this stock solution was dispersed with synthetic sewage (16 mL), activated sewage sludge (200 mL) and water, to final volume of 500 mL, to give the require concentration of 1000 mg/L. Analysis of the concentration, homogeneity and stability of the test material in the test preparations was not appropriate to the Test Guidelines. For the purpose of the test a reference material, 3,5-dichlorophenol was used.
RESULTS	
IC50	> 1000 mg/L
NOEC	1000 mg/L
Remarks – Results	The test validation criteria were satisfied. Observations made throughout the test period showed that at the test concentration of 1000 mg/L no undissolved test material was visible.
CONCLUSION	The notified chemical is not harmful to activated sludge micro-organisms.
TEST FACILITY	SafePharm Laboratories Ltd (2005g)