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May 2008

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

HPEXPF3NM

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)
Hewlett-Packard Australia Pty Ltd (ABN 74 004 394 763)
3 Richardson Place
Riverside Corporate Park
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: Identity of chemical, composition, introduction and use information, identity of sites

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
USA, EU, South Korea

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
HPEXPF3NM

OTHER NAME(S)
Heterocycle, 4-methyl,-4-oxide, methanesulfonate salt (name used in product MSDS)
Mmo-ms, nmmo-msa, mmno-msa

MOLECULAR WEIGHT
< 500 Da

ANALYTICAL DATA
Reference NMR, IR, MS, HPLC, UV spectra were provided (Safepharm Laboratories, 2005a).

3. COMPOSITION

DEGREE OF PURITY
> 90 %

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: White powder

Property	Value	Data Source/Justification
Melting Point/Freezing Point	100°C	Measured
Boiling Point	>360°C at 101.3 kPa	Calculated
Density	1450 kg/m^3 at 19.5°C	Measured

Vapour Pressure	$<1.6\times10^{-3}$ kPa at 25ºC	Measured
Water Solubility	~750-900 g/L at 20±0.5ºC	Measured
Hydrolysis as a Function of pH	$t_{1/2}$ > than 1 year at environmental pH range of 4-9	Measured
Partition Coefficient (n-octanol/water)	log Pow = < -2.26 at 22±1ºC	Measured
Surface tension	72.6±0.5 mN/m at 21.2±0.5°C	Measured
Adsorption/Desorption	log K_{oc} = -0.15-3.20	Estimated
Dissociation Constant	pKa = 4.24	Measured
Particle Size	Inhalable fraction (<100 μm): 19.0% Thoracic & respirable fractions (<10 μm): 4.69% MMAD* = Undetermined.	Measured. Too few particles were <10.2 μm to accurately assess MMAD.
Flash Point	Not determined	Not determined as the substance is high melting point solid
Flammability	Not significantly flammable.	Measured.
Autoignition Temperature	Does not self-ignite	Measured.
Oxidizing Properties	Not explosive	Determined based on the absence of structural alerts for oxidizers.
Explosive Properties	Not expected to be explosive. Oxygen balance < -200 (–94.22).	Calculated. The notified chemical does not contain any structural alerts for explosivity.

* MMAD = Mass Median Aerodynamic Diameter

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

Reactivity
The test substance is considered to be non-oxidizing, and non-explosive, and is not capable of causing fire or enhancing the risk of fire when in contact with combustible material. The substance is stable at room temperature and does not evolve any flammable gases in contact with water or humid air. The product is considered to be stable under normal environmental conditions. The notified chemical was shown to decompose from 127°C.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported at a concentration of < 20% as part of the ink in sealed containers

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	1-10	1-10	1-10	1 - 10	1 - 10

PORT OF ENTRY
Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
The cartridges and printheads containing the chemical will be supplied to various users.

TRANSPORTATION AND PACKAGING
The printheads (containing 100ml of ink) will be imported into Australia in a bundle within the printer box. Print cartridges (containing 800ml of ink) will be individually packaged in cardboard boxes and then imported into Australia in master cartons.

USE
The notified chemical is an ingredient within inks in inkjet reprographic processes for commercial use. All of the notified chemical will be imported as part of the ink in sealed containers at a typical concentration of < 20%.
OPERATION DESCRIPTION

No reformulation or repackaging of the product occurs in Australia. The product is likely to be stored for a very short time after importation and then sent to "Channel partners" who sell to business customers on behalf of the notifier. End-users will be commercial business workers and service engineers involved in maintenance of the printer.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Importation/waterside workers	10	4	70
Storage and transport	100	6	240
Business worker/Service engineer	1000	< 0.1	20

EXPOSURE DETAILS

Exposure for workers involved in importation, storage and transport is expected to be negligible as the notified chemical is double contained within sealed cartridges and printheads inside packaging boxes. Exposure is only possible in the unlikely event of accidental breakage of both the boxes and the cartridges/printheads contained within the boxes.

Business workers may come into dermal contact with ink (containing < 20% of the notified chemical) if the print substrate is handled before the ink dries completely, however, this would not be expected to occur frequently with modern print technology.

Assuming an average cartridge contains 800 ml, and given that the notified chemical is present at concentrations of <20% in the ink cartridge, each ink cartridge will contain up to 160 mg of the notified chemical. It is expected that approximately 23,500 pages will be printed from a single cartridge (Source: HP product website). Assuming that this equates to 5% ink coverage on each page, this translates to approximately **0.007 mg of notified chemical** per page.

A worst-case estimate for exposure to the notified chemical involves printing of graphics, whereby up to 100% of each page will be covered with the ink. In this situation, each page is estimated to contain up to **0.14 mg of the notified chemical**. Based on a 50% transfer on contact when handling printed materials (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 = <0.7%

∴ Exposure to fingertips per event = <0.7% of 0.14 mg = **$<9.8 \times 10^{-4}$ mg per event**.

Also, the expected area exposure per contact event = <**9.8×10^{-4} mg** ÷ 8 cm^2 = **$<1.2 \times 10^{-4}$ mg/cm^2**

For extensive contact with wet ink on printed material (i.e. >10 events per day) the daily exposure, assuming no washing between events, a 70 kg person and an estimate of 100% absorption, would be:

Daily exposure = (<**9.8×10^{-4}** (mg/event)×10) ÷ 70 = **~1.4×10^{-4} mg/kg bw/day**.

Exposure is not significant for workers involved in routine replacement of cartridges (containing 154 ml of notified chemical) and printheads (containing 20 ml of notified chemical) unless the cartridges were faulty or ruptured.

Exposure through inhalation of aerosols or vapour is unlikely as the notified chemical is a solid dissolved in ink solution, with a low vapour pressure (<0.0016 Pa at 25°C) and will be contained inside sealed containers under normal operating conditions.

The most likely route of exposure is via dermal contact during printer maintenance by service engineers, but this can be mitigated by the use of protective gloves.

The MSDS for the notified chemical recommends that if exposure occurs on the skin, the area should be

flushed with water for at least 15 minutes and contaminated clothing should be removed and laundered before reuse.

6.1.2. Public exposure

The print cartridges containing the notified chemical will not be available to the public. Public exposure to the notified chemical is therefore expected to be low, as it is fixed to the paper before becoming available to consumers.

6.2. Human health effects assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 >2500 mg/kg bw. Low toxicity
Rat, acute dermal toxicity	LD50 >2000 mg/kg bw. Low toxicity
Rabbit, skin irritation	Slightly irritating
Dermal corrosivity	No evidence of corrosivity
Rabbit, eye irritation	Slightly irritating
Mouse, skin sensitisation – Local lymph node assay	No evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL = 15 mg/kg/day
Mutagenicity – bacterial reverse mutation	Non mutagenic
Genotoxicity – in vitro chromosome aberration test	No conclusive evidence of genotoxicity
Genotoxicity – in vivo	Not performed

Toxicokinetics, metabolism and distribution.

Based on its low molecular weight and relatively high water solubility (74.8% - 90.1%), the notified chemical has the potential to be absorbed across biological membranes such as the gastrointestinal wall and respiratory membranes, including through aqueous pores.

Gastrointestinal tract absorption was demonstrated by the 28-day repeat oral dose toxicity study in rats where pathological effects were seen in various organs, although these were mostly confined to the highest dose (150-1000 mg/kg/day). Respiratory absorption is expected to be low in practice, as the notified chemical has a low vapour pressure (<0.0016 Pa at ambient temperature) and the proportion of respirable particles (<10 μM) is low (0.23%). Toxicity due to dermal absorption of the notified chemical is not expected to be high, based on the low lipophilicity (log P_{ow} = -2.26), and high LD_{50} value shown in the acute dermal toxicity test (LD50 > 2000 mg/kg/bw).

Acute toxicity

The notified chemical is of low toxicity via the oral and dermal routes. There were no signs of systemic toxicity or abnormalities found at necropsy.

Irritation and Sensitisation.

The notified chemical was found to be only slightly irritating to the eyes and skin of rabbits. All signs of dermal and ocular irritation were resolved within 48 hours after exposure, and no permanent changes occurred. There was also no evidence that the notified chemical is corrosive to human dermal tissue based on the Epiderm™ Skin Corrosivity Test.

There was no evidence of skin sensitisation in a mouse local lymph node assay.

Repeated Dose Toxicity

In a 28-day oral repeat dose oral gavage study in rats, adverse macroscopic, histopathological and clinical abnormalities were shown in all animals treated with 1000 mg/kg/day of the notified chemical, and in some animals treated at 150 mg/kg/day. The main effects were on the sex organs of both sexes (atrophy, abnormal cells), bone marrow and spleen (hyperaemia, loss of marrow function and extramedullary haemopoiesis), liver (congestion, enlargement), and kidneys (focal necrosis and scarring, increased leucocytes). A dose of 15 mg/kg/day produced some minor but statistically significant changes to a range of haematological values in rats of both sexes. Based on these results, the No Observed Adverse Effect Level (NOAEL) was established as 15 mg/kg/day.

Mutagenicity/Genotoxicity
The notified chemical did not induce mutations in a bacterial test and failed to induce significant chromosomal aberrations in mammalian cells *in vitro*. These results suggest that the notified chemical is not likely to be mutagenic to humans.

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
The main occupational exposure is via the skin and respiratory system for commercial business workers and printer service technicians, however based on the low acute toxicity and irritancy, the notified chemical is not likely to cause acute effects as a result of normal use or handling by workers. Based on the exposure amount estimated, the margin of exposure is high (> 10000); therefore the risk of adverse effects from repeated exposure is very low.

6.3.2. Public health
The risk to the public from exposure to the notified chemical is expected to be negligible, given its low toxicity profile and the fact that it will be encapsulated within a matrix and not be bioavailable upon contact with consumer products in which it is contained.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported in sealed cartridges and printheads containing up to 800 mL of the formulated ink (of which up to 20% is the chemical). There will be no release to the environment due to reformulation or repackaging.

RELEASE OF CHEMICAL FROM USE

The ink cartridges and printheads 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. Workers at large businesses will undertake installation and replacement. If leakage or spillage does occur, the ink will be contained with absorbent material and disposed of in landfill in accordance with federal, state and local regulations.

Cartridges are contained within the printer until the contents are used up and then they are removed and sent for recycling or disposed of to landfill. Printheads are designed to last the lifetime of the printer but if they do not they are sent back to the manufacturer in the USA for analysis.

Most of the notified chemical (> 98%) will be bound to printed paper, which will be disposed of to landfill, recycled or possibly incinerated.
RELEASE OF CHEMICAL FROM DISPOSAL

Used cartridges are sent to recycling or disposed of to landfill. During recycling of used cartridges, the cartridges will be broken down into component parts for recycling and residual ink (up to 2%; <200 kg per annum) left in empty cartridges is likely to be incorporated into recycled plastic products, as low grade dyes. These recycled products are expected to be disposed of to landfill at the end of their useful lives.

Printed paper, having the notified chemical thereon will be disposed of to landfill, recycled or possibly incinerated. Recycling of treated paper may result in the release of a proportion of the notified chemical to the aquatic compartment. Waste paper is re-pulped using a variety of chemical treatments, which result in fibre

separation and ink detachment from the fibres. The wastes are expected to go to trade waste sewers. Approximately 50% (NOLAN-ITU 2001) of the ink printed on paper will enter paper recycling and a proportion of the ink is expected to be recovered during recycling by adsorption to sludge. Any chemical adsorbed to sludge during the recycling process will be disposed of to landfill.

7.1.2 Environmental fate

The vast majority of the notified chemical will enter the environment from disposal of paper products on which ink containing the notified chemical will be printed. Approximately 50% (< 5 tonnes per annum) will be disposed of to landfill or possibly incinerated. The chemical is very water-soluble but due to its cationic nature it is expected to bind to soil and eventually degrade in-situ by abiotic and biotic processes to landfill gases including methane, hydrogen sulphide, ammonia, oxides of carbon, sulphur and nitrogen; and water vapour. If incinerated the notified chemical is expected to form oxides of carbon, sulphur and nitrogen; and water vapour. The other 50% (< 5 tonnes per annum) is expected to be released to sewer, after the deinking of paper during recycling. Assuming a worst case scenario, where the entire amount of chemical from paper recycling is released from sewage treatment plants then a maximum of 5 tonnes per annum will be released to the aquatic environment. The chemical is not readily biodegradable (for the details of the environmental fate studies please refer to Appendix C).

7.1.3 Predicted Environmental Concentration (PEC)

The Predicted Environmental Concentration arising from the industrial use pattern has been modelled for the worst case in which none of the notified chemical released in aqueous wastes from the application of end-use products is removed by; or degrades in, on-site waste water treatment and sewage treatment plants. As the notified chemical is to be used in industrial applications at paper recycling facilities located throughout Australia, it is anticipated that such releases will occur on 260 days into the Australian effluent volume. The details of the calculation based on these parameters are presented below:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1000	kg/year
Proportion expected to be released to sewer	50%	
Annual quantity of chemical released to sewer	5,000	kg/year
Days per year where release occurs	260	days/year
Daily chemical release:	19.231	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	20.496	million
Removal within STP	0%	
Daily effluent production:	4,099	ML
Dilution Factor – River	1.0	
Dilution Factor – Ocean	10.0	
PEC - River:	4.5	µg/L
PEC - Ocean:	0.45	µg/L

7.2. Environmental effects assessment

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	EC50 > 100 mg/L	Not harmful
Daphnia Toxicity	EC50 > 100 mg/L	Not harmful
Algal Toxicity	ErC50 49 mg/L	Harmful
Algal Toxicity	ErC50 > 100 mg/L	Not harmful
Inhibition of Bacterial Respiration	EC50 910 mg/L	Not harmful
Other		

The notified chemical is harmful to algae in one test. Acidity contributed to the toxicity shown in this test. Otherwise the notified chemical was shown to be practically non-toxic to aquatic organisms.

7.2.1 Predicted No-Effect Concentration

The Predicted No Effect Concentration (PNEC) was calculated from the worst-case value for the growth of algae (ErC50) and using a safety factor of 100 (three trophic levels of aquatic species were supplied).

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
ErC50	49	mg/L
Assessment Factor	100	
Mitigation Factor	1.00	
PNEC:	490	µg/L

7.3. Environmental risk assessment

Risk Assessment	PEC µg/L	PNEC µg/L	RQ
RQ - River	4.5	490	0.01
RQ - Ocean	0.45	490	<0.01

The Risk Quotient is 0.01 at sewage outfall. Therefore, the notified chemical is not expected to pose an unacceptable risk to the aquatic environment based on the current use pattern.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Based on the available data the notified chemical cannot be 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.

	Hazard category	***Hazard statement***
Aquatic Toxicity	3	Harmful to aquatic life

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

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

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

Recommendations

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced:
  - Avoid exposure to skin

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced:
  - Disposable gloves

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

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

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

Disposal

- The notified chemical should be disposed of by landfill.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by adsorbing with inert material (Vermiculite, sand, earth etc.) then collection and placing in suitable container for disposal.

Transport and Packaging

- Keep in a cool, dry location
- Keep container closed and away from extreme heat

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 even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

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

(1) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from an ingredient within inks in commercial inkjet reprographic processes, or is likely to change significantly;
  - the amount of chemical being introduced has increased from 10 tonnes, 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 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 100ºC

Method OECD TG 102 Melting Point/Melting Range
EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks Determined with differential scanning calorimeter to be 99.72 ºC
Test Facility Safepharm Laboratories (2005a)

Boiling Point >360ºC at 101.3 kPa

Method OECD TG 103 Boiling Point.
EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks Could not be determined experimentally as the substance decomposed before boiling from 127ºC at 103.52 kPa. The boiling point was calculated using an adaptation of the Stein and Brown Method (MPBP for Windows version 1.41, 2000 US EPA).
Test Facility Safepharm Laboratories (2005a)

Density 1450 kg/m^3 at 19.5°C

Method OECD TG 109 Density of Liquids and Solids.
EC Directive 92/69/EEC A.3 Relative Density.
Remarks Determined using the gas comparison pycnometer method
Test Facility Safepharm Laboratories (2005a)

Vapour Pressure ≤ 1.508×10^{-3} kPa at 25ºC

Method EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks Determined using the vapour pressure balance method. The values were too low and variable for a meaningful line of best fit. A regression slope (between 45-55°C) on a chosen data point was used to estimate a maximum value. Extrapolation to 25°C gave a maximum value of 1.508×10^{-3} kPa.
Test Facility Safepharm Laboratories Limited (2005b)

Water Solubility ~750-900 g/L at 20±0.5ºC

Method EC Directive 92/69/EEC A.6 Water Solubility.
Remarks Flask Method. The standard A6 methodology could not be used; due to the chemical's high water solubility, it was not possible to prepare samples of 5× saturation level. Visual inspection showed no undissolved material at 74.8% w/w and significant quantity of undissolved material at 90.1% w/w (the highest level tested). To convert to g/L, the density of the solution is required, at these concentrations. This value is not recorded and an estimation has been made based on a density of ~1 g/cm^3. The pH of the test solutions varied from 2.1-1.3 (decreasing with higher concentrations of substance).
Test Facility Safepharm Laboratories (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*
4	50±0.5	>120
7	50±0.5	>120
9	50±0.5	>120

Remarks Based on the results of the test the half-life is estimated to be greater than one year at 25°C at pH 4, 7 and 9.
Test Facility Safepharm Laboratories (2005a)

Partition Coefficient (n-octanol/water) log Pow = -2.26 at 22±1.0ºC

Method EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks Flask Method. The n-octanol saturated water was adjusted to nominal pH 9 (actual 8.7-8.9). The notified chemical is expected to be in its zwitterionic form with a net overall 0 charge. The notifier states that this will result in the highest n-octanol solubility (in comparison with its ionic form). The DEWHA accept this argument on the basis of the relative water solubilities of sulfanilic acid (Morrison & Boyd 1983) ions.
Test Facility Safepharm Laboratories (2005a)

Surface Tension 72.6 ± 0.5 mN/m at 21.2±0.5°C

Method EC Directive 92/69/EEC A.5 Surface Tension.
Remarks Ring method. Concentration: 1.05 g/L solution. The result was not corrected for Harkins-Jordan correction, but this correction is not required for the apparatus used (interfacial tension balance). The notified chemical is not considered surface active.
Test Facility Safepharm Laboratories (2005a)

Adsorption/Desorption – screening test log K_{oc} = -0.15-3.20

Method QSAR estimation.
Remarks The notified chemical has a permanent cationic charge, and will interact with the stationary phase of the HPLC by mechanisms other than partitioning. Accordingly the HPLC method is not valid and an estimation was made using the formula log Koc = 0.52 × log Pow + 1.02. However, the QSAR guidance document cautions that the equation may systematically underestimate the Koc of aliphatic amines and amino PAHs by 1 to 2 log units. Further comparisons with modelled and actual data for Chlormequat chloride, Difenzoquat metilsulfate and mepiquat chloride showed a maximum difference of 3.20 log units. As a worst case, this value was added to the calculated QSAR value (-0.15) above. The cationic nature is expected to have strong interaction with the clay particles in addition to adsorption to organic carbon.
Test Facility Safepharm Laboratories (2005a)

Dissociation Constant pKa = 4.24

Method OECD TG 112 Dissociation Constants in Water.
Remarks Potentiometric titration. From the structure, the counter ion is expected to be a weak conjugate base. No experimental determination was possible for the counter ion but its dissociation constant was estimated as -3.35.
Test Facility Safepharm Laboratories (2005a)

Particle Size

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

Range (μm)	*Mass (%)*
< 100 (inhalable)	19.0
<10.2 (thoracic)	4.69
< 5.4 (respirable)	0.23

Remarks The sieve method was used as a screening test to determine the proportion of particles with size < 100 μm. This was followed by the cascade impactor method for the definitive test to determine the proportion of particles with size < 10.2 μm.
Too few particles were of a size less than 10.2 μm to allow accurate assessment of mass mean aerodynamic diameter.
Test Facility Safepharm Laboratories (2005a)

Flammability Not determined

Method	EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks	Notified chemical self-extinguished 2 seconds after the flame was removed without propagating combustion.
Test Facility	Safepharm Laboratories (2005b)

Autoignition Temperature Does not self-ignite.

Method	EC Directive 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases). EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks	No self-ignition of the test substance was observed under the conditions of the test, where the substance was subjected to increasing temperature from ambient to 110ºC (10ºC higher than melting temperature)
Test Facility	Safepharm Laboratories (2005b)

Oxidizing Properties

Method	EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Remarks	Based on the chemical structure the result of the oxidising properties test has been predicted negative.
Test Facility	Safepharm Laboratories (2005b)

Explosive Properties

Method	EC Directive 92/69/EEC A.14 Explosive Properties
Remarks	Based on the calculated oxygen balance of the test material (-94.22) and the absence of structural alerts, the test material has been predicted to be negative for explosivity.
Test Facility	Safepharm Laboratories (2005b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

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

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 female	2000	0
2	3 female	2000	0

LD50	>2500 mg/kg bw
Signs of Toxicity	No signs of systemic toxicity. No abnormal changes to bodyweight.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks - Results	The LD_{50} value was estimated using a flow chart as per the OECD Guidelines 423.
CONCLUSION	The notified chemical of low toxicity via the oral route.
TEST FACILITY	SafePharm Laboratories (2005c)

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
Vehicle	Arachis oil BP
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations

RESULTS

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

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	No signs of dermal irritation.
Signs of Toxicity - Systemic	No signs of systemic toxicity.
Effects in Organs	No abnormalities noted at necropsy.
Remarks - Results	None.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	SafePharm Laboratories (2005d)

B.3. Dermal Corrosivity Potential

TEST SUBSTANCE: Notified chemical

METHOD: EpiDerm™ Skin Corrosivity Test (ECVAM validated method)
OECD TG 431 *In Vitro* Skin Corrosion: Human Skin Model Test

Vehicle: Applied as solid, moistened with sterile distilled water.

Remarks - Method: Test uses EpiDerm™ Skin Model Kit (MatTek, MA, USA) and uses the MTT reduction assay to determine corrosivity.
Investigators included isostearic acid, which was previously evaluated as non-corrosive (Fentem et. al., 1998), as an objective study comparison. In a preliminary study designed to avoid false negative results, the test material was found to not directly reduce MTT.
During dosing for the 60-minute exposure, the physical state of the test material gradually altered from a solid to a liquid form. Therefore, for the 60-minute tissue and for the two 3-minute tissues, fresh 25 mg aliquots of test material were prepared and applied without delay. This ensured the test material was applied to the tissue in the original solid form prior to wetting with 25 µl of sterile distilled water.

RESULTS

Substance	*Exposure Time (min)*	*% Mean Viable cells*
Negative control (Distilled water)	3	100
	60	100
Positive control (8.0 N Potassium hydroxide)	3	12.6
	60	9.2
Standard (isostearic acid)	3	93.0
	60	106.8
Test substance	3	**95.8**
	60	**87.6**

Remarks - Results: The relative mean tissue viability of the negative and positive controls fell within the acceptance criteria. In addition, the relative mean tissue viability of the standard fell within the predicted values for classification as a non-corrosive substance (≥50% after a 3-minute exposure and ≥15% after a 60-minute exposure compared to the negative control). These results confirmed the validity of this assay.

The mean relative viability of the test material-treated tissue was 95.8% and 87.6% after the 3- and 60-minute exposure times respectively, when compared to the negative control.

CONCLUSION: The notified chemical was found to be non-corrosive to human skin.

TEST FACILITY: SafePharm Laboratories (2004a)

B.4. 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: Applied as solid, moistened with distilled water.
Observation Period: 72 hours
Type of Dressing: Semi-occlusive.
Remarks - Method: No significant protocol deviations. The test material (as a 10% aqueous solution) has a pH of 2.8.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0	0.33	0.33	1	< 48 hrs	0
Oedema	0	0	0	0	0	0

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

Remarks - Results: Very slight erythema was noted at all treated skin sites one hour after exposure. At the 24-hour observation, two exposed skin sites continued to show very slight erythema, which had cleared by the 48 hour observation.

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

TEST FACILITY: SafePharm Laboratories (2004b)

B.5. Irritation – eye

TEST SUBSTANCE: Notified chemical

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

Species/Strain: Rabbit/New Zealand White
Number of Animals: 3
Observation Period: 72 hours
Remarks - Method: No significant protocol deviations. The test material (as a 10% aqueous solution) has a pH of 2.5. All animals were treated with local anaesthetic in each eye prior to treatment to minimise pain on application of the test material.

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	0.33	0.33	0.33	1	< 48 hrs	0
Conjunctiva: chemosis	0	0	0	0	0	0
Conjunctiva: discharge	0.33	0.33	0	1	< 48 hrs	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0.33	0	1	< 48 hrs	0

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

Remarks - Results: No effects on the cornea were observed. One animal exhibited iridial inflammation 24 hours after application of the test material, and appeared to resolve by the 48-hour observation. All three animals showed conjunctival redness and discharge at the 24-hour observation, and appeared normal 48 hours after treatment.

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

TEST FACILITY: SafePharm Laboratories (2005e)

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

TEST SUBSTANCE: Notified chemical

METHOD
OECD TG 429 Skin sensitisation: Local Lymph Node Assay.
EC Directive 2004/73/EC B.42, Local Lymph Node Assay in the mouse.

Species/Strain: Mouse/CBA/Ca
Vehicle: Dimethyl sulphoxide
Remarks - Method: No significant protocol deviations. The highest concentration that was suitable for dosing (i.e. a solution) was 25% in DMSO. A preliminary toxicity study at this concentration found no signs of systemic toxicity or excessive local irritation. Groups of 5 animals were used for each test substance concentration. Disintegrations per minute (DPM) were calculated per animal (two lymph nodes per animal). Positive control was α-hexylcinnamaldehyde as a 4:1 solution of acetone: olive oil (historical data performed less than 6 months before commence of this test).

RESULTS

Concentration (% w/w)	*Mean Proliferative response (DPM/animal)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	1380.73 ± 457.83	-
5	1624.36 ± 109.93	1.18
10	1788.07 ± 361.87	1.3
25	1976.51 ± 962.47	1.43
Positive Control		
5	-	1.52
10	-	2.63
25	-	5.07

Remarks - Results: No signs of systemic toxicity were noted and body weight changes of the test animals were comparable to those observed in the control group over the same period.
The Stimulation Index (SI) at all concentrations was < 3, which indicates that the test substance did not lead to significant stimulatory effects on mouse lymph node activity. The mean and standard deviation at 25% test concentration were substantially higher than the control group, but a statistical comparison (Unpaired *t* test) showed that the difference between the mean scores was not significant ($p>0.05$). The stimulation index of the positive control was 5.07 at 25%, indicating that the assay was reliable.

CONCLUSION: There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.

TEST FACILITY: SafePharm Laboratories (2005f)

B.7. Repeat dose toxicity

TEST SUBSTANCE: Notified chemical

METHOD
EC Directive 96/54/EC B7 Repeated Dose (28 days) Oral Toxicity.
OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents

Species/Strain: 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: none
Vehicle: Arachis oil BP
Remarks - Method: No recovery groups were included and no urinalysis was conducted. These deviations were not considered to invalidate the study. Although all animals in the high dose group either died or were prematurely

terminated, most of these were terminated just one day ahead of schedule, therefore it was considered that they had received adequate exposure and the integrity of the study was not affected.

RESULTS

Dose mg/kg bw/day	*Number and Sex of Animals*	*Mortality*
0	5F, 5M	0/10
15	5F, 5M	0/10
150	5F, 5M	0/10
1000	5F, 5M	4/10 (2M, 2F)*

* Early termination of 6 remaining animals on welfare grounds.

Mortality and Time to Death

Two animals died as a result of exposure at the highest dose - one female was found dead on day 18, and one male was found dead on day 28 of the study. On Day 25 and 27, one male and one female treated with 1000 mg/kg/day, were sacrificed for humane reasons due to severe toxicological symptoms, and therefore have been counted in the total mortality rate. On day 28, the remaining 6 animals in the high dose group were terminated on welfare grounds as at least 50% showed some signs of persistent toxicity.

Clinical Observations

There were no findings in all animals at 15 and 150 mg/kg/day doses, as well as the control group.
Animals treated with 1000 mg/kg/day began to firstly show transient episodes of excessive salivation, first in females on day 4 and in both sexes from day 6 onwards. By day 14, 90% of all test animals exhibited postural/movement changes such as hunched posture and tiptoed gait. Other clinically significant findings include pilo-erection in 100% of male animals after day 16, and pallor of the extremities in the majority of male and female rats from the third week of dosing. During the final week of dosing, animals exhibited abnormal respiration, decreased grooming behaviour and soiled fur, staining in the urine and around the eyes and ano-genital region, and instances of clonic convulsion, ptosis, and diarrhoea prior to death.

No toxicologically significant changes were observed at any dose level in functional performance and sensory reactivity tests.

A substantial reduction in food consumption was noted for animals of either sex treated with 1000 mg/kg/day, most noticeably from the second week of the study. This was accompanied by markedly reduced bodyweight gain compared to the controls.

Water consumption measurements showed that rats (particularly males) dosed at 1000 mg/kg/day had a substantial increase (80%) in levels of consumption compared to the controls and rats receiving lower doses. Noteworthy increases were also evident for males dosed at 150 mg/kg/day (29%).

Laboratory Findings

Haematology:

At 15 mg/kg/day, male and female rats showed different but statistically relevant changes to haematological values. Female rats showed slight increases in mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and in the number of circulating reticulocytes ($p<0.05$), whilst males showed some reduction in erythrocyte levels ($p<0.05$). At 150 mg/kg/day, reductions in mean erythrocyte levels were observed in males, and females had again showed elevated MCH, MCV along with an increased reticulocyte count. Both sexes showed elevations in MCHC and decreased lymphocyte numbers.

At a dose of 1000 mg/kg/day, all animals showed significantly decreased haemoglobin, erythocyte, haematocrit, and leucocyte levels, but MCH, MCHC, MCV and numbers of reticulocytes were greater than of the control group.
Females in all treatment dose groups showed a statistically significant ($p<0.05$) increase for activated partial thromboplastin time, however, there was no obvious dose-response relationship for the effect and no expected changes in platelet count and prothrombin time, therefore the results may not be of toxicological importance.

Clinical chemistry:

Male animals showed significant reductions in total protein and plasma albumin levels at 1000 mg/kg/day,

with some reduction in total protein levels evident at 150 mg/kg/day. No significant changes in blood protein levels were detected in females at all doses. Other changes included low cholesterol and elevated bilirubin levels in both sexes at 1000 mg/kg/day, and elevated bilirubin levels in females at 150 mg/kg/day.

Creatinine levels were reduced in males dose at 1000 mg/kg/day and females at 150 mg and 1000 mg/kg/day, but there was an absence of renal effects (e.g. increase in electrolyte, urea, glucose levels), therefore this finding may not present as being toxicologically significant in this study.

The haematological findings along with evidence of elevated bilirubin levels are consistent with non-immune, substance-induced haemolytic anaemia.

Effects in Organs

Organ weights:
Only animals treated with 1000 mg/kg/day showed any significant changes to absolute and relative (to bodyweight) organ weight, namely the liver (both sexes) and the adrenals, and kidney in males. There was a reduced thymus weight in each sex at 1000 mg/kg/day, and females had low ovary weight in comparison with controls.

Macroscopic findings:
Animals from the high dose group sacrificed on Day 28 had darkened spleens (6 animals) and small epididymides (1 animal). Animals in the high dose group that died prior to Day 28 had small seminal vesicles (1 animal), darkened salivary glands (1 animal) and dark liver and pallid stomach (1 animal).

Liver:
There was strong evidence of liver enlargement and pigment deposition (haemosiderin) in the sinusoids in at least 50% of sacrificed animals dosed at 1000 mg/kg/day. Liver congestion was found in the two animals found dead on day 18 and day 28 receiving the same dose. There were also signs of minimal focal necrosis in the liver lobules in one male rat dosed at 150 mg/kg/day. Mononuclear cell aggregation was found in females receiving 1000 mg/kg/day, but was not found in male counterparts.
As the liver is the primary metabolic and detoxication organ, certain symptoms such as hepatic enlargement is often seen in animals undergoing this type of treatment.

Bone marrow:
Hyperaemia of the marrow was evident in all rats of either sex at 1000 mg/kg/day, and a high level of adipose tissue infiltration into the bone marrow was present, particularly in male rats. These results indicate a decrease in the overall number of functional haemopoietic cells in the marrow compared to the controls.

Spleen:
A large concentration of haemosiderin pigment and signs of extramedullary haemopoiesis was found in 100% of animals of both sexes tested at 1000 mg/kg/day, accompanied by hyperaemia. These results suggest that the spleen has adapted in response to inadequate production of blood cells by the haemopoietic tissue within bone marrow.

Adrenals:
One rat of each sex dosed at 1000 mg/kg/day showed cortical hypertrophy of the adrenal gland.

Gastrointestinal tract:
Male rats tested at 1000 mg/kg/day showed signs of duodenal changes such as hypertrophy of mucosal cells. No effect was seen on female rats.

Kidneys:
Two male rats dosed at 1000 mg/kg/day exhibited symptoms including pigment deposition and focal necrosis, and one male showed clustering of basophils in the renal tubules. All four female rats dosed at 1000 mg/kg/day that were terminated at the end of the study as well as one female that was found dead on day 18 showed renal changes such as cortico-medullary mineralisation, cortical scarring and basophilic tubules.

Lungs:
Thrombosis of the pulmonary artery was observed in one rat of each sex dosed at 1000 mg/kg/day. Other findings such as perivascular oedema and lymphoid aggregation may not be related to the test substance as all

rats in every group (including controls) showed signs of pathology related to a respiratory infection.

Lymph nodes:
Findings were confined to rats receiving 1000 mg/kg/day of the test substance. Both female and male rats found dead on day 18 and 28 respectively, had dilated sinusoids of the mesenteric lymph nodes, and this condition was also present in two males and one female rat that were killed in extremis.

Thymus:
Lymphoid atrophy was observed in male and female rats being given 1000 mg/kg/day. There were no other findings in the control or in other test groups.

Thyroid:
80% of female rats receiving 1000 mg/kg/day showed evidence of thyroid changes that were absent in controls and lower dose groups. These included follicular cell hypertrophy, depletion of colloid and dilatation of follicles. The two latter findings were also observed in males treated with 1000 mg/kg/day; however, the relationship between the test substance and follicular cell hypertrophy is questionable as the majority of male rats displayed this characteristic across all dose groups, including the controls.

Epididymides:
100% of male rats (dosed at 1000 mg/kg/day) that were examined were found to have abnormal cells and reduced spermatozoal content. This observation was not found in other treatment groups.

Testes:
Testicular atrophy was seen in both gonads for male rats dosed at 1000 mg/kg/day, but not at any other dose level.

Uterus:
Uterine atrophy was observed in three female rats dosed only at 1000 mg/kg/day.

Remarks – Results
The initial treatment-related changes observed in rats suggest that the test substance either possesses irritant characteristics or was found to be unpalatable by the animals, and hence may have lead to reduced food intake, reduced body weight and disturbances in behavioural and physiological function may be attributable to inadequate nutrition.
Clearly, the majority of pathological findings were confined to rats given the highest treatment dose (1000 mg/kg/day). At this dose, significant effects were observed on blood chemistry and haemopoiesis, and liver functions. Noteworthy findings also include histopathological changes to sexual organs in both male and female rats at the highest dose.
There were limited organ changes in rats given 150 mg/kg/day, however, there were statistically significant effects on haematological values of rats of both sexes (e.g. reduced erythrocytes, reduced MCV and MCH levels). Some observable changes in haematological values were seen in rats dosed at 15 mg/kg/day, however were not sufficient in severity to be regarded as a serious adverse health effect.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 15 mg/kg bw/day in this study, based on haematological findings in rats of both sexes at this dose level.

TEST FACILITY	SafePharm Laboratories (2005g)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Plate incorporation procedure EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, TA102,
Metabolic Activation System	10% S9 fraction derived from phenobarbitone/β-naphthoflavone induced rat liver

Concentration Range in Main Test	a) With metabolic activation: 0, 50, 150, 500, 1500, 5000 µg/plate b) Without metabolic activation: 0, 50, 150, 500, 1500, 5000 µg/plate
Vehicle	Dimethyl sulphoxide
Remarks - Method	Investigators used *S. typhimurium* TA102 as an alternative to *E.coli* strains to detect cross-linking mutagenicity and base pair substitution (as listed in OECD TG 471 guidelines).

RESULTS

Remarks - Results	No significant increase in the frequency of revertant colonies was observed for any of the bacterial strains at any dose level, with or without metabolic activation. The positive control chemicals induced substantial increases in revertant colony numbers, confirming the sensitivity of the cultures and activity of the S9 mix.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	SafePharm Laboratories (2005h)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
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	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 fraction from phenobarbitone/β-naphthoflavone-induced rat liver.
Vehicle	Minimal Essential Media
Remarks - Method	No significant protocol deviations. In Test 1, the S9 fraction was used in 2% final concentration, while in Test 2 it was used in 1% final concentration.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 156.25, 312.5, 625, 1250*, 2500*, 5000*	4 hours	24 hours
Test 2	0*, 312.5, 625, 1250*, 2500*, 3750*, 5000	24 hours	24 hours
Present			
Test 1	0*, 156.25, 312.5, 625, 1250*, 2500*, 5000*	4 hours	24 hours
Test 2	0*, 156.25, 312.5, 625, 1250*, 2500*, 5000*	4 hours	24 hours

*Cultures selected for metaphase analysis.

RESULTS

Remarks - Results	Investigators note that the test material was more toxic than was observed in the preliminary toxicity test because there were no metaphase cells at 5000 µg/mL. There were two statistically significant levels of chromosome aberrations in this study. First was test group 2 at 5000 µg/mL in the presence of metabolic activators at the 4-hour exposure period test, and these were characterised mostly by chromatid gaps. The second noteworthy test was group 1 at 3750 µg/mL without metabolic activators in the 24-hour exposure test, and a mixture or chromatid breaks and gaps were observed. In general, the positive responses were well below that of historical standard positive control maximums for the exposure groups, and the

	investigators pointed out that a greater number of chromatid gaps, rather than breaks occurred. There was no significant increase in the numbers of polyploid cells in any exposure groups at all dose levels. The inconsistencies in the finding mean that no definite conclusions can be drawn from the results.
CONCLUSION	The notified chemical was not clastogenic to human lymphocytes treated *in vitro* under the conditions of the test.
TEST FACILITY	SafePharm Laboratories (2005i)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE: Notified Chemical

METHOD
- Inoculum: Activated sewage sludge
- Exposure Period: 28 days
- Auxiliary Solvent: None
- Analytical Monitoring: CO_2 analysis using TOC analyser
- Remarks - Method: Duplicate solutions of test substance containing 29.6 mg/L (≡ 10 mg C/L) and activated sewage sludge from Loughborough, Leicestershire UK, which treats predominantly domestic sewage were incubated in the dark for 28 days. A control; a reference substance (sodium benzoate) and a toxicity control containing the test substance and the reference substance were also run in duplicate. A second carbon absorber connected in series to the first absorber was analysed on day 0 and 29.

RESULTS

Test substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
1	1	1	42
6	0	6	65
10	0	10	80
14	9	14	79
22	10	22	78
28	20	28	81
29*	20	29*	88

* Corrected to include carryover from absorber 1

- Remarks - Results: All test substances were a light brown dispersion with no undissolved test material observable. The pH of all test preparations were between 7.5 and 7.8. Only minimal carry over of CO_2 was observed in the second absorber. The toxicity control showed 58% degradation after 28 days and showed that the test substance was not inhibitory to sewage sludge micro-organisms. The difference between duplicate vessels for the test substance was < 20%. Sodium benzoate attained 81% degradation after 28 days thereby confirming the suitability of the inoculum and test conditions.

CONCLUSION: The test substance cannot be considered readily biodegradable.

TEST FACILITY: Safepharm Laboratories Limited (2005k)

C.1.2. Bioaccumulation

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

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Notified Chemical

METHOD: In accordance with OECD TG 203 Fish, Acute Toxicity Test & EC Directive 92/69/EEC C.1 Acute Toxicity for Fish -semi-static.

Species	Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	~ 100 mg $CaCO_3$/L
Analytical Monitoring	Visual; HPLC
Remarks – Method	A range finding test was conducted on 3 fish exposed to 100 mg/L of test substance and a control. On the basis of range finding test, a definitive test was conducted by subjecting duplicate samples of seven fish to solutions of 100 mg/L of test substance as well as a control. Test solutions were renewed daily. pH range: 7.0-8.3 Oxygen: 9.7-10.2 mg O_2/L Temperature: 13.1-13.8°C

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*0 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	<0.088	7	0	0	0	0	0
100	98*	7	0	0	0	0	0

*Average of duplicate samples taken at 0 h, fresh media and from old media at 24 and 96 h. The range was 93.8-102 mg/L.

LC50	> 100 mg/L at 96 hours.
NOEC	100 mg/L at 96 hours.
Remarks – Results	The water temperature during acclimatisation was 12.8-13.7°C, which is just outside of the guideline of 14±1°C. It is unlikely that this affected the integrity or validity of the test. There were no other remarkable comments regarding the fish or solutions.

CONCLUSION The test substance is practically non-toxic to fish.

TEST FACILITY Safepharm Laboratories Limited (2005l)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified Chemical

METHOD In accordance with OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test -& EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - static.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	~250 mg $CaCO_3$/L
Analytical Monitoring	Visual: HPLC
Remarks - Method	A range finding test was conducted, by subjecting samples of 10 daphnids to a series of nominal concentrations of test substance of 0.010, 0.10, 1.0, 10 and 100 g/L and a control. On the basis of the range finding test, a limit test was conducted by subjecting four replicate samples of five daphnia to solutions of 100 mg/L of test substance as well as a control. A positive control was also run less than 2 months prior to the definitive test using a reference substance (potassium dichromate). pH range: 6.2 for the test solution; 8.0 for the control Oxygen: 8.6-8.7 mg O_2/L Temperature: 20.8-20.9°C

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	< 0.088	20	0	0
100	95.5*	20	0	0

*Average of all four samples.

LC50	> 100 mg/L at 48 hours
NOEC	100 mg/L at 48 hours
Remarks - Results	The test solutions were observed to be clear colourless solutions throughout the duration of the test. A concentration dependent difference between pH was observed throughout the test. This does not appear to have affected the test results. The positive control had a 48 hour EC50 of 1.2 mg/L. This within the normal range, with the average for all tested positive controls being 0.78 mg/L with SD of 0.24 mg/L.
CONCLUSION	The test substance is practically non-toxic to daphnia.
TEST FACILITY	Safepharm Laboratories Limited (2005m)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	The notified chemical
METHOD	In accordance with OECD TG 201 Alga, Growth Inhibition Test and EC Directive 92/69/EEC C.3 Algal Inhibition Test.
Species	*Scenedesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 6.25-100 mg/L Actual: 5.87-95.9 mg/L; Average value at time 0 and 72 hours.
Auxiliary Solvent	None
Water Hardness	Not Specified.
Analytical Monitoring	Particle Counter; HPLC
Remarks - Method	A range finding test was conducted by subjecting samples of ~1×10^4 algal cells/mL to a series of nominal test solutions of 1.0, 10 and 100 mg/L as well as a control. On the basis of the results, a definitive test was conducted by subjecting triplicate samples of ~ 1×10^4 cells/mL to test solutions detailed below and a control. A recovery test was also run. The temperature was maintained at 24±1°C. The pH was also measured.

RESULTS

Inhibition of Growth Rate and Biomass with respect to concentration of test substance and pH.

Nominal Concentration mg/L	Measured Concentration mg/L*	pH† at 0 hours	pH† at 72 hours	% Inhibition Biomass	% Inhibition Growth.
Control	< 0.0055	7.4	7.7	-	-
6.25	5.87	7.1	7.7	[12]	[4]
12.5	12.4	6.9	7.7	[4]	[2]
25	24.1	6.3	7.5	[9]	[4]
50	48.1	4.7	4.8	97	77
100	95.9	4.0	4.0	103	108

*Average value at time 0 and 72 hours. † Average of triplicate samples. Values in parentheses showed increase growth in comparison to the controls.

Biomass		*Growth*	
EbC50 mg/L at 72 h	*NOEC mg/L*	*ErC50 mg/L at 72 h*	*NOEC mg/L*
43	25	49 (95% CI 47-49)	25

Remarks - Results	The solutions were clear colourless solutions at 0 hours and at 72 hours green dispersion were observed in the lowest test concentrations and the control. The two highest test concentrations remained clear and colourless. The recovery test showed 97% recovery including for spiked algal tests. The pH declined with increasing test concentration. The effect of this pH difference on algal growth is not differentiated from the effect of the test concentrations. Noting that the USEPA Guidelines (USEPA 1996) indicate that if the test chemical is highly acidic and reduces the pH of the test solution below 5.0 at the first measurement, appropriate adjustments to pH should be considered; it is likely that the pH at 50 and 100 mg/L test concentrations was so low that it caused an immediate 'shock' which killed the algal cells and hence no adaptation occurred. The change in pH is likely to have caused the toxicity. A 95% confidence interval (CI) for inhibition of biomass could not be calculated.
CONCLUSION	The test substance is harmful to green algae.
TEST FACILITY	Safepharm Laboratories Limited (2005n)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE	The notified chemical
METHOD	In accordance with OECD TG 201 Alga, Growth Inhibition Test and EC Directive 92/69/EEC C.3 Algal Inhibition Test.
Species	*Scenedesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 100 mg/L Actual: 101 mg/L; Average value at time 0 and 72 hours.
Auxiliary Solvent	None
Water Hardness	Not Specified.
Analytical Monitoring	Particle Counter; HPLC
Remarks - Method	A range finding test was conducted by subjecting duplicate samples of ~1×10^4 algal cells/mL to a series of nominal test solutions of 0.10 1.0, 10 and 100 mg/L as well as a control. On the basis of the results, a definitive test was conducted by subjecting six replicate samples of ~1×10^4 cells/mL to test solutions detailed below and a control (triplicate). A recovery test was also run. The temperature was maintained at 24±1°C. The pH was also measured.

RESULTS

Inhibition of Growth Rate and Biomass with respect to concentration of test substance and pH.

Nominal Concentration mg/L	Measured Concentration mg/L*	pH† at 0 hours	pH† at 72 hours	% Inhibition Biomass	% Inhibition Growth.
Control	< 0.11	7.8	7.6	-	-
100	101	6.2	4.8	3	1

*Average value at time 0 and 72 hours. † Average of all samples.

Biomass		*Growth*	
<EbC50> mg/L at 72 h	*<NOEC> mg/L*	*<ErC50> mg/L at 72 h*	*<NOEC> mg/L*
> 100	100	> 100	100

Remarks - Results	The solutions were clear colourless solutions at 0 hours and at 72 hours green dispersions were observed. The recovery test showed 96% recovery for test without algae and 95% for spiked tests containing algae. The pH was lower in the test concentration and decreased significantly over time. Only a slight decrease in pH was observed in the control during the

duration of the test. The pH did not appear to affect the outcome of the test, as it dropped slowly over the duration of test to a value of just below 5.

CONCLUSION The test substance is practically non-toxic to green algae.

TEST FACILITY Safepharm Laboratories Limited (2006a)

C.2.5. Inhibition of microbial activity

TEST SUBSTANCE The notified chemical

METHOD In accordance with OECD TG 209 Activated Sludge, Respiration Inhibition Test and EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test

Inoculum Activated sewage sludge from Loughborough, Leicestershire UK, which treats predominantly domestic sewage.

Exposure Period 3 hours

Concentration Range Nominal: 32-3200 mg/L
Actual: Not determined

Remarks – Method A range finding test was conducted by measuring the Biological Oxygen Demand (BOD) of sewage sludge organisms. On the basis of the range finding test a definitive test was conducted by subjecting samples of sewage sludge to the concentrations detailed below. The hardness of the water was ~100 mg $CaCO_3$/L. A reference substance (3,5 dichlorophenol) was run as a positive control.

Inhibitory effect on the Respiration of Activated Sewage Sludge

Nominal Concentration mg/L		pH	% Inhibition
Control	R1	8.2	-
	R2	8.0	-
Test Substance	32	8.1	[2]
	100	8.0	[2]
	320	7.8	2
	1000	6.2	57
	3200	4.4	98
Reference Substance	3.2	8.2	20
	10	8.2	46
	32	8.3	80

RESULTS

IC50 910 mg/L

NOEC 320 mg/L

Remarks – Results The dissolved oxygen at the end of the test was below the guideline value of 2.5 mg O_2/L in the control and 32 and 320 mg/L test solutions and the 3.2 mg/L positive control. It was not considered to have altered the outcome of the test. The pH declined with increasing test concentration. The pH of the test solutions may have contributed to the inhibition of respiration of sewage sludge organisms. However, this is not considered to affect the integrity of the study and is expected to be representative of a wastewater treatment plant, where no pH adjustment of incoming effluent occurs.

CONCLUSION The test substance is practically non-inhibitory to sewage sludge organisms.

TEST FACILITY Safepharm Laboratories Limited (2005o)

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