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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

FSM-002M in PictureMate Photo Cartridge T5852

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

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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FULL PUBLIC REPORT

FSM-002M in PictureMate Photo Cartridge T5852

1. APPLICANT AND NOTIFICATION DETAILS

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

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication:
Chemical name; CAS number; Molecular formula; Structural formula; Molecular weight; Spectral data; Methods of detection and determination; Purity; % Weight of Hazardous and Non - Hazardous Impurities; Import volume; Concentration of notified chemical in products.

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

OTHER NAME(S)
Pyridine azo dye J-15

MARKETING NAME(S)
FSM-002M in PictureMate Photo Cartridge T5852

3. COMPOSITION

DEGREE OF PURITY
>80%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

ADDITIVES/ADJUVANTS
None

4. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as a component of inkjet printer inks (<5% concentration).

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

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

USE
The notified chemical will be used as a dye component of imported inkjet printer inks (<5%).

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

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, transport and storage

PORT OF ENTRY
Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
None known at this time. Potentially, the inkjet printer cartridges containing the notified chemical will be supplied to retailers and offices nationwide.

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a component of ready-to-use sealed plastic inkjet cartridges of 5-100 mL volumes. The cartridges are individually wrapped in plastic and cardboard packaging, and these will be imported in bulk in cardboard cartons. The cartridges will be transported by road.

5.2. Operation description
The cartridges will be transported and stored prior to national distribution where they will be used in office or home printing equipment. No reformulation or repackaging of the notified chemical will occur in Australia. The products containing the notified chemical will be delivered to the end-user in the same form in which they are imported. The cartridges will be installed or replaced into the inkjet printer by office workers, service technicians or consumers.

5.3. Occupational exposure
Number and Category of Workers

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

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

Both office workers and service technicians may be exposed (dermal or ocular) to the notified chemical in 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.

5.4. Release

RELEASE OF CHEMICAL AT SITE
Printer ink will be imported in ready-to-use cartridges (containing <5% notified chemical). No release

is expected as manufacturing and reformulation of the ink containing the notified chemical will not take place in Australia. Environmental release of the notified chemical is unlikely during importation, storage and transportation, and spillage during a transport accident is the most likely reason for environmental release. Individual container capacity, container and packaging specifications would limit the extent of release.

RELEASE OF CHEMICAL FROM USE

The ink cartridges are designed to prevent leakage and will not be opened during transport, use, installation or replacement. Therefore, release of ink containing the notified chemical to the environment is not expected under normal conditions of use. In the unlikely case of spills arising during installation and replacement, it is expected that the ink containing the notified chemical will be contained and collected with absorbent material and be subsequently disposed of to landfill. Cartridges are contained within the printer until the contents are used then they are removed and sent to a recycling and disposal centre or directly to landfill.

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

5.5. Disposal

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

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

5.6. 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. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20ºC and 101.3 kPa Red solid

Melting Point/Freezing Point > 360 ºC

METHOD	EC Directive 92/69/EEC A.1 Melting/Freezing Temperature. Differential scanning calorimetry
Remarks	The notified chemical was not observed to melt between 25 and 360 ºC.
TEST FACILITY	SafePharm Laboratories Ltd (2005a)

Boiling Point Not measured

Remarks	The boiling point would be > 360 ºC.
TEST FACILITY	SafePharm Laboratories Ltd (2005a)

Density 1510 kg/m^3 at 20 ± 1.0 ºC

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

Vapour Pressure < 2.1 x 10^{-8} kPa at 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 241°C.
TEST FACILITY SafePharm Laboratories Ltd (2006a)

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

METHOD Based on EC Directive 92/69/EEC A.6 Water Solubility.
Remarks Flask Method. Samples of the notified chemical could not be prepared at five times the saturation level, as recommended in the test guideline, as the saturation point was unable to be determined. No analysis could be performed due to the high solubility producing unfilterable mixtures. Therefore, the water solubility was estimated based on visual inspection.
TEST FACILITY SafePharm Laboratories Ltd (2005a)

Hydrolysis as a Function of pH

METHOD EC Directive 92/69/EEC C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$ days*
4	25	>365
7	25	>365
9	25	>365

Remarks The preliminary test showed less than 10% hydrolysis (by HPLC) after 5 days at 50ºC in buffers of pH 4, 7 and 9, which is estimated to be equivalent to a half-life of >1 year at 25ºC at any pH.
TEST FACILITY SafePharm Laboratories Ltd (2005a)

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

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

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

METHOD OECD TG 121 Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using HPLC.
Remarks Test was performed using the HPLC screening method at pH 7. The notified chemical eluted before the standard solution of acetanilide, indicating it is highly mobile in soil or sediment. As the test material eluted prior to the first calibration standard, a limit value has been reported for the adsorption coefficient, that of less than the valid calibration range.

The low adsorption properties of the test material containing acidic functional groups determined by the HPLC estimation method were consistent with the extremely high water solubility and low partition coefficient characteristics. Although the determined value is believed to accurately assess the affinity of the test material for the organic carbon content of soils and sewage sludge, the method guideline specifically requires the analysis of substances in an ionised form if

present within the environmentally relevant pH range of 5.5 to 7.5; the mobility of the test material in soil and sewage sludge may also be influenced by additional interactions other than partitioning not addressed by the test method, due to the anionic nature of the test material.

The alternative use of computer-based estimation programs and/or Quantitative Structure Activity Relationships (QSAR's) for materials of this nature are considered invalid as estimates are typically derived from the partition coefficient value. Therefore, once more the possible secondary interaction originating from the anionic charges present on the test material is not addressed.

TEST FACILITY SafePharm Laboratories Ltd (2005a)

Dissociation Constant Not determined

Remarks No determination was performed according to the OECD test guideline, as the notified chemical contains no modes of dissociation within the range and scope of the method. As the salts are of very strong acids, the notified chemical will remain dissociated throughout the environmental pH range of 4-9.

TEST FACILITY SafePharm Laboratories Ltd (2005a)

Surface Tension 68.4 mN/m at 22.8 ± 0.5°C

METHOD EC Directive 92/69/EEC A.5 Surface Tension.

Remarks By the ISO 304 ring method, the surface tension of a 1.01 g/L solution of the notified chemical was determined using an interfacial tension balance. The result was not corrected using the Harkins-Jordan correction table, as the correction was not considered applicable to the apparatus used. The notified chemical is not a surface active substance.

TEST FACILITY SafePharm Laboratories Ltd (2005a)

Particle Size

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

Range (µm)	*Mass (%)*
Proportion of test material having an inhalable particle size less than 100 µm	24.0
Proportion of test material having a thoracic particle size less than 10.2 µm	3.7
Proportion of test material having a respirable particle size less than 5.4 µm	0.8

Remarks Too few particles were of a size < 10.2µm to allow accurate assessment of mass median aerodynamic diameter.

TEST FACILITY SafePharm Laboratories Ltd (2005a)

Flash Point Not determined

Remarks Not applicable as notified chemical is a low volatility solid. The notified chemical would have a flash point < 290ºC.

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 (2006a)

Autoignition Temperature 290ºC

METHOD 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
TEST FACILITY SafePharm Laboratories Ltd (2006a)

Explosive Properties The notified chemical was determined not to have explosive properties

METHOD EC Directive 92/69/EEC A.14 Explosive Properties.
TEST FACILITY SafePharm Laboratories Ltd (2006a)

Reactivity Not determined

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

Oxidizing Properties Predicted to be negative

METHOD EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Remarks The notified chemical does not contain any chemical groups that would imply oxidising properties
TEST FACILITY SafePharm Laboratories Ltd (2006a)

7. TOXICOLOGICAL INVESTIGATIONS

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

7.1. Acute toxicity – oral

TEST SUBSTANCE — Notified chemical

METHOD — OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
EC Directive 2004/73/EEC B.1tris Acute Oral Toxicity – Acute Toxic Class Method.

Species/Strain — Rat/ Sprague Dawley CD (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*
1	3F	300	0
2	3F	300	1
3	3F	2000	1
4	3F	2000	1

LD50 — 2500 mg/kg bw
Signs of Toxicity — One animal treated with 300 mg/kg was found dead approximately 3 minutes after dosing.
Two animals treated at 2000 mg/kg were found dead 1 day after dosing.
Red staining of the urine and faeces were noted in surviving animals (300 mg/kg) four hours to three days after dosing. Animals appeared normal two or four days after dosing.
Hunched posture, diarrhoea stained red and noisy respiration were noted in animals treated at a dose level of 2000 mg/kg. Red staining of faeces, fur and urine were also noted. Surviving animals appeared normal seven, nine, or eleven days after dosing.
Effects in Organs — At necropsy, the animal that died after dosing with 300 mg/kg had red material in the stomach. Animals that died during the study that were dosed with 2000 mg/kg had abnormally red lungs, dark liver, dark kidneys, stomach stained red with red liquid or material present and small and large intestines stained red.
No abnormalities were observed at necropsy in animals killed at the end of the study.

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

TEST FACILITY — SafePharm Laboratories Ltd (2005b)

7.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical (88% purity)
METHOD	OECD TG 402 Acute Dermal Toxicity. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/Sprague-Dawley CD (Crl: CD(SD) IGS BR)
Vehicle	Moistened with distilled water
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations. Corrections were made for the purity of the test material.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	None
Signs of Toxicity - Systemic	None
Effects in Organs	None
Remarks - Results	Red coloured staining was noted at the treatment sites of all animals one to four days after dosing. This prevented evaluation of erythema.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	SafePharm Laboratories Ltd (2006b)

7.3. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 2004/73/EC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	Distilled water
Observation Period	7 days
Type of Dressing	Semi-occlusive.
Remarks - Method	One of the three animals was treated initially, where the test substance was applied to three sites for time periods of 3 minutes, 1 hour and 4 hours prior to its removal and evaluation of skin reactions. The other two animals were treated for 4 hours only.

RESULTS

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	1	1	1	<7 days	0
Oedema	0	0.7	0.7	1	<72 hr	0

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

Remarks - Results	No skin irritation was observed after 3 minute or the 1 hour exposure period on the single animal tested.
CONCLUSION	The notified chemical is slightly irritating to the skin.

TEST FACILITY SafePharm Laboratories Ltd (2005c)

7.4. 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
Observation Period 72 hours for 2 animals, 7 days for one animal
Remarks - Method A single rabbit was treated initially and an assessment of the initial pain reaction was made. After consideration of the ocular responses produced in the first treated animal, two additional animals were treated. In order to minimise pain on application of the test material, one drop of local anaesthetic (amethocaine hydrochloride 0.5%) was instilled into both eyes of the final animal 1-2 minutes prior to treatment. One treated eye was observed on day 7 to assess the reversibility of the ocular effect.

RESULTS

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

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

Remarks - Results Red coloured staining of the fur was noted around all treated eyes during the study.
Red coloured staining of the cornea was noted in one treated eye at the 24 and 48 hour observation. The staining did not affect evaluation of corneal effects.
An area of haemorrhage over the nictitating membrane was noted in one treated eye at the 24, 48 and 72 hour observations.
Two treated eyes appeared normal at the 72 hour observation and the remaining treated eye appeared normal at the 7 day observation.
The notified chemical is classified as a moderate irritant according to a modified Kay and Calandra classification system.

CONCLUSION The notified chemical is slightly/moderately irritating to the eye.

TEST FACILITY SafePharm Laboratories Ltd (2005d)

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

TEST SUBSTANCE Notified chemical (86.4% purity)

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

Species/Strain Mouse/CBA strain, female
Vehicle Dimethyl formamide
Remarks - Method A preliminary screening study on two animals, treated with 10% and 25% w/w notified chemical, was performed to determine its toxicity/irritancy

potential. The mice were treated with test substance on the dorsal surface of each ear daily for three days. A 25% test substance concentration was considered the highest concentration that could be prepared homogeneously to a visible acceptable level.

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

A laboratory historical positive control was used.

RESULTS

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

Remarks - Results

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

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

CONCLUSION

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

TEST FACILITY

NOTOX (2006)

7.6. Repeat dose toxicity

TEST SUBSTANCE

Notified chemical (88% purity)

METHOD

OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).

Species/Strain: Rat/Sprague-Dawley Crl:CD (SD) IGS BR

Route of Administration: Oral – gavage

Exposure Information: Total exposure days: 28 days
Dose regimen: 7 days per week
Post-exposure observation period: 14 days

Vehicle: Distilled water

Remarks - Method: No significant protocol deviations.
A 14-day repeated dose range finding study was performed at 500 and 1000 mg/kg bw/day, using 3 male and 3 female animals in each group. The test method was similar to the main study, and was used to select the doses for the main study.

RESULTS

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)	5M, 5F	150	0
IV (high dose)	5M, 5F	400	0
V (control recovery)	5M, 5F	0	0
VI (high dose recovery)	5M, 5F	400	0

Mortality and Time to Death

No mortality was observed during the treatment or recovery phases.

Clinical Observations

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

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

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Blood chemistry

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

Haematology

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

Urinalysis

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

Effects in Organs

Organ weights

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

Necropsy

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

Histopathology

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

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

A number of other effects were observed in the heart, liver, spleen, kidney, lung and bone marrow of some of the treated animals. However, effects of similar severity and in a similar number of control animals were also observed. In addition, many such effects are considered to be common in laboratory rats. As such, the effects were considered not to be of toxicological significance. No effects were found in other organs examined.

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

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

TEST FACILITY SafePharm Laboratories Ltd (2006c)

7.7. 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	Distilled water
Remarks - Method	No significant protocol deviations. As the notified chemical is an azo compound, the OECD test method strongly recommends the use of a modified Ames test with a reducing pre-incubation step to improve sensitivity (eg Prival and Mitchell, 1982). However, such a modification was not used in this test.

RESULTS

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

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

As a reductive pre-incubation step was not used in this study, the result

(non-mutagenic) is indicative only of the conditions of this particular Ames test. Many carcinogenic Azo dyes test negative in Ames tests without the use of a modified test (SCCNFP, 2002).

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

TEST FACILITY — SafePharm Laboratories Ltd (2005e)

7.8. Genotoxicity – in vitro

TEST SUBSTANCE — Notified chemical (88% purity)

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

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

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

*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	> 312.5	> 468.75	>5000	Negative
Test 2	> 312.5	> 156.25	>5000	Negative
Present				
Test 1	> 625	> 625	>5000	Negative
Test 2	-	625	>5000	Negative

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

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

TEST FACILITY — SafePharm Laboratories Ltd (2006d)

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

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

RESULTS

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

Remarks - Results	The toxicity control attained 27% degradation after 14 days and 30% degradation after 28 days, therefore confirming that the test material was not toxic to the sewage treatment micro-organisms used in the study. The standard material, sodium benzoate, attained 62% degradation after 14 days and 61% degradation after 28 days thereby confirming the suitability of the test method and culture conditions.
CONCLUSION	The test material cannot be considered to be readily biodegradable under the strict terms and conditions of OECD Guideline 301D.
TEST FACILITY	SafePharm Laboratories Ltd (2005f)

8.2. Ecotoxicological investigations

8.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test–semi static. EC Directive 92/69/EEC C.1 Acute Toxicity for Fish – semi static
Species	Rainbow trout (*Oncorhynchus mykiss*) [juvenile]
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	Chemical analysis at 0, 24, 48, 72 and 96 hours
Remarks – Method	Following a preliminary range-finding test, fish were exposed, in groups of ten, to an aqueous solution of the test material over a range of concentrations for a period of 96 hours at a temperature of approximately 14°C under semi-static test conditions. An amount of test material (1136 g) was dissolved in dechlorinated tap water and the volume adjusted to give a 1000 mg ai/L stock solution. Aliquots were each separately dispersed in a final volume of 20 L to give the test concentrations. The concentration and stability of the test material in the test preparations were verified by chemical analysis at 0 hours (fresh media), 24 and 96 (old media) hours. The LC50 value and associated confidence limits were calculated by the trimmed Spearman-Karber method using the ToxCalc computer software package. When only one partial response is shown, the trimmed Spearman-Karber method is appropriate.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	-	10	0	0	0	0	0
1.0	-	10	0	0	0	0	0
1.8	-	10	1	1	1	1	1
3.2	-	10	10	10	10	10	10
5.6	-	10	10	10	10	10	10
10	-	10	10	10	10	10	10

LC50	2.3 mg/L at 96 hours. 95% CI = 2.0 – 2.5 mg/L
NOEC	1.0 mg/L at 96 hours.
Remarks – Results	Throughout the duration of the test the test preparations were observed to be red solutions increasing in colouration as concentration increased. Analysis of the test preparations at 0, 24 and 96 hours showed measured test concentrations to range from 89% to 103% of nominal and so it was considered justifiable to calculate the LC50 values in terms of the nominal test concentration only. No sub-lethal effects were observed.
CONCLUSION	The notified chemical was found to be toxic to rainbow trout.
TEST FACILITY	SafePharm Laboratories Ltd (2005g)

8.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - Static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	An amount of test material (568 mg) was dissolved in reconstituted water and the volume adjusted to give a 1000 mg ai/L stock solution, from which the test concentrations were derived.

RESULTS

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

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

EC50	51 mg/L at 48 hours (95% confidence level of 43 – 60 mg/L)
NOEC (or LOEC)	18 mg/L at 48 hours
Remarks – Results	No effects were observed at test concentrations of less than 18 mg/L. Analytical monitoring at 0 and 48 hours showed measured test concentrations to range from 85% to 101% of nominal and so the results are based on the nominal test concentrations only. The 1.8 mg ai/L test sample at 48 hours showed a measured test concentration of 79%, which was below the 80% limit allowed by the test guidelines. However, as this test concentration was below the NOEC it was considered that this result did not affect the outcome or integrity of the study. The control was observed to be a clear, colourless solution throughout the duration of the test. The 1.8 to 180 mg ai/L test concentrations were observed to be clear, pink/red solutions, increasing in colouration with increasing concentration, throughout the duration of the test.
CONCLUSION	The notified chemical is harmful to *Daphnia magna*.
TEST FACILITY	SafePharm Laboratories Ltd (2005h)

8.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test. EC Directive 92/69/EEC C.3 Algal Inhibition Test.
Species	*Scenedesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 1.0, 3.2, 10, 32, and 100 mg/L Actual: 87-103% of Nominal
Auxiliary Solvent	Nil
Analytical Monitoring	HPLC
Remarks - Method	A preliminary range-finding test was conducted following the modified algal test method for coloured test substances. The results obtained indicated that despite the use of a reduced test volume and increased light intensity significant inhibition of growth was observed. Therefore, it was considered appropriate to conduct the test following the methods described above and further refined for coloured test substances, to differentiate between a reduced growth of algae due to a true toxic effect of the chemical or due to an indirect effect, a reduction in growth by light absorption of the coloured test substance (Memmert *et al* 1994). Following preliminary range–finding tests, *Scenedesmus subspicatus* was exposed to an aqueous solution of the test material for 72 hours under constant illumination and stirred continuously via magnetic stirrer at a temperature of 24±1°C. The test was conducted using two experimental methods performed in parallel. **Experiment A** The algae were exposed to test material concentrations of 3.2, 10, 32, 100 and 320 mg/L. Glass Petri dishes above the test vessels contained the culture medium alone. Therefore, inhibition of algal growth in these test vessels was due to a combination of both the toxic effects of the test material and reduction in light intensity. **Experiment B** The glass Petri dishes above the test vessels contained the test material solutions at concentrations of 3.2, 10, 32, 100 and 320 mg/L. The test vessels contained algal cells in culture medium alone. Therefore inhibition of algal growth was due to a reduction in light intensity alone. The difference between the inhibition values obtained in Experiment A and B can be interpreted as the true toxic effect of the test material on the algal cells. Pre-culture gave an algal suspension in log phase growth characterised by a cell density of 1.77 x 10^6 cells per mL. This suspension was diluted to a cell density of 2.00 x 10^4 cells per mL prior to use. One way analysis of variance incorporating Bartlett's test for homogeneity of variance and Dunnett's multiple comparison procedure for comparing several treatments with a control was carried out on the area under the growth curve data for Experiments A and B at 72 h for the control and all test concentrations to determine any statistically significant differences between the test and control groups.

RESULTS

Experiment A

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

Experiment B

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

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

Remarks - Results	Given that greater inhibition of growth was observed in Experiment B it was considered that the effect of the test material on algal growth was probably due to a reduction in light intensity alone, not the intrinsic toxic properties of the test material Analysis of the test preparation at 0 and 72 hours showed measured test concentrations to range from 97% to 107% of nominal and so it was considered justifiable to estimate the EC50 values in terms of the nominal test concentrations only.
CONCLUSION	The notified chemical is at worst harmful to *Scenedesmus subspicatus*.
TEST FACILITY	SafePharm Laboratories Ltd (2005i)

8.2.4. *Lemna* growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	Draft OECD TG *Lemna*, Growth Inhibition Test (April 2004)
Species	*Lemna minor*
Exposure Period	7 days
Concentration Range	Nominal: 100 mg/L Actual: 88.8-101% of nominal
Auxiliary Solvent	Nil
Analytical Monitoring	HPLC
Remarks - Method	Following a preliminary range-finding test, *Lemna minor* was exposed to an aqueous solution of the test material at a concentration of 100 mg/L for a period of 7 days, under constant illumination at a temperature of 24±2°C. The test solutions were renewed on days3 and 5. The number of fronds in each control and treatment group was recorded on days 0, 3, 5, and 7, along with observations on plant development. An amount of test material (227 mg) was dissolved in culture medium and the volume adjusted to 2 litre to give a 100 mg/L test solution. This method of preparation was repeated in order to provide the required test concentrations for the media renewal on days 3 and 5. Statistical analysis of the yield data was carried out for the control and all test concentrations using one way analysis of variance incorporating Bartlett's test for homogeneity of variance and Dunnett's multiple comparison procedure for comparing several treatments with a control.

RESULTS

Response Variable	*Measurement Variable*	*E_rC50 (mg/L)*	*NOEC (mg/L)*
Average Specific Growth Rate	Frond Number	>100	100
	Dry Weight	>100	100
Yield	Frond Number	>100	100
	Dry Weight	>100	100

Remarks - Results	Analysis of the freshly prepared test concentrations on Day 0 and the old or expired test concentrations on Days 3, 5 and 7 showed measured test concentrations to be near nominal and hence the results are based on nominal test concentrations only.
CONCLUSION	The notified chemical was not found to be harmful to *Lemna minor*.
TEST FACILITY	SafePharm Laboratories Ltd (2005j)

8.2.5 Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test US EPA Draft Ecological Effects Test Guidelines OPPTS 850.6500.
Inoculum	Activated sewage sludge.
Exposure Period	3 hours
Concentration Range	Nominal: 10, 32, 100, 320, 1000 and 3200 mg/L
Remarks – Method	Following preliminary range-finding tests, activated sewage sludge was exposed to an aqueous solution of the test material at a range of concentrations for a period of 3 h at 21°C with the addition of a synthetic sewage as a respiratory substrate. The rate of respiration was determined after 30 minutes and 3 hours contact time and compared to data for the control and a reference material, 3,5-dichlorophenol. An amount of test material (7273 mg) was dissolved in water and the volume adjusted to 1000 mL to give a 6400 mg/L stock solution from which serial dilutions were made. Analysis of the concentration, homogeneity and stability of the test material in the test preparations was not required by the Test Guidelines. For the purpose of the test a reference material, 3,5-dichlorophenol was used.
RESULTS	
IC50	>3200 mg/L
NOEC	32 mg/L
Remarks – Results	The test validation criteria were satisfied. Observations made throughout the test period showed that at the test concentration of 1000 mg/L no undissolved test material was visible. Validation criteria were satisfied for the test.
CONCLUSION	The notified chemical is not harmful to activated sludge micro-organisms.
TEST FACILITY	SafePharm Laboratories Ltd (2005k)

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

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

9.1.2. Environment – effects assessment

Aquatic ecotoxicity data were provided for three trophic levels, with fish demonstrating the highest level of sensitivity to the notified chemical. The following Predicted No-Effect Concentration has been calculated using an assessment factor of 100.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (fish)	2.3	mg/L
Assessment Factor	100.00	
PNEC:	23	µg/L

9.1.3. Environment – risk characterisation

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

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River:	0.33	23	0.015
Q - Ocean:	0.03	23	0.001

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

9.2. Human health

9.2.1. Occupational health and safety – exposure assessment

The notified chemical will be imported in pre-packed sealed cartridges. During transport and storage, workers will not be exposed to the notified chemical except in the unlikely event that packaging and cartridges are accidentally breached.

There is low potential for office workers to be exposed to the notified chemical in inks (<5%

concentration) when replacing spent cartridges. Accidental contact is expected to be minimal, but may occur.

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

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

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

Daily exposure = (<0.01 (mg/event)×10) ÷ 70 = <u>~0.0014 mg/kg bw/day</u>.

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.

Service technicians may occasionally experience skin contact with the notified chemical (<5% in the ink) during maintenance of inkjet printers. However, their exposure is likely to be limited to contact of inks with their fingertips during the handling and cleaning of printer components. Therefore, the exposure of these workers is expected to be minimal and infrequent.

9.2.2. Public health – exposure assessment

Public exposure through importation, transportation or storage is expected to be negligible. Such exposure could only occur in the unlikely event of an accident where crates, boxes, packaging and cartridges were ruptured, liberating inks containing the notified chemical.

The exposure of the public to the notified chemical through the use of inkjet printer inks is expected to be identical to that experienced by office workers during the changing of cartridges, printing onto paper and other media, and handling dried, printed pages. Members of the public may be expected to change inkjet printer cartridges less frequently than would office workers, as domestic applications are often smaller.

9.2.3. Human health – effects assessment

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).

Sulfonation of azo dyes appears to decrease their toxicity by enhancing their urinary excretion, and that of their metabolites. Highly sulfonated azo dyes are generally poorly absorbed from the intestine after oral intake. However, cleavage of the azo linkage takes place in the gastrointestinal tract, through the action of azo reductases. The sulfonated aromatic amines are rapidly absorbed. Given the red-stained urine, faeces and staining of organs seen in the acute oral toxicity study (SafePharm, 2005b) and repeated dose oral toxicity study (SafePharm, 2006c), and the systemic effects observed in the repeated dose oral toxicity study (SafePharm, 2006c), it is clear that the notified chemical can be absorbed from the gastrointestinal tract following oral exposure.

Ultimately, the metabolites of azo dyes are excreted in the urine and bile (Brown & DeVito, 1993, referenced in Øllgaard *et al*, 1998). Staining observed on cage liners in the repeated dose oral toxicity study (SafePharm, 2006c) could be indicative of the urinary excretion of

metabolites of the notified chemical.

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 25 mg/kg bw/day on the basis of the treatment related changes observed in the kidneys and/or stomach at 150 and 400 mg/kg bw/day.

In addition, the notified chemical was found to be moderately irritating to the eye, though not severe enough to warrant hazard classification, and a slight irritant to the skin.

The notified chemical was not a skin sensitiser when tested up to a concentration of 25% in a mouse local lymph node assay. Relatively few azo dyes have been demonstrated to be skin sensitisers (Øllgaard *et al* 1998). Therefore, the notified chemical is unlikely to cause skin sensitisation in exposed humans.

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

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

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

In addition, azo dyes are renowned for their content of impurities, particularly for the presence of component arylamines (SCCNFP, 2002; Øllgaard *et al* 1998). Such impurities are thought to contribute to their carcinogenicity, as these species may be more readily absorbed, and activated as carcinogens. The HPLC trace provided by the notifier indicates that the sample of the notified chemical contains a number of impurities. The impurities have been identified to be isomers of the notified chemical. As such, the impurities are unlikely to contribute to carcinogenicity of the notified chemical.

The notifier supplied test results showing that the notified chemical was found to be not mutagenic in bacteria (under the conditions of the Ames test used), and 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).

The Ames test performed was a standard test, according to OECD Test Guideline 471. However, the test guideline strongly recommends the use of alternative procedures for the detection of mutagenicity of azo dyes, as it is recognised that the standard procedure is not sufficiently sensitive for these chemicals. Modified tests, such as that of Prival and Mitchell, utilise a reductive pre-incubation step (during which the azo dye is reduced to amine species) before the test is carried out (Prival and Mitchell, 1982). This modified test is thought to yield a greater detection of mutagenic azo dyes. Because of this, NICNAS has required the notifier to perform a Prival and Mitchell modified Ames test and provide data when available. Based on the result of this study, further testing may be requested from the notifier.

Based on the available data, the notified chemical is not classified as a hazardous substance in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC

2004).

9.2.4. Occupational health and safety – risk characterisation

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 <5%, is not likely to be toxic at the highest levels of probable exposure (worst-case exposure estimate of ~0.0014 mg/kg bw/day, compared with NOEL of 25 mg/kg bw/day), although it may cause slight eye and skin irritation.

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

9.2.5. Public health – risk characterisation

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.

10. CONCLUSIONS – ASSESSMENT LEVEL OF CONCERN FOR THE ENVIRONMENT AND HUMANS

10.1. Hazard classification

Based on the available data the notified chemical cannot be classified as hazardous under the NOHSC *Approved Criteria for Classifying Hazardous Substances*. The notified chemical is hazardous to the environment. However, the hazard classification for environmental effects is not mandated in Australia.

and

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

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

10.2. 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.

10.3. Human health risk assessment

10.3.1. Occupational health and safety

There is No Significant Concern to occupational health and safety under the conditions of the occupational settings described.

10.3.2. Public health

There is No Significant Concern to public health when used in the proposed manner.

11. MATERIAL SAFETY DATA SHEET

11.1. Material Safety Data Sheet

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

11.2. Label

The label for products containing the notified chemical provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Labelling of Workplace Substances* (NOHSC 1994). The accuracy of the information on the label remains the responsibility of the applicant.

12. 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*, 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.

13. 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.
- additional mutagenicity test data is to be provided to NICNAS when available.

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical, intended as a component (<5%) 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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