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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

FSM-003B 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:

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

Director
NICNAS

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FULL PUBLIC REPORT

FSM-003B in PictureMate Photo Cartridge T5852

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Epson Australia Pty Ltd (ABN 91 002 625 783) of 3 Talavera Rd, 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 names, CAS number, Molecular formula, Structural formula, Molecular weight, Spectral data, Methods of detection and determination, Purity, % Weight of Non-Hazardous Impurities, and Import volume

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
UK (2006)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
FSM-003B in PictureMate Photo Cartridge T5852

OTHER NAME(S)
Black azo dye J-16

METHODS OF DETECTION AND DETERMINATION

METHODS	*Identity (detection):*	UV/Visible spectroscopy, Infrared spectroscopy, ^{1}H nuclear magnetic resonance (NMR) and electrospray ionisation liquid chromatography-mass spectrometry (ESI LC-MS).
	Purity (assay):	HPLC
TEST FACILITY	Fuji Photo Film Co. Ltd. (2005)	

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 manufactured and formulated into inks overseas. It will be imported into Australia as a component of inkjet printer inks, contained within inkjet printer cartridges.

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% notified chemical).

The inks will be used by 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 offices and retailers 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
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 that they will be imported.

The cartridges will be transported and stored prior to national distribution where they will be used in office or home printing equipment. 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 hrs per day	70 days per year
Storage and transport	100	6 hrs per day	240 days per year
Office workers, service technicians, consumers	10,000	<0.1 hrs per day	20 day per day

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. The cartridges will be distributed to a number of outlets, where the cardboard cartons will be opened and boxes containing individual cartridges will be stacked on shelves.

Both office workers and service technicians may be exposed to the notified chemical in ink while

changing printer cartridges, and service technicians may additionally be exposed during printer maintenance. Replacement of printer cartridges involves removal of the old printer cartridge from the printing machine and directly loading the new cartridge. 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 occur when handling printed media before the ink is adequately dried, especially when printing on non-absorbent materials. One kilogram of pure dye would be expected to print several million A4 paper sheets of coloured text or graphics. Under worst-case conditions, each piece of A4 paper could be assumed to incorporate 1 mg of notified chemical. After printed inks are dry, the notified chemical will be bound to the paper or other media, and is not expected to be readily available to cause exposure.

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 open during transport, use, installation or replacement. Therefore, release of ink containing the notified chemical to the environment is not expected under normal conditions of use. Installation and replacement spills will be contained with absorbent and disposed of in landfill.

Cartridges are contained within the printer until the contents are used then they are removed and disposed of or potentially sent to a recycling and disposal centre. In the latter scenario, the cartridges would then be broken down in to component parts for recycling. Residual ink (<2% of the notified chemical) left in empty cartridges would be separated from the cartridge and incinerated during the recycling of the cartridges.

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

5.5. Disposal

The total import volume of the notified chemical will ultimately be disposed as normal office/domestic waste that will end up in either landfill or be incinerated. Some waste paper printed with the ink may be disposed of directly to landfill with the notified chemical bound to the paper. Some will enter the paper recycling process.

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

5.6. Public exposure

The exposure of the public to the notified chemical in inkjet printer inks is expected to be identical to that experienced by office workers using the same ink.

6. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20ºC and 101.3 kPa Black solid powder, forming an opaque black solution in water.

Melting Point/Freezing Point >300ºC at 100.7 kPa

METHOD Differential scanning calorimetry according to EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks The substance decomposed without melting, starting at ~300°C.
TEST FACILITY SafePharm (2005a)

Boiling Point Not determined

Remarks The notified chemical decomposed prior to melting.
TEST FACILITY SafePharm (2005a)

Density 1,560 kg/m^3 at 22.2 ± 0.5ºC

METHOD EC Directive 92/69/EEC A.3 Relative Density.
Remarks The relative density of the notified chemical was determined by gas comparison pycnometry.
TEST FACILITY SafePharm (2005a)

Vapour Pressure <0.72 x 10^{-8} kPa at 25ºC

METHOD EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks Value shown is the highest extrapolated estimate, based on readings at 248ºC.
TEST FACILITY SafePharm (2006a)

Water Solubility 19.6-21.6% (w/w) at 20.0 ± 0.5ºC

METHOD The method used was 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 (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 (℃)*	*$t_{½}$ <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 (2005a)

Partition Coefficient (n-octanol/water) log P_{ow} at 22ºC <-3.16

METHOD EC Directive 92/69/EEC A.8 Partition Coefficient.

Remarks Analytical Method: HPLC analysis. No significant deviations from the test protocol (Shake Flask Method) were reported.

It was evident from the information obtained in the hydrolysis test and data relating to the pH of the test material in water that negligible hydrolysis of the sample solutions occurred during the course of the test.

TEST FACILITY SafePharm (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.

The low adsorption properties of the notified chemical containing acidic functional groups that were determined by the HPLC estimation method were consistent with the extremely high water solubility and low partition coefficient characteristics observed in other tests. While the K_{oc} value is believed to accurately represent 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. In addition, the mobility of the notified chemical 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, the possible secondary interaction originating from the anionic charges present on the test material are not addressed.

TEST FACILITY SafePharm (2005a)

Dissociation Constant Not determined

METHOD OECD TG 112 Dissociation Constants in Water.

Remarks No determination was performed according to the test guideline, as the notified chemical contains no modes of dissociation within the range and scope of the method. The notified chemical is expected remain dissociated throughout the environmental pH range of 4-9.

TEST FACILITY SafePharm (2005a)

Particle Size

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

Range (µm)	*Method used*	*Mass (%)*
<100 µm	100 µm sieve	60.2
<10.2 µm	Cascade impactor	8.47
<5.4 µm	Cascade impactor	1.36

Remarks Too few particles were of a size <10.2 µm to allow for accurate measurement of the mass median aerodynamic diameter (MMAD).

TEST FACILITY SafePharm (2005a)

Flash Point Not determined.

METHOD EC Directive 92/69/EEC A.9 Flash Point.

Remarks Not applicable as the notified chemical is a solid with low vapour pressure.

Flammability Not highly flammable.

METHOD EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks No ignition was observed in a dried sample of the notified chemical during 2 minutes of an applied Bunsen burner flame.
TEST FACILITY SafePharm (2006a)

Autoignition Temperature 379ºC

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

Explosive Properties Does not have explosive properties.

METHOD EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks Tests for thermal, shock and friction sensitivity gave negative results.
TEST FACILITY SafePharm (2006a)

Reactivity

Remarks Based on the chemical structure and experience in use the test material is predicted to be stable under normal conditions. The notified chemical decomposes above 300ºC.

Surface Tension 72.6 mN/m at 21ºC (not surface-active)

METHOD EC Directive 92/69/EEC A.5 Surface Tension.
Remarks By the ISO 304 ring method, the surface tension of a 0.996 g/L solution of the notified chemical was determined using an interfacial tension balance with the result not being 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 (2005a)

Oxidising Properties Negative (predicted)

METHOD EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Remarks Based on the chemical structure of the notified chemical, it not predicted to display oxidising properties.
TEST FACILITY SafePharm (2006a)

7. TOXICOLOGICAL INVESTIGATIONS

Endpoint and Result		*Assessment Conclusion*
Rat, acute oral	LD50 > 2,000 mg/kg bw	low toxicity
Rat, acute dermal	LD50 > 2,000 mg/kg bw	low toxicity
Rabbit, skin irritation		slight irritant
Rabbit, eye irritation		slight irritant
Mouse, skin sensitisation (Local Lymph Node Assay)		non-sensitiser
Rat, oral repeat dose toxicity - 28 days.		NOEL = 25 mg/kg bw/day NOAEL = 150 mg/kg bw/day
Genotoxicity - bacterial reverse mutation		non mutagenic
Genotoxicity – *in vitro* mammalian cytogenicity		non genotoxic

7.1. Acute toxicity – oral

TEST SUBSTANCE Notified chemical (85.6% pure)

METHOD — OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
EC Directive 92/69/EEC B.1tris Acute Oral Toxicity – Acute Toxic Class Method.

Species/Strain	Rat/Sprague-Dawley CD
Vehicle	Distilled water
Remarks - Method	Animals were evaluated for deaths or overt signs of toxicity 30 mins and 1, 2 and 4 hours after dosing, then once daily for fourteen days. At the end of the observation period, the animals were sacrificed and subjected to gross pathological examination.

RESULTS

Group	*Number and Sex of Animals*	*Dose* mg/kg bw*	*Mortality*
1	3F	300	0
2	3F	2,000	0
3	3F	2,000	0

* Administered to result in dose of the pure chemical shown.

LD50	>2,000 mg/kg bw
Signs of Toxicity	No signs of systemic toxicity were observed in animals treated with 300 mg/kg bw. At 2,000 mg/kg bw, hunched posture, pilo-erection and black-stained urine and faeces were observed. All adverse effects were reversible 3-4 days after dosing. No changes in the animals' bodyweight were observed at any dose level.
Effects in Organs	No abnormalities were observed at necropsy.
Remarks - Results	None.

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

TEST FACILITY — SafePharm (2005b)

7.2. Acute toxicity – dermal

TEST SUBSTANCE — Notified chemical (85.6% pure)

METHOD — OECD TG 402 Acute Dermal Toxicity – Limit Test.
EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal) – Limit Test.

Species/Strain	Rat/Sprague-Dawley CD
Vehicle	Dried arachis oil BP
Type of dressing	Semi-occlusive
Remarks - Method	The notified chemical was applied to ~10% of the total skin surface area of the test animal.

RESULTS

Group	*Number and Sex of Animals*	*Dose* mg/kg bw*	*Mortality*
1	5M	2,000	0
2	5F	2,000	0

* Administered to result in dose of the pure chemical shown.

LD50	>2,000 mg/kg bw
Signs of Toxicity - Local	No erythema, eschar or oedema was observed at the site of application. Grey-coloured staining was observed at all treatment sites, which persisted for 3-4 days in all animals.
Signs of Toxicity - Systemic	None. All animals showed the expected gains in body weight over the observation period.
Effects in Organs	No abnormalities were observed at necropsy.
Remarks - Results	None.

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

TEST FACILITY SafePharm (2006b)

7.4. 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	Three males
Vehicle	Distilled water
Observation Period	7 days
Type of Dressing	Semi-occlusive.
Remarks - Method	The test animals were exposed on the dorsal/flank area to the notified chemical for 3 minutes, 1 hour, or 4 hours. Observations were made at 1, 24, 48 and 72 hours, and at 7 days after exposure. The purity of the chemical is assumed to be the same as in the acute toxicity studies as it is the same batch.

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	0.67	1	1	72 hours	0
Oedema	0	0	0.67	1	48 hours	0

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

Remarks - Results The notified chemical induced a primary irritation index of 0.7.
All effects were fully reversible by 7 days after exposure.

CONCLUSION The notified chemical is a slight irritant to the skin.

TEST FACILITY SafePharm (2005c)

7.5. Irritation – eye

TEST SUBSTANCE Notified chemical

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

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 males
Observation Period	72 hours
Remarks - Method	The notified chemical was applied as a 10% w/w aqueous preparation, with a pH of 6.8. The purity of the chemical is assumed to be the same as in the acute toxicity studies as it is the same batch. Slight to moderate initial pain reactions were experienced by the test animals upon installation of the notified chemical solution. Local anaesthetic was administered to the third animal, 1 to 2 minutes before treatment.

RESULTS

Lesion	*Mean Score* Animal No.*	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*

	1	2	3			
Conjunctiva: redness	0	0	0	1	1 hour	0
Conjunctiva: chemosis	0	0	0	1	1 hour	0
Conjunctiva: discharge	0	0	0	1	1 hour	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

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

Remarks - Results: Black staining of the fur was observed around all treated eyes during the observation period.

CONCLUSION: The notified chemical is a slight irritant to the rabbit eye.

TEST FACILITY: SafePharm (2005d)

7.6. 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 Skin Sensitisation (Local Lymph Node Assay).

Species/Strain: Mouse/CBA/Ca

Vehicle: Dimethyl formamide (DMF)

Remarks - Method: The purity of the chemical is assumed to be the same as in the acute toxicity studies as it is the same batch. A laboratory historical positive control was used (recent relative to the study date).

A preliminary screening study on a single mouse, treated with 25% w/w notified chemical, was performed to determine its toxicity potential. This mouse was treated on the dorsal surface of the ear daily for three days.

In the main test, three groups of four mice were treated on the dorsal surface of the ear with 5, 10, or 25% (w/w) notified chemical in DMF for three consecutive days. A fourth group was treated with DMF alone. Five days after administration, all mice were injected with ^{3}H-methyl thymidine, five hours after this injection, the mice were sacrificed and their lymph node cells extracted.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (SI) (Test/Control Ratio)*
Notified chemical		
0 (vehicle control)	708.83	N/A
5	1091.19	1.54
10	1209.80	1.71
25	1710.91	2.41
α-hexylcinnamaldehyde (positive control)		
5	Unknown	2.64
10	Unknown	8.36
25	Unknown	12.94

Remarks - Results: No deaths or signs of systemic toxicity were observed in any animals during the study. Black staining of the fur and ears was noted one hour post-dosing on all three days.

Despite an apparent dose-dependency in the observed SI values, the notified chemical is considered to be a non-sensitiser as no SI value was greater than 3 (in accordance with the test guideline).

CONCLUSION: There was no evidence of induction of a lymphocyte proliferative

response indicative of skin sensitisation to the notified chemical.

TEST FACILITY SafePharm (2005e)

7.7. Repeat dose toxicity

TEST SUBSTANCE Notified chemical (>80% pure)

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).
US EPA Health Effects Test Guidelines, OPPTS 870.3050 Repeated Dose 28-Day Oral Toxicity Study in Rodents.

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

Route of Administration Oral – gavage

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

Vehicle Dried arachis oil BP

Remarks - Method Due to deterioration in the health of the animals at the highest dose level (750 mg/kg bw/day), the dosage of this group was ceased at Day 8, then recommenced at Day 10 at a reduced dose of 600 mg/kg bw/day. To sustain the integrity of the test, the 500 mg/kg bw/day dosage group also received a reduced dose (400 mg/kg bw/day) from Day 10.

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 I)	5M, 5F	150	0
IV (mid dose II)	5M, 5F	500 (days 1-9) 400 (days 10-28)	1M
V (high dose)	5M, 5F	750 (days 1-8) 600 (days 10-28)	5M, 3F
VI (control recovery)	5M, 5F	0	0
VII (high dose recovery)	5M, 5F	750 (days 1-8) 600 (days 10-28)	0

Mortality and Time to Death

One male of Group IV was found dead on Day 4. Three females and two males of Group V were killed *in extremis* on Day 12 and similarly another male was killed on Day 14. Two further males from this group were killed *in extremis* on Day 26.

Clinical Observations

Black staining of fur was observed in all animals treated with ≥150 mg/kg bw/day, persisting until Day 42 amongst high dose recovery animals. Black staining of faeces was observed in mid dose II and high dose groups, and incidents of pink-stained cage liners were observed in these animals.

In high dose animals, from Day 8, increased salivation after dosing and hunched posture was observed, along with isolated incidences of tip-toe gait, dehydration and tail elevation. These animals were not treated on Day 9 due to excessive bodyweight losses and increased water consumption. Following dosage reduction on Day 10, the condition of these animals continued to deteriorate, and hunched posture, dehydration, tiptoe gait, piloerection and emaciation led to the killing of 5 animals *in extremis*. Prior to termination, incidences of tail elevation and body tremors were observed. These effects were observed until the end of treatment in these groups. Following cessation of treatment, a complete regression of these signs was apparent.

Isolated incidences of hunched posture, noisy and decreased respiration were seen in mid dose II animals, but these effects were not observed in lower dose groups (≤150 mg/kg bw/day).

Functional Observations

A higher incidence of defecation and urination was observed in animals of groups mid dose II or higher during the first two weeks of treatment. No effects were observed in lower dose groups (≤150 mg/kg bw/day).

Reductions in food consumption were observed in high dose animals during the first two weeks of treatment. In these animals, and in mid dose II, substantial decreases in water consumption were also observed, persisting throughout the treatment period and for the first week following treatment (in the recovery group).

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Increased urine volume of lower specific gravity was detected for animals in animals of groups mid dose II or higher.

No changes in haematological parameters were observed.

Increases in blood cholesterol were evident for animals treated with ≥150 mg/kg bw/day. Females of Groups treated with ≥400 mg/kg bw/day showed elevated blood bilirubin. High dose males showed elevated creatinine levels. Males treated with ≥400 mg/kg bw/day showed reduced plasma chloride concentrations. No effects were observed in animals treated with 25 mg/kg bw/day.

Effects in Organs

General dark and black discolouration of various organs was observed in all animals treated with ≥150 mg/kg bw/day. The gastrointestinal tract also contained black contents at these dosage levels.

Liver: Males treated with ≥150 mg/kg bw/day were found to have increased liver weights. Histopathology showed centrilobular hepatocyte enlargement in animals of either sex treated with ≥400 mg/kg bw/day. Generalised hepatocyte enlargement was seen in four of the animals that were killed *in extremis*.

Kidney: High dose animals showed an increase in relative kidney weights relative to control animals. These animals were found to have hypertrophy and basophilia of the collecting duct epithelium, occasionally with associated dilatation of tubules and some groups of tubules with higher grades of basophilia. All females and one male of the mid dose II group also showed these changes in renal tubules. Partial regression of these effects was observed by the end of the recovery period.

Urinary bladder: Hyperplasia of the transitional epithelium was observed in two interim death males and two interim death high dose females, as well as in one female in each of the recovery control and treatment groups.

Adrenal glands: Vacuolisation of the cortical zona glomerulosa was observed in four males and one female of the interim death high dose animals. One male of the high dose recovery group also displayed similar vacuolisation.

Colon: Mucosal hypertrophy and basophilia were observed for three premature death males and in one premature death high dose female.

Stomach: Upon necropsy, a raised limiting ridge of the gastric epithelia was observed in animals treated with ≥150 mg/kg bw/day. Histopathology revealed acanthosis/hyperkeratosis of the limiting ridge, agglomeration of secretion in mucosal cells, and superficial mucosal atrophy/basophilia in all animals of the high and mid dose II groups, as well as in all females and three males of the mid dose I group. Regression of gastric changes was observed amongst the animals of the high dose recovery group.

Thymus: Lymphoid atrophy was observed in premature death animals - five males and two females of the high dose group – and in one surviving female of the high dose recovery group.

Mesenteric lymph node: Sinus histiocytosis was observed for two males and three females treated with the highest dose, and four of these animals had died prematurely.

Bone marrow: Generally severe adipose infiltration of the marrow, indicative of marrow hyperplasia, was seen amongst animals of both sexes in the high dose groups.

Seminal vesicles: Reduced secretory content was observed in the seminal vesicles for four males treated with the high dose, three of which died prematurely, and in two males of the mid dose II group, one of which died prematurely. One recovery control male

showed a similar effect, which was not observed in either of the surviving high dose recovery animals.

Remarks – Results

The majority of adverse effects were observed in animals treated with the higher doses, ≥400 mg/kg bw/day. The majority of the findings at these doses seemed to be related to the irritant nature of the notified chemical (eg stomach and renal tubule changes), or were adaptive in nature (eg hepatocyte enlargement).

Although treatment-related effects were observed at 150 mg/kg bw/day, these were considered to be lower in severity and more reversible than those observed at higher dosage levels. Changes in organs at this dose, and the changes observed microscopically, were considered adaptive changes. Due to the absence of degenerative changes, the effects observed at this dose were not considered adverse effects.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 150 mg/kg bw/day in this study, based on the absence of the serious adverse effects seen at higher doses.

The No Observed Effect Level (NOEL) was considered to be 25 mg/kg bw/day in this study, based on the absence of any toxicologically relevant effects at this dose.

TEST FACILITY SafePharm (2006c)

7.8. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

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

Species/Strain *S. typhimurium*: TA1535, TA1537, TA98, TA100
E. coli: WP2uvrA

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

Concentration Range in Main Test
a) With metabolic activation: 50-5,000 µg/plate
b) Without metabolic activation: 50-5,000 µg/plate

Vehicle DMSO

Remarks - Method 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	>5,000	>5,000	>5,000	None
Test 2	-	>5,000	>5,000	None
Present				
Test 1	>5,000	>5,000	>5,000	None
Test 2	-	>5,000	>5,000	None

Remarks - Results A blue colour in the plates was observed at doses of ≥50 µg/plate, but this did not interfere with the scoring of the revertant colonies. The positive controls yielded positive results, indicating that the test system was functioning appropriately.

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. It has been observed that many azo dyes are found to be non-

mutagenic in standard Ames tests, but mutagenic when a modified test is used (Øllgaard *et al*, 1998).

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 (2005f)

7.9. Genotoxicity – *in vitro*

TEST SUBSTANCE — Notified chemical (85.6% pure)

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) cell line
Metabolic Activation System	Phenobarbitone/β-naphthoflavone-induced rat liver S9 microsome mix
Vehicle	Eagle's Minimal Essential Medium (MEM)
Remarks - Method	Positive controls used were mitomycin C (0.05-0.1 µg/mL; used without metabolic activation) and cyclophosphamide (5 µg/mL; used with metabolic activation)

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 39.06*, 78.13*, 156.25*, 312.5, 625, 1250	6 hrs	24 hrs
Test 2	0*, 39.06, 78.13*, 117.19*, 156.25*, 234.38, 312.5	24 hrs	24 hrs
*Present***			
Test 1	0*, 39.06*, 78.13*, 156.25*, 312.5, 625, 1250	6 hrs	24 hrs
Test 2	0*, 39.06, 78.13*, 156.25*, 312.5*, 468.75, 625	6 hrs	24 hrs

*Cultures selected for metaphase analysis.
** S9 mix was used at 5% in Test 1 and 2% in Test 2.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	>1,250	>156.25	>1,250	negative
Test 2	>156.25	>156.25	>312.5	negative
Present				
Test 1	>1,250	>312.5	>1250	negative
Test 2	>1,250	>312.5	>625	negative

Remarks - Results — Low-level structural chromosomal aberrations, at higher levels than those observed in the negative controls, were observed in Test 1 at 39.06 µg/mL notified chemical in the absence of S9 mix, and at >156.25 µg/mL in the presence of S9. This result was also seen in Test 2 at 156.25 µg/mL with and without S9 mix. However, none of these apparent increases over control levels was found to be statistically significant, and in some 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 (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 microorganisms
Exposure Period	28 days
Auxiliary Solvent	Nil
Analytical Monitoring	Dissolved Oxygen
Remarks - Method	An amount of test material (117 mg) was dissolved in culture medium with the aid of ultrasonication for approximately 5 minutes and the volume adjusted to 100 mL to give a 1000 mg/L stock solution. An aliquot (30 mL) of this stock solution was dispersed in a final volume of 6 L of inoculated culture medium to give a test concentration of 5.0 mg/L. For the purposes of the test, a standard material, sodium benzoate, was used.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
3	6	3	59
7	11	7	73
14	11	14	80
21	11	21	81
28	11	28	82

Remarks - Results	The toxicity control attained 26% degradation after 14 days thereby confirming that the test material was not toxic to the sewage treatment microorganisms used in the study. The standard material, sodium benzoate, attained 80% degradation after 14 days and 82% degradation after 28 days thereby confirming the suitability of the test method and culture conditions.
CONCLUSION	The test material cannot be considered to be readily biodegradable under the strict terms and conditions of OECD Guideline 301D.
TEST FACILITY	SafePharm (2006e)

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	*Oncorhynchus mykiss*
Exposure Period	96 h
Auxiliary Solvent	Nil
Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	HPLC analysis of test concentrations.
Remarks – Method	Based on the results of the range-finding test, a "limit test" was conducted at a concentration of 100 mg/L to confirm that at the maximum concentration given in the OECD/EEC Test Guidelines, no mortalities or sub-lethal effects of exposure were observed. An amount of test material (4,680 mg) was dissolved in dechlorinated tap water and the volume adjusted to 1 L to give a 4,006 mg/L stock solution. An aliquot (500 mL) was further diluted in a final volume of 20 L and stirred using a flat bladed mixer for approximately 1 minute to give the 100 mg/L test concentration.

RESULTS

Nominal Concentration (mg/L)	Number of Fish	Mortality				
		3 h	24 h	48 h	72 h	96 h
100	20	0	0	0	0	1

LC50	>100 mg/L at 96 hours.
NOEC	100 mg/L at 96 hours.
Remarks – Results	The single mortality observed in the 100 mg/L test concentration at 96 h was considered to be due to natural causes and or handling stress and not a toxic effect. The control was observed to be a clear, colourless solution throughout the duration of the test. The 100 mg/L test preparation was observed to be a black coloured solution throughout the duration of the test. Analysis of the test preparation at 0, 24 and 96 h showed measured test concentrations to range from 100% to 114% of nominal and so it was considered justifiable to estimate the LC50 values in terms of the nominal test concentrations only.
Conclusion	The notified chemical is not harmful to *Oncorhynchus mykiss*.
Test Facility	SafePharm (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. EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia – static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Nil
Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	HPLC analysis of test concentrations.

Remarks - Method

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

An amount of test material (117 mg) was dissolved in dechlorinated tap water and the volume adjusted to 1 L to give the 100 mg/L test concentration.

RESULTS

Nominal Concentration (mg/L)	*Number of D. magna*	*Number Immobilised*	
		24 h	*48 h*
100	20	0	0

LC50 >100 mg/L at 48 hours

NOEC 100 mg/L at 48 hours

Remarks - Results

No immobilisation was observed at the test concentration of 100 mg/L. It was considered unnecessary and unrealistic to test at concentrations in excess of 100 mg/L. The control test media was observed to be a clear colourless solution and the 100 mg/L test media was observed to be a black coloured solution throughout the duration of the test.

Analysis of the test preparation at 0, 24 and 48 h showed measured test concentrations to range from 105 to 111% of nominal and so it was considered justifiable to estimate the LC50 values in terms of the nominal test concentrations only.

CONCLUSION

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

TEST FACILITY

SafePharm (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 increase 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 1.0, 3.2, 10, 32, and 100 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 1.0, 3.2, 10, 32, and 100 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 2.44×10^6 cells/mL. This suspension was diluted to a cell density of 2.48×10^4 cells/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 concentration to determine any statistically significant differences between the test and control groups.

RESULTS

Experiment A

Biomass		*Growth*	
E_bC50 mg/L at 72 h	*NOEC mg/L*	*E_rC50 mg/L at 72 h*	*NOEC mg/L*
2.7 (95% CI: 2.0-3.7)	1.0	34*	1.0

*It was not possible to calculate the 95% confidence limits for the E_rC50 value, as the data generated did not fit the models available for the calculation of confidence limits.

Experiment B

Biomass		*Growth*	
E_bC50 mg/L at 72 h	*NOEC mg/L*	*E_rC50 mg/L at 72 h*	*NOEC mg/L*
4.4 (95% CI: 3.6-5.5)	1.0	26 (95% CI: 18-37)	1.0

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 103% to 108% of nominal and so it was considered justifiable to estimate the EC50 values in terms of the nominal test concentrations only. It was considered unnecessary and unrealistic to test at concentrations in excess of 100 mg/L.

The cell concentration of the control cultures increased by a factor of 26-37 after 72 hours, which was in line with the OECD Guideline, which states that any enhancement must be at least by a factor of 16 after 72 hours.

CONCLUSION

The notified chemical is at worst harmful to *Scenedesmus subspicatus.*

TEST FACILITY SafePharm (2006f)

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: 1.0, 3.2, 10, 32, and 100 mg/L
Actual: 87-103% 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 range of concentrations for a period of 7 days, under constant illumination at a temperature of 24±2°C. The test solutions were renewed on days 2 and 5. The number of fronds in each control and treatment group was recorded on days 0, 2, 5, and 7, along with observations on plant development.

Amounts of test material (117 and 37 mg) were each separately dissolved in culture medium and the volume adjusted to 1 litre to give 100 and 32 mg/L test solutions respectively. A series of dilutions was made from these test solutions to give further test solutions. This method of preparation was repeated in order to provide the required test concentrations for the media renewal on days 2 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	3.2
	Dry Weight	>100	3.2
Yield	Frond Number	>100	3.2
	Dry Weight	59	3.2

Remarks - Results Analysis of the freshly prepared test concentrations on Day 0 and the old or expired test concentrations on Days 2, 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 is harmful to *Lemna minor*.

TEST FACILITY SafePharm (2006g)

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: 1,000 mg/L
Remarks – Method	Based on the results of a range-finding test, a "limit test" was conducted at a concentration of 1,000 mg/L (three replicates) to confirm that at this concentration no effect on respiration of the activated sewage sludge was observed. An amount of notified chemical (2,336 mg) was dissolved in water and the volume adjusted to 100 mL to give a 2000 mg/L stock solution. An aliquot (250 mL) of this stock solution was dispersed with synthetic sewage (16 mL), activated sewage sludge (200 mL) and water, to final volume of 500 mL, to give the require concentration of 1,000 mg/L. Analysis of the concentration, homogeneity and stability of the notified chemical in the test preparations was not appropriate to the Test Guidelines. For the purpose of the test a reference material, 3,5-dichlorophenol was used.
RESULTS	
IC50	>1,000 mg/L
NOEC	1,000 mg/L
Remarks – Results	The test validation criteria were satisfied. Observations made throughout the test period showed that at the test concentration of 1,000 mg/L no undissolved notified chemical was visible. Validation criteria were satisfied for the test.
CONCLUSION	The notified chemical is not harmful to activated sludge microorganisms.
TEST FACILITY	SafePharm (2005i)

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

Manufacture, reformulation and packaging into end-use containers occurs overseas, and release is not expected. After use, printed paper may be disposed of by incineration, to landfill or be recycled. The notified chemical which is 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	200.0	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	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 algae 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 (algae)	34	mg/L
Assessment Factor	100	
PNEC	340	µg/L

9.1.3. Environment – risk characterisation

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

Risk Assessment	PEC (µg/L)	PNEC (µg/L)	Q
Q - River	0.33	340	0.001
Q - Ocean	0.03	340	0.000

These quotients indicate that the proposed import volume and use pattern are expected to pose an acceptable 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. The design of the cartridges is expected to be such that they can be easily replaced without dermal exposure to ink. Accidental contact is expected to be minimal, but may occur. Workers are expected to avoid direct contact with inks to avoid staining of their skin and/or clothing.

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 binding of the notified chemical within the ink matrix on media is expected to be based on the other components of the ink (eg polymers) and the components and properties of the media (eg absorbency, hydrophobicity, paper coatings or other ingredients). The extent of binding is therefore likely to be a combination of how well integrated the notified chemical will become with these components upon drying of the ink. The components of the ink are likely to be more important for the purposes of risk assessment, as a wide range of media is available for use inkjet printers. It is expected that overall, inks will be designed to maximise fastness of dye components, to extend the life of inkjet prints. Therefore, minimal dye might be expected be released from dried prints upon contact with skin.

The most probable exposure of office workers to the notified chemical will be to wet ink on freshly printed media. 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. The exposure of these workers to the notified chemical might be expected to be quite frequent; however, these workers may service a great variety of different inkjet printers during a day, most of which would not contain inks formulated with the notified chemical. In addition, 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 extremely 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, as intact azo dyes often do not penetrate the skin because of their size and polarity (Øllgaard *et al* 1998). These 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).

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 (see below). The sulfonated aromatic amines are rapidly absorbed. Given the black-stained urine and dark staining of organs seen in the acute oral toxicity study (SafePharm, 2005b), and the systemic effects observed in the repeated dose oral toxicity study (SafePharm, 2006c), it is clear that the notified chemical can be absorbed, likely intact, 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). The pink 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 NOAEL in a 28-day oral repeat dose study in rats was 150 mg/kg bw/day on the basis of a lack of toxic effects at or below this dose level, and the NOEL was 25 mg/kg bw/day. Systemic adverse effects were observed in experimental animals following moderate to high oral doses of the notified chemical, suggesting that it may display weakly toxic properties.

In addition, the notified chemical was found to be a mild irritant, when administered in high

concentrations to the skin or eye. In addition, many of the adverse effects observed in the test animals of the repeat dose oral study were thought to be due to the irritant nature of the notified chemical.

The notified chemical was not a skin sensitiser, as shown in a mouse local lymph node assay. Several azo dyes have been demonstrated to be skin sensitisers in humans, using clinical patch tests, and others associated with causing allergic contact dermatitis (Øllgaard *et al* 1998). However, the structure and chemistry of the notified chemical do not resemble the structures of these sensitising azo dyes. Therefore, in combination with the negative mouse LLNA test result, the notified chemical is unlikely to cause skin sensitisation in exposed humans.

Mutagenicity:

Azo dyes as a class 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 enzymatic 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 catalysing reductive cleavage of the azo bond.

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, all of which potentially could be mutagenic. The structure of two of these arylamine species resembles those of known human carcinogens (SCCNFP, 2002; RoC, 2005). However, the significant structural modification of these species indicates that they are likely to be of lower concern as potential carcinogens (SCCNFP, 2002; US EPA, 2002).

In addition, azo dyes are renowned for their content of impurities, particularly for the presence of component arylamines (SCCNFP, 2002; Øllgaard *et al* 1998). Such impurities are thought to contribute to their carcinogenicity, as these species may be more readily absorbed, and activated as carcinogens. The HPLC trace provided by the notifier indicates that the sample of the notified chemical used contains low levels of contaminants that are more hydrophobic than the notified chemical itself (Fuji Photo Film Co. Ltd., 2005). The identity of these species is unknown, but these may be aromatic amine species.

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 it is available. Based on the result of this study, further testing may be requested from the notifier.

Conclusion:

Based on the currently available data, the notified chemical cannot be 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

The notified chemical will be imported in pre-packed sealed cartridges. In addition, the notified chemical is present in the ink at <5%, and 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 a NOAEL of 150 mg/kg bw/day). Therefore, discounting any possibility of a risk from carcinogenicity, the expected risk of the notified chemical to the health and safety of workers is expected to be minimal.

During most operations, the probable exposure of workers to the notified chemical is expected to be low, and thus the probable OHS risk is likely to be low. Transport, storage and retail workers are unlikely to be exposed to the notified chemical except when cartridges are accidentally breached. There is low potential for office workers to be exposed to the notified chemical when replacing spent cartridges, as the notified chemical is sealed within the cartridge, and the cartridges are designed to prevent leakage. Service technicians will generally experience only infrequent exposure to the notified chemical, at levels below the levels of exposure indicated by the worst-case estimate above.

Likewise, the exposure of workers to the notified chemical on dried, printed paper is expected to be low, as the dye should remain bound within the ink matrix. Therefore, the risk to workers handling dried inkjet prints containing the notified chemical is expected to be minimal if not negligible.

However, the OHS risk of the notified chemical cannot be established without a consideration of its likelihood of degradation into potentially carcinogenic aromatic amines. Breakdown of similar azo dyes to the notified chemical has been reported, following their exposure to heat or sunlight (Brown and DeVito, 1993, referenced in Øllgaard *et al* 1998). Given that inkjet prints are likely to be exposed to light for prolonged periods, and that preparations of the notified chemical may already contain traces of aromatic amine species, the risk presented by these species must be considered.

Chemicals which may release specific carcinogenic amines (as specified the Appendix of EU SCCNFP/0495/01) upon azo reduction are restricted in the EU (SCCNFP, 2002):

> "*Azo-dyes that may release, by reductive cleavage of one or more azo groups, one or more of the aromatic amines listed in Appendix, in concentrations above 30 ppm in the finished articles, according to the testing method specified in Appendix, may not be used in textile and leather articles which have the potential of coming into direct and prolonged contact with the human skin or oral cavity.*"

While the notified chemical is not expected to break down into one of the SCCNFP-specified arylamines, it is expected to break down into species that resemble these arylamines. These species may have very different properties to the notified chemical, and they may not be as readily bound, for example, within the matrices of a dried inkjet print. It is not possible to derive any conclusions without greater knowledge of their properties. Nevertheless, the expected exposure to these species is expected to be less than that specified by the SCCNFP (30 ppm), and is likely to be less frequent than "direct and prolonged". Therefore, the risk from these breakdown species is expected to be low.

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.*

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***
Environment	Acute Category 3	Harmful to Aquatic Life
	Chronic Category 3	Harmful to Aquatic Life with long lasting effects

10.2. Environmental risk assessment

On the basis of the PEC/PNEC ratio, the notified 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

The notified chemical is expected to present an acceptable risk to occupational health and safety under the conditions of the occupational settings described.

10.3.2. Public health

The notified chemical presents an acceptable risk to public health when used as a dye component of inkjet printer inks.

11. MATERIAL SAFETY DATA SHEET

11.1. Material Safety Data Sheet

The MSDS of the notified chemical and products containing the notified chemical provided by the notifier were in accordance with the NOHSC *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC 2003). They are 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 the product 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

- A copy of the MSDS should be easily accessible to employees.
- Service personnel should wear cotton or disposable gloves during routine maintenance and repairs of inkjet printers.
- 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 or eye contact with ink.

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.

12.1. Secondary notification

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

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical as a component of 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;
- 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.

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