File No: LTD/1316

June 2007

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

E-696 in Ink 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

TABLE OF CONTENTS

FULL PUBLIC REPORT

E-696 in Ink Cartridge T5852

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Epson Australia Pty Ltd (ABN 91 002 625 782)
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, Other name, Molecular Formula, Structural formula, Molecular weight, Spectral Data, Purity, Impurities, Import Volume

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT
None

NOTIFICATION IN OTHER COUNTRIES
VIIA Notification in the EU (UK) and a VIIA Notification in Switzerland

2. IDENTITY OF CHEMICAL

MARKETING NAME
Ink Cartridge T5852 (containing the notified chemical at <5%)

3. COMPOSITION

DEGREE OF PURITY
>80%

HAZARDOUS IMPURITIES
None

METHODS OF DETECTION AND DETERMINATION

UV-Visible, IR and ^{1}H-NMR Spectroscopy data were provided.

4. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported into Australia as a component in pre-packed, sealed ink cartridges at concentration of <5%.

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 is used in water-soluble ink at <5% for use with plain paper. It will be used in home or office printing equipment.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, transport and storage

PORT OF ENTRY
Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
The ink cartridges will be distributed to potentially thousands of offices and homes nation wide from the premises of retail distributors.

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a component of a ready to use sealed ink cartridge (plastic shell), containing between 5 and 100 ml of formulated ink and individually packaged in cardboard boxes. The cartridges will be transported by road.

5.2. Operation description
No reformulation or repackaging of the product occurs in Australia. The product is delivered to distributors as it is imported into Australia then sold to end-users. The cartridges will be installed/replaced by either service technicians, office workers, or the general public.

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 workers/Service technicians	10000	<0.1 hours per day	

Exposure Details
Office workers and service technicians will replace spent ink cartridges at workplaces. Replacement of printing cartridges involves removal of the old printing cartridge from the printing machine and directly loading the new cartridge. These workers may have dermal contact with very small quantities of the notified chemical if they touch the print heads when removing the sealing tape to replace spent cartridges. Exposure is also possible by handling printed papers before the ink is adequately dried or if printing to a non-absorbent substrate occurs as an error. After the ink is dry the notified chemical is bound to the paper and is not expected to be readily bioavailable. Dermal and possible ocular exposure could occur when handling faulty or ruptured cartridges.

5.4. Release

RELEASE OF CHEMICAL AT SITE
Printer ink will be imported in ready-to-use cartridges (containing <5% of the 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. 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 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

When changing ink cartridges, exposure to small amounts of the notified chemical may occur, however this task will be relatively infrequent. After the ink is dry the notified chemical is bound to the paper and is not expected to be bioavailable. While handling printed- paper or other substrates where the ink is only partially dried there may be small exposure of the skin to ink containing the notified chemical at <5%.

6. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20ºC and 101.3 kPa Black solid

Melting Point 358ºC with decomposition

METHOD	EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks	The test material has been determined to decompose prior to melting by differential scanning calorimetry. Statement of GLP
TEST FACILITY	Safepharm (2005a)

Density 1.81 kg/m^3 at 19.5ºC

METHOD	EC Directive 92/69/EEC A.3 Relative Density.
Remarks	Determined by gas comparison pycnometer. Statement of GLP
TEST FACILITY	Safepharm (2005a)

Vapour Pressure < 5.0 x 10^{-8} kPa at 25ºC

METHOD EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks Measured with a vapour pressure balance at 220 – 230°C and extrapolated. Statement of GLP
TEST FACILITY Safepharm (2005b)

Surface Tension 71.6 mN/m at 20.6 ± 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 0.887 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. Statement of GLP
TEST FACILITY Safepharm (2005a)

Water Solubility 54.0 – 56.0% w/w at 20 ± 0.5°C

METHOD EC Directive 92/69/EEC A.6 Water Solubility
Remarks Flask Method. The standard A6 Method was not applicable to this test material due to the high indeterminable saturation levels produced. It was therefore not possible to prepare samples at five times the saturation level as recommended in the guideline. No analysis could be performed due to the high solubility producing unfilterable mixtures and thus water solubility was estimated based on visual inspection.

During the shaking of samples 1 to 6 of the definitive test, there was a power failure overnight for an indeterminable period. However, the bath was at the correct shaking speed and temperature when checked in the morning after the event. As none of these samples had reached saturation, this was considered to have had a negligible effect on the test.

Statement of GLP
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 (°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 50°C in buffers of pH 4, 7 and 9, which is estimated to be equivalent to a half-life of >1 year at 25°C at any pH.
TEST FACILITY Safepharm (2005a)

Partition Coefficient (n-octanol/water) log P_{OW} <-3.71 at 21.6 ± 0.5°C

METHOD EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks No significant deviations from the test protocol (Shake Flask Method) were reported.
Statement of GLP
TEST FACILITY Safepharm (2005a)

Adsorption/Desorption – screening test log K_{oc} is <1.25 at 30°C.

METHOD EC Directive 2001/59/EC C.19 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography

Remarks Testing was performed at approximately neutral pH, with all salted functional groups ionised. This was based on advice previously received on the test materials of this type (*i.e.* salts of sulfonic acids). The notified chemical eluted before the reference substance Acetanilide.
Statement of GLP

TEST FACILITY Safepharm (2005a)

Dissociation Constant Not determined

Remarks There are at least 4 separate functional groups that have pKa's that overlap making experimental determinations technically impossible. Values for COOM and SO_3M are typically approx 4.2 and 0.7, respectively, from literature references. Also NH_2 and phenol groups range from 4.6 and 10, respectively.

The material is expected to remain ionised within the environmental pH range of 4-9.

Particle Size

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

Range (μm)	*Mass (%)*
<100	23.3
<10.2	3.6
<5.4	0.7

Remarks MMHD could not be determined. Mass of particles of < 100 μm was determined by the sieve method. Mass of particles of < 10.2 μm was determined by a cascade impactor.
Statement of GLP

TEST FACILITY Safepharm (2005a)

Flash Point Not determined

Remarks The substance is a solid of low volatility and hence the flash point test is not appropriate.

Flammability Limits Not highly flammable

METHOD EC Directive 92/69/EEC A.10 Flammability (Solids).

Remarks The test material has been determined to be not highly flammable as it failed to ignite in the preliminary screening test.
Statement of GLP

TEST FACILITY Safepharm (2005b)

Autoignition Temperature 288°C

METHOD 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.

Remarks Statement of GLP

TEST FACILITY Safepharm (2005b)

Explosive Properties Not explosive

METHOD EC Directive 92/69/EEC A.14 Explosive Properties.

Remarks The test material was subject to the following:
The BAM fall hammer test which is a test of mechanical sensitivity with respect to shock.

	The BAM friction test which is a test of mechanical sensitivity with respect to friction. The Koenen steel tube test which is a test of thermal sensitivity. The test material has been determined not to have explosive properties. Statement of GLP
TEST FACILITY	Safepharm (2005b)

Oxidizing Properties Predicted negative

METHOD	EC Directive 92/69/EEC A.17 Oxidising Properties (Solids).
Remarks	Based on the structure the result of the oxidising properties has been predicted negative using the structural details given in Betherick (1990). Statement of GLP
TEST FACILITY	Safepharm (2005b)

Reactivity Not highly reactive

Remarks	Based on the chemical structure and experience in use the test material is predicted to be stable under normal conditions.

7. TOXICOLOGICAL INVESTIGATIONS

Endpoint and Result	*Assessment Conclusion*
Rat, acute oral (LD50 estimated >2000 mg/kg bw)	low toxicity
Rat, acute dermal (LD50 >2000 mg/kg bw)	low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local Lymph Node Assay	no conclusive evidence of sensitisation
Rat, oral repeat dose toxicity - 28 days	NOAEL 150 mg/kg bw/day
Genotoxicity - bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro chromosome aberration test	non genotoxic

7.1. Acute toxicity – oral

TEST SUBSTANCE — Notified chemical.

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

Species/Strain	Rat/Sprague-Dawley CD
Vehicle	Arachis oil BP
Remarks – Method	There were no significant protocol deviations.

RESULTS

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

LD50	>2000 mg/kg
Signs of Toxicity	Signs of systemic toxicity noted in one animal during the day of dosing were pallor of the extremities, decreased respiratory weight, hunched posture, lethargy and laboured respiration. Black stained urine and faeces were also noted during the study in all animals, but disappeared by day 4. All animals showed expected gains in bodyweight over the study period.
Effects in Organs	No abnormalities were noted at necropsy.

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

TEST FACILITY — Safepharm Laboratories Ltd (2005c)

7.2. Acute toxicity – dermal

TEST SUBSTANCE — Notified chemical.

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

Species/Strain	Rat/Sprague-Dawley CD
Vehicle	The test material was moistened with arachis oil BP.
Type of dressing	Semi-occlusive
Remarks – Method	There were no significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5 per sex	2000	0/10

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	There were no signs of dermal irritation.
Signs of Toxicity - Systemic	There were no signs of systemic toxicity. All animals showed expected gains in bodyweight over the study period.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks – Results	Black staining was observed in all animals till up to 5 days for males and 7 days for females.

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

TEST FACILITY Safepharm Laboratories Ltd (2005d)

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	Moistened with distilled water
Observation Period	3 days
Type of Dressing	Semi-occlusive
Remarks – Method	There were no significant protocol deviations.

RESULTS

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

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

Remarks – Results Light grey-coloured staining was observed in all test animals.

CONCLUSION The notified chemical is slightly irritating to skin.

TEST FACILITY Safepharm Laboratories Ltd (2005e)

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	7 days
Remarks – Method	A Rabbit Enucleated Eye Test (REET) was performed prior to the *in vivo* test and indicated that the notified chemical is unlikely to have the potential to cause severe ocular irritation *in vivo*. There were no significant protocol deviations.

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	1	1	1	1	<7 days	0
Conjunctiva: chemosis	0	0	0	1	<24 hour	0
Conjunctiva: discharge	0.67	0.67	0.67	1	<72 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

A single application of the test material to the non-irrigated eye of three rabbits produced minimal to moderate conjunctival irritation. No other ocular effects were noted in treated eyes at the 7-day observation.

Black staining of the cornea and conjunctival membranes was noted in all treated eyes at the 24-hour observation, which faded to grey coloured staining at the 48 and 72-hour observations and disappeared by day 7. The staining did not affect evaluation of ocular effects.

Black staining of the fur, which on occasions had faded to grey coloured staining, was noted around all treated eyes throughout the study.

CONCLUSION

The notified chemical is slightly irritating to the eye.

TEST FACILITY

Safepharm Laboratories Ltd (2005f)

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

TEST SUBSTANCE

Notified chemical

METHOD

OECD 429 Skin Sensitisation: Local Lymph Node Assay
EC 2004/73/EC, Method B42: Skin Sensitisation (Local Lymph Node Assay).

Species/Strain: Mouse/CBA/Ca

Vehicle: 1% pluronic in distilled water

Remarks – Method: A preliminary screening test using one mouse was conducted to detect systemic/irritancy potential of the test substance. The mouse was treated with 25% test substance for 3 days and observed for 6 days.
There were no significant protocol deviations. The vehicle was chosen as it produced the highest concentration that was suitable for dosing.

RESULTS

Concentration	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
Negative control	527.15	NA
5% w/w	626.26	1.19
10% w/w	460.16	0.87
25% w/w	759.27	1.44
Positive Control*		
5% w/v	NR	2.6
10% w/v	NR	8.4
25% w/v	NR	12.9

*α-Hexylcinnamaldehyde as a solution in 70% ethanol in distilled water. NA, not applicable; NR, not reported.

Remarks – Results

The preliminary test did not show any signs of systemic toxicity. Black

staining of the fur and ears was noted 1 hour post dosing on days 1 to 3.

In the main study, no clinical symptoms and body weight changes were observed. The staining occurred in the same pattern as in the preliminary test in all test animals.

CONCLUSION — There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY — Safepharm Laboratories Ltd (2005g)

7.6. Repeat dose toxicity

TEST SUBSTANCE — Notified chemical.

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 preliminary 14-day repeated dose range finding study was performed at 750 and 1000mg/kg/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 doses for use in the main study.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
I (control)	5/sex	0	0/10
II (low dose)	5/sex	25	0/10
III (mid dose)	5/sex	150	0/10
IV (high dose)	5/sex	500	0/10
V (control recovery)	5/sex	0	0/10
VI (high dose recovery)	5/sex	500	0/10

Mortality and Time to Death

One male treated with 500 mg/kg/day was killed *in extremis* following signs of respiratory distress. At *post mortem* examination black discolouration of several tissues including the gastrointestinal tract was observed. The cause of such effects is uncertain. The test laboratory believes that it may have been attributable to small amounts of test material entering the respiratory tract possibly by reflux from the oesophagus after dosing. Given that similar effects were not observed in other animals, and the absence of histological correlation, the toxicological significance of the effects is uncertain.

The below sections will discuss only observations made in animals that survived to the completion of the study.

Clinical Observations

Black fur staining was evident throughout the treatment groups and persisted in recovery animals throughout the 14-day treatment-free period.

There were some changes in bodyweight gain, however, these were considered to be incidental. No treatment-related effects were detected in functional observations, food and water consumptions.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology: No treatment-related effects were detected.

Blood Chemistry: There were some statistically significant changes in the blood chemistry of the treated animals. However, these were considered to be of no toxicological significance, as a number of the effects were observed in isolation, or individual values were within expected ranges for animals of the strain and age used.

Urinalysis: Haemoglobin was seen in males and females treated with 500 mg/kg/day. Erythrocytes were observed in two males treated with 150 mg/kg/day.

Effects in Organs

Organ Weights: Females treated with 500 mg/kg/day showed an increase in adrenal weights, both absolute and relative to terminal bodyweight. Without supporting histopathological evidence the reason for this increase is unclear although its significance cannot be ignored.

Necropsy: A number of animals of either sex treated with 500 mg/kg/day showed black coloured contents in the kidneys and stomach. These observations were not considered to be indicative of toxicity, rather it was considered to be the result of excretion of the coloured notified chemical or its metabolites.

Histopathology:

Kidney: Hypertrophy of distal tubules and collecting ducts was observed in animals of either sex treated with 500 mg/kg/day. These rats also showed accumulations of red/purple pigment in the proximal tubular epithelium. These conditions regressed in recovery animals treated with the same dose following 14 days without treatment. The effects were considered to be treatment related.

Stomach: Agglomeration of secretion, frequently with associated superficial mucosal basophilia and/or mucous cell hypertrophy/hyperplasia, and acanthosis/hyperkeratosis of the limiting ridge were observed in rats of both sexes treated with 500 mg/kg/day, but not convincingly at any other treatment level. The condition had almost regressed after a 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, bone marrow and uterus of some of the treated animals. However, effects of similar severity were also observed in similar number of control animals. 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 500 mg/kg/day. These changes were considered to be the result of direct contact with the notified chemical.

CONCLUSION

The No Observed Adverse Effect Level was therefore considered to be 150 mg/kg/day, based on effects in the kidney and stomach.

TEST FACILITY	Safepharm Laboratories Ltd (2006h)

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 and TA100 *E. coli*: WP2 uvrA-
Metabolic Activation System	S9 fraction from phenobarbitone/ß-naphthoflavone-induced rat liver.
Concentration Range in	a) With metabolic activation: 50 - 5000 µg/plate.

Main Test	b) Without metabolic activation: 50 - 5000 µg/plate.
Vehicle	Sterile 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*
Present				
Test 1	>5000 µg/plate	>5000 µg/plate	>5000 µg/plate	None
Test 2	-	>5000 µg/plate	>5000 µg/plate	None
Absent				
Test 1	>5000 µg/plate	>5000 µg/plate	>5000 µg/plate	None
Test 2	-	>1500 µg/plate	>5000 µg/plate	None

Remarks – Results	The vehicle control plates gave counts of revertant colonies within the normal range. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with and without metabolic activation. Thus, the sensitivity of the assay and the efficacy of the S9-mix were validated. A blue/black colour was observed at and above 50 µg/plate, becoming darker with increasing concentration, on plates dosed in the absence of S9-mix. However, in the presence of metabolic activation the colour observed at and above 50 µg/ml was pink/purple, again increasing in intensity with increasing concentration. These observations did not prevent the scoring of revertant colonies. No toxicologically significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation. A small, but statistically significant increase in revertant colony frequency was observed in WP2uvrA- (without S9-mix) at 50 µg/plate in the range-finding test only. This response was less than two-fold the concurrent solvent control, with plate counts within the acceptable range of the test strain and non-reproducible in the main test. Therefore, this response was considered to be of no toxicological or biological importance.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Safepharm Laboratories Ltd (2005i)

7.8 Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosomal Aberration Test.
Species/Strain	Hamster
Cell Type/Cell Line	Chinese hamster lung cells
Metabolic Activation System	S9 fraction from phenobarbitone/ß-naphthoflavone-induced rat liver.

Vehicle	Minimal Essential Media (MEM)
Remarks – Method	No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Present			
Test 1	0*, 312.5, 625, 1250, 2500*, 3750*, 5000*	6 hours	24 hours
Test 2	0*, 312.5, 625, 1250*, 2500*, 3750, 5000*	6 hours	24 hours
Absent			
Test 1	0*, 312.5, 625, 1250, 2500*, 3750*, 5000*	6 hours	24 hours
Test 2	0*, 39.06, 78.13*, 117.19, 156.25*, 234.38*, 312.5	24 hours	24 hours

*Cultures selected for metaphase analysis.

RESULTS

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

* based on >50%decrease in mitotic index.

Remarks – Results	The vehicle control had frequencies of cells with aberrations within the range expected for the CHL cell line. All of the positive control materials induced highly significant increases in the frequency of cells with aberrations indicating the satisfactory performance of the test and of the activity of the metabolising system. In Test 1, in the absence of metabolic activation there was a slight increased total and percentage of cells with structural aberration (mainly chromosome exchange) at 5000 µg/mL and additional metaphases were scored to confirm the increase. The increase was within the in-house range for solvent controls and was considered to have no toxicological significance.
CONCLUSION	The notified chemical was not clastogenic to Chinese hamster lung cells treated in vitro under the conditions of the test.
TEST FACILITY	Safepharm Laboratories Ltd (2005j)

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical.
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I).
Inoculum	Activated sewage sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	The change in BOD of the test solutions was measured by autoreading using a data sampler, confirmed by HPLC.
Remarks – Method	No significant protocol deviations. Test concentration of the notified chemical was 100 mg/L.

RESULTS

Test substance		*Aniline*	
Day	*% degradation (BOD)*	*Day*	*% degradation*
7	-1	7	70
14	-1.67	14	76
21	-4.33	21	76
28	-9	28	76

Remarks – Results	All relevant OECD criteria were met. Aniline attained 76% degradation after 14 days thereby confirming the suitability of the inoculum and test conditions. A parallel analysis by HPLC indicated an average of 1% biodegradation of the test item, and a further parallel analysis of DOC indicated an average of 0% biodegradation.
CONCLUSION	The notified chemical cannot be classed as ready biodegradable.
TEST FACILITY	Kurume Laboratory (2005)

8.1.2. Bioaccumulation

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

8.2. Ecotoxicological investigations

8.2.1. Acute toxicity to fish

TEST SUBSTANCE — Notified chemical.

METHOD

EC Directive 92/69/EEC C.1 Acute Toxicity for Fish – semi-static.

Species	Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period	96 h
Auxiliary Solvent	None
Water Hardness	Ca. 100 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	No significant protocol deviations.

RESULTS

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 (2000 mg) was dissolved in dechlorinated tap water and the volume adjusted to give the 100 mg/L test concentration.

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	0	7	0	0	0	0	0
100	94	7	0	0	0	0	0

LC50	>100 mg/L at 96 hours.
NOEC	100 mg/L at 96 hours.
Remarks – Results	The control was observed to be a clear, colourless solution throughout the duration of the test. The 100 mg/L test preparation was observed to be dark blue/black solution throughout the duration of the test. Analysis of the test preparations at 0, 24 and 96 hours showed measured test concentrations to range from 92% to 98% of nominal and so the results are based on nominal test concentrations only.

CONCLUSION — The notified chemical is not harmful to Rainbow trout.

TEST FACILITY — Safepharm laboratories Ltd (2005k)

8.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical.
METHOD	EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia – 48 hours static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	Ca. 250 mg $CaCO_3$/L
Analytical Monitoring	HPLC
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 (100 mg) was dissolved in dechlorinated tap water and the volume adjusted to 1 L to give the 100 mg/L test concentration. No significant protocol deviations were reported.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	0	10	0	0
100	82	10	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 medium was observed to be a clear colourless solution and the 100 mg/L test medium was observed to be dark blue/black solutions throughout the test. Analysis of the test preparations at 0 and 48 hours showed measured test concentrations to range from 81% to 90% of nominal value and so the results are based on nominal test concentrations only.
CONCLUSION	The notified chemical is not harmful to *Daphnia magna*.
TEST FACILITY	Safepharm laboratories Ltd (2005l)

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: 101 - 137% 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 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 1.92 X 10^6 cells per mL. This suspension was diluted to a cell density of 2.25 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 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*
3.0 (95% CI: 2.3 – 3.9)	1.0	71*	1.0
Experiment B			
Biomass		*Growth*	
E_bC50 *mg/L at 72 h*	*NOEC* *mg/L*	*E_rC50* *mg/L at 72 h*	*NOEC* *mg/L*
6.0 (95% CI: 5.1 – 7.1)	3.2	18 (95% CI: 14 – 23)	3.2

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

Remarks - Results	Given that significant differences (greater than 10%) in the inhibition values between Experiments A and B were observed, it was considered that the effect of the test material on algal growth was not only due to a reduction in light intensity, but also due to the intrinsic toxic properties of the test material. Therefore, from classification purposes, the results determined from Experiment A should be used. Analysis of the test preparation at 0 and 72 hours showed measured test concentrations to range from 99% to 137% of nominal.
CONCLUSION	The notified chemical is harmful to *Scenedesmus subspicatus.*
TEST FACILITY	SafePharm Laboratories Ltd (2005m)

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: 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 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 4. The number of fronds in each control and treatment group was recorded on days 0, 2, 4, and 7, along with observations on plant development. Amounts of test material (100 and 32 mg) were each dissolved separately in culture medium and the volume adjusted to give 100 and 32 mg/L test solutions from which subsequent dilutions were made. This method of preparation was repeated in order to provide the required test concentrations for the media renewal on days 2 and 4. 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	>100	1.0

Remarks - Results	Analysis of the test preparations on Day 0 (fresh media) and on Days 2, 4 and 7 (old or expired media) showed measured test concentrations to be near nominal with the exception of the 1.0 mg/L test sample taken on Day 4 which showed a measured test concentration of 138% of nominal. Analysis of a frozen duplicate test sample showed a measured test concentration of 111% of 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 (2005n)

8.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical.
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sewage sludge from domestic sewage treatment plant.
Exposure Period	3 hours
Concentration Range Nominal	1000 mg/L
Remarks – Method	Oxygen consumption rates and percentage inhibition values for the control, test and reference materials (3,5-dichlorophenol) were measured after 30 minutes and 3 hours.
RESULTS	
IC50	>1000 mg/L
NOEC	1000 mg/L
Remarks – Results	The reference material gave a 3-Hour EC_{50} value of 7.3 mg/L.
CONCLUSION	The notified chemical is practically non-toxic to activated sewage sludge micro-organisms.
TEST FACILITY	Safepharm (2005o)

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. 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 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		
E_rC50 (Algae)	71	mg/L
Assessment Factor	100.00	
PNEC:	710	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	710	0.00046
Q - Ocean:	0.03	710	0.00005

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 for similar chemicals with the same use. 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).

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 seen in the acute oral toxicity study and the systemic effects and black coloured contents of the kidney observed in the repeated dose oral toxicity study, 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).

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 the treatment related changes observed in the kidney and stomach at the higher dose level of 500 mg/kg bw/day.

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

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

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

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

The notified chemical is not expected to be reductively cleaved to release any of the restricted aromatic amines specified in either the Appendix to EC Directive 76/769/EEC (EC, 2004) or the annexes of EU SCCNFP/0495/01 (SCCNFP, 2002). However, the notified chemical can be broken by azo reduction into a number of arylamine species. The mutagenic potential of these species is unknown, however, they are likely to have no or very low genotoxic and tumorigenic potential.

In addition, azo dyes are renowned for their content of impurities, particularly for the presence of component arylamines (SCCNFP, 2002; Øllgaard *et al* 1998). Such impurities are thought to contribute to their carcinogenicity, as these species may be more readily absorbed, and activated as carcinogens. The HPLC trace provided by the notifier indicates that the sample of the notified chemical used contains low levels (0.2%) of one impurity. The identity of the contaminant is unknown, but it may be an aromatic amine.

The notifier supplied test results showing that the notified chemical was found to be not mutagenic in bacteria (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 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

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 concentration of <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 150 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*
Environment		
Acute	2	Toxic to aquatic life
Chronic	2	Toxic to aquatic life with long lasting effects

10.2. Environmental risk assessment

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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