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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Magenta Dye 4

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Director
Chemicals Notification and Assessment

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Toxikos Pty Ltd (ABN: 30 095 051 791)
293 Waverly Road
MALVERN EAST VIC 3145

Hewlett-Packard Australia Pty Ltd (ABN: 74 004 394 763)
31-41 Joseph Street
BLACKBURN VIC 3130

NOTIFICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication:
Chemical name;
Other name;
Molecular formula;
Structural formula;
Molecular weight;
Spectral data;
Purity;
Identity of toxic or hazardous impurities;
Non-hazardous impurities;
Identity of Additives/adjuvants;
% Weight of additives;
Manufacture and import volume; and
Test facility details where this may identify original manufacturer

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows:
Adsorption/desorption properties;
Acute dermal toxicity;
Skin irritation;
Eye irritation;
Skin sensitisation;
Repeat dose toxicity;
Acute fish toxicity;
Algal growth; and
Inhibition of microbial activity.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
UK (2004)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Magenta Dye 4

METHODS OF DETECTION AND DETERMINATION

METHOD	Infrared (IR) Spectroscopy, ^{1}H Nuclear Magnetic Resonance (NMR) Spectroscopy, Mass Spectroscopy, Ultraviolet/Visible light (UV/VIS) Spectrometry.
Remarks	Reference spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
<60%

4. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported in ready to use sealed inkjet printing cartridges. The volume of the cartridges ranges up to 50 mL.

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

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

USE
The notified chemical is a dye used in preparations in inkjet reprographic processes. The notified chemical will be imported as a component of the dye, within sealed ink-jet cartridges at a typical concentration of <4%.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, transport and storage

PORT OF ENTRY
Not known.

IDENTITY OF MANUFACTURER/RECIPIENTS
The inkjet printing systems will be potentially supplied to offices nationwide.

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a component of a ready to use sealed inkjet cartridge. Cartridges containing the notified chemical are not a dangerous good, hazardous substance or scheduled poison, and thus no special transport or packaging requirements are necessary. 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 the end-user as it is imported into Australia. The sealed inkjet cartridges will be handled by service technicians or office workers replacing the spent cartridges in the printer

5.3. Occupational exposure

Number and Category of Workers

Category of Worker	*Number*	*Exposure Duration*	*Exposure Frequency*
Importation/Waterside workers	10	4 hours per day	70 days per year
Storage and transport	100	6 hours per day	240 days per year
Office worker/Service technician/Consumer	10 000	<0.1 hours per day	20 days per year

Exposure Details

The notified chemical is contained in sealed cartridges. The volume of the notified chemical in any single cartridge would typically be approximately 2 mL.

Office workers and customer service engineers will replace spent ink cartridges. 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 while replacing cartridges, or on handling printed paper or other inkjet printable substrates, particularly if the paper is handled before the ink is adequately dried or if printing to a non-absorbent substrate occurs by error. After the ink is dry the notified chemical is bound to the paper or other inkjet printable matrix 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

The notified chemical will be imported in sealed cartridges containing up to 50 mL of formulated ink (<4% of the chemical). There will be no release to the environment due to reformulation or repackaging.

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 use. These will be changed by office workers and the public. However, if leakage or spillage does occur, the ink will be contained with absorbent material, which will presumably be disposed of in landfill in the normal office garbage along with the empty cartridges and print heads.

The sealed cartridges are contained within the printer until they are removed for disposal. Residual ink (<10%) containing up to 12 kg of the notified chemical left in empty cartridges (<0.4%) will most likely be disposed of to landfill.

Most of the notified chemical (>99%) will be bound to 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 fiber separation and ink detachment from the fibers. The wastes are expected to go to trade waste sewers. The notifier estimated that about 50% of the ink printed on paper will enter paper recycling and up to 60% of the ink is expected to be recovered during recycling. Due to the low percentage of notified chemical in the ink and the widespread use, release to the aquatic compartment will be highly diffused. The notified chemical adsorbed to sludge during the recycling process will be disposed of to landfill

5.5. Disposal

No special precautions are required. The total import volume of the notified chemical will ultimately be disposed of to either landfill or be incinerated or recycled with paper.

5.6. Public exposure

Limited exposure may occur while changing inkjet cartridges, however this will be relatively infrequent and should only result in very limited exposure to small quantities of the notified chemical.

After the ink is dry the notified chemical is bound to the paper or other inkjet printable matrix and is not expected to be readily bioavailable. The public may be dermally exposed to notified chemical, while handling printed paper or other substrates, where the ink is only partially dried. The manufacturer has estimated public exposure to the notified chemical via the printed substrate. One kilogram of pure dye would be expected to produce several million sheets of A4 coloured text or graphics in the case of a paper substrate. Under worst-case conditions, each piece of A4 paper can be assumed to incorporate 1 mg of notified chemical. Based on a 50% transfer on contact when handling printed paper (assuming only partially dry ink), and the relative areas of finger ends and paper size, it is estimated that potential removal is <1% of the applied ink in each event.

6. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20ºC and 101.3 kPa Red-brown powder

Melting Point Decomposes without melting.

METHOD EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks The test substance was examined using differential scanning calorimetry by heating from 20ºC to 400ºC at a heating rate of 10ºC/min. There was an initial loss of moisture at 125ºC followed by decomposition around 340ºC.
TEST FACILITY Toxikos (2004a)

Boiling Point Decomposes without boiling.

METHOD EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks The test substance was examined using differential scanning calorimetry. Two samples were examined using an aluminium pin hole crucible. The samples were heated from 25ºC to 450ºC at heating rate of 10ºC/min. Each run showed a small endotherm around 130ºC and a significant exotherm above 320ºC. The endothermic peak was attributed to a form change or gassing and the exothermic peak was attributed to decomposition. A third sample was examined using a sealed gold crucible. The samples were heated from 25ºC to 450ºC at a heating rate of 5ºC/min to confirm the interpretation of the endothermic peak at 130ºC. No endothermic activity was observed over the temperature range scanned. A small exotherm occurred from around 200-240ºC followed by a pronounced exotherm from 270ºC to 310ºC and an exothermic tail to the test end. The large thermal mass of the gold crucible and the lower ramp rate contributed to the changes in temperatures for the exothermic peak. The trace suggests that the initial endotherm observed in the first two runs in the aluminium crucible was most likely due to gassing.
TEST FACILITY Toxikos (2004b)

Density 1730 kg/m^3 at 20ºC

METHOD EC Directive 92/69/EEC A.3 Relative Density.
Remarks The density was determined using a pycnometer.
TEST FACILITY Toxikos (2004a)

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

METHOD Method described in the Directive 92/69/EEC, Annex, Official Journal of the European Communities L383.
Remarks The melting and boiling points of the test substance were initially predicted to be 349°C and 643°C using the ProPred, Marrero & Gani Method. Two theoretical calculations were performed to estimate the vapour pressure. First, the predicted boiling point was used. The resulting vapour pressure value is $<10^{-18}$ kPa.

Secondly, the minimum boiling point to achieve a pressure above $>10^{-8}$ kPa was determined by iteration. Since no melting point was detected below the main decomposition exotherm (around 260°C) it was assumed that the predicted melting point (349°C) was accurate to within at least 100°C and the theoretical boiling point should therefore be of a similar level of accuracy. The notified chemical would have a theoretical boiling point much higher than 380°C and thus the vapour pressure is likely to be $<<10^{-8}$ kPa.

The Henry's Law constant (H) calculated from the molecular weight, measured water solubility, and the estimated vapour pressure according to the following equation: H = MW (g/mol) X Vapour Pressure (Pa)/Water Solubility (mg/L) was $<2.6 \times 10^{-8}$ Pa m^3/mol, indicating that the notified chemical is not likely to be volatile from water or moist soil (Mensink *et al*. 1995).

TEST FACILITY Toxikos (2004b)

Water Solubility 490-570 g/L at ambient temperature

METHOD Methods for Determination of Physico-Chemical Properties; Official Journal of the European Communities L383A, Vol. 35, 1992.

Remarks The test substance was weighed into centrifuge tubes and weighed amounts of distilled/deionised water added. The tubes were sealed and placed in a shaking constant temperature (30 ± 1ºC) bath overnight. Successive amounts of the test substance were added to each of the tubes over several days and the tubes were replaced in the water bath. The samples were visually inspected for the presence of solid test substance after allowing them to stand at room temperature for two days.

Three tests with concentrations ranging from 36.1 to 58.4% (w/w) were run. All three tests were observed to have formed a thick 'glue like' solution. The first test (36.1 to 49.3% (w/w)) had no evidence of solid substance, but the other two (45.3 to 58.4% w/w and 41.8 to 57.0% (w/w)) showed the presence of some solids.

The notified chemical is readily soluble (Mensink *et al.* 1995).

TEST FACILITY Toxikos (2004a)

Surface Tension 72.1 mN/m at 25 ± 1°C

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

Remarks Two solutions of the test substance were prepared with distilled/deionised water and equilibrated at 25 ± 1°C. The total time for preparation and equilibration was 51 and 30 minutes for test 1 and test 2, respectively. Tests 1 and 2 were run for 24 and 30 minutes, respectively using a Krüss Processor Tensiometer fitted with a Wilhelmy plate.

The results indicate that the notified chemical is not surface active.

TEST FACILITY Toxikos (2004a)

Hydrolysis as a Function of pH

METHOD Methods for Determination of Physico-Chemical Properties; Official Journal of the European Communities L383A, Vol. 35, 29th December 1992.

PH	*T (°C)*	*% hydrolysis after 7 days*
4	50	13
7	50	-15
9	50	16

Remarks Buffer solutions at pH 4, 7 and 9 were prepared in distilled/deionised water, which was degassed with helium before use. The tests were carried out at 50 ± 1ºC, 60 ± 1ºC and 70 ± 1ºC. The test solutions were analysed initially and over a period of several days using HPLC.

The test substance was found to undergo reversible changes at elevated temperatures forming two other components. These components reverted back to the main component of the test substance on standing at room temperature.

The notified chemical is not expected to hydrolyse significantly in the environmental pH range of 4 to 9.

TEST FACILITY Toxikos (2004a)

Partition Coefficient (n-octanol/water) log P_{ow} = <-2.7 at 25°C

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

Remarks Solutions of the test substance were prepared in n-octanol saturated distilled water. Concentrations of the test substance in water phases were determined spectrophotometrically by comparison to a calibrated curve prepared in distilled/deionised water containing 50% (v/v) n-octanol saturated water. The n-octanol phases were examined directly without dilution and no appreciable absorbance due to the test substance was obtained. The detection limit for the octanol phase was estimated to be 0.46 mg/L.

The low log P_{ow} is consistent with the high water solubility indicating a low affinity for the organic phase and component of soils and sediments.

TEST FACILITY Toxikos (2004a)

Adsorption/Desorption log Koc <5 at pH 2, <1.5 at pH 10 (based on Magenta Dye 3).

METHOD OECD Guideline for testing of chemicals 121. Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC) (2001).

Remarks The adsorption coefficient (K_{OC}) of the test substance (analogue chemical Magenta Dye 3) was estimated using a high performance liquid chromatography (HPLC) correlation technique. The test described has been validated for the quantitative estimation of logK_{OC} values in the range 1.5 to 5.0 (OECD 2001). In those instances where the statistical treatment of the experimental data produced a calculated logKoc value outside this range then the final result is quoted as either <1.5 or >5.0, as was the case here.

Although the notified chemical and the proposed analogue (previously assessed as LTD/1110) have differences, the above results are accepted based on the fact that both chemicals are likely to be highly ionised throughout the entire environmental pH range of 4 to 9.

TEST FACILITY Toxikos (2002a)

Dissociation Constant pKa_1 = 9.7
pKa_2 = 8.2
pKa_3 = 3.3

METHOD OECD TG 112 Dissociation Constants in Water.

Remarks The pKa was investigated spectrophotometrically using dilute aqueous solutions of the test substance. The pH of the test solutions was adjusted using potassium hydroxide and hydrochloric acid. A UV/Visible spectrum was obtained at each pH value. The ionic strength of the solution was maintained at a constant level by the addition of potassium chloride (0.15M).

In another test (Toxikos (2004c) only a one page summary provided) the notified chemical occurred in two forms and six experimental pKa values were found for each (between 3.10 and 9.04 with four in the acidic range and two in the neutral to

basic range).
TEST FACILITY Toxikos (2004a)

Particle Size Not determined.

Remarks Notified chemical is to be imported as part of a liquid formulation.

Flash Point Not determined.

Remarks Notified chemical is a solid.

Flammability Limits Not flammable.

METHOD EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks The test substance did not propagate combustion, and is not classified as highly flammable in terms of its burning characteristics.
TEST FACILITY Toxikos (2004b)

Autoignition Temperature 315 ± 5ºC

METHOD 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks A shallow endothermic peak was observed at oven air temperature from room temperature up to approximately 200ºC. At an oven air temperature of approximately 275ºC, exothermic activity was detected during the sample temperature increased at a maximum rate of approximately 443ºC/minute to attain a peak temperature of 530ºC. During the exotherm, the test substance attained a temperature of 400ºC at an oven air temperature of 315 ± 5ºC, which was defined as the relative self ignition temperature. On inspection after the test, decomposed material was found in sample basket.
TEST FACILITY Toxikos (2004b)

Pyrophoric Properties Not pyrophoric.

METHOD 92/69/EEC A.13 Pyrophoric Properties of Solids and Liquids
Remarks The test substance does not spontaneous ignite on contact with air at ambient temperature (20ºC) and is not classified as highly flammable in terms of its pyrophoric properties.
TEST FACILITY Toxikos (2004b)

Explosive Properties Not explosive.

METHOD EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks Mechanical sensitivity (shock):

The test substance has a limiting impact energy is greater than 40 Joules and is not classified as explosive in terms of its mechanical sensitivity with respect to shock.

Mechanical sensitivity (friction):

The test substance has a limiting load greater than 360 Newtons and is not classified as explosive in terms of its mechanical sensitivity in reference to friction.

Thermal sensitivity:

The test substance has a limiting diameter is less than 2mm and is not classified as explosive in terms of thermal sensitivity.

TEST FACILITY Toxikos (2004b)

Oxidising Properties Not oxidising.

METHOD	EC Directive 92/69/EEC A.17 Oxidising Properties (Solids).
Remarks	The results indicate that the test substance is attenuating the burning characteristics of cellulose rather than enhancing them. The test substance is not classified as an oxidising substance.
TEST FACILITY	Toxikos (2004b)

Reactivity

Remarks	The notified chemical is expected to be stable under normal environmental conditions of temperature and pressure.

7. TOXICOLOGICAL INVESTIGATIONS

Toxicological data provided for the notified chemical were: acute oral toxicity, bacterial reverse mutation, and chromosomal aberrations in cultured human peripheral blood lymphocytes. Data on acute dermal toxicity, skin and eye irritation, skin sensitisation and repeat dose toxicity were provided for a close analogue, Magenta Dye 3, previously notified as LTD/1110.

Endpoint and Result	*Assessment Conclusion*
Rat, acute oral LD50 >2500 mg/kg bw	low toxicity
Rat, acute dermal LD50 >2000 mg/kg bw (analogue)	low toxicity
Rabbit, skin irritation (analogue)	non-irritating
Rabbit, eye irritation (analogue)	slightly irritating
Guinea pig, skin sensitisation – adjuvant test (analogue)	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days. (analogue)	NOEL = 250 mg/kg bw/day
Genotoxicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosomal aberrations	non genotoxic

7.1. Acute toxicity – oral

TEST SUBSTANCE	Magenta Dye 4
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Sprague-Dawley CD
Vehicle	Distilled water.
Remarks – Method	No significant protocol deviation.

RESULTS

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

LD50	>2500 mg/kg bw
Signs of Toxicity	There were no signs of systemic toxicity. All animals showed expected gains in bodyweight and over the study period.
Effects in Organs	No abnormalities were noted at necroscopy.
Remarks - Results	Purple coloured faeces were noted in all animals one to seven days after dosing and purple coloured urine was noted in all animals one to four days after dosing.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Safepharm Laboratories (2004a)

7.2. Acute toxicity – dermal

TEST SUBSTANCE	Magenta Dye 3
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 Rat

Vehicle	Distilled water.
Type of dressing	Semi-occlusive.
Remarks – Method	No significant protocol deviation.

RESULTS

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

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	There were no signs of dermal irritation. Red coloured staining prevented evaluation of erythema at the treatment sites of all animals of six days after treatment. No erythema was observed in test animals after day six.
Signs of Toxicity - Systemic	There were no signs of systemic toxicity. All animals showed the expected gains in bodyweight over the study period.
Effects in Organs	No abnormalities were noted at necroscopy.

CONCLUSION — The analogue is of low toxicity via the dermal route.

TEST FACILITY — Safepharm Laboratories (2003a)

7.3. Irritation – skin

TEST SUBSTANCE — Magenta Dye 3.

METHOD — OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 animals
Vehicle	Distilled water.
Observation Period	72 hours
Type of Dressing	Semi-occlusive.
Remarks – Method	No significant protocol deviation.

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	0	0	-	0
Oedema	0	0	0	0	-	0

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

Remarks – Results	Pink/red coloured staining was noted at all treated sites throughout the study. The staining did not affect the evaluation of skin response.

CONCLUSION — The analogue is non-irritating to skin.

TEST FACILITY — Safepharm Laboratories (2002a)

7.4. Irritation – eye

TEST SUBSTANCE: Magenta Dye 3.

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
Observation Period: 7 days
Remarks – Method: No significant protocol deviation.

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	1.3	1	1	2	72 hours	0
Conjunctiva: chemosis	1	0.67	0.33	3	48 hours	0
Conjunctiva: discharge	0.67	0.33	0.33	3	24 hours	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

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

Remarks – Results: Pink coloured staining of the fur was noted surrounding all treated eyes during the study and persisted to Day 7. Pink coloured staining was noted in all treated eyes at the 1, 24, and 48-hour observations and persisted in two treated eyes at the 72-hour observation.

Staining prevented the evaluation of corneal and iridial effects in all treated eyes at the 1 and 24-hour observations and the evaluation of conjunctival redness in all treated eyes at the 1-hour observation and in two treated eyes at the 24-hour observation.

Slight to moderate conjunctival chemosis and severe discharge were noted in all treated eyes at 1 hour. Minimal chemosis and discharge were noted in two treated eyes at the 24-hour observation. Moderate conjunctival irritation was apparent in one treated eye at the 24-hour observation. Minimal conjunctival irritation was apparent in all treated eyes at the 48 and 72-hour observations. All treated eyes appeared normal at 7 days. Pink coloured staining of the fur around the treated eyes in all animals persisted until day 7.

CONCLUSION: The analogue is slightly irritating to the eye.

TEST FACILITY: Safepharm Laboratories (2002b).

7.5. Skin sensitisation

TEST SUBSTANCE: Magenta Dye 3.

METHOD: OECD TG 406 Skin Sensitisation - Maximisation Test.
EC Directive 96/54/EC B.6 Skin Sensitisation – Maximisation Test.

Species/Strain: Guinea pig/Albino Dunkin Hartley
PRELIMINARY STUDY: Maximum Non-irritating Concentration:
intradermal: staining precluded determination
topical: < 10% in arachis oil BP

MAIN STUDY
Number of Animals: Test Group: 10 Control Group: 5
INDUCTION PHASE: Induction Concentration:

	intradermal injection: 10% in arachis oil in BP topical application: 75% in arachis oil in BP
Signs of Irritation	Red coloured staining, which prevented evaluation of skin reactions, was noted at the intradermal induction and topical induction sites of all test groups animals. No skin reactions were noted at the intradermal induction sites of control group animals. Discrete or patchy erythema was noted at the topical induction sites of control group animals.
CHALLENGE PHASE	
1st challenge	topical application: 50% and 75%
Remarks – Method	No significant protocol deviation.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st challenge		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	50	0/9	0/9	-	-
	75	0/9	0/9	-	-
Control Group	50	0/5	0/5	-	-
	75	0/5	0/5	-	-

Remarks – Results

In the preliminary study following a 5% intradermal injection red coloured staining of injection site prevented evaluation of erythema until day 7. Red coloured staining of the dermal persisted at day 7. Following a 10% intradermal injection, erythema could not be assessed at Day 7 due to red coloured staining of the injection site, red coloured staining, and focal eschar. Intradermal injection of the formulation at 25% was not possible. Red coloured staining was also observed in animals following topical induction. At one hour, red coloured staining prevented the evaluation of erythema at 10, 25, and 50% (w/w) in one animal and 10 and 25% in another. Red coloured staining was observed at 24 hours and 48 hours, but did not prevent evaluation of erythema.

In the main study, at topical challenge, red coloured staining was noted at the challenge site of all test and control group animals during the study. This did not affect the evaluation of the skin reaction. No skin reactions were noted at the 24 and 48-hour observations in test or control animals challenged at 75% test compound in arachis oil BP. No skin reactions were noted at the 24 and 48-hour observations in test or control animals challenged at 50% test compound in arachis oil BP.

One test group animal was killed for humane reasons due to ill health on day 9.

CONCLUSION

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

TEST FACILITY

Safepharm Laboratories (2002c).

7.6. Repeat dose toxicity

TEST SUBSTANCE

Magenta Dye 3.

METHOD

Similar to OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.

Species/Strain	Rat/Sprague Dawley.
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	The dose in the main study was determined using a 14-day range finding study.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
I (control)	5/sex	0	0
II (low dose)	5/sex	15	0
III (mid dose)	5/sex	250	0
IV (high dose)	5/sex	1000	0
V (control recovery)	5/sex	0	0
VI (high dose recovery)	5/sex	1000	0

Mortality and Time to Death

No mortality was observed during the study.

Clinical Observations

In male and female high dose animals, red staining on the cage tray liners was observed from day 2 with some instances of generalised red staining of the fur from day 7. Within a few days of treatment cessation, these findings resolved. Red stained fur was also observed in males in the mid dose on day 23. Scab formation present on the neck of one low dose male between days 11 and 22, noisy respiration in a control female between days 27 and 28, and a damaged tail tip in one high dose recovery female between days 28 and 35 were all considered not to be of toxicological significance as they represented normal low incidence findings in rats of the strain and age used.

No treatment related behavioural differences were detected during open-field assessments. The inter and intra group differences in behaviour noted were considered to be normal variation for rats of the strain and age used. No treatment related changes were observed in measured functional performance parameters. No statistically significant intergroup differences were noted in the quantitative functional performance data. There were no treatment-related changes in sensory reactivity. All inter and intra group differences in sensory reactivity scores were considered a result of normal variation for rats of the strain and age used. Test animals showed weight gains similar to those of the control animals throughout the study. No statistically significant inter group differences in weekly body weight gain were observed.

No adverse effects on food consumption were observed during the study. Food efficiency in test animals was similar to control animals. No intergroup differences in water consumption were observed.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

High dose males and females showed an increase in blood aspartate aminotransferase. The observed effect was statistically significant ($p<0.01$) in males. High dose recovery females showed a statistically significant increase ($p<0.01$) in plasma potassium concentration. However based on the absence of observed histopathological effects in the kidney and the similar trend during treatment this finding was not considered of toxicological significance

High dose recovery males showed a reduced mean red corpuscular haemoglobin concentration while females showed a reduced eosinophil and neutrophil counts ($p<0.05$). Similar effects were not observed during the treatment phase and thus these minimal reductions were not considered toxicologically significant.

Dark red urine was observed for all high dose animals and the majority of animals in the mid dose. The low dose groups were unaffected. Within a few days of treatment cessation, these findings resolved in high dose recovery groups.

The high dose recovery females showed statistically significant increase ($p<0.05$) in urine volume of reduced specific gravity compared to controls. This difference was not observed in the non-recovery animals and hence were not considered of toxicological significance.

Effects in Organs

No toxicologically significant effects were detected in the organ weight parameters. No treatment related macroscopic abnormalities were observed. Histological investigation of the gastric mucosa showed agglomeration of secretions and mucous cell hyperplasia in high dose animals. Agglomeration of secretions was seen in two male rats and one female rat, with associated mucous cell hyperplasia observed at the mid dose. Acanthosis and hyperkeratosis of the limiting ridge were also observed at the high dose. There were indications of a slight regression of the conditions among the high dose recovery group compared with controls following an additional fourteen days without treatment. All remaining morphological changes seen in the heart (focal myocarditis of minimal severity or one or two foci in controls and treated animals), liver (scattered mononuclear cell foci in the majority of animals), spleen (extramedullary haemopoiesis), kidneys (isolated groups of basophilic tubules), thyroids (follicular cell hypertrophy), lungs (minimal severity bronchus associated lymphoid tissue in most animals, minor severity and low incident focal pneumonitis and accumulation of alveolar macrophages), bone (adipose infiltration of bone marrow in controls and treated animals), and uterus (dilatation of uterine horns) were those commonly observed in rats of the age and strain used.

Recovery high dose males showed a statistically significant ($p<0.05$) in absolute epididymides weight. The lack of histopathological correlation and the absence of a similar reduction in weight of this organ relative to bodyweight, suggested this finding was not of toxicologically significant.

Macroscopic abnormalities observed were restricted to darkened lungs in one control female. This was considered to represent a low incidence, normal association with exsanguination of animals.

Remarks – Results

The red staining observed on the cage tray-liners for high dose animals was presumably due to the micturation of red coloured urine. This finding was supported by urinalytical investigations when dark red urine was observed at high dose and the majority of animals in mid dose. Generalised red staining of the fur was also observed from day 7 among high dose animals and in one mid dose male on one occasion. In absence of histological renal changes, the abnormal coloured urine was considered evidence of the urinary excretion of the test substance and was not of toxicological significance.

Increased plasma enzyme aspartate aminotransferase was observed in high dose animals. The aetiology of this finding is uncertain and may be indicative changes of hepatocellular membrane permeability or tissue damage in other organs, such as the stomach.

The agglomeration of secretions and mucous cell hyperplasia observed in the gastric mucosa in high dose animals and in some mid dose animals are conditions that occasionally occur among control animals, thus these were only considered a treatment effect at the high dose. Acanthosis and hyperkeratosis of the limiting ridge were also observed high dose males. A slight regression of these conditions was observed during the recovery period.

CONCLUSION

The No Observed Effect Level (NOEL) was established as 250 mg/kg bw/day in this study, based on changes in the gastric mucosa at higher doses.

TEST FACILITY	Safepharm Laboratories (2003b).

7.7. Genotoxicity – bacteria

TEST SUBSTANCE	Magenta Dye 4
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	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100, TA102,
Metabolic Activation System	Phenobarbitone/β-naphthoflavone induced rat liver S9 fraction.
Concentration Range in Main Test	a) With metabolic activation: 50, 150, 500, 1500, 5000 μg/plate. b) Without metabolic activation: 50, 150, 500, 1500, 5000 μg/plate.
Vehicle	Distilled water.
Remarks - Method	

RESULTS

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

Remarks - Results	In a preliminary toxicity test, the notified chemical was non-toxic to TA100 at concentrations between 0 and 5000 μg/plate. The notified chemical was tested up to maximum recommended dose level of 5000 μg/plate. Pink colour was observed at and above 150 μg/plate, however this did not prevent scoring revertant colonies. There was no precipitation of the notified chemical at any dose. No significant increases in the frequency of the revertant colonies were recorded for any strain, at any dose level with and without the metabolic activation. The positive and negative controls gave the expected results confirming the sensitivity of the system.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Safepharm Laboratories (2004c)

7.8. Genotoxicity – in vitro

TEST SUBSTANCE	Magenta Dye 4
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	Cultured human peripheral blood lymphocytes.
Metabolic Activation System	Phenobarbitone/β-naphthoflavone induced rat liver S9 fraction.
Vehicle	Minimal Essential Media.
Remarks - Method	None.

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

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	>5000	≥1250	>5000	-
Test 2		≥1250	>5000	-
Present				
Test 1	>5000	≥5000	>5000	-
Test 2	>2500	>5000	>5000	-

Remarks - Results

The dose range used the preliminary test was 19.53 to 5000 µg/mL. The test was performed on cell culture using a 4-hour exposure time in the presence and absence of S9, followed by a 20-hour recovery time and continuous exposure of 24 hours with metabolic activation. Parallel flasks containing cultured medium without whole blood were established for three exposure conditions so that observation could be made on the precipitation of the notified chemical. No precipitation was observed in the parallel blood-free cultures at the end of the exposure periods in any group. Metaphase cells were present up to 5000 µg/mL in all exposure groups. There was no reduction in the mitotic index up to the maximum dose level at the 4-hour exposure time, however an approximate 50% inhibition of mitotic index was observed following 24-hour continuous exposure.

Test 1
Scoreable metaphases were present at 5000 µg/mL in the presence and absence of S9. No precipitation of the notified chemical was noted at any dose level tested. A dose related inhibition of mitotic index was observed in absence of S9. At 5000 µg/mL there was a 41% inhibition in the absence of S9. In the presence of S9, there was a slight reduction in mitotic index (14% inhibition). The notified chemical did not induce any statistically significant increase in frequency of cells with chromosomal aberrations either in the absence or presence of S9. The notified chemical did not induce a statistically significant increase in the number of polypoid cells at any dose level in the presence or absence of S9.

Test 2
Scoreable metaphases were present at 5000 µg/mL in the presence and absence of S9. No precipitation of the notified chemical was noted at any dose level tested. A dose related inhibition of mitotic index was observed in absence of S9. At 5000 µg/mL there was a greater than 50% inhibition in the absence of S9. In the presence of S9, there was no reduction in mitotic index. The notified chemical did not induce a statistically significant increase in the frequency of cells with chromosomal aberrations either in the absence or presence of S9. The notified chemical did not induce a statistically significant increase in the number of polypoid cells at any dose level in the presence or absence of S9.

CONCLUSION

The notified chemical was not clastogenic to human peripheral blood lymphocytes treated in vitro under the conditions of the test.

TEST FACILITY

Safepharm Laboratories (2004)

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

TEST SUBSTANCE	Magenta Dye 4.
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test.
Inoculum	Centrifuged, washed and resuspended activated sludge from a sewage treatment works that treats predominantly domestic sewage.
Exposure Period	28 days.
Auxiliary Solvent	None.
Analytical Monitoring	Chemical Oxygen Demand (COD).
Remarks – Method	In addition to the test substance (100 mg/L), blank and toxicity control (with test substance at 100 mg/L and sodium acetate at 200 mg/L) samples and samples containing a reference substance (sodium acetate at 200 mg/L) were measured. The pH in all of the test bottles was measured and adjusted to 7.4 ± 0.2, if necessary. The test bottle temperatures were maintained at 22 ± 2°C.

RESULTS

Test substance		*Sodium acetate*	
Day	*% degradation*	*Day*	*% degradation*
6	<6	6	68
10	<6	10	71
15	<6	15	67
21	<6	21	59
28	<6	28	52

Remarks – Results	At the end of test period, the temperatures in the bottles were within the range of 22 ± 2°C and the pH values were 7.4 in the control, 7.3 to 7.4 in reference substance and 7.4 to 7.6 in the test substance bottles. The results indicated that <6% of the test substance degraded in 28 days. Degradation of the reference substance (68% after 6 days) indicates that the test system was valid. The results for the toxicity control showed that the test substance did not inhibit the biodegradation of the reference substance although the mean oxygen consumption in the toxicity control bottles was marginally lower than that in the bottles with reference substance alone.
CONCLUSION	The notified chemical is not readily biodegradable according to the OECD criteria requiring >60% degradation within 10 days of commencement.
TEST FACILITY	Toxikos (2004d)

8.1.2. Bioaccumulation

No data on bioaccumulation were provided. The bioaccumulation potential is considered to be low due to the low log P_{ow} and high water solubility.

8.2. Ecotoxicological investigations

Results for an analogue chemical (previously assessed as LTD/1110) were provided for fish and algae toxicity and inhibition of microbial respiration tests, which are summarised below. The reports only included the main pages and not some of the annexes.

8.2.1. Acute toxicity to fish

TEST SUBSTANCE	Magenta Dye 3.
METHOD	OECD TG 203 Fish, Acute Toxicity Test - Static.
Species	Mirror carp (*Cyprinus carpio)*
Exposure Period	96 h
Auxiliary Solvent	None
Water Hardness	41.7 mg $CaCO_3$/L
Analytical Monitoring	The concentrations of the test substance in the test solutions were measured at 0 and 96 hours using a spectrophotometric method.
Remarks – Method	The test solution was prepared by adding a known amount of the test substance to 25 L of dilution water and stirring until the test substance was fully dissolved resulting in a deep black red coloured solution. A single nominal concentration of 1500 mg/L was tested.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*			
Nominal	*Actual**		*24 h*	*48 h*	*72 h*	*96 h*
0	<0.73	10	0	0	0	0
1500	1600	10	0	0	0	0

* The actual concentrations were provided in the notifier's draft assessment report but were not available in the test report provided (as no annexes were included).

LC50	>1500 mg/L at 96 hours.
NOEC	1500 mg/L at 96 hours (the highest concentration tested).
Remarks – Results	The results of the study showed that no mortalities were observed at the concentration tested. The dissolved oxygen concentration (7.8 to 8.2 mgO_2/L), pH (7.7 to 8.3) and temperature (22 ± 1°C) were all satisfactorily maintained. In the absence of the complete test report, the details of the measurements were not available.
CONCLUSION	The analogue chemical is practically non-toxic to fish.
TEST FACILITY	Toxikos (2002b)

8.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Magenta Dye 4.
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	226 mg $CaCO_3$/L
Analytical Monitoring	The concentrations of the test substance in the test solutions were measured at 0 and 48 hours using the High Performance Liquid Chromatography (HPLC) method. Samples were taken from the excess test solutions at the start and from one replicate of the control and test solution at the end of test.

Remarks – Method: The test solution was prepared by direct addition of the test substance to dilution water. After vigorous shaking the test solution was clear and deep magenta in colour. A single nominal concentration of 120 mg/L was tested.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual (Mean Measured)*		*24 h*	*48 h*
0	<2	20	0	0
120	120	20	0	0

LC50: >120 mg/L at 48 hours

NOEC: 120 mg/L at 48 hours (the highest concentration tested).

Remarks – Results: A change in the chromatogram pattern was observed in the 48-hour samples compared with the standards and the 0 hour samples, indicating a slight change in the structure or speciation of the molecule. However, quantification of the peak area was still possible using blank correction. The overall mean measured concentration was 100% of the nominal value. The nominal concentrations were used for reporting.

The dissolved oxygen concentration (9.0 mgO_2/L in control and 8.8 to 9.0 mgO_2/L in the test substance solutions) and pH (8.1 in control and 8.0 to 8.1 in test solutions) were satisfactorily maintained. The temperature was maintained at 20 ± 1°C (19.8, 19.6 and 19.6°C at 0, 24 and 48 hours).

No immobilised Daphnia or symptoms of toxicity were observed after 48 hours in any test vessel.

CONCLUSION: The notified chemical is practically non-toxic to *Daphnia magna*.

TEST FACILITY: Toxikos (2004e)

8.2.3. Algal growth inhibition test

TEST SUBSTANCE: Magenta Dye 3.

METHOD: OECD TG 201 Alga, Growth Inhibition Test.

Species: *Selenastrum capricornutum*

Exposure Period: 72 hours

Concentration Range Nominal: 1.0, 2.3, 5.0, 11, 25, 55 and 120 mg/L

Concentration Range Actual: <0.18, 0.98, 2.3, 4.9, 11, 24, 56, 120 mg/L

The actual concentrations were listed by the notifier but were not available from the test report provided. However, the summary of the test report indicated that the mean measured test concentrations ranged from 96 to 120% of the nominal exposure concentrations.

Auxiliary Solvent: None.

Water Hardness: Standard culture medium was used.

Analytical Monitoring: The concentrations of the test substance in the test solutions were measured at 0 and 72 hours and analysed spectrophotometrically.

Remarks – Method: A solution (2000 mL) of the highest nominal concentration (120 mg/L) was prepared by adding the test substance to sterile culture medium. The lower concentrations were prepared by adding aliquots of this solution to culture medium. The solutions were all clear and pink/red in colour

(increasing intensity with concentration).

Each replicate test vessel was inoculated to give an initial cell density of 1.00 x 10^4 cells/mL. The test vessels were incubated under shaded and illuminated conditions (two replicates each of the illuminated and shaded conditions).

RESULTS

	*Biomass**		*Growth**	
	EbC50 mg/L at 72 h	*NOEC Biomass mg/L at 72h*	*ErC50 mg/L at 72 h*	*NOEC Growth mg/L at 72h*
Exposed solution	6.8 (3.3-10.2)#	1.0	53.2##	1.0
Shaded solution	8.1 (7.1-9.2)#	1.0	41.3##	1.0

* All results are based on nominal test concentrations.
\# 95% confidence limit.
95% confidence limits for these values were not available in the test report.

Remarks – Results

The test temperature in the incubator was maintained within 24 ± 2°C (no detailed readings available). The pH values at the start of the test (7.6 in the controls and 7.5 to 7.9 in the test substance solutions) and at the end of the test (7.6 to 7.9 in the controls and 7.5 to 7.8 in test substance solutions) were all satisfactorily maintained.

The nominal concentrations were used for calculating the EC50 values. Graphical comparisons of the percentages of inhibition in the exposure and shaded vessels showed that these curves were essentially the same. Inhibition of growth rate in exposure vessels plotted (%E) against that in shaded vessels (%S) showed that the curve follows the theoretical line plotted when %E = %S. This result indicated that the light absorbing properties of the test substance were a significant factor in the inhibition and therefore, it is not possible to distinguish reduced growth due to toxic effects from those due to differences in illumination.

CONCLUSION

The report indicates that the test substance satisfies the exemption clause in Annex VI (Dir.93/21/EEC) and the 72-hour EC50 for algae should not be used as a basis for classification of the test substance (analogue chemical).

TEST FACILITY

Toxikos (2002c).

8.2.4. Inhibition of microbial activity

TEST SUBSTANCE

Magenta Dye 3.

METHOD

OECD TG 209 Activated Sludge, Respiration Inhibition Test.

Inoculum

Activated sludge obtained from a sewage treatment plant that treats predominantly domestic sewage.

Exposure Period

3 hours

Concentration Range Nominal

1.0, 3.2, 10, 32 and 100 mg/L

Remarks – Method

Test concentrations of the reference substance (3,5-dichlorophenol) were 1.0, 3.2, 10, 32 and 100 mg/L.

RESULTS

EC50

>100 mg/L at 3 hours

NOEC

100 mg/L at 3 hours

Remarks – Results

No significant effect on respiration was observed at any of the test concentrations used (% inhibition of the respiration rate < 10%). The

	EC50 of the reference substance at 3 hours was 13 mg/L (within the range of 5 to 30 mg/L), thus validating the test.
CONCLUSION	The analogue does not inhibit the respiration of activated sludge. These test results are consistent with the results of the ready biodegradability study (summarised in Section 8.1.1), which indicated that the notified chemical is not inhibitory to sewage sludge micro-organisms at 100 mg/L.
TEST FACILITY	Toxikos (2002d).

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

Environmental exposure of the notified chemical will result from the disposal of cartridges, printed-paper and any leaked ink containing the chemical during the use of the cartridges. The total import volume of the notified chemical will ultimately be either disposed of to landfill, incinerated or recycled with paper.

The notified chemical is not volatile, therefore, will not dissipate into air. It is water-soluble and is expected to remain within the aquatic environment but will not readily hydrolyse in natural waters at environmental pH values. The low log P_{ow} is consistent with the high water solubility indicating a low affinity for the organic phase and component of soils and sediments. It can be highly mobile in soil due to high water solubility. The adsorption data for the analogue chemical provided indicate that the notified chemical will be highly adsorbed at low pH and poorly adsorbed at high pH. However, it is expected to adhere to cellulose fibres on paper.

Although not readily biodegradable, it is anticipated that prolonged residence in an active landfill environment would eventually degrade the notified chemical due to abiotic or slow biotic processes. Incineration of waste paper and sludge will destroy the notified chemical with the generation of water vapour and oxides of carbon, nitrogen, and sulphur plus metal salts.

Recycling may take place in a number of centres throughout Australia. During the paper recycling process, waste paper is repulped using a variety of alkaline, dispersing and wetting agents, water emulsifiable organic solvents and bleaches. Trade sources estimate the washing process will recover 30-60% of the total amount of ink and therefore, at least 30% of the notified chemical in the recycled paper will be disposed of with sludge in landfill. However, a greater proportion can be expected to remain in the aqueous phase due to the high water solubility of the notified chemical.

A predicted environmental concentration (PEC) in the aquatic environment is estimated below using a worst-case scenario where the entire import volume (the maximum of 3000 kg) of the notified chemical will be used on paper and 50% of the printed paper will be recycled with 60% of the chemical remaining in the aqueous phase during the recycling process. Under this scenario 900 kg of the notified chemical per year will be discharged to sewer and if it is assumed that none is attenuated within the sewage treatment plants (STP), the daily release on a nationwide basis to receiving waters is estimated to be 2.47 kg/day.

Assuming a national population of 20 million and that each person contributes an average 200 L/day to overall sewage flows (Environment Australia, 2003), the worst-case predicted environmental concentration (PEC) in sewage effluent on a nationwide basis is estimated as 0.62 µg/L. Based on the respective dilution factors of 1 and 10 for inland and ocean discharges of effluents, the PECs of the notified chemical in freshwater and marine water may approximate 0.62 and 0.062 µg/L, respectively.

The notified chemical is not readily biodegradable. Its Henry's Law Constant of $< 2.6 \times 10^{-8}$

Pa m^3/mol (log H <-6.59) and log P_{ow} of <-2.7, which are both limit values were applied in the SIMPLETREAT model (European Commission, 2003) for modelling partitioning and losses in STPs. The results indicate that when 900 kg of the notified chemical is released into the aqueous phase of a STP, 0% released to air through volatilisation, 0% partitioned to biosolids and 100% (900 kg) partitioned to water. Therefore, the PECs of the notified chemical in effluent released, freshwater and marine water will be the same as the worst-case PECs estimated above (i.e. approximately 0.62 and 0.062 µg/L, respectively).

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 0.1 m of soil (density 1000 kg/m^3). Using these assumptions, irrigation with a concentration of 0.62 µg /L may potentially result in a soil concentration of approximately 6.2 x 10^{-3} mg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 3.1 x 10^{-2} mg/kg and 6.2 x 10^{-2} mg/kg, respectively.

Due to the low log P_{ow} and the high water solubility of the notified chemical, its potential for bioaccumulation is low in exposed aquatic organisms.

9.1.2. Environment – effects assessment

The results of the aquatic toxicity tests for the notified chemical and the analogue chemical are listed below.

Organism	*Duration*	*End Point*	*mg/L*
Fish (for the analogue)	96-h	LC50	>1600
Daphnia (for the notified chemical)	48-h	EC50	>120

The results obtained for algal growth inhibition (for the analogue chemical) are concluded to be influenced by the light absorbing properties of the chemical. As it was not possible to distinguish toxic effects from reduced growth due to light attenuation, the results of the algae toxicity study could not be used as a basis for classification of the chemical.

A predicted no effect concentration (PNEC - aquatic ecosystems) of >0.12 mg/L (>120 µg/L) has been derived by dividing the end point value of >120 mg/L by a worst-case scenario uncertainty (safety) factor of 1000 (as usable toxicity data are available only for two trophic levels). Use of this safety factor is also appropriate as the fish toxicity result was obtained using an analogue to the notified chemical of uncertain suitability for fish toxicity testing.

9.1.3. Environment – risk characterisation

Location	PEC* µg/L	PNEC µg/L	Risk Quotient (RQ)*
Australia-wide STPs			
Ocean outfall	0.062	>120	<5.1 x 10^{-4}
Inland River	0.62	>120	<5.1 x 10^{-3}

* PEC and the RQ values calculated assuming 100% of the notified chemical partitioned into water during the STP process (based on the SIMPLETREAT model).

The RQ values (PEC/PNEC) derived for the aquatic environment (assuming nationwide use, only 50% of the printed paper recycled and 60% of the notified chemical partitioned to water in STP) are considerably below 1 for both freshwater and marine water, indicating no immediate concern to the aquatic compartment. Based on the proposed use pattern the notified chemical is not expected to pose an unacceptable risk to the health of aquatic life. Bioaccumulation is not expected from the diffuse use pattern and low import volume. Based on low exposure potential from effluent for agricultural purposes, it is unlikely to result in unacceptable risk to soil organisms.

9.2. Human health

9.2.1. Occupational health and safety – exposure assessment

There is low potential for worker exposure to the notified chemical when replacing spent cartridges as the notified chemical is at low concentration (<4%) in the ink formulations which are sealed within the cartridge. Service technicians may occasionally experience dermal contact with the notified chemical during maintenance; however, the notified chemical is at low concentrations in the ink formulations. Exposure to the notified chemical on printed paper is low as the dye is bound to the paper matrix.

The notified chemical will be imported in pre-packed sealed cartridges. During transport and storage, workers are unlikely to be exposed to the notified chemical except when cartridges are accidentally breached.

9.2.2. Public health – exposure assessment

From the point of importation to the end use of the ink preparation containing the notified chemical, the ink preparation is either enclosed in a cartridge made for insertion in ink jet printers or is present on printed paper in a cured state. Public exposure through importation, transportation or storage is assessed as negligible. There is little potential for exposure during cartridge changes. Any exposure to the ink preparation that does occur is most likely to be dermal and of a minimal and transient nature. Ink containing the notified chemical on the printed page is bound to the paper and is not biologically available. Public exposure is assessed as low.

9.2.3. Human health – effects assessment

Based on the notified chemical's very low partition coefficient, high water solubility, and large molecular size, dermal absorption of the notified chemical is unlikely.

The notified chemical has low acute oral toxicity to rats. An analogue previously assessed as LTD/1110 (Magenta Dye 3) was found to have low acute dermal toxicity. The analogue was not irritating to the skin of the rabbit, and was not a skin sensitiser in the guinea pig maximisation test. The analogue was slightly irritating to the eye of the rabbit. In a 28-day repeat dose oral toxicity study in rats, a NOEL of 250 mg/kg bw/day was established based on the on changes in the gastric mucosa at higher doses.

The notified chemical was neither mutagenic nor genotoxic in a bacterial reverse mutation test and mammalian chromosome aberration test, respectively.

Based on the available data, the notified chemical is not classified as a hazardous substance in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC 2002).

9.2.4. Occupational health and safety – risk characterisation

The OHS risk presented by the notified chemical is expected to be low given that the notified chemical is present in the ink at <4%, is not determined to be hazardous and the ink is contained in enclosed cartridges.

9.2.5. Public health – risk characterisation

Members of the public are not likely to make contact with the notified chemical during cartridge changes unless the cartridge is ruptured or otherwise tampered with. Additionally the notified chemical is present at low concentrations in a formulation that is not classified as hazardous. Ink containing the notified chemical on the printed pages is bound to the paper and is not readily bioavailable.

Consumer exposure to the notified chemical via the printed paper has been estimated by the manufacturer. 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 can be assumed to incorporate 1mg of notified chemical. Based on a 50% transfer on contact when handling printed - paper (assuming only partially dry ink), and the relative areas of finger ends and paper size, it is estimated that potential removal is <1% of the applied ink in each event.

Estimated Exposure
Area of contact with finger ends (four fingers on one hand) = 8 cm^2
A4 sized paper substrate = ca. 600 cm^2
% Removal = (8/600) × 0.5 × 100 = < 1%
Therefore total removal to finger ends at point of contact would be < 1% of 1 mg notified chemical per event = < 0.01 mg

For extensive contact (i.e. > 10 events per day) the daily body burden, assuming no washing between events, 70 kg person and 100% absorption, would be < 0.01 × 10/70 = ca. 0.0014 mg/kg/day.

The NOEL from the 28-day repeat dose study for the analogue Magenta Dye 3 was 250 mg/kg bw/day.

The Margin of Exposure (NOEL/Estimated Exposure) is 178571. As the MOE exceeds 100, the notified chemical does not pose a regulatory concern.

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

10.1. Hazard classification

Based on the available data the notified chemical is not 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.

It is not possible to classify the notified chemical according to the GHS criteria for the environmental and human health end points.

10.2. Environmental risk assessment

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

10.3. Human health risk assessment

10.3.1. Occupational health and safety

There is Low Concern to occupational health and safety under the conditions of the occupational settings described.

10.3.2. Public health

There is Negligible Concern to public health when used as described in this notification.

11. MATERIAL SAFETY DATA SHEET

11.1. Material Safety Data Sheet

The MSDS of the product 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 the 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 as introduced:
  - Avoid ocular and dermal exposure
- 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.

Environment

Disposal

- The empty cartridges and ink containing the notified chemical should be disposed of in landfill in accordance with Federal, State and Local government regulations.

Emergency procedures

- Spills/release of the ink containing the notified chemical should be handled by containing the spill by soaking up with absorbent material (sawdust, sand or earth). Slowly vacuum or sweep the material/used absorbent into a bag or other sealable container for disposal.
- Do not allow material or contaminated packaging to enter drains, sewers or water courses. Do not flush into surface water or sanitary sewer system.

12.1. Secondary notification

The Director of Chemicals Notification and Assessment must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(2) of the Act:
  - if any of the circumstances listed in the subsection arise.

The Director will then decide whether secondary notification is required.

No additional secondary notification conditions are stipulated.

13. BIBLIOGRAPHY

Environment Australia (2003) Model and Guidance for Estimating Predicted Environmental Concentrations to Surface Water and Soil from Chemicals Released to the Environment Through a Sewage Treatment Plant. Chemical Assessment Section, Environment Australia, Canberra Australia.

European Commission (2003) Technical Guidance Document on Risk Assessment in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No. 1488/94 on Risk Assessment for Existing Substances and Directive 98/8/EC of the European Parliament and of the Council Concerning the Placing of Biocidal Products on the Market – Part II. Institute for Health and Consumer protection, European Chemicals Bureau, European Communities.

Mensink BJWG, Montforts M, Wijkhuizen-Maslankiewicz L, Tibosch H and Linders JBHJ (1995) Manual for Summarising and Evaluating the Environmental Aspects of Pesticides. National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands. Report No. 679101022.

NOHSC (1994) National Code of Practice for the Labelling of Workplace Substances [NOHSC:2012(1994)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

NOHSC (2002) Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2002)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

Safepharm Laboratories (2002a) [Analogue]: Acute Dermal Irritation in the Rabbit. SPL Project Number 780/213. Safepharm Laboratories Limited, Derby, UK (unpublished report submitted by notifier).

Safepharm Laboratories (2002b) [Analogue]: Acute Eye Irritation in the Rabbit. SPL Project Number 780/214. Safepharm Laboratories Limited, Derby, UK (unpublished report submitted by notifier).

Safepharm Laboratories (2002c) [Analogue]: Skin Sensitisation in the Guinea Pig – Magnusson and Kligman Maximisation Method. SPL Project Number 780/215. Safepharm Laboratories Limited, Derby, UK (unpublished report submitted by notifier).

Safepharm Laboratories (2003a) [Analogue]: Acute Dermal Toxicity (Limit Test) in the Rat. SPL Project Number 780/266. Safepharm Laboratories Limited, Derby, UK (unpublished report submitted by notifier).

Safepharm Laboratories (2003b) [Analogue]: 28 Day Repeated Dose Oral (Gavage) Toxicity Study in the Rat. SPL Project Number 780/287. Safepharm Laboratories Limited, Derby, UK (unpublished report submitted by notifier).

Safepharm Laboratories (2004a) [Notified Chemical]: Acute Oral Toxicity in the Rat – Acute Toxic Class Method. SPL Project Number 780/359. Safepharm Laboratories Limited, Derby, UK (unpublished report submitted by notifier).

Safepharm Laboratories (2004b) [Notified Chemical]: Reverse Mutation Assay "Ames Test" using *Salmonella typhimurium*. SPL Project Number 780/360. Safepharm Laboratories Limited, Derby, UK (unpublished report submitted by notifier).

Safepharm Laboratories (2004c) [Notified Chemical]: Chromosome aberration test in Human Lymphocytes *in vitro*. SPL Project Number 780/361. Safepharm Laboratories Limited, Derby, UK (unpublished report submitted by notifier).

Toxikos (2002a). Estimation of the Adsorption Coefficient on Soil HPLC Method. (unpublished report submitted by Toxikos Pty Ltd, Melbourne, Australia).

Toxikos (2002b) [Analogue]: Acute Toxicity to Mirror Carp (*Cyprinus carpio*). (unpublished report submitted by Toxikos Pty Ltd, Melbourne, Australia).

Toxikos (2002c) [Analogue]: Toxicity to the Green Alga (*Selenastrum capricornutum*). (unpublished report submitted by Toxikos Pty Ltd, Melbourne Australia).

Toxikos (2002d) [Analogue]: Effect on the Respiration Rate of Activated Sludge. (unpublished report submitted by Toxikos Pty Ltd, Melbourne Australia).

Toxikos (2004a) Physical/Chemical Characterisation of [Notified Chemical] (unpublished report submitted by Toxikos Pty Ltd, Melbourne Australia).

Toxikos (2004b) Determination of Physical and Chemical Properties: [Notified Chemical] (unpublished report submitted by Toxikos Pty Ltd, Melbourne Australia).

Toxikos (2004c) Experimental pKa Values for [analogue and notified chemical]. Summary Report. Intertek ASG, Manchester, UK (unpublished report submitted by notifier).

Toxikos (2004d) [Notified chemical]: Determination of 28-day Ready Biodegradability. (unpublished report submitted by Toxikos Pty Ltd, Melbourne Australia).

Toxikos (2004e) [Notified chemical]: Acute Toxicity to Daphnia Magna. (unpublished report submitted by Toxikos Pty Ltd, Melbourne Australia).

NOHSC (2003) National Code of Practice for the Preparation of Material Safety Data Sheets, 2nd edn [NOHSC:2011(2003)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

OECD (2001) OECD Guideline for Testing of Chemicals 121(22 January 2001): Estimation of the Adsorption Coefficient (Koc) on Soil and Sewage Sludge using HPLC.

United Nations (2003) Globally Harmonised System of Classification and Labelling of Chemicals (GHS). United Nations Economic Commission for Europe (UN/ECE), New York and Geneva.