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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Red Dye 2

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Director

Chemicals Notification and Assessment

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Hewlett Packard Australia Pty Ltd (ABN 74 004 394 763)
31-41 Joseph Street
Blackburn VIC 3130

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 identity;
Impurities;
Spectral data;
Percentage of dye in ink product;
Exact import volume; and
SPECIFIC USE

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows:
Flash point;
Dissociation constant; and
Bioaccumulation.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
EU, US and Switzerland

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Red Dye 2

3. COMPOSITION

DEGREE OF PURITY
High

4. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as a component of printing inks in pre-packed cartridges. The inks will contain <5% notified chemical.

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

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

USE
As a dye in printing equipment.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, Transport and Storage

PORT OF ENTRY
Melbourne VIC

IDENTITY OF MANUFACTURER/RECIPIENTS
Hewlett Packard Australia Pty Ltd
31-41 Joseph Street
Blackburn Victoria 3130

TRANSPORTATION AND PACKAGING
The notified chemical will be imported by ship as pre-packaged cartridges. The cartridges will be packed in sturdy cardboard boxes and would normally be transported and distributed to customers by road.

5.2. Operation Description
No reformulation or repackaging of the product occurs in Australia. The sealed ink-jet cartridge is delivered to the end-user in its original packaging. The ink-jet cartridge will be handled by service technicians and office workers when replacing spent cartridges in the printer.

5.3. Occupational Exposure
Number and Category of Workers

Category of Worker	*Number*	*Exposure Duration*	*Exposure Frequency*
Service technicians	Approx 10	8 h/day (approx.)	230 days/year (approx.)
Office workers	Approx 1000	5 - 10 minutes	Approx. 10 days/year

Exposure Details
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 film, 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 matrix and is not expected to be readily bioavailable.

Trained customer service engineers will maintain and clean printing machines.

5.4. Release

RELEASE OF CHEMICAL AT SITE
No release is expected as reformulation of the ink containing the notified chemical will not take place in Australia.

RELEASE OF CHEMICAL FROM USE
Release of the ink solution to the environment is not expected under normal use since the ink cartridges are designed to prevent leakage. If leakage or accidental spill occurs when changing spent cartridges, the ink will be contained with absorbent material, which will presumably be disposed of in landfill.

Ultimately, all of the notified chemical will be released to the environment. Printed paper to which the notified chemical will be bound will eventually be buried in landfill or incinerated. The chemical may also be released in effluent from de-inking processes. Residues left in empty cartridges (estimated as <10% of ink) will most likely be disposed of to landfill

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 wastes are expected to go to trade waste sewers. It is estimated that about 20% of the ink printed on paper will enter paper recycling and up to 60% of the ink is recovered during recycling.

The low percentage of notified chemical in the ink and the paper recycling process contributes to low and highly diffuse release of the chemical to the aquatic compartment.

5.5. Disposal

The disposal of uncured inks will be largely confined to residues contained in the cartridge systems that do not allow the replacement of individual colours. These residues are expected to remain in the cartridge housing and be disposed of by landfill.

5.6. Public Exposure

The notified chemical will not be manufactured, reformulated or packaged in Australia. The imported inkjet cartridges may be transported by air, ship, rail, or truck to their distribution location. The ink is contained in the cartridge and the physical design of the cartridge prevents handlers from accidentally touching the ink. The design also prevents leakage of ink. Contact with very small quantities of ink during changing cartridges or on handling incompletely dried printed material may occur.

The public may be exposed to the notified chemical in the event of an accident during transport involving extensive breakage of cartridges.

6. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa Red Crystalline powder.

Melting Point/Freezing Point > 400°C

METHOD EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
TEST FACILITY Hazleton UK (1992a).

Density 1668 kg/m^3 at 20°C

METHOD EC Directive 92/69/EEC A.3 Relative Density.
TEST FACILITY Hazleton UK (1992a).

Vapour Pressure 1.5 x 10^{-8} kPa at 25°C

METHOD OECD TG 104 Vapour Pressure.
EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks Determined using vapour pressure balance and extrapolated from the result at 205.75 °C
TEST FACILITY Hazleton UK (1992b).

Water Solubility 579 g/L at 20°C

METHOD EC Directive 92/69/EEC A.6 Water Solubility.
Remarks Shake flask method; Analytical Method: HPLC
TEST FACILITY Hazleton UK (1992a).

Hydrolysis as a Function of pH

METHOD OECD TG 111 Hydrolysis as a Function of pH.
EC Directive 92/69/EEC C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$ hours*
4	50	955
7	50	692
9	50	401

Remarks Test report not provided.
TEST FACILITY Hazleton UK (1992a).

Partition Coefficient (n-octanol/water) log Pow at 20°C = -3.8

METHOD EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks Shake flask method; Analytical Method: HPLC
TEST FACILITY Hazleton UK (1992a).

Adsorption/Desorption log K_{oc} = 3.46 at 21.5°C.

Soil Type	*Organic Carbon Content (%)*	*pH*	*Koc (mL/g)*
1		4.5-5.5	4300
2		5.5-6.5	1180
3		7.1-8.0	3070

METHOD OECD TG 106 Adsorption - Desorption Using a Batch Equilibrium Method.
Remarks Samples of three soil types characterised with respect to pH, organic carbon content, particle size distribution, cation exchange capacity and exchangeable cations, and moisture content were used in the tests. In the adsorption step, duplicate wet soil/test solution mixtures and a wet soil/0.01 M $CaCl_2$ solution as soil control were prepared for each soil type. A control consisting of test solution with no soil was also prepared. The samples were shaken continuously for 16 h. After equilibration, the samples were centrifuged to separate the phases and an aliquot was taken for analysis by HPLC. In the desorption step, the supernatant removed during the adsorption step was replaced with fresh 0.01 M $CaCl_2$ solution and the process was repeated as in the adsorption step. Between 1.22 and 11.1 % desorbed.

The result indicates that the average log Koc of 3.46 is considered to be of low to slight mobility in soil (McCall 1980).
TEST FACILITY Safepharm Laboratories Limited (1997b)

Dissociation Constant Not determined

Remarks The notified chemical contains aryl sulfonate groups which typically have pKa value of −1.0 to 1.0 and aryl amine groups which will have pKa values of 1.0 to 5.0. The notified chemical is in a salt form and will be fully dissociated in water.

Particle Size 11.7% of particles had a diameter less than 75 µm.

METHOD Dry sieving method.
TEST FACILITY Hazleton UK (1992a).

Surface Tension 46.1 mN/m at 20°C (90% saturated)
45.1 mN/m at 20°C (80% saturated)

METHOD EC Directive 92/69/EEC A.5 Surface Tension.
Remarks Concentration: 80% and 90% saturated. Based on the determined surface tensions,

the test substance is considered to be surface active.

TEST FACILITY Hazleton UK (1992a).

Flash Point Not determined

Remarks The notified chemical is solid.

Flammability Limits Not determined

Remarks The notified chemical will be imported as part of an aqueous solution.

Autoignition Temperature Not determined

Remarks The notified chemical will be imported as part of an aqueous solution.

Explosive Properties Not determined

Remarks Not expected to be explosive based on structure.

Reactivity Not determined

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

7. TOXICOLOGICAL INVESTIGATIONS

Endpoint and Result	*Assessment Conclusion*
Rat, acute oral LD50 > 2000 mg/kg bw	low toxicity
Rat, acute dermal LD50 > 2000 mg/kg bw	low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	severely irritating
Guinea pig, skin sensitisation - adjuvant test.	evidence of sensitisation.
Rat, oral repeat dose toxicity - 28 days.	NOAEL = 500 mg/kg/day bw
Genotoxicity - bacterial reverse mutation	mutagenic
Genotoxicity – in vitro chromosomal aberrations test in CHO cells	genotoxic
Genotoxicity – in vivo mouse bone marrow micronucleus test	non genotoxic

7.1. Acute toxicity – oral

TEST SUBSTANCE Notified chemical

METHOD OECD TG 401 Acute Oral Toxicity – Limit Test.
EC Directive 92/69/EEC B.1 Acute Toxicity (Oral) – Limit Test.

Species/Strain Rat/Sprague-Dawley.

Vehicle Purified water.

Remarks – Method A preliminary study was conducted with 2 rats/sex at doses of 500, 1000 and 2000 mg/kg bw.

RESULTS

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

LD50 > 2000 mg/kg bw

Signs of Toxicity None

Effects in Organs Red coloured urine persisting to Day 2.

Remarks – Results	No mortality was observed in the preliminary study.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Hazleton France (1992a)

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.
Vehicle	Purified water
Type of dressing	Semi-occlusive.
Remarks – Method	A preliminary study was conducted with 2 rats/sex at doses of 1000 and 2000 mg/kg bw.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Red colouration prevented observation of erythema during days 2 and 3.
Signs of Toxicity - Systemic	None
Effects in Organs	None
Remarks – Results	No mortality and signs of toxicity were observed during the study.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	Hazleton France (1992b)

7.3. Irritation – skin

TEST SUBSTANCE	Notified chemical.
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 males
Vehicle	Purified water
Observation Period	7 days.
Type of Dressing	Semi-occlusive.
Remarks – Method	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	2	0.67	1	2	72 hours	0
Oedema	0.33	0	1	1	72 hours	0

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

Remarks – Results	Discolouration of treated area by the test material was observed, and

resulted to imprecise scoring of erythema. In one animal, slight dryness of the skin at the application area was observed at 72-hour observation period, and absence of hair on the application area on day 7. Slight desquamation of the epidermis on the same animal was observed at 72 hours and persisted up to 7 days. Signs of irritation were resolved by day 7.

CONCLUSION — The notified chemical is slightly irritating to skin.

TEST FACILITY — Hazleton France (1992c)

7.4. Irritation – eye

TEST SUBSTANCE — Notified chemical.

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

Species/Strain — Rabbit/New Zealand White
Number of Animals — 3 males
Observation Period — 21 days
Remarks – Method — 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	2	2.67	2.33	3	21 days	2
Conjunctiva: chemosis	1.33	3	3	3	21 days	2
Corneal opacity	2	3.33	3.67	4	21 days	4
Iridial inflammation	1	1	1	1	21 days	1

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

Remarks – Results — Severe lesions observed at 72 hours which persisted up to 21 days include depilated area around the lids of the treated eye, neovascularisation are of the corneal surface, severe adherence of the test article to the cornea, deformation of the cornea, circumcorneal injections and congestion of the iris, permanent myosis with preservation of direct photomotor reflex, and presence of whitish humour on the side of the lids. The discolouration of the conjunctiva in all animals from 24-hour observation resulted to imprecise reading.

CONCLUSION — The notified chemical is severely irritating to the eye.

TEST FACILITY — Hazleton France (1992d)

7.5. Skin sensitisation

TEST SUBSTANCE — Notified chemical.

METHOD — OECD TG 406 Skin Sensitisation – maximisation test.
EC Directive 96/54/EC B.6 Skin Sensitisation - maximisation test.

Species/Strain — Guinea pig/Dunkin-Hartley
PRELIMINARY STUDY — Maximum Non-irritating Concentration:
intradermal: 2.5% w/w
topical: 9% w/w

MAIN STUDY

Number of Animals	Test Group: 10/sex Control Group: 10/sex
induction phase	Induction Concentration: intradermal injection: 2.5% w/w topical application: 9% w/w
Signs of Irritation	Signs of irritation including slight burnt aspect to the injection site were noted during the induction. The test article tinted the skin making erythema observation impossible.
CHALLENGE PHASE	
1st challenge	topical application: 9% w/w
Remarks – Method	During the preliminary study, the intradermal injection of 2.5% w/w, the test substance tinted the skin and erythema could not be scored. Treatment did not induce oedema. At topical application of 9%, similar effects to that during intradermal injection were observed. During the main study, the test substance tinted the skin making observation of erythema impossible. Therefore, the sensitisation reactions were determined by histological examination at 24 hr only.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: 1st challenge*	
		24 h	*48 h*
Test Group	9%	20/20	-
Control Group	9%	0/20	-

Remarks – Results	The macroscopic and histopathological examinations revealed pathological lesions of delayed hypersensitivity in all treated animals. No cutaneous abnormality was observed in the control group.
CONCLUSION	There was evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Hazleton France (1992e)

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
Route of Administration	Oral – gavage.
Exposure Information	Total exposure days: 28 days; Dose regimen: 7 days per week
Vehicle	Purified water.
Remarks – Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
I (control)	5/sex	0	0
II (low dose)	5/sex	50	0
III (mid dose)	5/sex	150	0
IV (high dose)	5/sex	500	0

Mortality and Time to Death

There were no unscheduled deaths during the study.

Clinical Observations

Staining of the tail in the mid and high dose groups were observed at weeks 2 to 4. Two females in low dose groups also had staining to the tail during the last week of treatment. All treated groups had dark and/or red faeces throughout the treatment period. Except for slightly higher amount of food consumed by treated males, no treatment-related effects on food consumption were observed. A slight increase in body weight gain for all treated groups over the treatment period.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Clinical chemistry

Slight decreases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by the dose response test were observed in treated males. Total bilirubin plasma concentration was increased in high dose groups.

Haematology

Slight increased in mean cell haemoglobin concentration (MCHC), decreased platelet counts and decreased percentage lymphocyte counts by the dose response test were observed in treated males. High dose group males also had higher percentage of neutrophil counts compared with controls.

Effects in Organs

Statistically significant increase in adrenal weights of low dose females was observed. Other than pink discolouration of stomachs from some high dose animals, no macroscopic effects in organ weights were observed. Sporadic histopathological changes were noted in both high dose animals and controls.

Remarks – Results

The slight increase in food consumption in treated males may be associated with the increase in body weight. The changes in MCHC and platelet count were considered by the study authors to be of insufficient magnitude to be of toxicological significance. The changes in lymphocyte and neutrophil counts were probably due to the shift in the type of white blood cells as total white blood cell count is comparable to controls. The increased in total plasma bilirubin concentration was attributed to the colourimetric method used for total bilirubin, which is based on the measurement of azobilirubin. The notified chemical is a diazo dye and therefore expected to affect the determination of total bilirubin using azobilirubin as endpoint.

The increase in adrenal weights were confined to low dose females and therefore not considered to be treatment related.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 500 mg/kg bw/day in this study.

TEST FACILITY	Hazleton Europe (1994a)

7.8. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1538, TA1535, TA1537, TA98, TA100
Metabolic Activation System	Aroclor 1254 - induced rat liver S9 fraction.
Concentration Range in Main Test	a) With metabolic activation: 0 - 5000 µg/plate. b) Without metabolic activation: 0 - 5000 µg/plate.
Vehicle	Water

Remarks – Method A preliminary study was conducted on TA100 without metabolic activation using test concentrations 0.05, 0.1, 0.5, 1.0 and 5.0 mg/plate. Toxicity was not observed at concentrations up to 5.0 mg/plate.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/plate) Resulting in:*			
	Cytotoxicity in PreliminaryTest	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Present				
Test 1	none	none	none	5000
Test 2	none	none	none	2500
Absent				
Test 1	none	none	none	2500
Test 2	none	none	none	2500

Remarks – Results A mutagenic effect was observed in TA98 and TA100 (up to 3.8 times of control in TA98 in the absence of metabolic activation) and the effect was reduced in the presence of S9. No precipitation or cytotoxicity was observed.

Appropriate positive controls induced marked increases in the number of revertant colonies, indicating that the test system responded appropriately.

CONCLUSION The notified chemical was mutagenic to bacteria under the conditions of the test.

TEST FACILITY Hazleton France (1992f)

7.9. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 In vitro Mammalian Chromosomal Aberration Test.
EEC Annex V B10

Cell Type/Cell Line Chinese Hamster Ovary cells

Metabolic Activation System Aroclor 1254 - induced rat liver S9 fraction

Vehicle Culture medium

Remarks – Method Experiment 1 consisted of 3 trials. Trial 1 was considered invalid due to technical errors during treatment. Trial 2 was considered invalid (for the absence of S9) since the positive control compound (NQO) failed to produce adequate number of analysable metaphase spreads. Trial 3 was performed in the absence of metabolic activation. The highest concentration chosen for analysis in Experiment 1 were 836.2 μg/ml (Trial 3) and 1900 μg/ml (Trial 2), which induced 67% and 68% mitotic inhibition in the absence and presence of S9, respectively. In Experiment 2, the highest concentrations chosen for analysis were 1000 μg/ml and 1700 μg/ml, which induced 69% and 81% mitotic inhibition in the absence and presence of S9, respectively.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Present			
Test 1	0, 1715, 1805, 1900	2 hr	20 hr
Test 2	0, 1228, 1445, 1700	2 hr	20 hr
Absent			
Test 1	0, 794.4, 836.2	20 hr	20 hr
Test 2	0, 902.4, 949.9, 1000	20 hr	20 hr

Above 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		1715	None	1715
Test 2		1228	None	1228
Absent			None	
Test 1		794.4	None	794.4
Test 2		902.4	None	902.4

Remarks – Results: Cultures treated with the test substance in both the absence and presence of S9 exhibited large increases in frequencies of aberrant cells. The presence of exchange type aberrations was significant, as these types of aberrations are known to lead to heritable damage. However, the large number of aberrations/aberrant cells were induced at dose levels inducing less than 50% mitotic inhibition and therefore, it is unlikely the aberrations were due to direct result of toxic effects.

CONCLUSION: The notified chemical was clastogenic to CHO cells treated in vitro under the conditions of the test.

TEST FACILITY: Hazleton Europe (1994b).

7.9. Genotoxicity – in vivo

TEST SUBSTANCE: Notified chemical.

METHOD: EC Directive 2000/32/EC B.12 – Mutagenicity (Micronucleus Test)
Species/Strain: Mouse/CD-1
Route of Administration: Oral – gavage.
Vehicle: Purified water.
Remarks – Method: No significant protocol deviations. The dose levels for the main study were selected from a dose range finding study.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time Hours*
1	5/sex	500	24 and 48 hours
2	5/sex	1000	24 and 48 hours
3	5/sex	2000	24 and 48 hours
4	5/sex	80 (CP)	24 hours

CP=cyclophosphamide.

RESULTS
Doses Producing Toxicity: None
Genotoxic Effects: Negative.
Remarks – Results: There were no remarkable body weight changes during the study.

No statistically significant increase in the frequency of micronuclei was observed in all dosed groups at any sampling time.

Appropriate positive controls induced marked increases in micronuclei, indicating that the test system responded appropriately.

CONCLUSION: The notified chemical was not clastogenic in this in vivo mouse bone marrow micronucleus test under the conditions of the test.

TEST FACILITY	Hazleton Europe (1996)

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical.
METHOD	Stated as "According to EC requirements".
Inoculum	Not stated.
Exposure Period	5 days
Auxiliary Solvent	None
Analytical Monitoring	BOD
Remarks – Method	For determination of microbial toxicity, nominal concentrations of the test substance ranging from 1 µg-10 g/L were incubated at 20°C for five days in darkened closed bottles connected to mercury manometers. The microbial toxicity (IC50) is that concentration of the test substance which lowers by 50% the Biochemical Oxygen Demand (BOD) of a reference substance over a 5 day period. The results indicate that the % inhibition based on the microbial toxicity reached a maximum of 26.6% at 100 mg/L of the test substance but declined to 21.8% at 10000 mg/L of the test substance. For BOD testing, duplicate test solutions at concentrations of 1 g/L and 100 mg/L were incubated at 20°C for five days in darkened closed bottles connected to mercury manometers. The test readings were very low despite the relatively high concentrations of test substance used. The reading at the lower concentrations (100 mg/L) is used as the maximum value for the BOD data analysis. The five-day BOD for the test substance was determined to be below 25 mg O_2/g and the theoretical BOD was calculated to be 1487 mg O_2/g. Chemical Oxygen Demand was also conducted using six flasks and 10 mL of the test substance solution was added to three of the flasks. Two blanks containing water and one control using potassium hydrogen phthalate as the reference were used in the test. Acidified mercuric sulphate solution, potassium dichromate and silver sulphate were added to the flasks. The mixture was heated to reflux for two hours and cooled to room temperature. The residual dichromate was then determined by titrating with standardised ferrous ammonium sulphate. The chemical oxygen demand was determined to be 864 mg O_2/g.
Remarks – Results	The five-day BOD of the notified chemical was determined to be below 25 mg O_2/g at test concentrations of 100 and 1000 mg/L. This is <2% of the theoretical BOD which was calculated to be 1487 mg O_2/g.
CONCLUSION	It is expected that the notified chemical cannot be classed as ready biodegradable
TEST FACILITY	Hazelton Europe (1994c)

8.1.2. Bioaccumulation

No bioaccumulation study was conducted. In view of the negative logPow and high water solubility, the bioaccumulation potential is considered to be low (Connell, 1990).

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	*Brachydanio rerio*
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	45.1 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	A 96 h semi-static toxicity test was conducted with the renewal of the test media at 24 h intervals. The test vessels were ten litre glass aquaria containing 10 L of test medium. A nominal concentration of 120 mg/L for the test substance was used in the test aquarium. The other aquarium without the test substance was used as a control. Ten *B. rerio* were placed in each aquarium. The contents of each test vessel were gently aerated. The fish were not fed during the test. The fish exhibiting toxic symptoms were recorded at 1, 24, 48, 72 and 96 h. The symptoms were classified as no effects, mild toxic effects (increased cough frequency, swimming position in test vessels different to controls), severe toxic effects (swimming abnormally or lying at the bottom of tank), and dead. Water quality parameters of temperature, dissolved oxygen and pH were measured throughout the test and were within acceptable limits.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
120	89	10	0	0	0	0	0

LC50	> 89 mg/L at 96 hours.
NOEC	89 mg/L at 96 hours.
Remarks – Results	The results of the tests were based on the mean measured concentrations of the test substance. The maximum concentration of the test substance causing no mortality to *B. rerio* after 96 h was observed to be 89 mg/L. As no toxic symptoms were observed the NOEC = 89 mg/L.

CONCLUSION — The notified chemical is at most slightly toxic to *Brachydanio rerio*.

TEST FACILITY — Hazelton Europe (1994d)

8.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE — Notified chemical

METHOD — OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - static.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None.
Water Hardness	Not stated.
Analytical Monitoring	UV/visible spectrophotometry.
Remarks – Method	Based on the results of the preliminary testing, measured concentrations ranging from 51.4-1000 mg/L were used for the definitive test. The test consisted of 4 replicates of a control and five test concentrations. Each replicate consisted of 5 daphnia except for test concentrations at 51.4, 108, 476.2 and 1000 mg/L where each of the 4 replicates contains 6 daphnia. The tests were run for 48 h and the number of immobile daphnia were recorded after 24 and 48 h. The tests were carried out in total darkness and no food were supplied during the test. Water quality parameters of temperature, dissolved oxygen and pH were measured throughout the test and were within acceptable limits.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*	*per replicate*	*24 h [acute]*	*48 h [acute]*
	0	5 in 3 vessels, 6 in 1 vessel	0	0
	51.4	5 in 3 vessels, 6 in 1 vessel	0	0
	108	5 in 3 vessels, 6 in 1 vessel	0	0
	226.8	5 in 3 vessels, 6 in 1 vessel	0	1
	476.2	5 in 3 vessels, 6 in 1 vessel	0	1
	1000	5 in 3 vessels, 6 in 1 vessel	0	4

LC50	3510 mg/L at 48 hours (CI: 1334-4690)
NOEC	100 mg/L at 48 hours

Remarks – Results	All results were based on measured concentrations. The number of daphnia immobilised during the test were statistically analysed using Probit analysis. A 24 h EC50 was not determined as no daphnia died during the first 24 h of the test.
CONCLUSION	The notified chemical is very slightly toxic to *Daphnia magna*.
TEST FACILITY	Institute of Freshwater Ecology (1992)

8.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical.
METHOD	EC Directive 92/69/EEC C.3 Algal Inhibition Test.
Species	*Selenastrum capricornutum*
Exposure Period	72 hours
Concentration Range	
Nominal	10, 20, 40, 80, 160 mg/L
Actual	5.8, 14, 29, 65, 130 mg/L
Auxiliary Solvent	None.
Water Hardness	Not stated.
Analytical Monitoring	HPLC
Remarks – Method	The test was conducted by exposing growing algal cultures to the nominal concentrations of the test substance ranging from 10-160 mg/L for a period of 72 h. 100 mL of each solution were added to three flasks. Three further flasks were prepared containing culture medium only and served as controls. Each set of flasks were inoculated with the test organisms. A further three flasks were prepared with test concentration of 160 mg/L and were not inoculated. At approximately 24 h intervals after the start of the inoculation, samples were taken for cell counting using a haemocytometer. The algal cultures were incubated in a temperature controlled, illuminated incubator for a period of 72 h. At the end of the exposure, test conditions such as pH, temperature and light intensity were found to be within the range of acceptability.

RESULTS

Biomass		*Growth*	
E_bC_{50} *mg/L at 0 - 72 h*	*NOEC mg/L*	E_rC_{50} *mg/L at 0 - 72 h*	*NOEC mg/L*
63.6	5.8	> 130	> 130

Remarks – Results	All results were based on the measured concentrations of the test substance. Losses of the test substance over the duration of the test were greater at lower nominal concentrations. Mean measured exposure concentration as a percentage of the nominal concentrations ranged from 58% to 81%.
CONCLUSION	The notified chemical is very slightly toxic to algae.
TEST FACILITY	Hazleton Europe (1994e)

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

Most of the dye will be bound to paper and eventually be disposed by landfill. However, some

paper will be recycled and due to the high water solubility of the dye, a greater proportion will remain in the aqueous phase. Recycling may take place in a number of centres throughout Australia. The predicted concentration in sewage effluent on a nationwide basis is estimated as 0.21 μg/L.

Fate
The substance is not expected to bioaccumulate due to its high water solubility. Abiotic or slow biotic processes are expected to be largely responsible for the degradation of the notified chemical as it is not readily biodegradable. Incineration of waste paper will destroy the compound with the generation of water vapour and oxides of carbon, sulphur and nitrogen. As a consequence of its anionic nature, the notified chemical is likely to be low to slight in soil mobility through adsorption onto soil particles and sediments as indicated by its log Koc = 3.46.

9.1.2. Environment – effects assessment

In summary the aquatic toxicity data indicate:

Zebra fish (*Brachydanio rerio)*: 96 h LC50	>89 mg/L
Daphnia magna: 48 h LC50	3510 mg/L
Algae *(Selenastrum capricornutum)*: 72 h E_bC50	63.3 mg/L

Using the lowest LC50 of 63.3 mg/L for algae, a predicted no effect concentration (PNEC) of 0.63 mg/L has been derived by dividing the LC50 value by a safety factor of 100 since toxicity data are available for all three trophic levels.

9.1.3. Environment – risk characterisation

The notified chemical will enter environmental compartments indirectly by disposal of waste paper (for recycling, to landfill or for incineration) and by direct release from discarded printer cartridges at landfill sites. Based on the import volume, method of packaging and low concentration in ink, release of the notified chemical to the environment is expected to be low and widespread. Waste from the recycling process includes sludge which is dried and disposed of to landfill, and any of the notified chemical partitioned to the supernatant water will be released to sewer.

The PEC/PNEC ratio for the aquatic environment, assuming nationwide use, is 3.3×10^{-4} (0.21/630) and 3.3×10^{-5} (0.021/630), for freshwater and marine water, respectively. These values are significantly less than 1, indicating no immediate concern to the aquatic compartment. This value is expected to be much lower given that not all paper to which the ink is applied will be recycled thus limiting the exposure of the notified chemical to sewer.

9.2. Human health

9.2.1. Occupational health and safety – exposure assessment

Office workers and customer service engineers may be intermittently exposed to the notified chemical contained in the ink cartridge when replacing the spent ink cartridge, and during repair maintenance and cleaning of ink jet printers. Customer service engineers may potentially come in contact with the notified chemical more often than office workers. Exposure is expected to be controlled through the design of the ink cartridges and the printing machines. Customer service engineers often wear cotton disposable gloves. Pre-packed ink cartridges are sealed and worker exposure to the ink is minimised by the use of the replacement procedures recommended by the manufacturer.

Exposure may occur upon handling printed matter. However, very little printing ink is used per sheet of paper and it would not be separately available for exposure or dermal uptake as it is fused and fixed to the printed surface.

Waterside, warehouse and transport workers are unlikely to be exposed to the notified chemical unless the packaging is breached.

9.2.2. Public health – exposure assessment

The printing ink will be available for use in home printers. The public will have dermal

exposure to the notified chemical in the printing ink when inserting or removing a damaged cartridge and clearing paper jams. The ink is contained in the cartridge and the physical design of the cartridge prevents handlers from dermal exposure. The cartridge design also prevents leakage of ink.

Public exposure will also occur by dermal contact with printed media treated with ink containing <5 % notified chemical.

9.2.3. Human health - effects assessment

The notified chemical was of low acute oral and dermal toxicity in rats. It was slightly irritating to skin and a severe eye irritant in rabbits. Severe eye lesions observed at 72 hours which persisted up to 21 days include depilated area around the lids of the treated eye, neovascularisation are of the corneal surface, severe adherence of the test article to the cornea, deformation of the cornea, circumcorneal injections and congestion of the iris, permanent myosis with preservation of direct photomotor reflex, presence of whitish humour on the side of the lids, discolouration of the conjunctiva. Macroscopic and histopathological examinations revealed pathological lesions indicative of skin sensitisation in all treated animals in an adjuvant study in guinea pigs.

The notified chemical was mutagenic in bacteria and clastogenic in *in vitro* chromosomal aberration assay using chinese hamster ovary cells. However, it was not clastogenic in *in vivo* mouse bone marrow micronucleus test in the absence and presence of metabolic activation.

In a 28-day oral repeat dose toxicity study in rats, staining of the tail in the mid and high dose groups were observed at weeks 2 to 4 and in two females in low dose groups during the last week of treatment. All treated groups had dark and/or red faeces throughout the treatment period. The slight increase in food consumption in treated males may be associated with the increase in body weight. The changes in lymphocyte and neutrophil counts were probably due to the shift in the type of white blood cells since the total white blood cell count is comparable to controls. The increase in total plasma bilirubin concentration in high dose males was due to the colourimetric method used for total bilirubin, which is based on the measurement of azobilirubin. The notified chemical is a diazo dye and therefore expected to affect the determination of total bilirubin using azobilirubin as endpoint. Slight increased MCHC and decreased platelet count was confined to treated males and of limited significance. The No Observed Adverse Effect Level (NOAEL) was established as 500 mg/kg bw/day (the highest dose tested) in this study.

On the basis of the data supplied, the notified chemical would be classified as a hazardous substance according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999) and warrants the risk phrases: R41 – Risk of serious damage to eyes and R43 – May cause sensitisation by skin contact.

9.2.4. Occupational health and safety – risk characterisation

The loading and removal of a cartridge into or from its containment area in a printer can be readily accomplished without any contact with ink. Skin contact with the ink may occur if an attempt is made to insert or remove a damaged cartridge or to correct a paper-jam.

The cartridges are not refilled. Spent cartridges contain on average <10% of remaining ink. The remaining ink contained within the cartridge cannot be removed without breaking the cartridge. Ink on paper will be bound to the paper and is unlikely to be transferable to a person's skin.

Although the notified chemical is a severe eye irritant and skin sensitiser, the risk of adverse effects arising from exposure to the notified is low due to the low potential for exposure and low concentration of notified chemical in the printing ink.

Based on the expected low exposures, the health risk posed to office workers and customer service engineers, by the notified chemical is very low. In addition, the occupational health risk to waterside, warehouse and transport workers is negligible, considering the small quantities in

individual ink cartridges and the low hazard presented by the chemical.

9.2.5. Public health – risk characterisation

Given that the manner of exposure for the public is similar to that for office workers performing the same tasks, the risk from public exposure to the notified chemical is considered to be low.

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

10.1. Hazard classification

Based on the available data the notified chemical is classified as hazardous under the NOHSC *Approved Criteria for Classifying Hazardous Substances*. The classification and labelling details are:
R41 – Risk of serious damage to eyes; and
R43 – May cause sensitisation by skin contact.

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

Eye irritant Category 1
Symbol: Corrosive
Signal word: Danger
Hazard statement: Causes severe eye damage

Skin sensitiser Category 1
Symbol: Exclamation Mark
Signal word: Warning
Hazard statement: May cause allergic skin reaction

Chronic hazards to the aquatic environment Category III
Symbol: No symbol used
Signal word: No signal word
Hazard statement: Harmful to aquatic life with long lasting effects

10.2. Environmental risk assessment

On the basis of the PEC/PNEC ratio:

The chemical is not considered to pose a risk to the environment based on its reported use pattern.

10.3. Human health risk assessment

10.3.1. Occupational health and safety

There is Low 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 as a component of printing inks.

11. MATERIAL SAFETY DATA SHEET

11.1. Material Safety Data Sheet

The MSDS of the products containing the chemical provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 1994a). 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 chemical provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Labelling of Workplace Substances* (NOHSC, 1994b). The accuracy of the information on the label remains the responsibility of the applicant.

12. RECOMMENDATIONS

REGULATORY CONTROLS
Hazard Classification and Labelling

- The NOHSC Chemicals Standards Sub-committee should consider the following health hazard classification for the notified chemical:

  – R41 – Risk of serious damage to eyes; and
  – R43 – May cause sensitisation by skin contact.

- Use the following risk phrases for products/mixtures containing the notified chemical:
  – ≥10%: R41 – Risk of serious damage to eyes.
  – 5%≤conc <10%: R36 – Irritating to eyes.
  – ≥1%: R43 – May cause sensitisation by skin contact.

CONTROL MEASURES
Occupational Health and Safety

No special precautions are required for the notified chemical when used at low quantities as a component of ink cartridges for printers. However, in the interests of good occupational health and safety, the following guidelines and precautions should be observed for use of printing inks containing the notified chemical:

- Avoid contact with skin.
- Printers should be located in well-ventilated areas.
- Service personnel should wear cotton or disposable gloves when replenishing spent ink cartridges and servicing printers.

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

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

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the 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

- Do not allow material or contaminated packaging to enter drains, sewers or water courses.

Disposal

- The notified chemical should be disposed of in landfill or be destroyed through incineration.

Emergency procedures

- Spills/release of the notified chemical should be handled by collecting the cartridge intact and landfilled. Contain the spill and absorb with sawdust, sand or earth. Place used absorbent in suitable sealed containers and follow state or local regulation for the disposal of the waste.

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.

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