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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Cyan Dye 2

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Director

Chemicals Notification and Assessment

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Toxikos Pty Ltd
PO Box 74
CAULFIELD EAST VIC 3145

Hewlett Packard Australia Pty Ltd
31-41 Joseph St
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 Name
- CAS Number
- Molecular Formula
- Structural Formula
- Molecular Weight
- Spectral Data
- Purity
- Impurities
- Additives/Adjuvants
- Manufacture details
- Test facility details
- Toxicological/Ecotoxicological Report references

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
USA, EU Switzerland

2. IDENTITY OF CHEMICAL

OTHER NAME(S)
None

MARKETING NAME(S)
Cyan Dye 2

METHODS OF DETECTION AND DETERMINATION

ANALYTICAL METHOD	IR, UV-Vis and MS
Remarks	
TEST FACILITY	Confidential

4. INTRODUCTION AND USE INFORMATION

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

The notified chemical is imported into Australia as part of sealed inkjet printing systems (cartridge and printhead). The volume of the cartridges ranges from 30-90 mL and printheads range up to 10 mL. Cartridges will be delivered to consumers by road transport.

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

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

USE

Cyan 2 is a dye used in preparations in inkjet reprographic processes.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, Transport and Storage

PORT OF ENTRY

Not known

IDENTITY OF RECIPIENTS

The inkjet printing systems will be potentially supplied to offices nationwide.

TRANSPORTATION AND PACKAGING

The inkjet printing system containing the notified chemical is not a dangerous good, hazardous substance or scheduled poison, and therefore no special transport or packaging requirements are necessary. Cartridges are 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 printing system 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	40 days per year
Storage and transport	100	6	240 days per year
Office worker/Service technician/Consumer	10 000	<0.1	20

Exposure Details

The notified chemical is contained in sealed cartridges. The volume of the notified chemical in any single coloured (non-black) cartridge will range from 2-15 mL. Normal handling, involving

replacement of the cartridge would not normally result in exposure. Exposure would only result if the cartridge were faulty or ruptured.

5.4. Release

RELEASE OF CHEMICAL AT SITE
The notified chemical will be imported in sealed cartridges containing up to 90 g of formulated ink (with a maximum of 4% of the chemical). The size of the print head will range to a maximum of 10 g. There will be no release to the environment due to reformulation or repackaging.

RELEASE OF CHEMICAL FROM USE
The ink cartridges 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. However, if leakage or spill does occur, the quantity of ink released will be small and will be contained with absorbent material. These will presumably be disposed of to 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. The disposal of uncured inks will be largely confined to residues contained in colour printing systems, which do not allow the replacement of individual colours. Environmental exposure will result from the disposal of printed-paper, discarded cartridges and any accidental leakage of the cartridges during use.

The notifier has not provided an estimate of the amount of residue in the spent cartridge, but expects up to 90 % of the notified substance will be bound to printed paper which will be disposed of to landfill, recycled or incinerated. Based on a maximum import volume of 1 tonne, up to 100 kg of the notified chemical will be sent to landfill as residue in empty toner cartridges.

The remaining 90% of the notified chemical (up to 900 kg) bound to paper is expected to be recycled, disposed of to landfill or incinerated. If recycled, all of the developer containing the notified chemical will be removed from the paper/pulp during the deinking stage of the recycling process and the notified chemical will remain in the aquatic phase or end up in the resultant sludge, which will be disposed of to landfill.

5.5. Disposal
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
The public will be exposed to the dye after use, when it is expected to be fixed to the paper. 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.

6. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa Purple granular solid

Melting Point/Freezing Point >300°C

METHOD EC Directive 92/69/EEC A.1 Melting Temperature (Metal Block apparatus)
Remarks
TEST FACILITY Confidential

Boiling Point >400°C at 101.3 kPa

METHOD Theoretical Assessment
Remarks
TEST FACILITY Confidential

Density 1850 kg/m^3

METHOD EC Directive 92/69/EEC A.3 Relative Density.
Remarks
TEST FACILITY Confidential

Vapour Pressure << 10^{-3} Pa at 25°C (Estimated theoretically).

METHOD The vapour pressure was estimated based on a theoretical assessment that recognised that within a homologous series of organic compounds, the boiling point rises and the vapour pressure at a given temperature falls with increasing molecular weight. A comparison made using a number of organic compounds showed that a compound with a molecular weight of the test substance would be expected to have a very high boiling point (e.g. > 400°C) and a correspondingly lower vapour pressure. By comparing the test substance with other compounds in the series examined, it was deduced that the vapour pressure at 25°C would be substantially less than 10^{-3} Pa. Therefore, no experimental measurement was attempted.
Remarks
TEST FACILITY Confidential

Water Solubility >470 g/L at 25°C

METHOD EC Directive 92/69/EEC A.6 Water Solubility (Flask Method).
Remarks Three tests containing the test substance and distilled water were centrifuged at 3000 rpm for 30 minutes and then allowed to equilibrate at 25°C ± 1°C in a constant temperature bath. All three tests appeared to be viscous solutions with no undissolved test substance. Portions of each test were weighed out and the concentrations of the test substance were measured spectrophotometrically by comparison to a calibration graph prepared in distilled water.

The test substance is readily soluble (Mensink *et al.* 1995).
TEST FACILITY Confidential

Hydrolysis as a Function of pH The test substance is hydrolytically stable at pH 4, 7 and 9

METHOD EC Directive 92/69/EEC C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.
Remarks The submission indicates that the last stage of the process for the production of the test substance involves heating to 40°C in the presence of sodium hydroxide and this is monitored to ensure that hydrolysis is complete. Therefore, monitoring the hydrolysis of the test substance at alkaline pH was not considered as necessary.

As acid hydrolysis proceeds by a different mechanism to alkaline hydrolysis, the hydrolysis at pH 4 was studied. Duplicate tests of the test substance were prepared in pH 4 buffer solution and placed in an oven at 50°C. The tests were examined initially and over a period of 8 days. Test solutions were analysed using HPLC. No hydrolysis of the test substance at pH 4 was observed over the 8-day period. Therefore, it was considered not necessary to carry out further studies at pH 7.

The test substance can be considered to be hydrolytically stable at pH 4, 7 and 9 (Mensink *et al.* 1995).
TEST FACILITY Confidential

Partition Coefficient (n-octanol/water) log P_{ow} = -2.6

METHOD EC Directive 92/69/EEC A.8 Partition Coefficient (Shake-flask Method).
Remarks Solutions of the test substance were prepared in n-octanol saturated distilled water. Concentrations of the test substance in the water phases were determined by comparison to a calibration curve prepared in n-octanol saturated distilled water.

The n-octanol phases were examined directly without dilution and the concentration of the test substance determined by comparison to a calibration curve prepared in methanol/n-octanol (water saturated).

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 Confidential

Adsorption/Desorption Not determined.

Remarks The high water solubility and the low log Pow indicate that the test substance can be highly mobile in soil. However, experience shows that it should adsorb due to the anionic character.

Dissociation Constant Not determined.

Remarks The test substance contains sulfonic acid groups which are expected to remain ionised throughout the environmental pH range of 4 to 9.

Particle Size Not available

Flash Point Not applicable

Flammability Limits Not classified as flammable

METHOD EC Directive 92/69/EEC A.10 Flammability (Solids).
EC Directive 92/69/EEC A.11 Flammability (Gases).
EC Directive 92/69/EEC A.12 Flammability (Contact with Water).

Remarks

TEST FACILITY Confidential

Autoignition Temperature No ignition below 400°C

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

Remarks

TEST FACILITY Confidential

Explosive Properties Not explosive

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

Remarks The test substance did not explode when exposed to heat, mechanical shock or friction.

TEST FACILITY Confidential

ADDITIONAL TESTS

Surface Tension 71.4mN/m at 25ºC ± 1ºC

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

Remarks Solutions of the test substance were prepared by weighing 0.0995 g of the test substance into a 100 mL volumetric flask and made up to the mark with distilled water. This was treated ultrasonically for 5 minutes to dissolve all of the test substance and allowed to cool to room temperature before making the measurements. Two tests were conducted using a Krüss Processor Tensiometer fitted with a Wilhelmy plate. The two tests were done at 25ºC ± 1ºC with preparation times of 16 and 64 minutes before the surface tension was measured.

The results indicate that the test substance is not surface active.

TEST FACILITY Confidential

Oxidizing Properties Not oxidising

METHOD EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).

Remarks A reduced testing programme was employed as the results indicate that the test substance attenuated the burning characteristics of cellulose rather than enhanced them.

TEST FACILITY Confidential

7. TOXICOLOGICAL INVESTIGATIONS

Endpoint and Result	*Assessment Conclusion*
Rat, acute oral	Discriminating dose 2000 mg/kg
Rat, acute dermal	LD50 > 2000 mg/kg bw
Rabbit, skin irritation	Not irritating
Rabbit, eye irritation	Slightly irritating
Guinea pig, skin sensitisation - adjuvant test	Not sensitising
Rat, oral repeat dose toxicity - 28 days.	NO(A)EL 50 mg/kg bw/day
Genotoxicity - bacterial reverse mutation	Not mutagenic
Genotoxicity – in vitro chromosome aberration	Not genotoxic

7.1. Acute toxicity – oral

TEST SUBSTANCE	Cyan Dye 2
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Method EC Directive 92/69/EEC B.1 Acute Toxicity (Oral) – Fixed Dose Method
Species/Strain	Rat/Alpk:AP$_f$SD
Vehicle	Deionised water
Remarks - Method	Main test phase was preceded by a sighting phase where a single female was dosed with 500 mg/kg of the test substance. The animal survived and showed no signs of toxicity. An additional female was dosed with 2000 mg/kg to further investigate the toxicity. Again, the animal survived and showed no signs of toxicity. The initial fixed dose-level for the main phase was selected as 2000 mg/kg.

RESULTS

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

Discriminating dose	2000 mg/kg bw
Signs of Toxicity	There were no mortalities and no signs of evident toxicity. The faeces of one male and one female and the urine of one female were stained blue by the test substance for up to 2 days after dosing. The tail, fur and skin of the animals were also stained blue in all cases, with staining of the tail persisting throughout the study in some animals.
Effects in Organs	Darkening of the kidneys was observed in all animals and one female displayed red spots/areas in the glandular region of the stomach.
Remarks - Results	The effects seen in the stomach are indicative of an irritant effect.
CONCLUSION	The notified chemical administered at a dose of 2000 mg/kg failed to produce any lethality. The discriminating dose is therefore 2000 mg/kg and the notified chemical is of low toxicity.
TEST FACILITY	Confidential

7.2. Acute toxicity - dermal

TEST SUBSTANCE	Cyan Dye 2
METHOD	OECD TG 402 Acute Dermal Toxicity. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/Alpk:AP$_f$SD

Vehicle	The test substance was moistened with a small amount of deionised water to give a dry paste.
Type of dressing	Occlusive
Remarks - Method	

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Animals were stained blue by the test substance for up to 5 days which prevented the evaluation of erythema in one male and one female on day 2. Three males and four females showed signs of slight skin irritation which had resolved by day 11.
Signs of Toxicity - Systemic	No systemic effects were noted.
Effects in Organs	Speckling of the thymus was observed in one male and one female.
Remarks - Results	Effects seen in the thymus are believed to be a spontaneous finding unrelated to treatment.

CONCLUSION — The acute dermal median lethal dose of Cyan Dye 2 is estimated to be in excess of 2000 mg/kg. The notified chemical is of low toxicity via the dermal route.

TEST FACILITY — Confidential

7.4. Irritation – skin

TEST SUBSTANCE — Cyan Dye 2

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
Vehicle	The test substance was moistened with a small amount of deionised water to give a dry paste.
Observation Period	3 days
Type of Dressing	Occlusive
Remarks - Method	

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	0.3	0.3	0.3	1	1 day	0

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

Remarks - Results	No erythema was observed in any of the animals throughout the study. Very slight oedema was observed in all three animals for 1 day after application. The notified chemical stained the skin of all animals blue throughout the study.

CONCLUSION — Discolouration of the skin in all animals persisted until the end of the

study, however, due to the absence of significant erythema or oedema, the notified chemical is determined to be not irritating to the skin.

TEST FACILITY Confidential

7.5. Irritation - eye

TEST SUBSTANCE Cyan Dye 2

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 21 days
Remarks - Method

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	ND	ND	ND	ND	ND	0
Conjunctiva: chemosis	0	0	0	1	< 1 day	0
Conjunctiva: discharge	0	0	0	3	< 1 day	0
Corneal opacity	0	0	0	ND	ND	0
Iridial inflammation	0	0	0	0	-	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.
ND: Not determined

Remarks - Results

Approximately ¼ of the test substance was displaced from the conjunctival sac of each animal immediately after application.
The application of the test substance caused practically no or slight initial pain (1-2 on a scale of 0-5).
The test substance stained the eye of each rabbit, which prevented the evaluation of corneal opacity in two animals approximately one hour application and conjunctival redness in all animals for up to 19 days.
Slight conjunctival chemosis and a severe discharge were observed in all three animals approximately one hour after application.
One animal also displayed Harderian discharge, convoluted eyelids and dried secretion around the periorbital skin. All overt signs of irritation had completely resolved in this animal 14 days after application.

CONCLUSION A full assessment of irritation was not possible due to staining by the test substance.
However, as staining dissipated by the end of the study period, and considering the lack of other signs of irritation during the study, the notified chemical is considered to be a slight irritant to the rabbit eye.

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7.6. Skin sensitisation

TEST SUBSTANCE Cyan Dye 2

METHOD OECD TG 406 Skin Sensitisation – Magnuson & Kligman Maximisation method.
EC Directive 96/54/EC B.6 Skin Sensitisation
US EPA, Health Effects Guidelines, OPPTS 870.2600 Skin sensitisation

Species/Strain	Guinea pig/Dunkin Hartley
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: 3% w/v topical: 75% w/v
MAIN STUDY	
Number of Animals	Test Group: 20 Control Group: 10
INDUCTION PHASE	Induction Concentration: intradermal injection 3% w/v topical application 75% w/v
Signs of Irritation	
CHALLENGE PHASE	
1st challenge	topical application: 75%, 50%, 25%, 10% w/v
2nd challenge	topical application:

Remarks - Method

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: 1st challenge*	
		24 h	*48 h*
Test Group	75%	1/20*	1/20*
	50%	*	*
	25%	0/20*	0/20*
	10%	0/20*	0/20*
Control Group	75%	0/10*	0/10*
	50%	*	*
	25%	0/10*	0/10*
	10%	0/10*	0/10*

Remarks - Results

* Animals were stained blue by the test substance in all animals treated with the 50% w/v preparation and in many animals treated with the 75%, 25% or 10% w/v preparations of the test substance.

Histopathological examination revealed minor differences between control and treated groups however the differences are insufficient to diagnose the compound as a sensitiser.

A positive control study using hexylcinnamaldehyde demonstrated the sensitivity of the test system.

CONCLUSION

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

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7.7. Repeat dose toxicity

TEST SUBSTANCE

Cyan Dye 2

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	
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	Water

Remarks - Method

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
I (control, main)	5M/5F	0	0
II (control, recovery)	5M/5F	0	0
III (low dose)	5M/5F	50	0
IV (mid dose)	5M/5F	250	0
V (high dose)	5M/5F	1000	0
VI (high dose, recovery)	5M/5F	1000	0

Mortality and Time to Death

No deaths occurred during the study period.

Clinical Observations

Staining of the coat or tail was observed in both groups treated at 1000 mg/kg/ bw/day with tail staining also observed in males of the 250 mg/kg/ bw/day group. Blue coloured faeces was noted for groups treated at 50, 250, and 1000 mg/kg/ bw/day for weeks 1 to 5.

Salivation was observed once in one male and several times in 3 females in the recovery group treated at 1000 mg/kg/ bw/day.

Bodyweights

There was no effect of treatment on bodyweight during dosing and no significant differences in bodyweight gains between treated animals and concurrent controls during the recovery period.

Food consumption

There was no effect of treatment on food consumption.

Functional Observations and Motor Activity

Slight decrease in hind limb grip strength for the males in the group treated at 250 mg/kg bw/day and in landing foot splay for females in the group treated at 250 mg/kg bw/day.

Clinical Pathology

Haematology

Statistically significant reductions were observed for neutrophil, monocyte, eosinophil, basophil, and large unstained cell counts in recovery group males. These changes were however not observed in the main study animals and the minor statistical significance is believed to arise as a result of high control values in several recovery males.

Blood chemistry

Statistically significant increases in albumin, total protein and cholesterol were observed in main study males of the 1000 mg/kg/ bw/day group while increased triglycerides were observed in females. Minor reductions of chloride levels for males receiving 1000 mg/kg/ bw/day and of sodium in females receiving 1000 mg/kg/ bw/day were also observed. These changes were not however present in the opposite sex or recovery group animals and were considered incidental to treatment. Differences in enzyme activity in some recovery animals were observed which are attributed to extreme control values.

Urinalysis

No treatment related effects on urinary parameters were discernible from the tests performed.

Effects in Organs

Organ weights

While the weight of some organs in the 1000 mg/kg/ bw/day were statistically different from concurrent controls , the absence of a dose-response, equivalent effects in the other sex or the recovery group, the findings were considered to be incidental to treatment.

Macroscopic findings
A dose-related discolouration of tissues and/or intestinal contents was observed in both males and females at day 29. Most animals treated at 1000 mg/kg/ bw/day displayed discolouration of the majority of abdominal organs together with the salivary gland and the cervical lymph node in some cases. Discolouration at 50 mg/kg/ bw/day was however confined to the intestinal contents and kidneys in some animals only. Discoloration of abdominal organs of all animals and extra-abdominal tissues in some animals persisted at doses of 1000 mg/kg/ bw/day to day 43.

Microscopic findings
Increased incidence at day 29 of tubular pigmentation and vacuolation in the kidney was observed in both males and females at 1000 mg/kg/ bw/day and tubular pigmentation in 2/5 females at 250 mg/kg/ bw/day with the effects in the 1000 mg/kg/ bw/day group persisting to day 43. Additionally there was an increased incidence of basophilia of the intestinal Goblet cell mucin in males and females at 1000 mg/kg/day and in males of the 250 mg/kg/day group, however these effects had resolved by day 43.

Remarks – Results
Staining effects observed in 250 and 1000 mg/kg/ bw/day treatment groups were considered to be due to the physical properties of the test substance and do not indicate toxicity.
Neither hind limb grip strength or foot splay effects observed in males and females respectively treated at 250 mg/kg bw/day were observed at 1000 mg/kg bw/day and are therefore considered to be incidental to treatment.
Urine specific gravity and test strip indices were not examined for the groups treated at 250 or 1000 mg/kg/day.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 50 mg/kg bw/day in this study, based on renal tubular changes at 1000 and 250 mg/kg bw/day and increased incidences of basophilia of intestinal Goblet cell mucin in the 1000 mg/kg bw/day which was unresolved following recovery.

TEST FACILITY Confidential

7.8. Genotoxicity - bacteria

TEST SUBSTANCE Cyan Dye 2

METHOD OECD TG 471 Bacterial Reverse Mutation Test – *Salmonella typhimurium*
OECD TG 471 Bacterial Reverse Mutation Test – *Escherichia coli*
EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.
Plate incorporation procedure/Pre incubation procedure

Species/Strain *S. typhimurium*: TA1535, TA1537, TA98, TA100
E. coli: WP2 uvrA (pKM101), WP2 (pKM101)

Metabolic Activation System

Concentration Range in Main Test
a) With metabolic activation: 100-5144 µg/plate.
b) Without metabolic activation: 100-5144 µg/plate.

Vehicle Deionised water

Remarks - Method

RESULTS

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

Remarks - Results In two separate assays the test substance failed to produce significant

reproducible increases in the observed number of revertant colonies in any of the tester strains used either in the presence or absence of S9-mix.

CONCLUSION — The notified chemical was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY — Confidential

7.9. Genotoxicity – in vitro

TEST SUBSTANCE — Cyan Dye 2

METHOD — OECD TG 473 In vitro Mammalian Cytogenic Test.
EC Directive 92/69/EEC B.10 In vitro Mammalian Cytogenic Test.

Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 (Sprague Dawley – phenobarbitol/β-naphthoflavone)
Vehicle	Supplemented RPMI-1640 media
Remarks - Method	

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	8.3, 50, 100, 250, 500*, 1000, 2500*, 5144*	3	3
Test 2	50, 100, 250*, 500, 1000*, 2500*, 3750, 5144	20	20
Present			
Test 1	8.3, 50, 100, 250, 500*, 1000, 2500*, 5144*	3	3
Test 2	100, 500*, 1000, 2500*, 5144*	3	3

*Cultures selected for metaphase analysis.

RESULTS

Remarks - Results — No statistically or biologically significant increases in the percentage of aberrant cells, above solvent control values were recorded for any cultures treated with Cyan Dye 2 in either the presence or absence of S9-mix.
The sensitivity of the test system and the metabolic activity of the S9 mix employed were demonstrated by the increases in the frequencies of aberrant cells induced by the positive control agents, mitomycin C and cyclophosphamide.

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

TEST FACILITY — Confidential

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

TEST SUBSTANCE — Cyan Dye 2

METHOD — OECD TG 301 F Ready Biodegradability: Manometric Respirometry

	Test and Official Journal of the European Communities, L383 A, Part C.4-D, Biodegradation: Determination of Ready Biodegradability – Manometric Respirometry.
Inoculum	Centrifuged, washed and resuspended activated sludge from a predominantly domestic sewage treatment works
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Chemical Oxygen Demand (COD)
Remarks - Method	In addition to the test substance, blank samples and samples containing a reference substance (sodium acetate) were measured.

RESULTS

Test substance		*Sodium Acetate*	
Day	*% degradation*	*Day*	*% degradation*
5	<6	5	55
10	<6	10	58
15	<6	15	60
20	<6	20	58
28	<6	28	58

Remarks - Results	The reference substance attained a maximum level of biodegradation of 60%. The level of biodegradation after the period of the 10-day window was slightly lower than the minimum 60% expected for a biodegradable substance. However, this was still considered to be high enough to confirm that the activated sludge contained viable organisms and therefore the validity of the study.
CONCLUSION	The test substance is not readily biodegradable according to the OECD criteria requiring > 60% within 10 days of commencement.
TEST FACILITY	Confidential

8.1.2. Bioaccumulation

No bioaccumulation data were provided. However, if there is any release to the aquatic compartment bioaccumulation is not expected due to the high water solubility and the low log P_{ow} of the notified chemical.

8.2. Ecotoxicological investigations

8.2.1. Acute toxicity to fish

TEST SUBSTANCE	Cyan Dye 2
METHOD	OECD TG 203 Fish, Acute Toxicity Test and EC Directive 92/69/EEC C.1 Acute Toxicity for Fish - Static
Species	Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	41.3 mg $CaCO_3$/L
Analytical Monitoring	Spectrophotometric analysis
Remarks – Method	Samples were taken from the centre of the test solution for spectrophotometric analysis of concentration. Oxygen content, pH and temperature were all satisfactorily maintained.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*			
Nominal	*Actual*		*24 h*	*48 h*	*72 h*	*96 h*

Dilution water control	-	10	0	0	0	0
180	180	10	0	0	0	0

LC50	> 180 mg/L at 96 hours.
NOEC	> 180 mg/L at 96 hours (highest test concentration used).
Remarks – Results	The mean measured concentration was 100% of the nominal concentration and the percentage loss in the measured concentration over the test period was < 1%. Due to the intense colouration of the test solutions it was not possible to assess the fish for any symptoms of toxicity other than mortality.

CONCLUSION	The test substance is practically non-toxic to fish.
TEST FACILITY	Confidential

8.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Cyan Dye 2
METHOD	EC Directive 84/449/EEC C.2 Acute Toxicity for Daphnia and OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Static Test
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	193 mg $CaCO_3$/L
Analytical Monitoring	Spectrophotometric analysis
Remarks - Method	The concentration of the test substance in the test solutions was measured at the beginning and end of the exposure period. Assessments of daphnia immobilisation were made at 24 and 48 hours.

RESULTS

Concentration (Test Substance) mg/L Nominal	*Number of D. magna*	*% Immobilised*	
		24 h	*48 h*
Control	20	0	0
180	20	0	0

LC50	> 180 mg/L at 48 hours
NOEC	> 180 mg/L at 48 hours (highest test concentration used).
Remarks - Results	Oxygen content, pH and temperature were all satisfactorily maintained. The mean measured concentration of the test substance during the exposure period was 106% of the nominal value of 180 mg/L and the percentage loss in the measured concentration over the test period was 5%.

CONCLUSION	The test substance is practically non-toxic to daphnia.
TEST FACILITY	Confidential

8.2.3. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE	Cyan Dye 2
METHOD	OECD TG 202 Daphnia sp. Reproduction Test – Semi Static
Species	*Daphnia magna*
Exposure Period	21 days

Auxiliary Solvent	None
Water Hardness	Varied between 204-206 (new) and 209-210 (old) mg $CaCO_3$/L in dilution water control but it was not possible to determine the hardness value of the test solution due to the intensity of its colour.
Analytical Monitoring	Spectrophotometric analysis
Remarks - Method	Samples were taken from the centre of the new and old test solutions for spectrophotometric analysis of concentration. Oxygen content, pH and temperature were all satisfactorily maintained. The overall mean measured concentrations (of the new and old test solutions) ranged from 95% to 102% of nominal values therefore, the results are based on nominal concentrations of the test solutions.

RESULTS

Concentration mg/L Nominal	*Number of D. magna*	*% Mortality*			
		24 h	*48 h*	*14 d*	*21 d*
Dilution water control	10	0	0	0	0
5	10	0	0	0	0
10	10	0	0	0	1
20	10	0	0	0	1
40	10	0	0	4	6
80	10	0	0	9	10

21 day EC50	33 mg/L
(For reproduction)	
Overall	
NOEC	10 mg/L at 21 days
For length (of adults)	
NOEC	40 mg/L at 21 days
For reproduction	
NOEC	10 mg/L at 21 days

Remarks - Results

At the 80 mg/L concentration, two daphnia were observed to be slow and pale on day 11, 50% mortality occurred on day 12 which increased to 100% mortality by day 15.

The LC50 values were calculated using an in-house computer program "LC50" using Stephan's Method. Both reproduction and growth (length) data were tested for normality using Shapiro and Wilk's test for non-normality. The reproduction data (normal at the 5% significance level) analysed using Student's T-test with Bonferroni's adjustment following analyses of variance. The length data (not normal at the 5% significance level) were analysed using a non-parametric test, the Wilcoxon Rank Sum. Mortality was adjusted for in the statistical analysis.

CONCLUSION — Based on the NOEC value for reproduction the test substance is very slightly toxic to *Daphnia magna* adults (Mensink 1995).

TEST FACILITY — Confidential

8.2.4. Algal growth inhibition test

TEST SUBSTANCE	Cyan Dye 2
METHOD	OECD TG 201 Alga, Growth Inhibition Test, EC Directive 92/69/EEC C.3 Algal Inhibition Test and RCC In den Leppsteinwiesen 19, D-64380 Inhibition of Algal Growth Caused by Coloured Test Samples, Memmert *et al.*, 1995
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 (Mean measured)	1.1, 2.6, 5.3, 12, 27, 60 and 130 mg/L
Auxiliary Solvent	None
Water Hardness	Standard test medium was used.
Analytical Monitoring	Spectrophotometric analysis
Remarks - Method	The test method was selected due to its suitability for coloured solutions enabling to determine whether the effects on algae is caused by the test substance or a reduction in light due to colour. Four replicate cultures of the control and each test concentration were used with two replicates of the exposure and shaded test vessels for each test concentration. One blank (no algal medium) was incubated concurrently for each control and test concentration.

RESULTS

	Growth - E_rC50	*Biomass - E_bC50*	*NOEC(Growth)*	*NOEC (Biomass)*
	mg/L at 72 h	*mg/L at 72 h*	*mg/L at 72 h*	*mg/L at 72 h*
Exposure solutions	37	5.7	1.0	1.0
Shaded solutions	33	5.6	<1.0	<1.0

Remarks - Results	The mean measured concentrations of the test solutions ranged from 106% to 113% of the nominal exposure concentrations. The toxicity results are based on the nominal concentrations. Following advice specifically for coloured substances, growth rate data were used in calculation of EC50 values and for all subsequent comparisons. Graphical comparisons of the percentages of inhibition in the exposure and shaded vessels showed that these inhibition curves were essentially the same. Inhibition of growth rate in exposure vessels plotted against that in shaded vessels showed that the curve follows the theoretical line plotted when the quotient of the inhibition of growth curves is equal to one for all test concentrations.
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.
TEST FACILITY	Confidential

8.2.5. Inhibition of microbial activity

TEST SUBSTANCE	Cyan Dye 2
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.

	Ecological and Toxicological Association of Dyestuffs Manufacturers (ETAD) Method 103: Screening test for the assessment of the possible inhibitory effect of a test chemical on aerobic waste water bacteria.
Inoculum	Activated sludge obtained from a sewage treatment plant that treats sewage predominantly domestic origin
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, 3.2, 10 and 30 mg/L.

RESULTS

IC50	> 100 mg/L
NOEC	100 mg/L (highest concentration tested)
Remarks – Results	No significant effect on respiration was observed at any of the test concentrations used (% inhibition of the respiration rate < 10%). The IC50 of the reference substance was 7 mg/L, thus validating the test.

CONCLUSION	No microbial inhibition was observed at any of the test concentrations.
TEST FACILITY	Confidential

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

The total import volume of the notified chemical will ultimately be either disposed of to landfill, incinerated or recycled with paper. 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.

Recycling may take place in a number of centres throughout Australia. A predicted environmental concentration (PEC) in the aquatic environment is estimated below using a worst-case scenario where the entire import volume (1000 kg) is released to sewer during recycling and not removed during sewage treatment processes (Environment Australia 2003). Assuming a national population of 19,500,000 and that each person contributes an average 200 L/day to overall sewage flows, the daily release on a nationwide basis to receiving waters is estimated to be 2.74 kg/day, the predicted concentration in sewage effluent on a nationwide basis is estimated as 0.7 μg/L.

Amount entering sewer annually	20 kg	
Population of Australia 19.5 million		
Amount of water used per person per day		200 L
Number of days in a year	365	

Estimated PEC

0.703 μg/L

Based on dilution factors of 1 and 10 for inland and ocean discharges of STP-treated effluents, the PECs of the notified chemical in freshwater and marine water may approximate 0.703 or 0.0703 μg/L, respectively.

Fate

The potential for bioaccumulation is low due to the low log P_{ow} and the high water solubility, which is further reduced by the low levels of aquatic exposure. Although not readily biodegradable, it is anticipated that prolonged residence in an active landfill environment would eventually degrade the notified substance due to abiotic or slow biotic processes. Incineration of waste paper and sludge will destroy the compound with the generation of water vapour and oxides of carbon, nitrogen and sulphur plus metal salts.

9.1.2. Environment – effects assessment

The results of the aquatic toxicity tests are listed below.

Organism	*Duration*	*End Point*	*mg/L*
Fish	96-h	LC50	>180
Daphnia	48-h	EC50	>180

A predicted no effect concentration (PNEC - aquatic ecosystems) of > 0.18 mg/L (> 180 μg/L) has been derived by dividing the end point value of > 180 mg/L by a worst-case scenario uncertainty (safety) factor of 1000 (as toxicity data are available only for two trophic levels).

9.1.3. Environment – risk characterisation

The notified chemical will enter environmental compartments indirectly by disposal of waste paper (to landfill or for recycling or 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 (4 %), 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 $< 3.9 \times 10^{-3}$ and $< 3.9 \times 10^{-4}$ 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

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.

There is low potential for worker exposure to the notified chemical when replacing spent cartridges as the ink formulations are in a liquid form and therefore are unlikely to generate residual dusts. Service technicians may occasionally experience skin contact with the notified chemical during maintenance, however, the notified chemical is at low concentrations (<5%) in the ink formulations. Exposure to the notified chemical on printed paper is low as the dye is bound to the paper matrix.

9.2.2. Public health – exposure assessment

Public exposure through importation, transportation or storage is assessed as negligible. There is little potential for exposure during cartridge changes. 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

The notified chemical has a molecular weight greater than 900 and a low octanol/water partition coefficient, indicating a low degree of lipophilicity and low potential to cross biological membranes.

The notified chemical was found to be of low acute oral toxicity with the discriminating dose of

2000 mg/kg bw/day failing to cause any lethality. Acute dermal toxicity studies demonstrated that the notified chemical is also of low toxicity via the dermal route with the LD_{50} for the rat estimated to be > 2000 mg/kg.

Dermal irritation studies found the notified chemical to cause discolouration of the skin which persisted throughout the study. Slight oedema was also observed for one day after the application. The notified chemical was determined to be not irritating to the skin due to the absence of significant erythema and oedema. A full assessment of eye irritation was not possible due to the staining effect of the substance, which prevented the evaluation of some test parameters. Slight conjunctival chemosis and severe discharge were observed in all animals approximately one hour after application. As the staining dissipated by the end of the study period and in the absence of any other irritating effects, the notified chemical is considered only slightly irritating to the eye.

Staining effects observed throughout a 28 day repeat dose oral toxicity study in rats were considered to be due to the physical properties of the substance and do not indicate toxicity. No compound related effects on body or organ weights, food consumption, urine, clinical biochemistry, or haematological parameters. Renal tubular changes and increased incidence of basophilia in Goblet cell mucin were observed in all animals of the 1000 mg/kg bw/day group and in some animals treated with 250 mg/kg bw/day with renal tubular changes in the high dose group persisting throughout the recovery period. Salivation during the dosing period was also observed in the 1000 mg/kg bw/day group. The NO(A)EL was established as 50 mg/kg bw/day.

Skin sensitisation studies on guinea pigs revealed no evidence of reactions indicative of skin sensitisation to the notified chemical.

No genotoxic effects were observed in a Bacterial Reverse Mutation Test or an *in vitro* human lymphocyte cytogenic assay.

Hazard classification for health effects.
Cyan Dye 2 is not determined to be hazardous in accordance with the *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 polymer 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 bioavailable.
Therefore, the risk to public health from exposure to the notified chemical is considered 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 not classified as hazardous under the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2003).

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 in the intended manner.

11. MATERIAL SAFETY DATA SHEET

11.1. Material Safety Data Sheet

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

11.2. Label

The label for the notified chemical and products containing the chemical provided by the notifier were 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

- Skin protection is essential when handling the notified chemical.
- A copy of the MSDS should be easily accessible to employees.
- If products and mixtures containing Cyan Dye 2 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 notified chemical should be disposed of to either landfill or be incinerated or recycled with paper in accordance with local, state or national legislation.

Emergency procedures

- Spills/release of the notified chemical should be handled by containing, adsorbing and cleaning up spillage and transferring to a container for disposal. Wash the spillage area clean.
- Do not allow spilled/released chemical or washings to enter drains, sewers or watercourses.

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(1) of the Act; if

- over 1 tonne per annum of the notified chemical is used in Australia Test reports on adsorption/desorption and dissociation constant are required to be submitted for the notified chemical should import volumes reach this quantity.

or

(2) 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.

13. BIBLIOGRAPHY

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