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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

S178095

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

Director
Chemicals Notification and Assessment

TABLE OF CONTENTS

FULL PUBLIC REPORT

S178095

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT
TOXICOS PTY LTD. 293 Waverly Road, Malvern East, VIC 2145

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication:
Chemical name
Other names
CAS number
Molecular and structural formulae
Molecular weight
Spectral data
Purity
Identity of toxic or hazardous impurities
Non-hazardous impurities
Additives/adjuvants
Manufacture and Import volume
Methods of detection and determination

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)
No previous notification.

NOTIFICATION IN OTHER COUNTRIES
UK, Japan and USA.

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
S178095

3. COMPOSITION

DEGREE OF PURITY **Non-Confidential**
High

All compositional information is exempt.

4. INTRODUCTION AND USE INFORMATION

USE
S178095 is a dye used in preparations in ink-jet reprographic processes. The preparation is sold contained within a cartridge for ink-jet printers.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, Transport and Storage

PORT OF ENTRY
Melbourne.

TRANSPORTATION AND PACKAGING
S178095 is imported into Australia in sealed ink-jet cartridges. The volume of a single coloured (non-black) cartridge ranges from 2-15 mL. Cartridges will be delivered to consumers by road transport.

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 ink-jet cartridge will be handled by service technicians or office workers replacing the spent cartridges in the printer.

5.3. 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 as ink cartridges are designed to prevent leakage. These will be changed by the public. However, if leakage or spill does occur, the ink will be contained with absorbent material which will presumably be disposed of in landfill.
Ultimately, practically all the notified chemical will be released to the environment. Paper to which the notified chemical will be bound will eventually be buried in landfill or incinerated, or the chemical may be released in effluent from de-inking processes. Residues left in empty cartridges 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. However, this will be in a highly diffuse manner.

5.4. 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.

6. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa S178095 is a brown solid in powder form.

MELTING POINT >300°C

METHOD EC Directive 92/69/EEC A.1 Melting/Freezing Temperature
Remarks
TEST FACILITY Analytical Sciences Group (2000)

BOILING POINT >>400°C at 101.3 kPa

METHOD Not determined experimentally.
Remarks A theoretical appraisal of the expected boiling point has been made by comparison to the

literature data.
TEST FACILITY Analytical Sciences Group (2000)

DENSITY 1670 kg/m^3 at 20ºC

METHOD OECD TG 109 Density of Liquids and Solids. (Pyncometer)
TEST FACILITY Analytical Sciences Group (2000)

VAPOUR PRESSURE <<10^{-3} Pa at 25ºC

METHOD Not determined experimentally.
Remarks A theoretical appraisal of the expected boiling point and relatedness to vapour pressure has been made by comparison to the literature data.

The chemical is ionic. These compounds have strong inter-molecular bonds with lower vapour pressures than non-ionic compounds of similar molecular weights.
TEST FACILITY Analytical Sciences Group (2000)

WATER SOLUBILITY 140 g/L at 25ºC

METHOD OECD TG 105 Water Solubility (Flask method).
Remarks S178095 was found to produce gel formation at concentrations above 14% w/w in distilled water.
TEST FACILITY Analytical Sciences Group (2000)

HYDROLYSIS AS A FUNCTION OF PH <10% hydrolysis at pH 7.0 and 9.0 at 50ºC
>10% at pH 4.

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

pH	*T (°C)*	*$t_{½}$ days*
4	70	1.6
4	60	3.8
4	50	10.2
4	25	145.2

Remarks The chemical is expected to be hydrolytically stable within the environmental pH range.
TEST FACILITY Analytical Sciences Group (2000)

PARTITION COEFFICIENT (n-octanol/water) log Pow at 20ºC = <-2.0

METHOD OECD TG 107 Partition Coefficient (n-octanol/water), Shake Flask Method.
Remarks The log P_{OW} of <-3.4 quoted in the report is outside the range (-2 to 4) that can be determined by the method used. The partition coefficient is therefore considered to be lower than the limit value of the test, that is <-2.
TEST FACILITY Analytical Sciences Group (2000)

. SOIL ADSORPTION

TEST SUBSTANCE Notified Chemical

METHOD Proposed OECD Test Guideline 121 for the Testing of Chemicals.
Estimation of the adsorption coefficient (K_{OC}) on soil and on sewage sludge using HPLC.
Remarks - Method The test has been validated for the quantitative estimation of Log K_{OC} values in the range 1.5-5.0.

RESULTS LogKoc <1.5
Remarks - Results The retention time of the notified substance was compared to several

standards with the most mobile being acetanilide (log K_{OC} = 1.25) and the least mobile being *pp*DDT (log K_{OC} = 5.63). The notified chemical had a retention time less than acetanilide. Because this test is only validated for a lowest quantitative estimation of log K_{OC} of 1.5, the result is given as <1.5.

CONCLUSION The notified substance would have a high mobility in soil so would not be expected to partition significantly to soil, sludge or sediment.

TEST FACILITY Brixham Environmental Laboratory, (2001b).

DISSOCIATION CONSTANT pKa =…

METHOD

Remarks The dissociation constant was not provided. The notifier stated the compound was expected to dissociate at pH greater than 7. Functional groups in the notified chemical are very strong and Environment Australia expects this chemical would remain fully dissociated at environmental pH.

TEST FACILITY

FLAMMABILITY LIMITS None

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

Remarks The substance did not propagate combustion.
The substance did not evolve highly flammable gases on contact with water.

TEST FACILITY Analytical Sciences Group (2000)

AUTOIGNITION TEMPERATURE None

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

Remarks The substance did not spontaneously ignite.

TEST FACILITY Analytical Sciences Group (2000)

EXPLOSIVE PROPERTIES Not explosive

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

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

TEST FACILITY Analytical Sciences Group (2000)

REACTIVITY Not reactive

METHOD EC Directive 92/69/EEC A.14 Thermal Sensitivity.

Remarks An analysis of the chemical structure of S178095 shows that it does not possess oxidising properties and will not give rise to highly exothermic reactions when in contact with other substances, particularly flammable ones, in the manner in which oxidising substances do.

TEST FACILITY Analytical Sciences Group (2000)

ADDITIONAL TESTS

ADDITIONAL SOLUBILITY DATA >30 g/100 mL DMSO at 25°C

METHOD OECD TG 116 Fat Solubility of Solid and Liquid Substances.
Remarks S178095 was insoluble in ethanol, n-hexane, acetone, toluene, n-octanol, chloroform.
TEST FACILITY Analytical Sciences Group (2000)

SURFACE TENSION OF AQUEOUS SOLUTIONS 72.0±0.1 mN/m at 25±1 ºC

METHOD OECD TG 115 Surface Tension of Aqueous Solutions.(Krüss Process Tensiometer fitted with a Wilhelmy plate)
Remarks Concentration: 0.985 g/L. The chemical is not surface active.
TEST FACILITY Analytical Sciences Group (2000)

7. TOXICOLOGICAL INVESTIGATIONS

7.1. Acute toxicity – oral

TEST SUBSTANCE	S178095, Magenta Solid, Batch No 41
METHOD	OECD Guidelines for the Testing of Chemicals No 423 "Acute Oral Toxicity- Acute Toxic Class Method' (22 March 1996) EC Directive 96/54/EEC B.1 tris Acute Toxicity (Oral) – Acute Toxic Class Method
Species/Strain	Rat/Male and Female Sprague- Dawley CD
Vehicle	Distilled Water
Remarks - Method	

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 Female	2000	0
2	3 Male	200	0

LD50	>2500 mg/kg bw
Signs of Toxicity	None
Effects in Organs	None
Remarks - Results	Red-coloured staining of the faeces was noted in all animals one to three days after dosing. Red-coloured staining of the urine was noted in males one day after dosing. No other abnormalities were noted during the study.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Safepharm Laboratories Limited, 2001(a)

7.2. Acute toxicity - dermal

TEST SUBSTANCE	S178095, Magenta Solid, Batch No 41.
METHOD	OECD TG 402 Acute Dermal Toxicity. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/Sprague-Dawley CD
Vehicle	Distilled water
Type of dressing	Semi-occlusive.
Remarks - Method	

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 Male	2000	0
2	5 Female	2000	0

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	None
Signs of Toxicity - Systemic	None
Effects in Organs	None

Remarks - Results: No dermal irritation observed, although pink staining affected accurate evaluation.

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

TEST FACILITY: Safepharm Laboratories Limited, 2001(b)

7.4. Irritation – skin

TEST SUBSTANCE: S178095/41

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

Species/Strain: Rabbit/New Zealand White
Number of Animals: Three male
Vehicle: Distilled water
Observation Period: 72 hours
Type of Dressing: Semi-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	0
Oedema	0	0	0	0	0	0

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

Remarks - Results: Slight pink-coloured staining was noted at all treated skin sites during the study. The staining did not affect evaluation of skin responses.

CONCLUSION: The notified chemical is non-irritating to skin.

TEST FACILITY: Safepharm Laboratories Limited 2001(c)

7.5. Irritation - eye

TEST SUBSTANCE: S178095/41

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

Species/Strain: New Zealand White
Number of Animals: three
Observation Period: 14 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	1	2	1.7	2	72 hours	0
Conjunctiva: chemosis	0.8	1.8	0.8	2	72 hours	0
Conjunctiva: discharge	0.8	1.2	1	2	72 hours	0
Corneal opacity	0	0	0	0	N/A	0
Iridial inflammation	0	0	0	0	N/A	0

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

Remarks - Results	Pink-coloured staining of the cornea, iris and conjunctivae was noted during the study. One treated eye appeared normal at the 7-day observation and two treated eyes appeared normal at the 14-day observation.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	Safepharm Laboratories Limited, 2001(d)

7.6. Skin sensitisation

TEST SUBSTANCE	S178095/41
METHOD	OECD TG 406 Skin Sensitisation - Magnusson and Klingman Maximisation Method. EC Directive 96/54/EC B.6 Skin Sensitization.
Species/Strain	Guinea pig/Dunkin Hartley
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: 10%w/w (Red coloured staining prevented accurate evaluation of erythema) topical: 50% w/w
MAIN STUDY	
Number of Animals	Test Group: 10 Control Group: 5
induction phase	Induction Concentration: intradermal injection 5% w/w topical application 50% w/w
Signs of Irritation	
CHALLENGE PHASE	
1st challenge	topical application: 25 and 50% w/w
Remarks - Method	Red-coloured staining prevented accurate evaluation of erythema in test group following intradermal and topical induction.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st challenge		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	25% w/w	0	0	0	0
	50% w/w	0	0	0	0
Control Group	25% w/w	0	0	0	0
	50% w/w	0	0	0	0

Remarks - Results	Red-coloured staining was noted at the challenge sites of all test group animals during the study. This did not affect evaluation of skin responses.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Safepharm Laboratories Limited, 2001(e)

7.7. Repeat dose toxicity

TEST SUBSTANCE	S178095/41

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/CD®BR
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days; Dose regimen: 7 days per week; Post-exposure observation period: 14 days (recovery period)
Vehicle	Reverse osmosis water
Remarks - Method	
RESULTS	Doses: 0,25,250,1000 mg/kg/d Number of animals: 5/sex/dose, plus 5/sex recovery animals at 0, 1000 mg/kg/day.

Mortality and Time to Death

There were no deaths.

Clinical Observations

During the treatment period pink staining of the tail, pink staining of the cage tray and dark faeces were observed for animals of all treated groups. Pink staining of the coat, particularly on the paws, head, dorsal surface and perigenital areas was also observed for animals receiving 250 or 1000 mg/kg/day. The pink staining of the cage paper and tails was still present at the end of the recovery period for animals previously treated at 1000 mg/kg/day; the staining of the coats and discoloured faeces had resolved.

There was no evidence of neurotoxicity during the weekly functional observational battery tests.

Overall bodyweight gain and food conversion efficiency were slightly low for males receiving 1000 mg/kg/day when compared to the controls. This was not apparent in the recovery period.

Food consumption was unaffected by treatment.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology

After four weeks of treatment:
Low mean cell volume and mean cell haemoglobin for males and low mean cell haemoglobin for females receiving 1000 mg/kg/day;
Low mean cell haemoglobin concentration for females receiving 1000 mg/kg/day;
High red blood cell, total white blood cell and lymphocyte counts for males receiving 1000 mg/kg/day;
High platelet counts for females receiving 1000 mg/kg/day;
Short activated partial thromboplastin times for males receiving 1000 mg/kg/day.
After two weeks of recovery:
Mean cell haemoglobin was still slightly low for males;
Platelet counts were still high and activated partial thromboplastin times were short for females.

Blood Chemistry

After four weeks treatment:
High urea and cholesterol concentrations for females receiving ≥250 mg/kg/day, and high creatine concentrations for females receiving ≥25mg/kg/day;
High calcium concentrations for animals receiving 1000 mg/kg/day;
High potassium concentrations for males receiving 1000 mg/kg/day;
High total protein and albumin concentrations for animals receiving 1000 mg/kg/day.
Low albumin/globulin ratios for females receiving 1000 mg/kg/day.
After two weeks of recovery:
Urea, creatinine and cholesterol concentrations were still slightly higher than those of the controls for females previously treated at 1000 mg/kg/day;
Other changes observed during the treatment period showed a full recovery.

Urinalysis
After four weeks treatment:
Low volume and pH and a high specific gravity for males receiving 1000 mg/kg/day;
Abnormal colouration of the urine (pink, orange or red) was observed for all animals receiving 250 or 1000 mg/kg/day and one female receiving 25 mg/kg/day.
After two weeks recovery:
The urine was still abnormally coloured, but other changes were no longer apparent.

Effects in Organs
Organ Weights
After four weeks of treatment:
Kidney and spleen weights were high for animals which received 1000 mg/kg/day;
Liver weights were high for females that received 250 or 1000 mg/kg/day;
Heart and ovary weights were also high, compared to controls, for females which received 1000 mg/kg/day.
After two weeks of recovery:
kidney and spleen weights were still slightly high for animals previously treated at 1000 mg/kg/day and liver and ovary weights were still high for previously treated females.

Macroscopic Examination
After four weeks of treatment:
Pink colouration of the kidneys for all animals which received 250 or 1000 mg/kg/day;
Pink colouration of the mesenteric lymph nodes for one male which received 1000 mg/kg/day;
Pink colouration of the contents of the gastro-intestinal tract for three males which received 1000 mg/kg/day;
Pale spleen was observed in one male which received 1000 mg/kg/day.
After two weeks recovery:
Pink colouration of the kidneys was still apparent after two weeks of recovery for one male and four females previously treated at 1000 mg/kg/day.

Histopathology
Perivascular inflammatory changes in the kidney, liver, lungs and spleen in the majority of animals receiving 1000 mg/kg/day and occasional animals receiving 250 mg/kg/day;
Condensed cortical tubular cytoplasm in the kidney was also seen in animals which received 250 or 1000 mg/kg/day and in a single female which received 25 mg/kg/day;
Glycogen depletion was observed for animals which received 1000 mg/kg/day.
After two weeks recovery:
Glycogen depletion had resolved;
All other microscopic changes were still present, indicating that the recovery period of two weeks was not long enough for the changes to reverse.

Remarks – Results
The low body weight gain and low food conversion efficiency observed for males receiving 1000 mg/kg/day were considered to be non-specific indicators of toxicity. Increases in plasma urea, creatinine and potassium concentrations, and the changes in the pH, SG and volume of the urine are in agreement with the observed increased weight and perivascular lymphoid aggregations, indicating that the kidney is a target organ. Similarly, elevated plasma cholesterol, triglyceride, albumin concentrations, increased weights (in females), portal inflammation and glycogen depletion identify the liver as a target organ. Low mean haemoglobin, mean cell haemoglobin concentration and mean cell volume, and high red blood cell and platelet counts for animals receiving 1000 mg/kg/day suggest a potential effect on the haemopoetic system, however, no associated changes in femoral bone marrow were observed. The elevated total white blood cell and leucocyte changes in males receiving 1000 mg/kg/day are probably associated with the inflammatory changes seen at histopathology examination. Reduced activated partial thromboplastin times, and the condensed cytoplasm change were not considered to be biologically significant. The significance of the increased heart weight for females and the raised calcium levels for animals receiving 1000 mg/kg/day is unclear. Persistence of many of the changes at the end of the recovery period indicates that a period of two weeks was not long enough fo the changes to reverse.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 25 mg/kg bw/day in this study, based on urinalysis, macroscopic examination and histopathology which revealed adverse affects at the next highest dose level.

TEST FACILITY Huntingdon Life Sciences Ltd, 2001(a).

7.8. Genotoxicity - bacteria

TEST SUBSTANCE S178095/41

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
Plate incorporation procedure (first test)
Preincubation (second test).

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, *E. coli*: WP2 uvrA,(pKM101).
Metabolic Activation System	S9 fraction from Aroclor-induced rat liver microsomes.
Concentration Range in Main Test	a) With metabolic activation: 50, 150, 500, 1500, 5000 µg/plate. b) Without metabolic activation: 50, 150, 500, 1500, 500 µg/plate.
Vehicle	Distilled Water
Remarks - Method	Positive controls were Benzo[a]pyrene and Sodium azide. There were three replicates for each treatment.

RESULTS

Remarks - Results	No substantial increases in revertant colony numbers over control counts were obtained with any of the tester strains following exposure ti S178095 at any concentration in either the presence or absence of S9 mix. No visible thinning of the background lawn of non-revertant cells was obtained following exposure to S178095.

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

TEST FACILITY Huntingdon Life Sciences Ltd, 2001(b)

7.9. Genotoxicity – in vitro

TEST SUBSTANCE S178095/41

METHOD OECD TG 473 In vitro Mammalian Chromosomal Aberration Test.

Cell Type/Cell Line	*Human Lymphocytes*
Metabolic Activation System	Aroclor-induced rat liver microsomes, S9 fraction.
Vehicle	Distilled water.
Remarks - Method	The positive controls were Mitomycin C and Cyclophosphamide. There were two replicates of each treatment, including positive controls and solvent controls.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Present			
Test 1	0, 39.06, 78.13, 156.25, 312.5, 625, 1250*, 2500*, 5000*	3 hours	19 hours
Test 2	0, 625, 1250*, 2500*, 5000*	3 hours	19 hours
Absent			
Test 1	0, 69.06, 78.13, 156.25, 312.5, 625, 1250*, 2500*, 5000*	3 hours	19 hours
Test 2	0, 625, 1250*, 2500*, 5000*	19 hours	19 hours

RESULTS	First Test Toxicity Data: In both the presence and absence of S9 mix, S178095 was caused no reduction in the mitotic index. The quantitative analysis for polyploidy showed no increase in the number of polyploid metaphase figures when compared to the solvent control. Metaphase analysis: In both the presence and absence of S9 mix, S178095 caused no statistically significant increases in the proportion of cells with chromosomal aberrations at any dose level, when compared with the solvent control. Both positive control compounds caused large, statistically significant increases (P<0.001) in the proportion of aberrant cells. Second Test Toxicity Data: In both the presence and absence of S9 mix, S178095 caused not substantial reduction in the mitotic index. The quantitative analysis for polyploidy showed no increase in the number of polyploid metaphase figures when compared to the solvent control. Metaphase analysis: In both the presence and absence of S9 mix, S178095 caused no statistically significant increases in the proportion of cells with chromosomal aberrations at any dose level, when compared with the solvent control. Both positive control compounds caused large, statistically significant increases (P<0.001) in the proportion of aberrant cells.
Remarks - Results	
CONCLUSION	The notified chemical was not clastogenic to human lymphocytes treated in vitro under the conditions of the test.
TEST FACILITY	Huntingdon Life Sciences Ltd, 2001(c)

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 301F Ready Biodegradability: Manometric Respirometry Test.

Inoculum	Activated sludge.
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	
Remarks - Method	The experimental design consisted of both an inoculum blank control and an abiotic control which contained the activated sludge with all organisms killed by the addition of $HgCl_2$. The purpose of these controls was to ensure the test substance was present in a medium that has the same amount of particulate matter as the test bottles so any removal mechanisms could be accounted for.

RESULTS

Test substance		*Sodium Acetate*	
Day	*% degradation*	*Day*	*% degradation*
15	8	15	67
28	<8	28	67

Remarks - Results: Degradation was calculated through measuring COD and BOD. The total bottle contents were analysed for the test substance at the end of the test period. The result of this analysis indicated that a mean of 100% nominal of the initial concentration was present at the end of the study. Further HPLC analysis also indicated the notified chemical had not undergone any structural change during the course of the study.

Degradation in the control was considered acceptable and the test is considered valid.

CONCLUSION: The notified chemical is not readily biodegradable.

TEST FACILITY: Brixham Environmental Laboratory, (2001a).

8.1.2. Bioaccumulation

CONCLUSION: No test was conducted. However, given the high water solubility, low fat solubility and minimal expected exposure of the chemical to the aquatic environment, bioaccumulation is not expected to occur.

8.2. Ecotoxicological investigations

8.2.1. Acute toxicity to fish

TEST SUBSTANCE

METHOD OECD TG 203 Fish, Acute Toxicity Test – static.
Species Mirror carp (*Cyprinus carpio*)
Exposure Period 96 hours
Auxiliary Solvent None
Water Hardness 47.3 mg $CaCO_3$/L
Analytical Monitoring The mean measured concentrations ranged from 98-106% of the nominal values. The percentage loss in measured concentrations over the test period ranged from <1 to 11%. On the basis of the analytical data, the nominal concentrations were used for the calculation and reporting of the results.
Remarks – Method No deviations to the protocol are reported.

RESULTS Results were calculated using the moving average angle method.

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual (96 h)*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
Dilution water control	<2.6	10		0	0	0	0
320	310	10		0	0	0	0
560	540	10		0	0	0	0
1000	990	10		0	0	0	0
1800	1800	10		0	0	0	0
3200	3200	10		0	0	1	6

LC50 3000 mg/L at 96 hours.
NOEC (or LOEC) 1800 mg/L at 96 hours.
Remarks – Results It was not possible to assess sub-lethal effects during the study due to the intensity of colour in the test solutions. Mortality was assessed daily when fish were netted into a beaker of dilution water taken from each test vessel.

CONCLUSION The notified substance is practically non-toxic to fish.

TEST FACILITY Brixham Environmental Laboratory, (2001c).

8.2.2. Acute/chronic toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation - static.
Species *Daphnia magna*
Exposure Period 48 hours
Auxiliary Solvent None
Water Hardness 201 mg $CaCO_3$/L
Analytical Monitoring Mean measured concentration of the test substance ranged from 96-104% of the nominal exposure concentrations. The toxicity results are therefore based on the nominal exposure concentrations.
Remarks - Method No deviations from the protocol are reported.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual (48 h)*		*24 h*	*48 h*
Control	<0.52	20	0	0
5.0	5.4	20	0	0
11	11	20	0	1
25	24	20	0	0
55	54	20	0	0
120	120	20	0	1

LC50	>120 mg/L at 48 hours
NOEC (or LOEC)	120 mg/L at 48 hours
Remarks - Results	There was one mortality observed in both the 11 and 120 mg/L test concentrations. These immobilities are considered random and no dose response can be identified.
CONCLUSION	The notified chemical is practically non toxic to aquatic invertebrates.
TEST FACILITY	Brixham Environmental Laboratory, (2001d).

8.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	Official Journal of the European Communities, L 383 A, Part C.3, Algal Inhibition Test.
Species	*Selenastrum capricornutum*
Exposure Period	72 hours
Concentration Range Nominal	1.0, 2.3, 5.0, 11, 25, 55 and 120 mg/L
Concentration Range Actual	1.0, 2.2, 5.0, 11, 31, 57 and 130 mg/L
Auxiliary Solvent	None
Water Hardness	Not stated.
Analytical Monitoring	Mean measured concentrations of the test substance ranged from 100 to 124% of the nominal exposure concentration. Toxicity results are based on the nominal exposure concentrations of the test substance.
Remarks - Method	The method appears based on the standard OECD and EEC test guidelines.

RESULTS

Biomass		*Growth*	
Exposure solutions mg/L at 72 h	*Shaded solutions mg/L at 72 h*	*Exposure solutions mg/L at 72 h*	*Shaded solutions mg/L at 72 h*
EbC50 = 3.1	EbC50 = 4.6	ErC50 = 44.0	ErC50 = 36.9
NOEC <1.0	NOEC = 1.0	NOEC <1.0	NOEC = 1.0

Remarks - Results	The test solutions exhibited a pink through to red colouration which increased in intensity with increasing nominal concentration. The culture medium control was clear and colourless.
CONCLUSION	Effects on biomass may be considered the most sensitive endpoint. However, this effect may be argued to be a result of lower light intensity due to the colouration of the test solution and not a toxic effect of the notified substance.
TEST FACILITY	Brixham Environmental Laboratory, (2001e).

8.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified substance.
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sludge.
Exposure Period	3 hours
Concentration Range	1.0, 3.2, 10, 32 and 100 mg/L

Nominal	
Remarks – Method	Results are based on nominal concentrations.

RESULTS

IC50	>100 mg/L
NOEC	100 mg/L
Remarks – Results	Less than 10% inhibition was observed in all tested concentrations. The reference substance, 3,5-dichlorophenol, caused substantial inhibition of the respiration rate of the activated sludge with an IC50 around 7 mg/L indicating the sludge was responding normally and confirming the viability of the sludge organisms.

CONCLUSION	The notified chemical may be considered practically non toxic to microbes.
TEST FACILITY	Brixham Environmental Laboratory, (2001f).

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

If isolated, the low octanol-water partition coefficient and high water solubility indicate the notified chemical will be predominantly distributed in water, where it will become diluted and dispersed. Adsorption data indicates a minimal partitioning to the organic phase of soils and sediment.

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 spent cartridges and containers at landfill sites. Some waste paper may be disposed of directly to landfill with the notified chemical bound to the paper. It is anticipated that prolonged residence in an active landfill environment would eventually degrade the notified substance. Incineration of waste paper will destroy the compound with the generation of water vapour and oxides of carbon, nitrogen and sulphur.

In addition to landfill, some of the ink printed on paper will enter the paper recycling process. During such processes, waste paper is repulped using a variety of alkaline, dispersing and wetting agents, water emulsifiable organic solvents and bleaches. These agents enhance fibre separation, ink detachment from the fibres, pulp brightness and the whiteness of paper. De-inking wastes are expected to go to trade waste sewers. 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 remain associated with sludge and share its fate. Although the notified chemical is highly water soluble, it is uncertain how much may partition to the supernatant water during de-inking operations while the chemical is in its ink formulation.

Bioaccumulation is not expected due to the notified chemicals low log K_{ow}, indicating low lipid solubility, and large molecular weight (greater than 1200 g/mol) which inhibits passage through cell membranes.

Based on the import volume, method of packaging and low concentration of the notified chemical in ink, release of the notified chemical to the environment is expected to be low but widespread.

It is difficult to determine a predicted environmental concentration (PEC) as there is no indication of quantities of waste paper sent to the various streams (landfill, incineration or de-inking). However, some worst case assumptions may be made. If it assumed 2000 kg of the notified chemical ends up in de-inking effluent per annum, and de-inking operations occur on 300 days of the year, then about 7 kg per day of the notified chemical will be released in this manner around the country. If this all goes to a single coastal STP with a daily effluent outflow of 250 ML, the concentration in the effluent will be 28 ppb and may be expected to be diluted by a factor of 10 to around 3 ppb upon release to receiving waters.

9.1.2. Environment – effects assessment

The notified chemical is practically non-toxic to fish and daphnia. Indirect effects may be anticipated to algae in the event that levels are high enough to lead to discolouration of receiving water.

9.1.3. Environment – risk characterisation

The notified chemical will be used as an ingredient of printing ink formulations, with most expected to eventually be disposed of in landfill. The compound is not readily biodegradable (<8% over 28 days), has a low partition coefficient and a high water solubility. In its isolated form, these characteristics all indicate that most of the material would eventually partition to water. However, the notified chemical will share the fate of the wastepaper it is applied to in its ink formulation. Where the waste paper is disposed of to landfill or incinerated, release to water is expected to be minimal. There is potential for release to water due to de-inking procedures during paper recycling.

Release will be widespread, probably over an extended period of time and therefore in minimal quantities. However, worst case assumptions suggest a PEC for water of 3 ppb (see 9.1.1). This is three orders of magnitude less than the concentration at which indirect effects were seen on algae (EbC50 = 3.1 ppm).

These considerations indicate minimal risk to the environment when the notified chemical is used as a component of ink in the manner and levels indicated by the notifier.

9.2. Human health

9.2.1. Human health – exposure assessment

OCCUPATIONAL HEALTH AND SAFETY

The most likely exposure route for the notified chemical is dermal. Contact may occur if residues of the ink are left in the printer or on the cartridge. Exposure would then take place when the cartridge is changed or the copier serviced.

PUBLIC HEALTH

The public may be exposed to the notified chemical following transport accidents involving the breakage of cartridges. Each broken cartridge will release up to 15 ml of ink. The total volume of spilled ink is likely to be small and readily contained for adsorption onto an inert material for mechanical collection, together with broken cartridges, for disposal as land-fill. Any contact is likely to be dermal and of a minimal and transient nature.

Members of the public are unlikely to contact the notified chemical as an environmental contaminant. Spent cartridges with unused residues will be disposed of as land-fill. Discarded printed paper will either be recycled or sent as land-fill.

In the course of the use of the cartridges, consumers may make dermal contact with the ink preparation containing the notified chemical where an attempt is made to repair some mechanical mishap involving the cartridges in the printer. This possibility is remote and spent cartridges will be easily replaced by new ones without any contact with the ink content. On printed paper the notified chemical will be contained in a cured ink preparation and will be inaccessible to human contact. The potential for exposure of the public to the notified chemical is therefore negligible.

9.2.3. Human health - effects assessment

9.2.3.1 SUMMARY OF TOXICOLOGICAL INVESTIGATIONS

Endpoint and Result	*Assessment Conclusion*
Rat, acute oral LD50 >2500 mg/kg bw	low toxicity
Rat, acute dermal LD50 >2000 mg/kg bw	low toxicity
Rabbit, skin irritation	non irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation - adjuvant test/non-adjuvant test.	no evidence
Rat, oral Repeat Dose Toxicity - 28 Days.	NOAEL 25 mg/kg.bw/day
Genotoxicity - bacterial reverse mutation	Non mutagenic*
Genotoxicity – in vitro Mammalian Chromosomal Aberration Test	Non genotoxic*

9.2.3.2 DISCUSSION

S178095 is a high molecular weight azo dye, which has high solubility in water, and is insoluble in organic solvents. The octanol-water partition coefficients is < two. While these factors would mitigate against bioaccumulation of the compound, pink staining of skin and organs in toxicity testing would seem to indicate some deposits in the cell cytoplasm. Pink

staining of the urine and faeces suggest that the notified chemical can be metabolised and removed from the body.

The notified chemical was shown to be of low acute toxicity by both oral and dermal routes. It is non-irritating to the skin and only mildly irritating to the eye. Although red staining of the skin prevented accurate evaluation of erythema following topical and intradermal induction, the compound was found to be non-sensitising in guinea pigs.

In a 28-day dose test rat study, low body weight gain and associated low food conversion efficiency observed for males receiving 1000 mg/kg/day were considered to be non-specific indicators of toxicity. Changes were observed in blood parameters, kidney, liver, spleen, heart and ovary weights, and microscopic changes in the kidney, liver, spleen and lungs. Many of the changes were still apparent at the end of the recovery period indicating that a period of two weeks was not long enough for the changes to reverse. Based on histopathological changes and associated changes in clinical chemistry parameters at 250 mg/kg/d, the NOAEL for the study was 25 mg/kg/d.

Azo compounds undergo enzymatic reduction *in vivo* involving cytochrome P450 (Ballantyne 1995). Methods used to test for mutagenicity need to take account of this biotranformation. Although some debate still exists on the most appropriate methods of ensuring reduction of the compound, a 30 minute pre-incubation step, the use of uninduced hamster liver S9 rather than Arachlor induced rat liver S9, and flavin mononucleotide rather then riboflavin are among the changes to the standard Ames method that have been recommended (Gatehouse et al, 1994, Prival and Mitchell, 1982). Not all these protocols were followed in testing this compound. However, non-mutagenicity or decreased mutagenicity is predictable for S178095 because of the large substituent groups on the azobenzene rings.

S178095 showed no evidence of clastogenic activity in *in vitro* cytogenetic testing.

According to NOHSC (1999), S178095 does not meet the criteria for classification as a hazardous substance.

9.2.4. Human health – risk characterisation

9.2.4.1 OCCUPATIONAL HEALTH AND SAFETY

Staining of tissue during toxicity testing indicates that this chemical may sorb to or penetrate the skin. S168746 belongs to a class of chemicals known to include many skin sensitisers. The possibility of skin sensitisation resulting from dermal contact with the notified chemical can therefore not be dismissed on the basis of a single negative test. However, the amount of the notified chemical to which a worker may be exposed is low, both because of the low volume involved in a likely contact scenario, and because the concentration of the notified chemical in the ink is <5%. Provided proper instruction in the handling of inks, particularly in clean-up procedures in the event of contact, are given to workers via MSDS, labels and instruction manuals, the risk to workers may be considered to be low.

9.2.4.2 PUBLIC HEALTH

From the point of importation to the end use of the ink preparation containing the notified chemical, the ink preparation is either enclosed in a cartridge made for insertion in ink jet printers or is present on printed paper in a cured state. The notified chemical is therefore inaccessible to contact by the public and will remain so unless a cartridge (new or spent) is damaged by some mechanical means such an unlikely transport accident. The possibility of cartridge damage is slight. Any public exposure to the ink preparation that does occur is most likely to be dermal and of a minimal and transient nature. The notified chemical is present in the ink preparation at a concentration of up to 5%. The notified chemical is of low toxicity. The notified chemical has a high molecular weight and is not likely to penetrate biological membranes in contact with it. The low likelihood of public exposure to the ink preparation containing the notified chemical, the low concentration of the notified chemical in the ink preparation and the low toxicity of the notified chemical, suggest that it will not pose a significant risk to public health when used in the proposed manner.

10. CONCLUSIONS

10.1. Environment

On the basis of the available information, the overall environmental hazard of the notified chemical is expected to be low.

10.2. Health hazard

Based on the available data the notified chemical is not classified as hazardous under the NOHSC Approved Criteria for Classifying Hazardous Substances.

10.3. Human health

10.3.1. Human health – Occupational health and safety

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

10.3.2. Human health – public

There is No Significant Concern to public health when used in the proposed manner.

11. RECOMMENDATIONS

CONTROL MEASURES

Occupational Health and Safety

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical when changing cartridges or servicing equipment:
  - Cotton gloves

- 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.

Disposal

- The notified chemical should be disposed of into landfill.

Emergency procedures

- Spills/release of the notified chemical should be contained as described in the MSDS (ie. Contain with absorbent material and transfer to a sealable waste container) and the resulting waste disposed of in landfill.

11.1. Secondary notification

No additional secondary notification conditions are stipulated.

12. MATERIAL SAFETY DATA SHEET

The MSDS for the notified chemical was provided in a format consistent with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC,1994).

This MSDS was provided by the applicant as part of the notification statement. It is reproduced here as a matter of public record. The accuracy of this information remains the responsibility of the applicant.

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