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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Black Dye 2

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Director
Chemicals Notification and Assessment

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Black Dye 2

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Hewlett-Packard Australia Pty Ltd
31 – 41 Joseph St
BLACKBURN VIC 3130

Toxikos Pty Ltd
293 Waverly Road
MALVERN EAST VIC 3145

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 No., identity of the product containing the notified chemical, molecular weight, molecular and structural formulae, spectral data, impurities and import volume.

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None.

NOTIFICATION IN OTHER COUNTRIES
USA, EU, Switzerland.

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Black dye 2.

METHODS OF DETECTION AND DETERMINATION

ANALYTICAL METHOD: Infrared, ultraviolet/visible, nuclear magnetic resonance and mass spectroscopy.

3. COMPOSITION

DEGREE OF PURITY
> 60%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
A number of related by-products are present as components of the notified chemical.

ADDITIVES/ADJUVANTS
None.

4. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
In inkjet cartridges in cardboard boxes.

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

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

USE
As a dye for use in inkjet reprographic processes.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, Transport and Storage

PORT OF ENTRY
Unknown.

IDENTITY OF MANUFACTURER/RECIPIENTS
Notifier or notifier's agent.

TRANSPORTATION AND PACKAGING
The inkjet cartridges will normally be packaged in small cardboard boxes packed in larger cardboard boxes.

5.2. Operation Description

The notified chemical is imported from overseas as a component of printer ink. The printer ink is contained in a sealed cartridge which itself is packaged in cardboard.

The cartridges will be transported and stored prior to national distribution where they will be used in office or home printing equipment. The cartridges will be installed/replaced either by office workers, service technicians or consumers.

5.3. Occupational exposure

Number and Category of Workers

Category of Worker	*Number*	*Exposure Duration*	*Exposure Frequency*
Importation	10	4 hr	40 days/yr
Storage & Transport	100	6 hr	240 days/yr
Office worker / service technician / consumer	10000	< 0.1 hr	20

Exposure Details
Exposure to the notified chemical during the importation transport and storage of the printer cartridges is not expected except in the unlikely event of an accident where the sealed cartridge and its packaging may be breached.

Office workers and service technicians may be exposed to the notified chemical when changing printer cartridges with service technicians also potentially exposed during printer maintenance. However, the cartridges are designed to deposit ink on the paper with little remaining in the cartridge or in the printer itself.

Users of the printers may be exposed to the notified chemical during handling of printed paper, particularly if the paper is handled before the ink is adequately dried or if printing to a non-absorbent substrate occurs by error. After the ink is dry the notified chemical is bound to the paper matrix and is not expected to be readily bioavailable.

5.4. Release

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported in sealed cartridges containing up to 50 g of formulated ink (with a maximum of 1.5% of the chemical). 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. 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 98 % 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 20 kg of the notified chemical will be sent to landfill as residue in empty ink cartridges.

The remaining 98% of the notified chemical (up to 980 kg) bound to paper which 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

Members of the public may be exposed to the notified chemical through handling of the printed paper. The notifier has calculated that each printed page contains 1 mg of dye. However, once printed onto paper and dried, the notified chemical is bound and unavailable for release except if the paper is handled before the ink is adequately dried or if printing to a non-absorbent substrate occurs by error.

6. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20ºC and 101.3 kPa Black flaky solid.

Melting Point/Freezing Point > 300ºC

METHOD EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks Differential Scanning Calorimetry (DSC) was used. Decomposition related features were seen at 150°C and 250°C.

Density 1730 kg/m^3 at 20ºC

METHOD EC Directive 92/69/EEC A.3 Relative Density.
Remarks Pycnometer method. Reference liquid n-hexane.

Vapour Pressure < 10^{-5} kPa over 20–50ºC.

METHOD EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks A weight-loss effusion manometer (a variation of the vapour pressure balance

described in the test guidelines), that utilises the relationship stated by the Knudsen equation between the vapour pressure of a substance and the rate at which it effuses through a small orifice into a vacuum, was used. The resulting weight loss was measured as the change of displacement of a small pan that was suspended from a spring in a vacuum.

The results demonstrated the presence of components with higher vapour pressures than that of the main bulk of the test substance. Some decomposition of the test substance on heating was also evident. Therefore, the relatively rapid rate of effusion indicated initially was ignored and the subsequent very slow rate was considered as being the characteristic of the test substance.

Applying the Knudsen equation, the vapour pressure at 115.3°C (over 45.6 hours) was measured to be 1.28 x 10^{-6} kPa (9.6 x 10^{-6} mm Hg). Previous experience had shown that extrapolation down to temperatures 100 K below the measured range involves a reduction in the vapour pressure by a factor of 10^2 to 10^3, so that in the required temperature range of 20–50°C, the vapour pressure would be in the region of 1.33 x 10^{-9} kPa (10^{-8} mm Hg). Therefore, the vapour pressure was predicted to be < 1.33 x 10^{-6} kPa (10^{-5} mm Hg) over 20–50°C.

Water Solubility 25 g/L at 20°C

METHOD EC Directive 92/69/EEC A.6 Water Solubility (Flask Method).

Remarks Determination of the water solubility was difficult as the test substance had a tendency to form a gel. The test samples were centrifuged, weighed portions removed from the tops of the centrifuge tubes and diluted with distilled water. The concentration of the test substance was determined by spectrophotometry at 24, 48 and 72 hours by comparison to a calibration curve prepared for distilled water and results indicated a mean water solubility of 2.5% w/w adjusted for the 2.2% water content.

As all the test solutions had formed a gel it was not possible to measure the pH. A number of further tests were carried out as follows to confirm the water solubility. Accurately measured amounts of the test substance was diluted to 10 mL with distilled water, mixed well and treated ultrasonically for 20 minutes. The tests were examined visually after standing overnight at room temperature. The observations indicated that the resulting concentrations of 0.9 and 2.0 % w/v were in solution, 2.6% w/v was partially gelled and the 3.2 and 3.8% w/v were gelled. These results indicate a water solubility of 2.0 to 2.6 % w/v thus support the previous solubility results of 2.5% w/v.

Fat (or n-octanol) Solubility < 0.02 mg/100 g HB 307 at 37°C

METHOD EC Directive 92/69/EEC A.7 Fat Solubility.

Remarks The standard fat HB 307 was used. The tests were started at 30°C and 50°C then equilibrated at 37°C. After centrifuging, the tests were filtered through a Millipore filter (HPLV, 0.45 µm). The fat filtrates were examined spectrophotometrically and the concentration of the test substance was determined by comparison to a calibration curve prepared in methanol. The absorbances for all tests (except one of 8 tests) were less than the detection limit, i.e. ≤ 0.02 mg/100 g
The test substance has low fat solubility.

Hydrolysis as a Function of pH

METHOD Official Journal of the European Communities (1984) Test C10

pH	*T (°C)*	*% degradation*
4	50	< 10 after 120 hours
7	50	< 10 after 120 hours
9	50	< 10 after 120 hours

Remarks The tests were carried out at 50°C ± 0.5ºC in buffer solutions that were de-oxygenated before use by bubbling with helium for approximately 5 minutes.
Test solutions were analysed spectrophotometrically. Hydrolysis in pH 4, 7 and 9 buffers was less than 10% after 120 hours days therefore no further testing was conducted. As the test substance was unlikely to hydrolyse, the flasks were not sampled and analysed after 2.4 hours. The water baths used to maintain the temperature were operated at a tolerance of ± 0.5ºC, however, this deviation was not considered to have had any significant effect on the results obtained.

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

Partition Coefficient (n-octanol/water) log Pow at 20ºC = -3.7

METHOD EC Directive 92/69/EEC A.8 Partition Coefficient (Shake-flask Method).
Remarks The concentrations of the test substance were measured spectrophotometrically. The concentration in the water phase was determined by comparison to a calibration curve prepared in distilled water. Log Pow was calculated by comparing the absorbance at 575 nm in the n-octanol phases with the calibration curve prepared in methanol.

The spectra from the n-octanol phases differed to those from methanol and distilled water. This was explained to be possibly due to differences in solvent or the preferential partitioning of an impurity in the test substance.
The report indicates that if an impurity has been selectively extracted into the n-octanol phase, the log P value will be less than the determined value of –3.7. A difference was also noted between the spectra of the water phases and calibration standards.

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

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 will adsorb due to the anionic character.

Dissociation Constant Not determined.
Remarks The test substance is expected to remain at least partially ionised throughout the environmental pH range of 4 to 9, although it contains carboxylate groups which may protonate in this range.

Particle Size Not determined.

Surface Tension 72.2 mN/m at 25ºC

METHOD EC Directive 92/69/EEC A.5 Surface Tension.
Remarks Solutions of the test substance at 1% and 0.1% w/v were prepared in distilled water approximately 5 minutes before the measurements were made. A White Electrical Instrument Company torsion balance calibrated using a 0.5000 g weight, which gives a theoretical reading of 61.3 mN/m, was used.
The results obtained were very similar to the surface tension of distilled water (i.e. not surface active).

Flash Point Not determined.

Flammability Limits Not flammable.

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

EC Directive 92/69/EEC A.12 Flammability (Contact with Water).
EC Directive 92/69/EEC A.13 Pyrophoric Properties of Solids and Liquids.

Autoignition Temperature 340°C

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

Explosive Properties Not explosive.

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

Oxidizing Properties Not oxidizing.

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

Reactivity

Remarks Expected to be stable under normal environmental conditions.

7. TOXICOLOGICAL INVESTIGATIONS

Endpoint and Result	*Assessment Conclusion*
Rat, acute oral LD50 > 2000 mg/kg bw	low toxicity
Rat, acute dermal LD50 > 2000 mg/kg bw	low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	irritating
Guinea pig, skin sensitisation - adjuvant test.	evidence of sensitisation (notified chemical) no evidence of sensitisation (black ink containing notified chemical)
Rat, oral repeat dose toxicity - 28 days.	NOAEL = 1000 mg/kg/day
Genotoxicity - bacterial reverse mutation	non mutagenic
Genotoxicity – in vivo mouse micronucleus	non genotoxic

7.1. Acute toxicity – oral

TEST SUBSTANCE — Notified chemical.

METHOD — OECD TG 401 Acute Oral Toxicity – Limit Test.
- Species/Strain: Rat/Wistar.
- Vehicle: Corn oil. Dose volume 10 mL/kg.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5/sex	2000	None.

- LD50: > 2000 mg/kg bw
- Signs of Toxicity: No toxicologically significant signs.
- Effects in Organs: No toxicologically significant signs.
- Remarks – Results: Black or brown staining of faeces and urine was observed in all animals up to day 3; staining of the tail was observed throughout the study in all animals; staining of the coat was observed on day 1 in all animals;

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

7.2. Acute toxicity - dermal

TEST SUBSTANCE — Notified chemical.

METHOD — OECD TG 402 Acute Dermal Toxicity – Limit Test.
- Species/Strain: Rat/Wistar
- Vehicle: Deionised water.
- Type of dressing: Occlusive.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5/sex	2000	None.

- LD50: > 2000 mg/kg bw
- Signs of Toxicity - Local: Slight to moderate erythema and oedema in the first 4 days of the study.
- Signs of Toxicity - Systemic: None.
- Effects in Organs: None.
- Remarks – Results: Black/brown staining of the application site was seen throughout but did not obscure visual observation of irritation.

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

7.3. Irritation – skin

TEST SUBSTANCE Notified chemical.

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 3
Vehicle Deionised water.
Observation Period 6 days.
Type of Dressing Occlusive.

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	0.33	0	0	2	1 day	0
Oedema	0.67	0	0	2	2 days	0

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

Remarks – Results Brown staining persisted for up to six days but did not obscure visual observation of irritation; slight oedema was seen in all animals at 1 hr after application.

CONCLUSION The notified chemical is slightly irritating to skin.

7.4. Irritation - eye

TEST SUBSTANCE Notified chemical.

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 3
Observation Period 9 days.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	2	2.33	1.33	3	7 days	0
Conjunctiva: chemosis	1	0.67	0.33	2	48 hours	0
Conjunctiva: discharge						
Corneal opacity	0.33	1.33	0	2	72 hours	0
Iridial inflammation	0	0.33	0	1	24 hours	0

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

Remarks - Results Black staining obscured observations for up to one day and persisted for up to 9 days; slight pain was observed on instillation.

CONCLUSION The notified chemical is irritating to the eye.

7.5. Skin sensitisation

7.5.1 Notified chemical

TEST SUBSTANCE	Notified chemical.	
METHOD	OECD TG 406 Skin Sensitisation – maximisation test.	
Species/Strain	Guinea pig/Dunkin Hartley.	
PRELIMINARY STUDY	Not described.	
MAIN STUDY		
Number of Animals	Test Group: 20	Control Group: 10
INDUCTION PHASE	Induction Concentration intradermal injection, 1% (w/v) topical application, 75% (w/v)	
Signs of Irritation	Not stated.	
CHALLENGE PHASE		
1st challenge	topical application: 3% (w/v) topical application: 10% (w/v)	

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	3%	20/20	20/20		
	10%	20/20	20/20		
Control Group	3%	3/10	1/10		
	10%	8/10	2/10		

Remarks - Results

Draize scores for animals testing positive to the notified chemical were as follows: All control animals which tested positive had a score of 1 except for one at 24 hours and 3% challenge concentration which had a score of 2.

For the test animals and challenge concentration of 3% (w/v) there were 13 scores of 3, 6 of 2 and 1 of 1 at 24 hours and 17 of 3, 1 of 2 and 2 of 1 at 48 hours.

For the test animals and challenge concentration of 10% (w/v) there were 15 scores of 3, 3 of 2 and 2 of 1 at 24 hours and 17 of 3 and 3 of 1 at 48 hours.

Skin staining did not obscure visual observation of reactions.

CONCLUSION

There was evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.

7.5.2 Typical ink containing the notified chemical

TEST SUBSTANCE	Formulated Commercial Ink.	
METHOD	OECD TG 406 Skin Sensitisation – maximisation test.	
Species/Strain	Guinea pig/Dunkin Hartley.	
PRELIMINARY STUDY	Maximum Non-irritating Concentration: Intradermal, 5% (w/v) Topical, 100% (w/v)	
MAIN STUDY		
Number of Animals	Test Group: 10	Control Group: 5
INDUCTION PHASE	Induction Concentration intradermal injection, 25% (w/v) topical application, 100% (w/v)	
Signs of Irritation	Not stated.	
CHALLENGE PHASE		

1st challenge	topical application: 75% (w/v) topical application: 100% (w/v)

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	75%	0/10	0/10		
	100%	0/10	0/10		
Control Group	75%	0/5	0/5		
	100%	0/5	0/5		

Remarks - Results Black/grey staining did not obscure visual observations.

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

7.6. Repeat dose toxicity

TEST SUBSTANCE Notified chemical.

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

Species/Strain	Rat/Alpk:APfSD
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	Deionised water.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
I (control)	6/sex	0	None.
II (low dose)	"	50	"
III (mid dose)	"	200	"
IV (high dose)	"	1000	"
V (control recovery)	"	0	"
VI (high dose recovery)	"	1000	"

Clinical Observations

Scattered spontaneous observations. No treatment-related observations. Reductions in bodyweight of low and high dose females were thought to be due to abnormally low initial values. There was a slight reduction in food consumption and efficiency of food utilisation in these groups. Some differences in body weight gain in treated males were either minimal or showed no dose-response relationship. Discoloured faeces were observed for the mid and high dose groups.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Clinical chemistry: slightly elevated glucose and slightly reduced calcium in high dose males.

Haematology: No findings.

Urinalysis: No toxicologically significant findings. Urine discolouration was observed, particularly in high dose animals.

Effects in Organs

Organ weights: Increased kidney to bodyweight ratios of high dose females and a smaller and not statistically significant increase in males also recovery group males. Slight increase in brain weight of high dose males was

not reflected in an increased brain/bodyweight ratio.

A minimal increase in incidence of unilateral hydronephrosis in high dose males was observed. Otherwise, there was a black or grey discolouration of the mucosal surface of the non-glandular region of the stomach in one mid dose female and in 1 male and 3 females of the high dose group.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 1000 mg/kg bw/day in this study, based on the observation that slightly increased kidney weights in high dose animals has no histopathological correlates.

7.7. Genotoxicity - bacteria

TEST SUBSTANCE	Notified chemical.
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Plate incorporation procedure.
Species/Strain	*S. typhimurium*: TA1538, TA1535, TA1537, TA98, TA100. *E. coli*: WP2 uvrA (pKM101).
Metabolic Activation System	Rat liver S9 fraction.
Concentration Range in Main Test	a) With metabolic activation: 0 - 6250 µg/plate. b) Without metabolic activation: 0 - 6250 µg/plate.
Vehicle	DMSO.

RESULTS

Remarks - Results	No cytotoxicity was observed but precipitation was observed at 1000 µg/plate. No significant increases in the numbers of revertant colonies either in the presence or absence of metabolic activation. Positive controls were used and in all cases resulted in large increases in revertants, confirming the sensitivity of the test system.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.

7.8. Genotoxicity – in vivo

TEST SUBSTANCE	Notified chemical.
METHOD	OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
Species/Strain	Mouse/C57BL/6JfCD-1/Alpk
Route of Administration	Oral – gavage.
Vehicle	Corn oil.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
1	5/sex	3130	24 hours
2	"	5000	24, 48 and 72 hours

RESULTS

Doses Producing Toxicity	None.
Genotoxic Effects	None.
CONCLUSION	The notified chemical was not clastogenic in this in vivo mouse bone marrow micronucleus test under the conditions of the test.

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

A ready biodegradability test was not conducted. However, the results of a modified Zahn-Wellens inherent biodegradability test was provided (included under Section 8.3 A).

8.1.2. Bioaccumulation

No bioaccumulation data were provided. However, if there is any release to the aquatic compartment the potential for bioaccumulation is considered to be low due to the high water solubility and the low log P_{ow} of the notified chemical (Connell, 1990).

8.2. Ecotoxicological investigations

8.2.1. Acute toxicity to fish

TEST SUBSTANCE	Black Dye 2 The test sample (T137) used was a solution of the test substance Black Dye 2 (3.6%) in water (96.4%). The test solutions were prepared with T137 to be equivalent to a concentration of 100 mg of the test substance/L.
METHOD	EC Directive 84/449/EEC C.1 Acute Toxicity for Fish – Static test.
Species	Rainbow trout (*Salmo gairdneri*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	52 mg $CaCO_3$/L
Analytical Monitoring	Spectrophotometric analysis
Remarks – Method	The concentration of T137 in the test solutions was measured at the beginning and end of the exposure period. An aerated static procedure was used as previous experience on similar test substances of relatively low toxicity has, in general, being stable in aqueous solution and not expected to be lost by volatilisation or foaming.

RESULTS

Concentration (Test Substance) mg/L Nominal	*Number of Fish*	*Mortality*			
		24 h	*48 h*	*72 h*	*96 h*
Control	10	0	0	0	0
100	10	0	0	0	0

LC50	> 100 mg of test substance/L at 96 hours.
NOEC (or LOEC)	> 100 mg of test substance/L at 96 hours (highest test concentration used).
Remarks – Results	The pH and temperature were all satisfactorily maintained. The mean concentration of T137 was measured to be 98% of the nominal concentration. Symptoms of toxicity other than mortality could not be observed due to the intense colour of the test solutions.
CONCLUSION	The test substance is practically non-toxic to fish.

8.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Black Dye 2 The test sample (T137) used was a solution of the test substance Black Dye 2 (3.6%) in water (96.4%).

METHOD	EC Directive 84/449/EEC C.2 Acute Toxicity for Daphnia – Static Test
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	166 mg $CaCO_3$/L
Analytical Monitoring	Spectrophotometric analysis
Remarks - Method	The test solutions were prepared with T137 to be equivalent to a concentration of 100 mg of the test substance/L. The concentration of T137 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*	*% Mortality*	
		24 h	*48 h*
Control	20	0	0
100	20	0	0

LC50	> 100 mg/L at 48 hours
NOEC	> 100 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 101% of the nominal value of 100 mg/L.

CONCLUSION	The test substance is practically non-toxic to Daphnia.

8.2.3. Algal growth inhibition test

TEST SUBSTANCE	Black Dye 2
METHOD	OECD TG 201 Alga Growth Inhibition Test and EC Directive 92/69/EEC C.3 Algal Inhibition Test.
Species	*Selenastrum capricornutum*
Exposure Period	72 hours
Concentration Range Nominal	0.14, 0.31, 0.68, 1.5, 3.3, 7.3 and 16 mg/L
Concentration Range Actual (Mean measured)	0.16, 0.36, 0.77, 1.7, 3.5, 7.6 and 16 mg/L
Auxiliary Solvent	None
Water Hardness	Standard test medium was used.
Analytical Monitoring	Spectrophotometric analysis
Remarks - Method	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	6.4	1.3	0.14	0.14
Shaded solutions	5.2	1.5	0.31	0.14

Remarks - Results	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 quotient of the inhibition of growth curves is higher than 0.9 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.

8.2.4. Inhibition of microbial activity

A. Effect on respiration rate of activated sludge
B. Effect on nitrifying ability of activated sludge
C. Effect on sludge digestion and determination of colour removal under anaerobic conditions

TEST SUBSTANCE

Black Dye 2
The test sample (T137) used was a solution of the test substance Black Dye 2 (3.6%) in water (96.4%).

METHOD

A. Ecological Method No. 103 of the Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry (ETAD) which is a screening test that formed the basis of the OECD TG 209 Activated Sludge, Respiration Inhibition Test.
B. The Assessment of the Nitrifying Ability of Activated Sludge (Tentative Methods) Department of the Environment, UK.
C. Modification of the method "Amenability of Sewage Sludge to Anaerobic Digestion Department of the Environment, UK".

Inoculum	A & B. Activated sludge C. Raw feed sludge and sludge from a heated digester (collected from a local sewage treatment works.
Exposure Period	A. 3 hours B. 4 hours C. 15 days
Concentration Range Nominal	A & B. 2800 mg T137/L (equivalent to 100 mg test substance/L) C. 3,450, 6.900 and 17,275 mg of T137/L (equivalent to approximately 124, 248 and 622 mg test substance/L)
Remarks – Method	B. The sludge was incubated under aerobic conditions in an inorganic medium containing ammonium ion. C. The raw and digester sludges were mixed (at 1:3 ratio) and placed in incubation bottles of batch digestion units and allowed to stand approximately for 30 minutes at 35ºC before connection to the gas measuring devices. At the end of the test a sample of the supernatants from each control and test units were taken for clolorimetric analysis.

RESULTS

A. IC50	> 100 mg/L
B. Concentration of oxidised nitrogen in the presence of the test substance	14 mg/L
Remarks – Results	A. A 17% inhibition was observed however, was considered as unlikely to be significant in terms of sewage treatment practice where the substance concentration will be considerably lower. The IC50 of the 3,5-dichlorophenol was 18 mg/L, thus validating the test. B. Concentration of oxidised nitrogen in the presence of the test substance was the same as the concentration for control.

C. A slight inhibition was observed over the first few days but no significant inhibition over the whole study period. A mean decolourisation of 92% was achieved.

CONCLUSION — The test substance does not cause significant inhibition of the respiration rate or the nitrifying ability of activated sludge and is unlikely to affect sludge digestion.

8.3A. Biochemical/chemical oxygen demand (BOD_5/COD) and bioelimination

TEST SUBSTANCE — Black Dye 2
The test sample (T137) used was a solution of the test substance Black Dye 2 (3.6%) in water (96.4%).

METHOD — BOD_5 and COD – Method of the Department of the Environment, UK
Bioelimination – A modification of the Zahn-Wellens Test (OECD TG 302B)

Inoculum — BOD_5 - A bacterial inoculum prepared from a treated domestic sewage effluent
Bioelimination – Activated sludge

Exposure Period — BOD_5 – 5 days
Bioelimination – 28 days

Auxiliary Solvent — None

Analytical Monitoring — Spectrophotometric analysis

Remarks - Method — A standard dispersion of T137 in distilled water at a nominal strength of 28 g/L (equivalent to 1 g/L of test substance) was used to determine BOD_5, COD and bioelimination.
Bioelimination was determined by colorimetric analysis.

RESULTS

BOD (5 days) g O_2 /g	*COD g O_2 /g*	*BOD/COD*
< 0.1	1.26	<0.08

Remarks - Results — BOD_5 - The results of the BOD test showed mean differences between test samples and controls (at both the 25-times and 250-times dilution rates) that corresponded to BOD levels considered to be below the realistic detection limits of the method.
Bioelimination – The mean bioelimination (Day 5-28) determined by colour removal was 14%.

Several DOC values were estimated due to faults of the DOC instrument, which appeared to be giving inconsistent results.

CONCLUSION — The test substance does not undergo any significant biodegradation under the aerobic conditions of the BOD_5 test and only minor decolourisation occurred in the Zahn-Wellens Test.

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

The total import volume of the notified chemical will ultimately either be 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 chemical due to abiotic or slow biotic processes. Incineration of waste paper and sludge will destroy the chemical with the generation of water vapour and oxides of carbon, nitrogen and sulphur.

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	>100
Daphnia	48-h	EC50	>100

A predicted no effect concentration (PNEC - aquatic ecosystems) of > 0.1 mg/L (> 100 µg/L) has been derived by dividing the end point value of > 100 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 (1.5%), 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 < 7.03×10^{-3} and < 7.03×10^{-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 (< 1.5%) in the ink formulations. Exposure to the notified chemical on printed paper is low as the dye is bound to the paper matrix although some dermal exposure may occur if the paper is handled prior to complete drying.

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 except if the paper is handled prior to complete drying. Public exposure is assessed as low.

9.2.3. Human health - effects assessment

The notified chemical was of low acute oral and dermal toxicity in rats, was a slight skin and a moderate eye irritant, was a skin sensitiser in guinea pigs and was neither mutagenic in bacteria nor clastogenic in mouse bone marrow cells. In a 28-day study, rats were administered the notified chemical by oral gavage and the NOAEL was 1000 mg/kg/day, as slight kidney weight effects at the top dose were not supported by histopathological evidence. The notified chemical is classified as a skin sensitiser according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (1999). However, a black ink containing the notified chemical was not a skin sensitiser in guinea pigs and would not be so classified. The major difference between the skin sensitisation studies appears to be the high topical induction dose (75% (w/v)) in the study using the notified chemical alone as the sensitisation response was very strong even at a low challenge dose of 3% (w/v).

9.2.4. Occupational health and safety – risk characterisation

The OHS risk presented by the notified chemical is expected to be low given that the notified chemical is contained in enclosed cartridges and the ink would not be classified as hazardous.

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 and is not classified as hazardous at these concentrations. 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 to be low.

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

10.1. Hazard classification

Based on the available data the notified chemical is classified as hazardous under the NOHSC *Approved Criteria for Classifying Hazardous Substances*. The classification and labelling details are:

R43: May cause sensitisation by skin contact

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

For the environment it is not possible to categorise the notified chemical according to the OECD (2002) Globally Harmonised System for the Classification and Labelling of Chemicals.

10.2. Environmental risk assessment

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

10.3. Human health risk assessment

10.3.1. Occupational health and safety

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

10.3.2. Public health

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

11. MATERIAL SAFETY DATA SHEET

11.1. Material Safety Data Sheet

The MSDS of the ink containing the notified chemical provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 1994a). It is published here as a matter of public record. The accuracy of the information on the MSDS remains the responsibility of the applicant.

11.2. Label

The label for the ink containing the notified chemical provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Labelling of Workplace Substances* (NOHSC, 1994b). The accuracy of the information on the label remains the responsibility of the applicant.

12. RECOMMENDATIONS

CONTROL MEASURES

Occupational Health and Safety

- A copy of the MSDS should be easily accessible to employees.
- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances*, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Environment

Disposal

- The 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 clearing 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

- Import volumes are projected to exceed 1 tonne/annum, test reports on adsorption/desorption and dissociation constant will need to be submitted in addition to other requirements.

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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