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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Chemical in S195178

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health and Ageing, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment and Water Resources.

For the purposes of subsection 78(1) of the Act, this Full Public Report may be inspected at our NICNAS office by appointment only at 334-336 Illawarra Road, Marrickville NSW 2204.

This Full Public Report is also available for viewing and downloading from the NICNAS website or available on request, free of charge, by contacting NICNAS. For requests and enquiries please contact the NICNAS Administration Coordinator at:

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Director
NICNAS

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Toxikos Pty Ltd (ABN: 30 095 051 791)
293 Waverley Road
Malvern East VIC 3145

And

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

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication:
Chemical name; Other names; Molecular formula; Structural formula; Molecular weight; Spectral data; Methods of detection and determination; Purity; Impurities; Identity of additives/adjuvants; Manufacture/import volume; Use details.

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
EU - VIIA

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
S195178 (<5% notified chemical)

CAS NUMBER
None assigned

MOLECULAR
WEIGHT
>500 g/mol

ANALYTICAL DATA
Reference NMR, IR, HPLC, GC, UV/vis, ES-MS spectra and elemental analyses were provided.

3. COMPOSITION

DEGREE OF PURITY > 75%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Black granular powder

Property	Value	Data Source/Justification
Melting Point	>300°C	Measured
Boiling Point	>400°C at 101.3 kPa	Estimated on the basis of high molecular weight and high melting point of the notified chemical.
Density	1590 kg/m^3 at 20 ± 0.5°C	Measured
Vapour Pressure	0.0027 kPa at 25°C	Estimated (Modified Watson correlation)
Water Solubility	~380-400 g/L at 20°C	Visual assessment
Solvent Solubility	>29.5% w/w DMSO 10.4 - 12.4% w/w ethanol <0.03 µg/mL n-hexane 0.14 µg/mL acetone	Visual assessment and measured
Hydrolysis as a Function of pH	t½ >1 year at pH 4 and 7 t½ = 629.4 days (15106 hrs) at pH 9	Extrapolated from measured data
Partition Coefficient (n-octanol/water)	log P_{ow} < -3.5 at 25°C	Measured
Surface Tension	72.1 mN/m at 25°C	Measured
Adsorption/Desorption	log K_{oc} > 5 (pH 3) and log K_{oc} < 1.5 (pH10) at 20.6-23.9°C	Measured
Dissociation Constant	pKa 1 ~10.9 pKa 2 ~2.7	Measured
Particle Size		The notified chemical is imported in an aqueous solution and remains as such throughout its lifetime in Australia.
Flash Point		Low vapour pressure solid
Flammability	Not highly flammable	Measured
Autoignition Temperature	253 ± 5 °C	Measured
Pyrophoric Properties	Not pyrophoric	Measured
Explosive Properties	May pose risk of explosion if heated under confinement.	Measured (Thermal sensitivity test)
Accelerated Storage Test	Stable (shelf life >2 years)	Measured
Oxidising Properties	Not oxidising	Analogue data

DISCUSSION OF PROPERTIES

For full details of tests on physical and chemical properties, please refer to Appendix A.

Reactivity

The notified chemical is expected to have an ambient shelf life of up to two years. The notified chemical will not liberate flammable gases on contact with water (NPIL Hazards Group, 2006).

Classification

While the notified chemical is not classified as an explosive in accordance with the Australian Dangerous Goods Code (FORS, 1998) there was shown to be the possibility of explosion when heated under confinement, and therefore the following precautionary risk phrase can be applied:
R44 Risk of explosion if heated under confinement.

5. INTRODUCTION AND USE INFORMATION

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

The notified chemical will be imported into Australia as a component (<5%) of inkjet printer ink within sealed inkjet printer cartridges.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	<10	<10	<10	<10	<10

PORT OF ENTRY: All major ports in Australia

IDENTITY OF MANUFACTURER/RECIPIENTS
The inkjet cartridges will be used in home and office printers nationwide.

TRANSPORTATION AND PACKAGING
The notified chemical will be transported in its original sealed cartridge packaging and stored in boxes for subsequent transport to suppliers. Cartridges will be transported by road.

USE
The notified chemical will be used as a colourant in ink preparations for home and office inkjet printers at concentrations of <5%.

OPERATION DESCRIPTION
No reformulation or repackaging of the notified chemical will occur in Australia. The sealed cartridge will be delivered to service technicians, office workers and the general public in its original packaging and these will only be handled when replacing used cartridges in printers.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

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

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 cartridge and its packaging may be breached.

Both office workers and service technicians may be exposed (dermal or ocular) to the notified chemical in ink while changing printer cartridges, and service technicians may additionally be exposed during printer maintenance. Dermal exposure to small quantities of the notified chemical may occur if the print heads are touched while replacing the cartridges. In addition, dermal and possibly ocular exposure could occur when handling faulty or ruptured cartridges. Such exposure is only expected to be infrequent in nature.

Dermal exposure of workers may also occur when handling printed media before the ink is adequately dried, especially when printing on non-absorbent materials. Assuming an average cartridge capacity of 20 mL, when printing text (5% ink coverage per page), it is expected that approximately 700 pages will be printed from a single cartridge. Given that the notified chemical is present at concentrations of <5% in the ink cartridge, each ink cartridge will contain up to 1 gram of the notified chemical. Distributed across 700 pages, this translates to approximately **1 mg of notified chemical** per page.

A worst-case estimate for exposure to the notified chemical involves printing of graphics, whereby up to 100% of each page will be covered with the ink. In this situation, each page is estimated to contain up to **20 mg of the notified chemical**. Based on a 50% transfer on contact when handling printed materials (assuming partially dry ink), and the relative contact area of fingertips and paper size:

Area of contact with finger ends (four fingers on one hand) = 8 cm^2
A4 sized paper = ~600 cm^2
% Removal = (8/600) × 0.5 × 100 = <0.7%
∴ Exposure to fingertips per event = <0.7% of 20 mg = **<0.14 mg per event**.
Also, the expected area exposure per contact event = <140 μg ÷ 8 cm^2 = **<17.5 μg/cm^2**

For extensive contact with wet ink on printed material (i.e. >10 events per day) the daily exposure, assuming no washing between events, a 70 kg person and an estimate of 10% absorption, would be:

Daily exposure = [(<0.14 (mg/event)×10) ÷ 70] ÷ 10 = **~0.002 mg/kg bw/day**.

The probable exposure is likely to be lower than this worst case estimate, given that exposure is likely to be avoided to minimise staining of skin and clothing, and washing following skin contact is likely to occur.

A more realistic exposure estimate, assuming 10% transfer when handling printed materials and only one finger exposure (2cm^2) is as follows:

% Removal = (2/600) × 0.1 × 100 = <0.03%
∴ Exposure to fingertips per event = <0.03% of 20mg = **<0.007 mg per event**
The expected area exposure per contact event = <7 μg ÷ 2 cm^2 = **<3.5 μg/cm^2**
Daily exposure, using the assumptions above, would be:
[(<0.007 (mg/event)×10) ÷ 70] ÷ 10 = **~0.0001 mg/kg bw/day**.

It is not possible to estimate the level of exposure that is expected for the smaller arylamine species that might be derived from the notified chemical.

After printed inks are dry, the notified chemical will be bound to the paper or other media, and is not expected to be readily available.

6.1.2. Public exposure

The exposure of the public to the notified chemical in inkjet printer inks is expected to be identical, or of a lesser extent, than that experienced by office workers using the same ink.

6.2. Human health effects assessment

The results from toxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	low toxicity oral LD50 >2000 mg/kg bw
Rat, acute dermal toxicity	low toxicity LD50 >2000 mg/kg bw
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay Assay 1	no evidence of sensitisation
Mouse, skin sensitisation – Local lymph node assay Assay 2	evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL 150 mg/kg bw/day NOEL 15 mg/kg bw/day
Genotoxicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosomal aberration test	non genotoxic

Toxicokinetics, metabolism and distribution:

Many azo dyes are unlikely to be absorbed through the skin because of their size and polarity (Øllgaard, 1998). Smaller species may undergo percutaneous absorption, dependent on their properties. There was no evidence from the toxicological investigations to suggest that the notified chemical was absorbed following dermal exposure. Absorption through the skin is not expected to be significant, given its relatively high molecular weight (>500 Da), high water solubility (>10 g/mL), and low partition coefficient (log P< -1). These parameters demonstrate its low lipophilicity, indicating that it is not likely to cross the stratum corneum (European Commission, 2003). However, bacterial skin microflora have been reported to be able to break down azo dyes into smaller species, which may be more readily absorbed, through azo reduction (SCCNFP, 2002).

Given the black coloured urine and colouration of organs and the gastrointestinal tract seen in the acute oral toxicity study and repeated dose oral toxicity study, and the systemic toxic effects observed in the repeated dose oral toxicity study, it is likely that the notified chemical can be absorbed from the gastrointestinal tract following oral exposure.

Ultimately, the metabolites of azo dyes are excreted in the urine and bile (Brown & DeVito, 1993, referenced in Øllgaard, 1998 and Fuji, 2007). Sulfonation of azo dyes appears to decrease their toxicity by enhancing their urinary excretion, and that of their metabolites. Black coloured urine and staining observed on cage liners in the repeated dose oral toxicity study were indicative of the urinary excretion of metabolites of the notified chemical.

General toxicity:
The notified chemical when applied directly to the skin was found to be slightly irritating to the eyes and skin. There were no local dermal effects observed in the acute dermal toxicity test.

In a 28-day oral repeat dose study in rats, the NOAEL was 150 mg/kg bw/day, based on the absence of the serious adverse effects that were seen at higher doses. Most of the observed effects were considered by the investigators to be due to the irritant nature of the notified chemical. The NOEL was 15 mg/kg bw/day, based on the absence of any toxicologically relevant effects at this dose.

Sensitisation:
Two local lymph node assays were performed on the notified chemical. Assay 1 indicated that the notified chemical was not a skin sensitiser at concentrations up to 10%, whilst Assay 2 displayed evidence of skin sensitisation at all concentrations tested (5 – 25%). Note that it was not possible to derive an accurate EC3 value for sensitisation potency from this assay. The two assays mainly differed in the vehicles used, with Assay 1 using 1% pluronic (a non-ionic surfactant) in distilled water, and Assay 2 using dimethyl sulfoxide (DMSO). It is well documented that the choice of vehicle for LLNA studies has significant bearing upon the results of such studies (McGarry, 2007). It has been suggested that if a test substance is soluble in dimethyl formamide (DMF) or DMSO, these should be considered the preferred vehicles for use in LLNA in preference to 1% Pluronic L92 (Ryan, 2002). In addition, DMSO is amongst the recommended vehicles listed in OECD test guideline 429 for the LLNA, whilst pluronic is not. Therefore, Assay 2 is considered to be more predictive of human sensitisation. Thus it is concluded that the notified chemical should be classified as a potential skin sensitiser.

Mutagenicity:
Azo dyes are a concern for their potential induction of mutagenicity and carcinogenicity. The azo linkage is the most labile portion of an azo dye molecule, and it is readily enzymatically metabolised in mammals, including man (SCCNFP, 2002). Liver azo reductase enzymes reductively cleave the molecule into component amines. Some metabolism may also occur in the cells of the bladder wall, and during percutaneous absorption. Skin and anaerobic intestinal bacteria are also capable of reductive cleavage of the azo linkage.

Azo reduction is thought to contribute to the carcinogenicity of many azo dyes. The notified chemical is not expected to be reductively cleaved to release any of the restricted aromatic amines specified in either the Appendix to EC Directive 76/769/EEC (EC, 2004) or the annexes of EU SCCNFP/0495/01 (SCCNFP, 2002). However, the notified chemical can be broken by azo reduction into a number of arylamine species, many of which could potentially be mutagenic. The structures of these arylamine species resemble known human carcinogens (SCCNFP, 2002; RoC, 2005), and one is described by a USEPA category of concern for carcinogenicity. The significant structural modifications of these species does not indicate that they are likely to be of lower concern as potential carcinogens (SCCNFP, 2002; US EPA, 2002).

In addition, azo dyes are renowned for their content of impurities, particularly for the presence of component arylamines (SCCNFP, 2002; Øllgaard, 1998). Such impurities are thought to contribute to their carcinogenicity, as these species may be more readily absorbed and activated as carcinogens. The analytical data provided by the notifier indicates that the sample of the notified chemical contains a number of impurities, most of which are structurally similar to the notified chemical. As such, these impurities may possibly contribute to carcinogenicity of the notified chemical.

The notifier supplied test results showing that the notified chemical was found to be not mutagenic in bacteria (under the conditions of the Ames test used), and it did not induce chromosomal aberrations in mammalian cells *in vitro*. The Ames test performed was a standard test, according to OECD Test Guideline 471. However, the test guideline strongly recommends the use of alternative procedures for the detection of mutagenicity of azo dyes, as it is recognised that the standard procedure may not be sufficiently sensitive for these chemicals. Modified tests, such as that of Prival and Mitchell, utilise a reductive pre-incubation step (during which the azo dye is reduced to amine species) before the test is carried out (Prival and Mitchell, 1982). This modified test is thought to yield a greater detection of mutagenic azo dyes. As such, mutagenicity of the notified chemical cannot be ruled out on the basis of the studies performed.

Overall, these results do not rule out the notified chemical as a possible carcinogen, as reductive metabolism may

be significant *in vivo*. In general, the degree of correlation between mutagenicity study results and the carcinogenicity of azo dyes as a class is poor, likely due to their complex metabolism *in vivo* (Brown and DeVito, 1993, referenced in Øllgaard, 1998).

Based on the potential to induce skin sensitisation, the notified chemical is classified as hazardous to health in accordance with the *Approved Criteria for Classifying Hazardous Substances* (NOHSC 2004).

The following risk phrase should be applied:
R43: May cause sensitisation by skin contact.

6.3. Human health risk characterisation

6.3.1. Occupational health and safety

Dermal and accidental ocular exposure are the main routes of worker exposure to the notified chemical at concentrations of <5%. Skin or eye irritation is unlikely to occur during such exposure, given that the notified chemical is slightly irritating to the skin and eyes, and is present at concentrations below the irritation cut off (ie. <20%). Acute effects from exposure to the notified chemical are unlikely to occur, given its low acute toxicity (oral and dermal).

The notified chemical, present in inks at concentrations of <5%, is not likely to be toxic at the highest levels of probable exposure (worst-case exposure estimate of ~0.002 mg/kg bw/day, compared with the NOEL of 15 mg/kg bw/day). The notified chemical is not expected to cause mutagenicity, given that exposure is likely to be low, absorption following dermal contact is expected to be insignificant, and the anticipated short duration of skin contact will limit the breakdown of the notified chemical to arylamine species by skin bacteria.

The notified chemical is expected to be sensitising at the concentrations present in inks (<5%), based on the results of the LLNA (Assay 2). However, it was not possible to define an EC3 value for sensitisation potency from this assay. Whilst accurate estimations of exposure to the notified chemical are not available, under normal circumstances, exposure is expected to be minimal, as workers are unlikely to make deliberate contact with inks containing the notified chemical. Accidental exposure would involve low level contact with inks on the fingertips. Such low exposure levels are unlikely to be sufficient to induce skin sensitisation. As such, the risk of sensitisation is expected to be low, however, it cannot be ruled out in situations other than the proposed uses, where significant repeated dermal exposure might occur.

Given that exposure of workers to the notified chemical is expected to be low, the OHS risk is not considered to be unacceptable.

6.3.2. Public health

The unlikely but potential public exposure to the notified chemical through accidents during importation, transportation or storage is assessed as negligible. The exposure and hazard of the notified chemical to members of the public during use of inkjet printers are expected to be identical or similar to that experienced by office workers. Therefore, the risk of the notified chemical to the health of the public is not considered to be unacceptable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical is not manufactured or reformulated in Australia. It will be imported and distributed within ready-to-use cartridges (containing <5% notified chemical). Release to the environment is not expected during importation and transport. However, release may occur in the unlikely event of a spill where the printer cartridge is ruptured.

RELEASE OF CHEMICAL FROM USE
Release of the ink solution to the environment is not expected under normal use, as the cartridges are of high integrity and are designed to prevent leakage. In the unlikely case of spills arising during installation and replacement, it is expected that the ink containing the notified chemical will be contained and collected with absorbent material and be subsequently disposed of to landfill. Up to an estimated 5% of the ink formulation containing the notified chemical will remain in the used printer cartridge, and cartridges are expected to be disposed of as household waste to landfill.

Most of the notified chemical (95%) will be used for its intended purpose as ink, and will be bound to printing paper. It is anticipated that 50% of paper to which the notified chemical is bound will be recycled, of which 60% may be released in effluent from de-inking processes. Waste paper is generally pulped using chemical treatments that result in the separation of the dye from the paper fibres. During this process, a proportion of the notified chemical may be recovered through adsorption to sludge, with the remainder released to trade waste sewers. While most may partition to water, due to the low percentage of the notified chemical in these inks and the widespread use, release to the aquatic compartment from any given recycling plant will be low, based on worst case assumptions. Any notified chemical absorbed to sludge during the recycling process will be disposed of to landfill.

The remaining 50% of the paper to which ink is bound is claimed to be either buried in landfill or incinerated, but landfill is more likely.

RELEASE OF CHEMICAL FROM DISPOSAL
The majority of the annual import volume of the notified chemical will ultimately be disposed of as normal office/domestic waste that will end up in either landfill or enter the paper recycling process. During paper recycling, much of the notified chemical may be released to the sewer, with the remainder being adsorbed to sludge, which is said to be disposed by landfill or incineration, but more likely landfill.

Residual ink (~5% of the notified chemical) left in the empty cartridges will be disposed of through the usual channels for handling domestic waste.

7.1.2 Environmental fate

For the details of the environmental fate studies, please refer to Appendix C. The notified chemical, as a component of ink, is expected to remain fixed to the paper throughout its useful life. Assuming that 5% of the chemical will remain in empty cartridges, then 95% (<9500 kg per annum) will be used for its intended purpose as ink. Approximately 50% of paper is recycled, meaning <4750 kg will be disposed during paper recycling, of which 60% (<2850 kg per annum) is expected to release ink during the process. This is likely to occur at recycling plants throughout Australia over 260 working days. Residual chemical in the empty cartridges will be landfilled or recycled, with any recycled product likely to also be landfilled at the end of its useful life. In landfill the notified chemical is likely to be mobile based on its K_{OC} value, once the paper substrate or cartridge has degraded.

The notified chemical has very high water solubility and highly variable log K_{OC} values, depending on the pH conditions in which it enters the environment. Under alkaline pH conditions, most of the notified chemical is not expected to be recovered through adsorption to sludge, and instead will partition to the water phase. The notified chemical is neither readily hydrolysable nor biodegradable, and is therefore expected to persist in the environment. However, its low log P_{OW} value and high molecular weight indicate low potential for bioaccumulation.

The remainder of the paper products will be disposed of via landfill.

7.1.3 Predicted Environmental Concentration (PEC)

The PEC was calculated based on the assumption that 50% of printed paper is recycled, of which 60% is

released from de-inking. A worst case scenario is considered in which there is no recovery of the notified chemical from de-inking. The amount released to the sewer is calculated as follows:

10,000 kg x 0.95 x 0.5 x 0.6 = 2850 kg notified chemical.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	10,000	kg/year
Proportion expected to be released to sewer	28.50%	
Annual quantity of chemical released to sewer	2,850.00	kg/year
Days per year where release occurs	260	days/year
Daily chemical release:	10.96	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	20.496	million
Removal within STP	0%	
Daily effluent production:	4,099	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	2.67	µg/L
PEC - Ocean:	0.27	µg/L

7.2. Environmental effects assessment

The results from ecotoxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	EC50 >120 mg/L	Non-toxic
Daphnia Toxicity	EC50 >120 mg/L	Non-toxic
Algal Toxicity	EC50 >120 mg/L	Non-toxic
Inhibition of Bacterial Respiration	EC50 >120 mg/L	Non-toxic
Other		

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

7.2.1 Predicted No-Effect Concentration

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50 (Fish)	120.00	mg/L
Assessment Factor	100.00	
Mitigation Factor	1.00	
PNEC:	1,200.00	µg/L

A predicted no effect concentration (PNEC – aquatic ecosystems) of 120 mg/L (1,200 µg/L) has been derived by dividing the end point value of 120 mg/L by a worst-case scenario uncertainty (safety) factor of 100 (as usable toxicity data are available for three trophic levels).

7.3. Environmental risk assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River:	2.67	1,200	**0.002**
Q - Ocean:	0.27	1,200	**0.0002**

From PEC/PNEC (2.67 µg/L ÷ 1,200 µg/L) ratio, a value of 0.002 is the calculated risk quotient for the aquatic environment. The notified chemical is therefore not expected to pose an unacceptable risk to the aquatic environment. This calculation has been based on a worst case scenario and adsorption to sludge (where it occurs) is expected to remove some of the notified chemical from effluent. The notified chemical released to the aquatic environment is expected to remain persistent in solution due to its low biodegradability potential.

The high molecular weight, high water solubility and low log P_{OW} of the notified substance indicate a low potential for bioaccumulation.

Although some of the notified chemical may be released from landfill into waterways, due the chemical's low ecotoxicity, it is unlikely to pose a risk to the aquatic environment. Consequently the notified chemical is not expected to pose an unacceptable risk to the environment.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available data the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)]. The classification and labelling details are:

R43 May cause sensitisation by skin contact

and

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

	Hazard category	***Hazard statement***
Skin sensitiser	1	May cause allergic skin reaction

Human health risk assessment
Under the conditions of the occupational settings described, the risk to workers is not considered to be unacceptable.

When used in the proposed manner the risk to the public is not considered to be unacceptable.

Environmental risk assessment
On the basis of the PEC/PNEC ratio:

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

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- The Office of the ASCC, Department of Employment and Workplace Relations (DEWR), should consider the following health hazard classification for the notified chemical:
  - R43: May cause sensitisation by skin contact.

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - Concentration ≥ 1%: R43 May cause sensitisation by skin contact.

- Products containing more than 1% notified chemical and available to the public must carry the following safety directions on the label:
  - Avoid contact with skin

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical:
  - Avoid contact with eyes and skin.
  - Avoid heating the notified chemical when under confinement.

- Service personnel should wear cotton or disposable gloves when removing spent printer cartridges containing the notified chemical and during routine maintenance and repairs.

- 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 *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Public Health

- The following measures should be taken to minimise public exposure to the notified chemical:
  - Avoid skin contact with ink.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by containment with absorbent materials like sand or soil and preventing entry into drains, sewers or water courses. Contaminated wastes should be placed in a sealed container and disposed of adequately.

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director of NICNAS must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(1) of the Act; if
  - the notified chemical is imported in any form other than in inkjet cartridges.

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from use in ink preparations for inkjet printers, or is likely to change significantly;
  - the amount of chemical being introduced has increased from 10 tonnes per annum, or is likely to increase, significantly;
  - if the chemical has begun to be manufactured in Australia;
  - additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

Material Safety Data Sheet
The MSDS of the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point >300ºC

Method	EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks	Determined using a metal block apparatus (Gallenkamp Melting Point Apparatus).
Test Facility	Intertek ASG (2006a)

Density 1590 kg/m^3 at 20 ± 0.5ºC

Method	EC Directive 92/69/EEC A.3 Relative Density.
Remarks	Measured using a Micromeritics Pycnometer 1330 TC, which was calibrated using glycerol.
Test Facility	Intertek ASG (2006a)

Vapour Pressure 0.0027 kPa at 25ºC (theoretical estimate)

Method	OECD TG 104 Vapour Pressure. EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks	Vapour pressure was estimated using a modified Watson Correlation, known as the Grain-Watson correction for solids. The calculations assumed values of 300ºC for the melting point and 400ºC for the boiling point.
Test Facility	Intertek ASG (2006a)

Water Solubility 380-400 g/L at 20ºC

Method	Visual assessment
Remarks	A series of weights of the notified chemical were dissolved in vials and the formation of a very thick gel was observed at 380 g/L. Based on this result the water solubility was estimated to be around 380-400 g/L.
Test Facility	Intertek ASG (2006a)

Solvent Solubility
> 29.5 % w/w dimethyl sulfoxide (DMSO) at ambient temperature
10.4 - 12.4 % w/w ethanol at ambient temperature
< 0.03 µg/mL n-hexane at ambient temperature
0.14 µg/mL acetone at ambient temperature

Method	The solubilities of the notified chemical in DMSO and ethanol were determined visually. Spectrophotometric analysis was used to determine its solubility in n-hexane and acetone.
Test Facility	Intertek ASG (2006a)

Hydrolysis as a Function of pH

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

pH	*T (°C)*	*$t_{½}$ <hours or days>*
4	25°C	> 1 year
7	25°C	> 1 year
9	25°C	629.4 days

Remarks	Since < 10% hydrolysis occurred after 5 days, no further tests were conducted for the test material at pH 4 and 7. Further testing at pH 9 at 50°C, 60°C and 70°C was performed with the results used to extrapolate the half-life to 25°C.
Test Facility	Intertek ASG (2006a)

Partition Coefficient (n-octanol/water) log Pow < -3.5 at 20ºC

Method	OECD TG 117 Partition Coefficient (n-octanol/water). EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks	Shake Flask Method. The absorbance of the n-octanol phase at 571 nm was < 0.036. By preparing test solutions in distilled water, a test concentration of 0.14 mg/L was determined to correspond with the detection limit. Therefore the concentration of the test substance in n-octanol was determined to be <0.14.
Test Facility	Intertek ASG (2006a)

Surface Tension 72.1 mN/m at 25ºC

Method	OECD TG 115 Surface Tension of Aqueous Solutions. EC Directive 92/69/EEC A.5 Surface Tension.
Remarks	Determined using a tensiomenter and a notified chemical concentration of 1 g/L. Based on the results, the notified chemical is not considered to be surface-active.
Test Facility	Intertek ASG (2006a)

Adsorption/Desorption – screening test
log K_{oc} (pH 3) > 5 and
log K_{oc} (pH 10) < 1.5 at 20.6-23.9°C

Method	OECD TG 121 Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography.
Remarks	The test has been validated for quantitative estimation of log K_{OC} values in the range 1.5-5. At pH 3, neither test concentration (10 and 20 mg/L) eluted from the column, therefore they were assigned an estimated log K_{OC} of > 5.0. At pH 10, both test concentrations eluted from the column before the reference substance, sodium nitrate, therefore they were assigned an estimated log K_{OC} of < 1.5.
Test Facility	Brixham Environmental Laboratory (2006a)

Dissociation Constant
pKa 1 ~ 10.9
pKa 2 ~ 2.7

Method	OECD TG 112 Dissociation Constants in Water.
Remarks	The pKa values were determined at ambient temperature (~23°C). Due to the complex nature of the test substance, the pKa values should be considered approximate. The test substance cannot be deemed to be in a molecular state at any time and is expected to always be ionised at environmentally relevant pH (range 4-9).
Test Facility	Intertek ASG (2006a)

Flammability Not highly flammable

Method	EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks	The notified chemical did not propagate combustion.
Test Facility	Syngenta (2006a), Syngenta (2006b)

Autoignition Temperature 253 ± 5 ºC

Method	EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks	The notified chemical attained a temperature of 400 °C (defined as the Relative Self-Ignition Temperature) at an oven air temperature of 253 ± 5°C. From inspection, the test sample appeared unchanged after the test.
Test Facility	Syngenta (2006a)

Pyrophoric Properties Not pyrophoric

Method	EC Directive 92/69/EEC A.13 Pyrophoric Properties of Solids and Liquids.
Remarks	The notified chemical did not spontaneously ignite on contact with air.
Test Facility	Syngenta (2006a)

Explosive Properties Not explosive

Method	Based on EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks	The notified chemical did not explode when exposed to heat, mechanical shock or friction. Some effects were seen as a result of the application of heat (using a 2mm orifice diameter), however, these were not considered to be explosions according to the definition of the test method.
Test Facility	Syngenta (2006a)

Explosive Properties May pose risk of explosion if heated under confinement.

Method	Based on EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks	The thermal sensitivity test was repeated using a tube with a 1 mm orifice diameter and achieved a positive result in one of the duplicate tests. Thus the notified chemical can pose a risk of explosion if heated under confinement.
Test Facility	Syngenta (2006b)

Stability Testing Stable (shelf life >2 years).

Method	Duplicate samples of the notified chemical were transferred into vials, sealed and stored for 14 days in an oven at 54 ± 2 °C. A HPLC comparison was conducted between the tested samples and samples stored at room temperature.
Remarks	The samples were stable over the test period. According to the testing laboratory, this indicates an ambient shelf-life of at least two years.
Test Facility	Intertek ASG (2006a).

Oxidizing Properties Not an oxidising substance.

Method	EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Remarks	An analogue of the notified chemical has been shown not to possess oxidising properties, and rather, appeared to retard combustion during the test. The notified chemical has a lower proportion of oxygen than the analogous substance and so it is determined to be no more oxidising than the analogous substance.
Test Facility	NPIL Hazards Group (2006).

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Sprague-Dawley CD (Crl:CD (SD) IGS BR)
Vehicle	Distilled water
Remarks - Method	No significant protocol deviations

RESULTS

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

LD50	>2000 mg/kg bw
Signs of Toxicity	There were no signs of systemic toxicity.
Effects in Organs	Black coloured kidneys were observed in all Group II animals.
Remarks - Results	Black staining of the fur, urine and faeces was noted throughout the study.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Safepharm Laboratories (2006a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test.
Species/Strain	Rat/Sprague-Dawley CD (Crl: CD (SD) IGS BR)
Vehicle	Moistened with arachis oil BP.
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5 male	2000	0
II	5 female	2000	0

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	Black staining was noted at treatment sites up to 2 days after application. This did not affect evaluation of dermal reactions.
Signs of Toxicity - Systemic	No systemic toxicity observed.
Effects in Organs	No abnormalities were noted upon necropsy.
Remarks - Results	None
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	Safepharm Laboratories (2006b)

B.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 males
Vehicle Moistened with distilled water
Observation Period 72 hours
Type of Dressing Semi-occlusive.
Remarks - Method No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0.3	0.3	0	1	48 hr	0
Oedema	0	0	0	0	0 hr	0

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

Remarks - Results Black/purple staining was noted at all treated skin sites until the 48 hour observation. The staining did not affect the evaluation of the skin responses.

Very slight erythema was observed in one animal 1 hour after patch removal, which persisted until the 48 hour observation time. Very slight erythema was noted after 24 hours in another animal but this was not observed at the 48 hour observation time.

No evidence of skin irritation was observed in the third animal throughout the study.

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY Safepharm Laboratories (2006c)

B.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 males
Observation Period 72 hours
Remarks - Method No significant protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0.7	0.7	0.7	2	72 hr	0
Conjunctiva: chemosis	0	0	0	1	24 hr	0
Conjunctiva: discharge	0.3	0.3	0.3	2	48 hr	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

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

Remarks - Results	Black staining of the fur was noted around all treated eyes throughout the study. Moderate conjunctival irritation was noted in all treated eyes 1 hour after treatment with minimal conjunctival irritation at the 24 and 48 hour observation points.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	Safepharm Laboratories (2006d)

B.5. Skin sensitisation – mouse local lymph node assay (LLNA) Assay 1

TEST SUBSTANCE	Notified chemical
METHOD	OECD 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain	Mouse/ CBA/Ca
Vehicle	1% pluronic in distilled water
Remarks - Method	The vehicle used is not recommended by the OECD test guideline.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	548.95	-
2.5	717.84	1.31
5	821.01	1.50
10	432.71	0.79
*Positive Control (α-Hexylcinnamaldehye)**		
5 (v/v in acetone)	-	3.53
10 (v/v in acetone)	-	5.39
25 (v/v in acetone)	-	8.23

*Based on historical control data (< 2 months from experiment).

Remarks - Results	Black staining was observed on the fur and ears of all test animals one hour after dosing on days 1 to 3. The results were considered to be negative, given that the Stimulation Index was <3 at all tested concentrations.
CONCLUSION	There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	Safepharm Laboratories (2006e)

B.6. Skin sensitisation – mouse local lymph node assay (LLNA) Assay 2

TEST SUBSTANCE	Notified chemical
METHOD	OECD 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain	Mouse/ CBA/Ca
Vehicle	Dimethyl sulfoxide (DMSO)
Remarks - Method	No significant protocol deviations

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	828.26	-
5	3195.99	3.86
10	3045.60	3.68
25	5587.31	6.75
*Positive Control (α-Hexylcinnamaldehye)**		
5 (v/v in DMSO)	-	1.39
10 (v/v in DMSO)	-	3.81
25 (v/v in DMSO)	-	5.84

*Based on historical control data (< 1 year from experiment).

Remarks - Results: Black staining was observed on the fur and ears of all test animals after dosing on days 1 to 3. The results were considered to be positive, given that the Stimulation Index was >3 at all tested concentrations.

CONCLUSION: There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY: Safepharm Laboratories (2007a)

B.7. Repeat dose toxicity

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
- Species/Strain: Rat / Sprague-Dawley Crl:CD (SD) IGS BR
- Route of Administration: Oral – gavage
- Exposure Information: Total exposure days: 28 days; Dose regimen: 7 days per week; Post-exposure observation period: None
- Vehicle: Distilled water
- Remarks - Method: No significant protocol deviations. Dosage levels were chosen based on a dose range-finding study.

RESULTS

Dose mg/kg bw/day	*Number and Sex of Animals*	*Mortality*
0	5M, 5F	0
15	5M, 5F	0
150	5M, 5F	0
750	5M, 5F	0

Mortality and Time to Death
No mortality was observed during the study.

Clinical Observations
From day 2 onwards, both sexes treated with 750 mg/kg bw/day had black coloured faeces and black staining was detected on the tray liners of their cages. From day 3 onwards, animals of either sex treated with 150 mg/kg bw/day had transient incidences of black/dark faeces on their tray-liners.

Incidents of increased salivation were noted for both sexes in the 750 mg/kg bw/day group (from day 4 onwards for females and day 13 for males) and staining of the mouth and fur by the test substance during the treatment period were noted.

Animals of either sex treated with 750 mg/kg bw/day showed some statistically significant increases in

forelimb and hindlimb grip strength in comparison to control values. Statistically significant increases in the final 20% of total activity was also noted for both sexes at this dose level, as well as in females at the lower dose level of 150 mg/kg.

Statistically significant increases in bodyweight gain were noted in female rats treated with 750 mg/kg during week 1 and week 3 and in 150 mg/kg females during week 1. Male animals treated with 750 mg/kg bw/day and 150 mg/kg bw/day displayed statistically significant increases in bodyweight gain during week 3 of the study.

Some increases in food consumption were observed, mainly with the 750 mg/kg bw/day dose groups. Substantial increases in water intake, when compared to controls, were evident for animals of either sex treated with 750 mg/kg bw/day.

No significant changes were observed during the behavioural assessments or the sensory activity assessments.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Clinical chemistry
Animals of both sexes treated with 750 mg/kg bw/day showed increases in plasma bilirubin and reductions in sodium and chloride levels. Male animals in this dose group also showed increases in cholesterol, calcium and plasma glucose. Females in this dose group also showed increases in albumin and decreases in plasma alkaline phosphatase.

Some statistically significant reductions in plasma sodium and chloride levels were detected in males treated with 150 mg/kg bw/day. However, all individual values were within normally expected ranges for these parameters and differences were considered to have arisen incidentally.

Haematology
Statistically significant increases in mean cell haemoglobin and mean cell volume and reductions in methaemoglobin levels were observed in male rats treated at all dose levels. Such changes were considered to be of no toxicological significance given that the individual values were within expected ranges for these parameters, and because similar effects were not observed in female animals.

Urinalysis
Males and females treated with 750 mg/kg bw/day displayed an increase in black coloured urine. In addition, haemoglobin was present in the urine of all males and the majority of females (4/5) in this dose group. One male and one female animal treated with 150 mg/kg bw/day also exhibited haemoglobin in their urine, which had a light brown colouration. A reduction in the volume of urine, which was of increased specific gravity was reported for males in this dose group. Haemoglobin was also detected in the urine of one female treated with 15 mg/kg bw/day.

Effects in Organs
Organ weights
Statistically significant elevations in spleen weights, both absolute and relative to terminal bodyweight, were observed in males treated with 750 mg/kg bw/day and 150 mg/kg bw/day.

Males treated with 750 mg/kg bw/day were noted to have lower absolute and relative testes weights when compared to controls and also showed an increase in heart weights.

Necropsy
Animals of either sex in the 750 mg/kg bw/day dose group displayed black contents in the gastro-intestinal tract (colon, rectum and stomach in males; caecum, colon and rectum in females) and dark discolouration of the kidneys. One male exhibited a small and flaccid left testis at necropsy and males in this dose group showed dark discolouration of the testes. The right adrenal of one female from this group was observed to be damaged on removal.

Some isolated abnormalities were observed in the 15 mg/kg bw/day dose group, however, these were considered to be unrelated to treatment.

Histopathology
Adrenals: Treatment-related cortical vacuolation was apparent for males treated with 750 mg/ kg bw/ day.

Caecum: Animals of either sex in the 750 mg/kg bw/day dose group showed mucosal hypertrophy, higher grades of severity of mononuclear cell infiltration of the lamina propria, and prominent secretory granules in mucosal cells.

Stomach: Treatment-related agglomeration of secretion in the mucosa adjacent to the limiting ridge and acanthosis/hyperkeratosis of the limiting ridge were detected in animals of either sex in the 750 mg/kg bw/day dose group. Agglomeration of secretion was also noted in males treated with 150 mg/kg bw/day.

Kidneys: Animals treated with 750 mg/kg bw/day showed treatment-related hypertrophy of the epithelium of distal tubules and collecting ducts.

Testis: High dose male rats were observed to have testicular atrophy of varying degrees of severity.

Epididymis: A treatment-related reduction in spermatozoal content was observed in males treated with 750 mg/kg bw/day.

Remaining morphological changes were those commonly observed in laboratory rats of the age and strain used. There were no significant differences in incidence or severity between the control and treatment groups that were considered to be of toxicological significance.

Remarks – Results
Several adverse effects were observed in animals treated with 750 mg/kg bw/day of the notified chemical. The majority of the findings at these doses seemed to be related to the irritant nature of the notified chemical (eg changes in the adrenals, caecum, stomach, kidneys, blood chemistry values, and organ weights).

Although treatment-related effects were observed at 150 mg/kg bw/day, these were considered to be lower in severity and were not degenerative in nature. Thus, the effects observed at this dose were not considered adverse effects.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 150 mg/kg bw/day in this study, based on the absence of the serious adverse effects that were seen at higher doses.

The No Observed Effect Level (NOEL) was considered to be 15 mg/kg bw/day in this study, based on the absence of any toxicologically relevant effects at this dose.

TEST FACILITY	Safepharm Laboratories (2007b)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA$^-$
Metabolic Activation System	S9 fraction from phenobarbitone / β-naphthoflavone induced rat liver
Concentration Range in Main Test	50-5000 µg/plate
Vehicle	Distilled water
Remarks - Method	Manual colony counts were performed at doses at or above 500 µg/plate due to intense test material colouration. The positive controls used for the assays performed without metabolic activation with TA98, TA100, and TA1535 were not those recommended by the test method. For TA100 and TA1535, N-ethyl-N'-nitro-N-nitrosoguanidine was used, and for TA98, 4-Nitroquinoline-1-oxide was used.

A preliminary test was performed using TA100 and WP2uvrA$^-$ only, both with and without metabolic activation.

As the notified chemical is an azo compound, the OECD test method strongly recommends the use of a modified Ames test with a reducing pre-incubation step to improve sensitivity (eg Prival and Mitchell, 1982). However, such a modification was not used in this test.

RESULTS

Remarks - Results

No test material precipitate or significant increases in the frequency of revertant colonies were observed on the plates at any of the doses tested in either the presence or absence of metabolic activation.

Some reductions in the bacterial background lawn of TA1535 and TA1537 in the main experiment in the absence of metabolic activation were observed at the highest and second highest tested concentrations. These reductions were not statistically significant.

Given that a reductive pre-incubation step was not used in this study, the result (non-mutagenic) is indicative only of the conditions of this particular Ames test. Many carcinogenic azo dyes test negative in Ames tests without the use of a modified test (SCCNFP, 2002).

CONCLUSION

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

TEST FACILITY

Safepharm Laboratories (2006f)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 473 In vitro Mammalian Chromosome Aberration Test.

Species/Strain: Human (2 male and 2 female donors)

Cell Type/Cell Line: Human peripheral blood lymphocytes

Metabolic Activation System: S9 fraction from phenobarbitone / β-naphthoflavone induced rat liver

Vehicle: Double deionised water

Remarks - Method: No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1 [a]	0*, 39, 78, 156, 313, 625, 1250*, 2500*, 5000*	3 hr	20 hr
Test 2 [b]	0*, 125, 250, 500, 1000, 2000*, 3000*, 4000*, 5000	20 hr	20 hr
Present			
Test 1 [a]	0*, 39, 78, 156, 313, 625, 1250*, 2500*, 5000*	3 hr	20 hr
Test 2 [a]	0*, 39, 78, 156, 313, 625, 1250*, 2500*, 5000*	3 hr	20 hr

*Cultures selected for chromosomal aberration analysis.

[a] Experiments performed using pooled male donor blood.

[b] Experiments performed using pooled female donor blood.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	N/A	5000	>5000	Negative
Test 2	N/A	>4000	5000	Negative

Present				
Test 1	N/A	>5000	>5000	Negative
Test 2	N/A	>5000	>5000	Negative

Remarks - Results

Cultures treated with the test substance, in the presence or absence of metabolic activation, showed no statistically or biologically significant increase in the percentage of aberrant cells as compared to the controls. The positive controls showed significant increases in aberrant cells, demonstrating the sensitivity of the test system.

CONCLUSION

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

TEST FACILITY

Central Toxicology Laboratory (2006)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test.
Inoculum	Activated sludge obtained from domestic sewer.
Exposure Period	28 days
Auxiliary Solvent	None specified
Analytical Monitoring	Respirometer (BOD) Spectrophotometer (COD)
Remarks - Method	Test samples were run along with blanks, positive controls (sodium benzoate as the reference) and toxicity controls. Oxygen take up during microbial respiration was measured as a decrease in pressure, from which the Biochemical Oxygen Demand (BOD) was calculated. Oxygen uptake was recorded every 2 hours during the 28 day period and calculated as a percentage of the measured Chemical Oxygen Demand (COD) (evaluated spectrophotometrically) for the notified chemical and reference substance.

RESULTS

Test substance		*Sodium benzoate (reference substance)*	
Day	*% Degradation*	*Day*	*% Degradation*
5	4	5	65
10	6	10	70
15	5	15	74
20	6	20	74
28	9	28	76

Remarks - Results	The COD value for the test substance was 1.19 g O_2/g substance. The BOD value for the test substance indicated negligible biodegradation. The COD and BOD values for sodium benzoate showed that the inoculum and test procedure were valid in this analysis.
CONCLUSION	The test material cannot be regarded as readily bio-degradable.
TEST FACILITY	Brixham Environmental Laboratory (2006b)

C.1.2. Bioaccumulation

The notified substance has low potential to bioaccumulate. This is based on the high water solubility of the test substance and the low value for the partition coefficient.

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test - static. EC Directive 92/69/EEC C.1 Acute Toxicity for Fish -static.
Species	Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	None specified
Water Hardness	47.7 mg $CaCO_3$/L

Analytical Monitoring	Visual; HPLC
Remarks – Method	Due to the intensity of colouration in the test solutions, observations for mortality were tested in separate vessels. In addition, it was not possible to assess symptoms of toxicity during the study. During the exposure period the temperature was maintained between 15°C, the oxygen content at 94-99% saturation, and the pH 7.5-7.8.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	<0.34	10	0	0	0	0	0
120	120	10	0	0	0	0	0

LC50	>120 mg/L at 96 hours.
NOEC (or LOEC)	120 mg/L at 96 hours.
Remarks – Results	HPLC confirmed 100% recovery of the nominal concentration of the test substance at 0 and 96 hours.

CONCLUSION The test substance is non-toxic to Rainbow trout (*Oncorhynchus mykiss*).

TEST FACILITY Brixham Environmental Laboratory (2006c)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - static.
EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - static.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None specified
Water Hardness	244 mg $CaCO_3$/L
Analytical Monitoring	Visual; HPLC
Remarks - Method	Due to the intensity of the colouration in the test solutions, assessment of mortality in the nominal 25 to 120 mg/L solution was only possible at 48 hours, and observation for symptoms of toxicity was not possible at any time. During the exposure period the temperature was maintained between 19.4-20.1°C, the oxygen content at 90-92% saturation, and the pH 7.9-8.1.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	<0.31	20	0	0
1.1	1.5	20	0	0
2.3	2.8	20	0	0
5.1	5.8	20	0	0
11	12	20	0	0
25	25	20	0	0
55	53	20	0	0
120	120	20	0	0

LC50	>120 mg/L at 48 hours
NOEC	120 mg/L at 48 hours
Remarks - Results	HPLC confirmed 96-136% recovery of the nominal concentration of the test substance at 0 and 48 hours. No symptoms of toxicity were observed

at nominal test concentrations of 1.1 to 12 mg/L.

CONCLUSION The test substance is non-toxic to *Daphnia magna.*

TEST FACILITY Brixham Environmental Laboratory (2006d)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test.
EC Directive 92/69/EEC C.3 Algal Inhibition Test.

Species	*Selenastrum capricornutum*
Exposure Period	72 hours
Concentration Range	Nominal: 1.0-120 mg/L Actual: 1.0-120 mg/L
Auxiliary Solvent	None specified
Water Hardness	Not specified
Analytical Monitoring	Coulter counter; HPLC
Remarks - Method	Two replicate cultures of nominal cell density of 1×10^4 per mL in direct contact with 1.0, 2.3, 5.0, 11, 25, 55 and 120 mg/L of test substance were prepared (Test A). Further tests of the same concentrations were prepared in duplicate, but with the algae only shaded by the test substance and not in direct contact (Test B). Two control replicates were also run for each test. During the exposure period the temperature was maintained between 23.9-24.1°C and the pH 7.5-8.2.

RESULTS

Test A (in direct contact and shaded)

Biomass		*Growth*	
E_bC50	*NOEC*	*E_rC50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
0.98	<1.0	5.7	<1.0

Test B (shaded only)

Biomass		*Growth*	
E_bC50	*NOEC*	*E_rC50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
0.91	<1.0	4.82	<1.0

Remarks - Results HPLC confirmed 92-100% recovery of the nominal concentration of the test substance at 0 and 72 hours. Algal cell densities for the exposure and shaded solutions followed the same patterns across the nominal concentrations, however, the lowest densities were consistently found in the shaded solutions.

Graphical comparisons of the percentages of inhibition in the exposure and shaded vessels showed that these curves were essentially the same. Inhibition of growth rate in exposure vessels plotted (%E) against that in shaded vessels (%S) showed that this curve follows closely the theoretical line plotted when %E = %S, with a mean S/E > 0.9. In addition, when the inhibition of growth rate in exposure vessels and shaded vessels are plotted against nominal concentration, %E-%S was within 10%. At the lowest tested concentration (1.0 mg/L) the S/E value was 0.82 but this is not considered to invalidate the test.

This result indicated that the light absorbing properties of the test substance were a significant factor in the inhibition and therefore, it is not

possible to distinguish reduced growth due to toxic effects from those due to differences in illumination. The report indicates that the test substance satisfies the exemption clause in Annex VI (Dir.93/21/EEC) and the 72 hour EC50 for algae should not be used as a basis for classification of the test substance.

CONCLUSION The test substance is non-toxic to *Selenastrum capricornutum.*

TEST FACILITY Brixham Environmental Laboratory (2006e)

C.2.4. Duckweed growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 221 Lemna sp., Growth Inhibition Test.

Species	*Lemna minor*
Exposure Period	7 days
Concentration Range	Nominal: 5.0-120 mg/L Actual: 4.5-120 mg/L
Auxiliary Solvent	None specified
Water Hardness	Not reported
Analytical Monitoring	HPLC
Remarks - Method	Three replicates, each containing 4 plants (12 fronds), were subjected to nominal test concentrations of 5.0, 11, 25, 55 and 120 mg/L. In addition, 6 control replicates were also run. Plant and frond numbers were counted on days 3, 5 and 7. Growth rate was also measured. During the exposure period the temperature was maintained between 23.2-23.7°C and the pH 6.0-6.7.

RESULTS

Biomass		*Growth*	
E_bC50 *mg/L at 7 days*	*NOEC* *mg/L*	*E_rC50* *mg/L at 7 days*	*NOEC* *mg/L*
>120	25 (frond number)	>120	25 (frond number)
>120	5 (dry weight)	>120	5 (dry weight)

Remarks - Results HPLC confirmed 90-100% recovery of the nominal concentration of the test substance at 0 and 7 days. The EC50 for frond number, dry weight and growth rate are all >120 mg/L. Some plants in the 55 and 120 mg/L test solutions were observed to be small. The mean frond number and growth rate (frond number) in the 55 and 120 mg/L test solutions deviated significantly from the controls, hence a NOEC value of 25 mg/L. The plant dry weight and growth rate (dry weight) were significantly less than the controls at all test concentrations except for 5 mg/L.

CONCLUSION The notified chemical is very slightly toxic to the Duckweed *Lemna minor* under the conditions of this test.

TEST FACILITY Brixham Environmental Laboratory (2006f)

C.2.5. Inhibition of microbial activity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 209 Activated Sludge, Respiration Inhibition Test.
EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test

Inoculum Activated sludge (obtained from local sewage treatment works, Devon,

	UK).
Exposure Period	3 hours
Concentration Range	Nominal: 1-100 mg/L Actual: not tested
Remarks – Method	Nominal test concentrations of 1.0, 3.2, 10, 32 and 100 mg/L were prepared. Two controls and reference substance (3,5-dichlorophenol) were also run.

RESULTS

IC50	>100 mg/L
NOEC	100 mg/L
Remarks – Results	All test concentrations produced <10% reduction in the respiration rate of activated sludge in this test. The reference substance caused 90% inhibition in activated sludge thus validating the test procedure.

CONCLUSION	The notified chemical cannot be classified as inhibiting microbial activity.
TEST FACILITY	Brixham Environmental Laboratory (2006g)

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