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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Chemical B in Bonjet Black 818

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:

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

Director
NICNAS

TABLE OF CONTENTS

FULL PUBLIC REPORT 3
1. APPLICANT AND NOTIFICATION DETAILS 3
2. IDENTITY OF CHEMICAL 3
3. COMPOSITION 3
4. PHYSICAL AND CHEMICAL PROPERTIES 4
Discussion of Observed Effects 4
5. INTRODUCTION AND USE INFORMATION 4
6. HUMAN HEALTH IMPLICATIONS 5
6.1. Exposure assessment 5
6.1.1. Occupational exposure 5
6.1.2. Public exposure 6
6.2. Human health effects assessment 6
6.3. Human health risk characterisation 8
6.3.1. Occupational health and safety 8
6.3.2. Public health 9
7. ENVIRONMENTAL IMPLICATIONS 9
7.1. Environmental Exposure & Fate Assessment 9
7.1.1 Environmental Exposure 9
7.1.2 Environmental fate 10
7.1.3 Predicted Environmental Concentration (PEC) 10
7.2. Environmental effects assessment 10
7.2.1 Predicted No-Effect Concentration 10
7.3. Environmental risk assessment 11
8. CONCLUSIONS – SUMMARY OF RISK ASSESSMENT FOR THE ENVIRONMENT AND HUMAN HEALTH 11
8.1. Hazard classification 11
8.2. Human health risk assessment 11
8.2.1. Occupational health and safety 11
8.2.2. Public health 11
8.3. Environmental risk assessment 11
9. MATERIAL SAFETY DATA SHEET 12
10. RECOMMENDATIONS 12
11. REGULATORY OBLIGATIONS 12
12 . BIBLIOGRAPHY 13
Appendix A: Physico-Chemical Properties 16
Appendix B: Toxicological Investigations 19
B.1. Acute toxicity – oral 19
B.2. Acute toxicity – dermal 19
B.3. Irritation – skin 19
B.4. Irritation – eye 20
B.5. Skin sensitisation 20
B.6. Repeat dose toxicity (14 day) 21
B.7. Repeat dose toxicity (28 day) 22
B.8. Genotoxicity – bacteria 23
B.9. Genotoxicity – *in vitro* 25
B.10. Genotoxicity – *in vivo* 25
Appendix C: Environmental Fate and Ecotoxicological Investigations 27
C1. Environmental Fate 27
C.1.1. Ready biodegradability 27
C.1.2. Bioaccumulation 27
C2. Ecotoxicological Investigations 27
C.2.1. Acute toxicity to fish 27
C.2.2. Acute/chronic toxicity to aquatic invertebrates 28
C.2.3. Algal growth inhibition test 29
C.2.4. Inhibition of microbial activity 30

FULL PUBLIC REPORT

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Epson Australia Pty Ltd (ABN 91 002 625 783) of 3 Talavera Rd, North Ryde NSW 2113

NOTIFICATION CATEGORY
Standard: Chemical other than polymer.

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication:
Chemical names, CAS number, Molecular formula, Structural formula, Molecular weight, Spectral data, Methods of detection and determination, Purity, % Weight of Impurities, Confidential use details and Import volume

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
UK (2002)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Bonjet Black 818

OTHER NAME(S)
Bonjet Black 817-E

MOLECULAR WEIGHT
>500 Da

ANALYTICAL DATA
Reference ^{1}H-NMR, IR, and UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
>85% – for the mixture of two chemicals in Bonjet Black 818

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (>1% by weight)
None

ADDITIVES/ADJUVANTS
The notified chemical is Chemical B in Bonjet Black 818. A second major component of this dye, "Chemical A", is notified as STD/1036. These two chemicals are synthesised in the same mixture to form the dye product Bonjet Black 818.

4. PHYSICAL AND CHEMICAL PROPERTIES

The physicochemical properties (except where otherwise noted with an asterisk) were determined for a mixture of two chemicals, Bonjet Black 817-E, equivalent to Bonjet Black 818. For full details of tests for physical and chemical properties, please refer to Appendix A.

Appearance at 20ºC and 101.3 kPa Dark brown powder

Property	Value	Data Source/ Justification
Melting Point/Boiling Point	Decomposed without melting from 175ºC	Measured
Density	1,420 kg/m^3 at 21ºC	Measured
Vapour Pressure	5.2×10^{-5} kPa at 25ºC	Measured
Water Solubility	54.2 g/L at 20ºC	Measured*
Hydrolysis as a Function of pH	t½ > 1 year at 25ºC (pH 4, 7, 9)	Estimated*
Partition Coefficient (n-octanol/water)	log P_{ow} = -1.89 at 45ºC	Measured*
Surface Tension	60.1 mN/m at 22.0ºC	Measured
Adsorption/Desorption	log K_{oc} <1.25 at 40ºC	Measured*
Dissociation Constant	Not determined. QSAR estimates: pKa = -0.53-11.74.	Numerous ionisable groups*
Particle Size	Inhalable fraction (<100 μm): 54.3% Respirable fraction (<10 μm): 19.3%	Measured
Flash Point	Not determined	Solid with low vapour pressure
Flammability	Not highly flammable	Measured
Autoignition Temperature	342ºC	Measured
Explosive Properties	Not expected to be explosive	Estimated*
Oxidising Properties	Not oxidising	Measured
Moisture Content	Mean moisture content = 8.368% (w/w)	Measured

* Value determined for the notified chemical, not for the mixture Bonjet Black 817-E.

Discussion of Observed Effects

Reactivity

The notified chemical is not oxidising, and it is stable under normal conditions. It is expected to burn if involved in a fire, evolving noxious fumes (eg oxides of carbon, nitrogen, sulfur and phosphorus).

5. INTRODUCTION AND USE INFORMATION

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

The notified chemical will be imported as a component of inkjet printer inks (<5%), contained within individually packaged inkjet printer cartridges.

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

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

PORT OF ENTRY

Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS

None known at this time. Potentially, the inkjet printer cartridges containing the notified chemical will be supplied to offices and retailers nationwide.

TRANSPORTATION AND PACKAGING

The notified chemical will be imported as a component of ready-to-use sealed plastic inkjet cartridges of 5-100 mL volumes. The cartridges are individually wrapped in plastic and cardboard packaging, and these will be imported in bulk in cardboard cartons. The cartridges will be transported by road.

USE

The notified chemical will be used in inkjet printer inks as a component of the dye product Bonjet Black 818. Both it and "Chemical A in Bonjet Black 818" (STD/1036) will each be present in the inks at <5%. The inks will be imported within inkjet printer cartridges, which will be used for office and general printing work by the public. Sealed ink cartridges containing the notified chemical will be used as necessary to replace spent cartridges in inkjet printers.

OPERATION DESCRIPTION

No reformulation or repackaging of the notified chemical will occur in Australia. The products containing the notified chemical will be delivered to the end-user in the same form that they will be imported.

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 or replaced into the inkjet printer by office workers, service technicians or consumers.

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*
Importation/Waterside workers	10	4 hrs per day	70 days per year
Storage and transport	100	6 hrs per day	240 days per year
Office workers, service technicians, consumers	10,000	<0.1 hrs per day	20 days per day

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 cartridge and its packaging may be breached. The cartridges will be distributed to a number of outlets, where the cardboard cartons will be opened and boxes containing individual cartridges will be stacked on shelves.

There is low potential for office workers to be exposed to the notified chemical in inks (<5% concentration) when replacing spent cartridges. Both office workers and service technicians may be exposed to the notified chemical in ink while changing printer cartridges. Replacement of printer cartridges involves removal of the old printer cartridge from the printing machine and directly loading the new cartridge. Dermal exposure to small quantities of the notified chemical may occur if the print heads are touched while replacing the cartridges, but workers are expected to avoid direct contact with inks to avoid staining of their skin and/or clothing. In addition, dermal and possibly ocular exposure could occur when handling faulty or ruptured cartridges. Overall, the design of the cartridges is expected to be such that they can be easily replaced without dermal exposure to ink. Accidental contact is expected to be minimal.

Dermal exposure of office workers to the notified chemical from dried inks on printed paper is expected to be minimal, as the dye will be largely bound to the paper within the matrix of the dried ink. The extent of binding of the notified chemical within the ink matrix on media is expected to be based on the other components of the ink (eg polymers) and the components and properties of the media (eg absorbency, hydrophobicity, paper coatings or other ingredients). The binding is therefore likely to be a combination of how well integrated the notified chemical will become with these components upon drying of the ink.

The most probable exposure of office workers to the notified chemical will be to wet ink on freshly printed media, especially when printing on non-absorbent materials. The extent of dermal exposure of workers to wet ink on paper or other non-absorbent substrate has been estimated by the notifier:

One kilogram of pure dye would be expected to print several million A4 paper sheets of text or

graphics. Under worst-case conditions, each piece of A4 paper could be assumed to incorporate 1 mg of notified chemical. Based on a 50% transfer on contact when handling printed paper or other substrate (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 = <1%
∴ Exposure to fingertips per event = <1% of 1 mg = <u><0.01 mg per event</u>.

For extensive contact with wet ink on paper or other substrate (i.e. >10 events per day) the daily exposure to the notified chemical, assuming no washing between events, a 70 kg person and conservative estimate of 100% absorption, would be:

Daily exposure = (<0.01 (mg/event)×10) ÷ 70 = <u>~0.0014 mg/kg bw/day</u>.

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

Service technicians may occasionally experience skin contact with the notified chemical (<5% in the ink) during maintenance of inkjet printers. The exposure of these workers is likely to be limited to contact of inks with their fingertips during the handling and cleaning of printer components. Therefore, the exposure of these workers is expected to be minimal and infrequent.

6.1.2. Public exposure

The exposure of the public to the notified chemical through the use of inkjet printer inks is expected to be identical to that experienced by office workers during the changing of cartridges, printing onto paper and other media, and handling dried, printed pages. Members of the public may be expected to change inkjet printer cartridges less frequently than would office workers, as domestic applications are often smaller.

Public exposure through importation, transportation or storage is expected to be negligible. Such exposure could only occur in the extremely unlikely event of an accident where crates, boxes, packaging and cartridges were ruptured, liberating inks containing the notified chemical.

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	LD_{50} 300-600 mg/kg bw: Harmful
Rat, acute dermal toxicity	LD_{50} >2,000 mg/kg bw: Low toxicity
Rabbit, skin irritation	Slightly irritating
Rabbit, eye irritation	Slightly irritating
Guinea pig, skin sensitisation – adjuvant test	No evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOEL 15 mg/kg bw/day
Genotoxicity – bacterial reverse mutation	Non-mutagenic
Genotoxicity – *in vitro* chromosome aberration	Genotoxic
Genotoxicity – *in vivo* mouse micronucleus test	Non-genotoxic

Toxicokinetics, metabolism and distribution:

The notified chemical is likely to be absorbed via the oral route, as indicated by the deaths in the 14-day oral toxicity study, the signs of toxicity observed in the 28-day repeated dose oral study and the deaths in the mouse micronucleus assay at the highest dose level. It is not clear if the notified chemical is absorbed intact or degraded prior to gastrointestinal absorption, perhaps through the action of intestinal microflora (SCCNFP, 2002). Observations of black urine would support the hypothesis that it is absorbed and excreted largely unchanged. Significant dermal absorption of the notified chemical via the dermal route is not expected, based on its low partition coefficient ($logP_{ow}$ = -1.89).

The azo linkage is the most labile portion of an azo dye molecule, and it is readily metabolised in mammals, including man (SCCNFP, 2002). Liver azo reductase enzymes reductively cleave the

molecule into component amines. Some metabolism may also occur in other cells, in particular those of the bladder wall, and during percutaneous absorption. Anaerobic intestinal bacteria are also capable of catalysing reductive cleavage of the azo bond. On this basis, azo dyes should be assessed for toxicity and classified similarly to their component amines (DFG, 1988, quoted in Golka *et al*, 2004). Bacterial skin microflora have been reported to be able to break down azo dyes into smaller amine species through azo reduction, and these may be more readily absorbed through the skin than would the parent dye (SCCNFP, 2002).

The notified chemical is likely to have only limited potential for broad physiological distribution once absorbed, due to its polarity. Ultimately, the metabolites of azo dyes are excreted in the urine and bile (Brown and DeVito, 1993, referenced in Øllgaard *et al*, 1998). Supporting this prediction for the notified chemical is the black-stained urine and faeces that were observed in both of the oral gavage studies.

General toxicity:

The notified chemical displayed moderate acute oral toxicity (LD50 = 300-600 mg/kg bw), inferred from the result of the 14-day rat oral gavage study, and a dose of 750 mg/kg bw killed two of seven mice within 24 hours during the micronucleus assay. Low dermal toxicity (LD50 >2,000 mg/kg bw) was observed in rats.

The NOEL in a 28-day oral repeat dose study in rats was 15 mg/kg bw/day, based on the absence of any treatment-related effects at this dose level. In this study, the adverse effects of the notified chemical at 150 mg/kg bw/day on the spleen persisted until the end of the 14-day treatment-free period. Treatment-related effects in the two oral gavage studies either caused death within 24 hours (≥600 mg/kg bw) or took several days of treatment to manifest (≥150 mg/kg bw), which appears to indicate a dose-dependent mode of toxicity.

Toxicity for reproduction:

In the rat 28-day oral repeat dose study, a slow-onset testicular atrophy was observed in male rats treated with 150 mg/kg bw/day Bonjet Black 817-E. This was observed in only two males of the treatment group (at the end of the treatment period), but in all males of the corresponding 14-day recovery group, with worsening severity. This suggests that continuing testicular damage, possibly irreversible, was initiated during treatment that was not apparent until weeks later in the majority of exposed individuals. The nature of the testicular damage that was induced by the notified chemical is not known.

Irritation and Sensitisation:

The notified chemical was slightly irritating to both rabbit eye and rabbit skin.

The notified chemical was not found to be sensitising in a guinea pig maximisation test. Supporting this finding is the absence of structural moieties within the notified chemical that are known alerts for sensitisation (Barratt *et al*, 1994), and it lacks of similarity with other azo dyes that are known to be skin sensitisers in humans (Øllgaard *et al*, 1998).

Mutagenicity:

Azo dyes as a class are a concern for their potential induction of mutagenicity and carcinogenicity (Combes and Haveland-Smith, 1982). Reductive cleavage or degradation into component aromatic amines is one of the mechanisms leading to the genotoxicity of azo dyes (SCCNFP, 2002). The aromatic amines that arise from the azo reduction and cleavage of azo dyes are thought to be activated as mutagens through their *N*-oxidation by cytochrome P450 isozymes. The *N*-hydroxylarylamines that are formed may be further glucuronated (activated) or acetylated (inactivated), which may influence their mutagenicity (Bartsch, 1981). Under acidic pH, they form reactive nitrenium ions that can alkylate bases in DNA, particularly the nucleophilic centres in guanine. This mechanism is thought to contribute to the carcinogenicity of many azo dyes, and as a result, azo dyes should be assessed for toxicity and classified similarly to their component amines (DFG, 1988, quoted in Golka *et al*, 2004).

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 arylamine species which resemble these, and which could potentially be mutagenic. The structure of one of these arylamine species resembles a known human carcinogen (SCCNFP, 2002; RoC, 2005). The structural modification of this species does not indicate that it may be of lower

concern as potential carcinogen (SCCNFP, 2002; US EPA, 2002). In addition, a second azo reduction product may have the potential to be genotoxic, based on a plethora of published mutagenicity data for analogous chemicals.

The notifier supplied test results showing that Bonjet Black 817-E was found to be non-mutagenic in bacteria (under the conditions of the Ames test used), but it induced chromosomal aberrations in mammalian cells *in vitro*. In an *in vivo* mouse micronucleus study, Bonjet Black 817-E did not significantly increase the formation of micronuclei in bone marrow polychromatic erythrocytes.

Azo dyes are renowned for their content of impurities, particularly for the presence of component arylamines (SCCNFP, 2002; Øllgaard *et al*, 1998). The identity of these species is known for Bonjet Black 818. The identified impurities are likely either to display similar genotoxic properties to the notified chemical, or to have a lower propensity for genotoxicity.

Overall, the available data does not rule out the notified chemical as a possible carcinogen. 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 *et al*, 1998).

Hazard classification:

Based on the 14-day and 28-day oral gavage studies, the notified chemical is classified as hazardous under the NOHSC *Approved Criteria for Classifying Hazardous Substances*.

R22	Harmful if swallowed
R48/22	Harmful: Danger of serious damage to health by prolonged exposure if swallowed
R62	Possible risk of impaired fertility

6.3. Human health risk characterisation

6.3.1. Occupational health and safety

The notified chemical will be imported in pre-packed sealed cartridges. In addition, the notified chemical is present in the ink at <5%, and is not likely to be toxic at the highest levels of probable exposure (worst-case exposure estimate of ~0.0014 mg/kg bw/day, compared with a NOEL of 15 mg/kg bw/day). Therefore, discounting any possibility of a risk from carcinogenicity, the expected risk of the notified chemical to the health and safety of workers is expected to be minimal.

During most operations, the probable exposure of workers to the notified chemical is expected to be low, and thus the probable OHS risk is likely to be low. Transport, storage and retail workers are unlikely to be exposed to the notified chemical except when cartridges are accidentally breached. There is low potential for office workers to be exposed to the notified chemical when replacing spent cartridges, as the notified chemical is sealed within the cartridge, and the cartridges are designed to prevent leakage. Service technicians will generally experience only infrequent exposure to the notified chemical, at levels below the levels of exposure indicated by the worst-case estimate above.

Likewise, the exposure of workers to the notified chemical on dried, printed paper is expected to be low, as the dye should remain bound within the ink matrix. Therefore, the risk to workers handling dried inkjet prints containing the notified chemical is expected to be minimal if not negligible.

However, the OHS risk of the notified chemical cannot be established without a consideration of its likelihood of degradation into potentially carcinogenic aromatic amines. Breakdown of similar azo dyes to the notified chemical has been reported, following their exposure to heat or sunlight (Brown and DeVito, 1993, referenced in Øllgaard *et al* 1998). Given that inkjet prints are likely to be exposed to light for prolonged periods, and that preparations of the notified chemical may already contain traces of aromatic amine species, the risk presented by these species must be considered.

Chemicals which may release specific carcinogenic amines (as specified the Appendix of EU SCCNFP/0495/01) upon azo reduction are restricted in the EU (SCCNFP, 2002):

> "*Azo-dyes that may release, by reductive cleavage of one or more azo groups, one or more of the aromatic amines listed in Appendix, in concentrations above 30 ppm in the finished articles, according to the testing method specified in Appendix, may not be used in textile and leather articles which have the potential of coming into direct and prolonged contact with the human skin or oral cavity.*"

While the notified chemical is not expected to break down into one of the SCCNFP-specified arylamines, it is expected to break down into species that resemble these arylamines. These species may have very different properties to the notified chemical, and they may not be as readily bound, for example, within the matrices of a dried inkjet print. It is not possible to derive any conclusions without greater knowledge of their properties. Nevertheless, the expected exposure to these species is expected to be less than that specified by the SCCNFP (30 ppm), and is likely to be less frequent than "direct and prolonged". Therefore, the risk from these breakdown species is expected to be low.

6.3.2. Public health

The exposure and hazard of the notified chemical to the members of the public during the 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 assessed to be low. The unlikely but potential public exposure through accidents during importation, transportation or storage is assessed as negligible.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The chemical is imported and sold within a printer cartridge, with no reformulation or repackaging in Australia. No release of the notified chemical will occur except in the unlikely event of spills, where the cartridge is ruptured.

RELEASE OF CHEMICAL FROM USE

During use, the notified chemical will be fixed to the paper substrate. At the end of the paper substrate's useful life, it will be disposed of or recycled. During paper recycling it will be de-inked with some of the notified chemical being adsorbed to the pulp and the remainder released to trade waste sewers. The pulp containing some of the ink will be manufactured into lower grade paper and cardboard products, which will likewise be disposed of or recycled.

It is expected that up to 0.5% (5 kg per annum) of the ink containing the notified chemical will remain in the printer cartridge. Most will be disposed as household waste, however approximately 20% is expected to be sent for cartridge recycling. The inks may be incorporated into low-grade inks for colouring items such as recycled plastic products. At the end of these plastic products' useful lives, they will likely be discarded.

RELEASE OF CHEMICAL FROM DISPOSAL

Paper substrates having the notified chemical fixed thereon will be disposed of to landfill, recycled or possibly incinerated. During recycling, some of the notified chemical will be released to sewer. In the sewage treatment plant (STP) some will adsorbed to sludge for disposal by landfill or possibly incineration, with the remainder going to sewage outfall. Most of the residue in empty cartridges will be disposed to landfill. If the ink is recycled to low-grade ink and incorporated into recycled products, then it is likely to be disposed of to landfill at the end of the recycled products' useful lives.

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 for its useful life. Assuming that 0.5% of the chemical will remain in empty cartridges, then 99.5% (995 kg per annum) will be used for its intended purpose as ink. Approximately 50% of paper (NOLAN-ITU, 2001) is recycled meaning ~500 kg will be disposed during paper recycling. This is likely to occur at recycling plants throughout Australia over 260 working days. Assuming a worse case scenario where none of the chemical adsorbs to pulp or sludge, then a Predicted Environmental Concentration (PEC) is calculated as 0.47 µg/L at sewage outfall (see table below). The remainder of the paper products will be landfilled or possibly incinerated. Residual chemical in the empty cartridges will be landfilled or recycled, with any recycled product likely to also be landfilled and 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. It is expected to eventually degrade by biotic and abiotic processes to landfill gases including methane, ammonia, hydrogen sulphide, oxides of carbon, nitrogen and sulphur; and phosphates and water vapour. During incineration, the notified chemical is expected to be combusted to phosphates, oxides of sulphur, nitrogen and carbon, and water vapour.

7.1.3 Predicted Environmental Concentration (PEC)

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1,000	kg/year
Proportion expected to be released to sewer	50	%
Annual quantity of chemical released to sewer	500	kg/year
Days per year where release occurs	260	days/year
Daily chemical release:	1.92	kg/day
Water use	200	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:	0.47	µg/L
PEC - Ocean:	0.05	µg/L

7.2. Environmental effects assessment

Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	EC50 >100 mg/L	Non-toxic
Daphnia Toxicity	EC50 >100 mg/L	Non-toxic
Algal Toxicity	ErC50 ≥11 mg/L	Moderately toxic
Inhibition of Bacterial Respiration	EC50 >1,000 mg/L	Non-toxic

7.2.1 Predicted No-Effect Concentration

A PNEC of ≥110 µg/L was calculated from the lowest EC50 for algae and dividing by a safety (assessment) factor of 100, as toxicity data are available for three trophic levels.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
Assessment Factor	100	
PNEC	≥110	µg/L

7.3. Environmental risk assessment

Risk Assessment	PEC (µg/L)	PNEC (µg/L)	Q
Q - River:	0.47	≥110	<0.01
Q - Ocean:	0.05	≥110	<0.01

The risk quotient for sewage outfall is <0.01. 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 – SUMMARY OF RISK ASSESSMENT FOR THE ENVIRONMENT AND HUMAN HEALTH

8.1. 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:

R2 Risk of explosion by shock, friction, fire or other sources of ignition
R22 Harmful if swallowed
R48/22 Harmful: Danger of serious damage to health by prolonged exposure if swallowed
R51/53 Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
R62 Possible risk of impaired fertility

S35 This material and its container must be disposed of in a safe way.
S36/37Wear suitable protective clothing and gloves
S61 Avoid release to the environment. Refer to special instructions/safety data sheets.

and

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

	Hazard category	***Hazard statement***
Human Health	4	Harmful if swallowed
Human Health	1B	May damage fertility
Environment	Acute 3	Hazardous to the aquatic environment

8.2. Human health risk assessment

8.2.1. Occupational health and safety

Under the conditions of the occupational settings described, the risk to workers is considered acceptable.

8.2.2. Public health

When used in the proposed manner the risk to the public is considered acceptable.

8.3. 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.

9. MATERIAL SAFETY DATA SHEET

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

10. RECOMMENDATIONS

REGULATORY CONTROLS
Hazard Classification and Labelling

- The Office of the ASCC, Department of Employment and Workplace Relations (DEWR), should consider the following health, environmental and physicochemical hazard classifications for the notified chemical:
  - *R2 Risk of explosion by shock, friction, fire or other sources of ignition*
  - *R22 Harmful if swallowed*
  - *R48/22 Harmful: Danger of serious damage to health by prolonged exposure if swallowed*
  - *R51/53 Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment*
  - *R62 Possible risk of impaired fertility*
  - *S35 This material and its container must be disposed of in a safe way.*
  - *S36/37 Wear suitable protective clothing and gloves*
  - *S61 Avoid release to the environment. Refer to special instructions/safety data sheets.*

CONTROL MEASURES
Occupational Health and Safety

- A copy of the MSDS should be easily accessible to employees.
- Service personnel should wear cotton or disposable gloves during routine maintenance and repairs of inkjet printers.
- 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.

Public Health

- Products containing the notified chemical should be labelled with the following safety direction:
  - Avoid skin or eye contact with ink.

Disposal

- The notified chemical should be disposed of by as domestic waste.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by soaking up with damp cloth and placing in a suitable container for disposal. For large spills physically contain (by diking, etc.) and adsorb with inert material (sand, vermiculite etc) and place in suitable container for disposal.

11. REGULATORY OBLIGATIONS

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 regardless of whether the notified chemical has been listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director 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 importation volume exceeds one tonne per annum notified chemical; or
- if the notified chemical is imported in any fashion other than within an inkjet ink cartridge.
- any additional mutagenicity test data is to be provided to NICNAS when it is available.

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical as a component of inkjet printer inks has changed, or is likely to change significantly;
- the amount of chemical being introduced has increased, 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.

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Appendix A: Physico-Chemical Properties

All physicochemical properties (except where otherwise noted) were determined for a mixture of two chemicals, Bonjet Black 817-E, equivalent to Bonjet Black 818. Where a property was determined for the notified chemical alone, HPLC was used to separate the isomers.

Melting Point/Boiling Point Decomposed without melting from 175ºC

METHOD OECD TG 102 Melting Point/Melting Range.
EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks Measured using differential scanning calorimetry.
TEST FACILITY Safepharm (2000a)

Density 1,420 kg/m^3 at 21±0.5ºC

METHOD OECD TG 109 Density of Liquids and Solids.
EC Directive 92/69/EEC A.3 Relative Density.
Remarks Measured using a gas comparison pycnometer.
TEST FACILITY Safepharm (2000a)

Vapour Pressure 5.2×10^{-8} kPa at 25ºC

METHOD OECD TG 104 Vapour Pressure.
EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks Determined using a vapour pressure balance. The balance readings were low and variable. Consequently, the reading, which gave the highest reliable vapour pressure, was chosen. (A slope of -1500 K was imposed for that run which was most likely to have been fully degassed).
TEST FACILITY Safepharm (2000b)

Water Solubility 54.2 g/L at 20±0.5ºC

METHOD OECD TG 105 Water Solubility.
EC Directive 92/69/EEC A.6 Water Solubility.
Remarks Flask Method. Determined for the notified chemical, not for the mixture, Bonjet Black 817-E. The solution pH was determined to be 7.14-7.18.
TEST FACILITY Safepharm (2000a)

Hydrolysis as a Function of pH t½ >1 year at 25ºC (pH 4, 7, 9)

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)*	*t½*
4	25	>1 year
7	25	>1 year
9	25	>1 year

Remarks A preliminary test at 50°C showed less than 10% hydrolysis after 5 days. This is equivalent to a half-life of >1 year at 25°C.
Determined for the notified chemical, not for the mixture Bonjet Black 817-E.
TEST FACILITY Safepharm (2000a)

Partition Coefficient (n-octanol/water) log P_{ow} = -1.89 at 24±0.5ºC

METHOD OECD TG 117 Partition Coefficient (n-octanol/water).
EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks Shake Flask Method. The pH of the solution was 6.95.
Determined for the notified chemical, not for the mixture Bonjet Black 817-E.
TEST FACILITY Safepharm (2000a)

Surface Tension 60.1 mN/m at 22.0ºC

METHOD OECD TG 115 Surface Tension of Aqueous Solutions.
EC Directive 92/69/EEC A.5 Surface Tension.
Remarks Determined using the ring method and a notified chemical concentration of 1.02 g/L. The notified chemical was not considered to be surface-active.
TEST FACILITY Safepharm (2000a)

Adsorption/Desorption log K_{oc} <1.25 at 40°C

METHOD OECD TG 106 Adsorption - Desorption Using a Batch Equilibrium Method.
Remarks Determined for the notified chemical, not for the mixture Bonjet Black 817-E. The notified chemical eluted before the reference substances.
TEST FACILITY Safepharm (2000a)

Dissociation Constant Not determined. QSAR estimates provided.

Remarks The notified chemical contains numerous ionisable groups, making direct measurement of its dissociation constants impractical. The notifier has provided estimates derived using QSAR modelling (method unspecified), and the pKa values for different groups ranged from –0.53 to 11.74.

Particle Size

METHOD OECD TG 110 Particle Size Distribution/Fibre Length and Diameter Distributions.

Range (μm)	*Mass (%)*
<100 μm	54.3
<10 μm	19.3

Remarks The inhalable fraction (<100 μm) was determined by sieve, and the respirable fraction was determined using a cascade impactor. Too few particles were present to allow accurate assessment of the mass median aerodynamic diameter (MMAD).
TEST FACILITY Safepharm (2000a)

Flammability Not highly flammable

METHOD EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks The pile of Bonjet Black 817-E failed to ignite during 2 minutes' application of a bunsen flame.
TEST FACILITY Safepharm (2000c)

Autoignition Temperature 342ºC

METHOD 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks The test cube contained black charred material at the conclusion of the test.
TEST FACILITY Safepharm (2000c)

Explosive Properties Not expected to be explosive

METHOD Estimated, based on the structural formula.
TEST FACILITY Safepharm (2000c)

Oxidising Properties Not oxidising

METHOD EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Remarks Mixtures of the notified chemical and cellulose failed to support combustion.
TEST FACILITY Safepharm (2000c)

Moisture Content Mean moisture content = 8.368% (w/w)

METHOD Measured gravimetrically. ~1 g of the notified chemical was weighed into loss bottles and heated to 105ºC. The loss of mass was considered due to lost moisture.

TEST FACILITY Safepharm (2000c)

Appendix B: Toxicological Investigations

All toxicological investigations were performed using a mixture of two chemicals, Bonjet Black 817-E, equivalent to Bonjet Black 818.

B.1. Acute toxicity – oral

The notified chemical displayed moderate acute oral toxicity, inferred from the result of the 14-day rat oral gavage study (see below; Safepharm, 1997a). In this study, an LD50 of 300-600 mg/kg bw was implied in rats, based on less than 50% survival after a single dose of 600 mg/kg bw, and >50% survival at 300 mg/kg bw/day. From these results, Bonjet Black 817-E is considered harmful following an acute oral exposure.

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Bonjet Black 817-E
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal) – Limit Test.
Species/Strain	Rat/Sprague-Dawley Crl:CD BR
Vehicle	Distilled water
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	5M, 5F	2,000	0

LD50	>2,000 mg/kg bw
Signs of Toxicity - Local	Very slight erythema and small superficial scattered scabs were observed in female animals only. Black-stained fur was noted in all animals one to four days after dosing.
Signs of Toxicity - Systemic	No signs of systemic toxicity were noted.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks - Results	None.
CONCLUSION	The test substance is of low toxicity via the dermal route.
TEST FACILITY	Safepharm (2000d)

B.3. Irritation – skin

TEST SUBSTANCE	Bonjet Black 817-E
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	Three males
Vehicle	Distilled water
Observation Period	7 days
Type of Dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			

Erythema/Eschar	0	1	0	1	72 hours	0
Oedema	0	0	0	0	-	0

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

Remarks - Results: Very slight erythema was observed in all animals 1 hour after treatment. Grey/green skin staining was observed at all treated sites. Loss of skin elasticity was observed in one animal at 72 hours, but this had reversed by 7 days. No corrosive effects were observed.

CONCLUSION: The test substance was slightly irritating to the skin.

TEST FACILITY: Safepharm (2000e)

B.4. Irritation – eye

TEST SUBSTANCE: Bonjet Black 817-E

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

Species/Strain: Rabbit/New Zealand White
Number of Animals: Three (2 males, 1 female)
Observation Period: 7 days
Remarks - Method: A significant initial pain reaction was observed upon instillation of the test substance into the eye of Animal No. 1. Therefore, one drop of local anaesthetic eye drops was administered to both eyes of the following two animals before the test substance was administered.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1.3	1.0	1.0	2	72 hours	0
Conjunctiva: chemosis	0.3	0.3	0.3	2	24 hours	0
Conjunctiva: discharge	0	0	0	2	1 hour	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

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

Remarks - Results: Residual test material was observed around the treated eyes of all animals throughout the study.

Dark purple-coloured staining was observed in all treated eyes during the study, which prevented accurate evaluation of corneal or iridial effects one hour after treatment. This staining also prevented scoring of conjunctival redness in two treated eyes one hour after treatment.

CONCLUSION: The test substance was slightly irritating to the eye.

TEST FACILITY: Safepharm (1997b)

B.5. Skin sensitisation

TEST SUBSTANCE: Bonjet Black 817-E

METHOD: OECD TG 406 Skin Sensitisation – Maximisation Study.
EC Directive 96/54/EC B.6 Skin Sensitisation - Maximisation Study.

Species/Strain: Guinea pig/albino Dunkin Hartley
PRELIMINARY STUDY: Maximum Non-irritating Concentration:
intradermal: 5% (w/v) in distilled water

topical: 25% (w/v) in distilled water

MAIN STUDY

Number of Animals: Test Group: 10 males; Control Group: 5 males

INDUCTION PHASE: Induction Concentration:
intradermal: 5% (w/v) in distilled water or 1:1 distilled water/FCA mix
topical: 10% (w/v) in distilled water

Signs of Irritation: The intradermal injection sites could not be evaluated due to the degree of staining induced by the test material. Very slight erythema was noted at the intradermal injection site of one control animal at 24 hours.

CHALLENGE PHASE

1st challenge: topical: 10% and 5% in water

Remarks - Method: No significant protocol deviations.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions from challenge after:*	
		24 h	*48 h*
Test Group	10%	0	0
	5%	0	0
Control Group	10%	0	0
	5%	0	0

Remarks - Results: No skin reactions were observed at the 24- or 48-hour observations.

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

TEST FACILITY: Safepharm (1997c)

B.6. Repeat dose toxicity (14 day)

TEST SUBSTANCE: Bonjet Black 817-E

METHOD: 14-day oral (gavage) range-finding toxicity study of the 28-day oral toxicity study.

Species/Strain: Rat/Sprague-Dawley Crl:CD BR

Vehicle: Distilled water

Exposure Information: Total exposure days: 14 days
Dose regimen: 7 days per week
Post-exposure observation period: None

Remarks - Method: The test material (10 mL at various concentrations) was administered to each animal for up to 14 consecutive days, by gavage. Control animals were treated with only 10 mL distilled water. An additional control group was used as a preliminary study, concurrently with the 150 mg/kg bw/day group.

At the end of the treatment period, all surviving animals were sacrificed and subjected to an external and internal macroscopic examination.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
1 (control)	3M, 3F	0	0
2	3M, 3F	150	0
3 (control)	3M, 3F	0	0
4	3M, 3F	300	1F (day 5), 1F (day 8)*
5	3M, 3F	600	1M, 3F (day 2)**

* After the second death at 300 mg/kg bw/day, the surviving animals of this group were sacrificed on day 8.

** The two surviving animals treated with 600 mg/kg bw/day were sacrificed on day 2.

LD50	300-600 mg/kg bw (based on less than 50% survival after a single dose of 600 mg/kg bw, and >50% survival at 300 mg/kg bw/day)
Signs of Toxicity	No clinical signs were observed in animals treated at 150 mg/kg bw/day. Black-stained or dark faeces were observed in animals of both 150 and 300 mg/kg bw/day dose groups, but this was considered to be due to the coloured test material and thus not of toxicological significance. With the exception of the two female decedents, all animals treated with 300 mg/kg bw/day appeared normal until day 7, when hunched posture developed. Bodyweight loss was observed in these animals by day 7. The two females found dead on days 5 and 8 showed a deterioration in physical condition prior to their deaths, including bodyweight loss, stained external fur, hunched posture, lethargy, piloerection, laboured respiration, decreased respiratory rate, dehydration, emaciation, tiptoe gait and swelling of the snout.
Effects in Organs	The four animals found dead after a single dose of 600 mg/kg bw/day were found to have swelling of the tongue, enlarged salivary glands, dark staining of the glandular gastric epithelium and dark gastrointestinal contents. Female decedents at 300 mg/kg bw/day showed similar changes at necropsy, while also showing black staining of the uterus and/or non-glandular gastric epithelium. All surviving animals at 300 or 600 mg/kg bw/day showed no macroscopic abnormalities. Males treated with 150 mg/kg bw/day showed enlarged and/or dark spleens, and one showed patchy pallor and accentuated lobular pattern of the kidneys. One female also showed a dark spleen at this dose level.
Remarks - Results	The results were sufficient to indicate that test substance should be considered as harmful via the oral route.
CONCLUSION	On the basis of this study, the dose levels for the 28-day repeat-dose oral toxicity study were chosen to be 1.5, 15 and 150 mg/kg bw/day.
TEST FACILITY	Safepharm (1997a)

B.7. Repeat dose toxicity (28 day)

TEST SUBSTANCE	Bonjet Black 817-E
METHOD	EC Directive 92/69/EEC B.7 Repeated Dose (28 Days) Toxicity (Oral). Japanese Ministry of Health and Welfare (MHW) Guidelines (1986) for a twenty-eight day repeat dose oral toxicity study.
Species/Strain	Rat/Sprague-Dawley Crl:CD BR
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Distilled water
Remarks - Method	No significant protocol deviations. The dose levels for this study were selected on the basis of a 14-day oral range-finding toxicity study (above under *B.6. Repeat dose toxicity (14 day)*).

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
I (control)	5M, 5F	0	0
II (control recovery)	5M, 5F	0	0
III (low dose)	5M, 5F	1.5	0

IV (mid dose)	5M, 5F	15	0
V (high dose)	5M, 5F	150	0
VI (high dose recovery)	5M, 5F	150	0

Mortality and Time to Death

No deaths occurred during the study.

Clinical Observations

No clinical signs of toxicity were observed during the study. Black-stained urine and faeces were observed throughout the treatment period, but these were considered due to the coloured test substance, and not toxicologically relevant. Stained urine was not observed during the recovery period.

Animals treated with 150 mg/kg bw/day showed a reduction in bodyweight gain during week 3 (males) and week 4 (males and females) of treatment. Females at this dose showed reduced food intake during week 4. These findings were reversible during the 14-day recovery treatment-free period.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology: Animals treated with 150 mg/kg bw/day showed a reduction in haemoglobin, erythrocyte count and haematocrit, and increases in mean corpuscular volume, total leucocyte count, reticulocyte count, methaemoglobin, mean corpuscular haemoglobin (females only) and elevated neutrophil count (males only). Many of these changes persisted up to 14 days of recovery, including the reduced erythrocyte count and increased mean corpuscular volume, mean corpuscular haemoglobin and reticulocyte count.

Clinical chemistry: Slight increases in plasma bilirubin concentrations were observed in animals treated with 150 mg/kg bw/day, and females at this dose showed increased potassium and plasma aspartate aminotransferase activity. These effects were reducible after 14 days of recovery.

Urinalysis: No adverse effects on urinary parameters were found.

Effects in Organs

Animals of both sexes treated with 150 mg/kg bw/day showed significant increases in spleen weight, both absolute and relative to terminal bodyweight. This effect persisted in females after 14 days recovery. Microscopic examination revealed increased severity of splenic extramedullary haemopoiesis and haemosiderin deposition relative to controls. Haemosiderin deposition was also observed in the liver and renal tubules of females treated with 150 mg/kg bw/day. All occurrences of haemosiderin deposition persisted until the end of the recovery period.

Males of the 150 mg/kg bw/day recovery group showed reduced absolute and relative testes weight at the end of the recovery period. Upon microscopic examination, testicular atrophy was observed in two males of the 150 mg/kg bw/day treatment group and in all males of the corresponding recovery group, indicating it was a progressive, irreversible effect.

Remarks – Results

No adverse effects were observed in animals of either sex treated with 1.5 or 15 mg/kg bw/day.

It was considered that the evidence of haemolytic anaemia observed in high dose animals led to the spleen functioning as an additional site for red blood cell formation and this hypothesis explains the increased spleen weights and extramedullary haematopoiesis.

CONCLUSION

The No Observed Effect Level (NOEL) was established as 15 mg/kg bw/day in this study, based on the absence of treatment-related effects at this dose level.

TEST FACILITY Safepharm (1997a)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE Bonjet Black 817-E

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test

	using Bacteria. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	Phenobarbitone/β-naphthoflavone-induced rat liver S9 mix
Concentration Range in Main Test	a) With metabolic activation: 50, 150, 500, 1500 and 5000 µg/plate b) Without metabolic activation: 50, 150, 500, 1500 and 5000 µg/plate
Vehicle	Distilled, sterile water
Remarks - Method	Positive controls used: -S9: N-ethyl-N'-nitro-N-nitrosoguanidine, 9-aminoacridine and 4-nitroquinoline-1-oxide. +S9: 2-Aminoanthracene and benzo[a]pyrene.

RESULTS

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

Remarks - Results

A grey colour in the plates was observed at doses of 50 µg/plate, which became intense purple at ≥500 µg/plate, but this did not interfere with the scoring of the revertant colonies. The positive controls yielded positive results, indicating that the test system was functioning appropriately.

As 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. It has been observed that many azo compounds are found to be non-mutagenic in standard Ames tests, but mutagenic when a modified test is used (Øllgaard *et al*, 1998). Therefore, this negative result is considered inconclusive as a modified test (eg Prival and Mitchell, 1982) was not used.

CONCLUSION

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

TEST FACILITY

Safepharm (2000f)

B.9. Genotoxicity – *in vitro*

TEST SUBSTANCE	Bonjet Black 817-E
METHOD	OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test. EC Directive 2000/32/EC B.10 Mutagenicity - *In vitro* Mammalian Chromosome Aberration Test.
Cell Type/Cell Line	Human lymphocytes
Metabolic Activation System	Phenobarbitone/β-naphthoflavone-induced rat liver S9 mix
Vehicle	Minimal Essential Medium (MEM)
Remarks - Method	A continuous 24-hour exposure without S9 was not performed, as a clear positive response was observed using a 4-hour exposure. Likewise, a second experiment was not performed because a clear response was observed in the first test.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent	0*, 78.13, 156.25, 312.5, 625*, 1250*, 2500*	4	24
Present	0*, 78.13, 156.25, 312.5, 625*, 1250*, 2500*	4	24

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	>2500	≥1250	>2500	Positive
Present	>2500	≥1250	>2500	Positive

Remarks - Results	The test substance induced dose-related, statistically significant increases in the frequency of cells with chromosomal aberrations, in both the presence and absence of metabolic activation. Without activation, a positive response was seen only at the top dose; with activation a dose-response was observed at 1,250 and 2,500 μg/mL.
CONCLUSION	The test substance was clastogenic to human lymphocytes treated *in vitro* under the conditions of the test.
TEST FACILITY	Safepharm (2001)

B.10. Genotoxicity – *in vivo*

TEST SUBSTANCE	Bonjet Black 817-E
METHOD	OECD TG 474 Mammalian Erythrocyte Micronucleus Test. EC Directive 2000/32/EC B.12 Mutagenicity – *in vivo* Mammalian Erythrocyte Micronucleus Test.
Species/Strain	Mouse/albino Crl:CD-1(ICR)BR
Route of Administration	Oral – gavage
Vehicle	Distilled water
Remarks - Method	A dose range-finding test was performed using doses of 500-1000 mg/kg bw, and a maximum tolerated dose (MTD) of 750 mg/kg bw was selected. Due to a lack of differences in toxic response between sexes of animals in the range-finding test, the main test was performed using only male mice.

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Sacrifice Time (hours)*
I (vehicle control)	7M	0	24
II (vehicle control)	7M	0	48

III (low dose)	7M	187.5	24
IV (mid dose)	7M	375	24
V (high dose)	7M	750	24
VI(high dose)	7M	750	48
V (cyclophosphamide)	5M	50	24

RESULTS

Doses Producing Toxicity	Premature deaths were observed in the 24-hour 750 mg/kg bw test material treatment group, where two animals died prior to sampling of their bone marrow. The only clinical sign observed was hunched posture, seen in the surviving animals at 750 mg/kg bw of both the 24- and 48-hour test groups.
Genotoxic Effects	There was a small but statistically significant increase in the frequency of micronucleated polychromatic erythrocytes (mPCE) in the 24-hour, 750 mg/kg bw group when compared with its control. This was attributed to a low concurrent control value, and the frequency of mPCE obtained at this dose was thought to be within the upper limit of the historical vehicle control range. Indeed, the level of mPCEs found after treatment with the test substance is in fact below the expected 0.2% rate of spontaneous mPCE formation in mouse bone marrow (EHC 51, 1982).
Remarks - Results	The reduced PCE/NCE ratio seen in this group may also indicate bone marrow toxicity, which is known to stimulate erythropoiesis and increase the spontaneous frequency of mPCE. The statistical power of the analysis was reduced in the 24-hour, 750 mg/kg bw test group, due to the two premature deaths. The impact of this on the analyses performed is uncertain. The negative test result, while requiring justification, is reasonably convincing. There is no evidence of a strongly positive clastogenic test result.

CONCLUSION The test substance was not clastogenic under the conditions of this *in vivo* mouse micronucleus test, although the test data have some weaknesses.

TEST FACILITY Safepharm (2005)

Appendix C: Environmental Fate and Ecotoxicological Investigations

All environmental fate and ecotoxicological investigations were performed using a mixture of two chemicals, Bonjet Black 817-E, equivalent to Bonjet Black 818.

C1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Bonjet Black 817-E
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test.
Inoculum	Sewage Sludge from sewage treatment plant (STP) at Belper Derbyshire, UK treating predominantly domestic sewage.
Exposure Period	28 Days
Auxiliary Solvent	None specified
Analytical Monitoring	CO_2 analyser
Remarks - Method	Duplicate test samples were run with a final concentration of 10 mg carbon/L. Duplicate blanks and reference material (sodium benzoate) were run. A further single toxicity test was run containing the test substance and reference substance. Two CO_2 absorbers were connected in series. Samples were taken on days 0, 1, 2, 3, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 27, 28 and 29 for the first absorber and days 0 and 29 for the second.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
1	0	1	11
3	5	3	50
10	3	10	76
14	4	14	79
28	7	28	91
29*	10	29	94

* Day 29 values corrected to include any carry over of CO_2 detected in absorber 2.

Remarks - Results	The toxicity control attained 36% degradation after day 28 thereby confirming that the test material was non-toxic to sewage sludge organisms.
CONCLUSION	The test substance cannot be regarded as readily biodegradable.
TEST FACILITY	Safepharm (2000g)

C.1.2. Bioaccumulation

The notified chemical has a low log K_{ow} value and is therefore unlikely to bioaccumulate.

C2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Bonjet Black 817-E
METHOD	OECD TG 203 Fish, Acute Toxicity Test EC Directive 92/69/EEC C.1 Acute Toxicity for Fish –semi static.
Species	Rainbow Trout (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	~100 mg $CaCO_3$/L

Analytical Monitoring	Visual; HPLC
Remarks – Method	A preliminary range finding study was conducted using three fish per test concentration of 1.0, 10 and 100 mg/L of test substance. A control was also run. Based on the findings of the range finding test a "Limit Test" was run by subjecting duplicates of ten fish to 100 mg/L of test substance. A single control was also run. The test solutions were changed daily. Temperature: 14.0°C pH: 7.4-8.0 Dissolved oxygen: 9.2-9.7 mg O_2/L Light 16 hours light, 8 hours dark with 20 minute transition.

RESULTS

Nominal Concentration (mg/L)	*Number of Fish*	*Mortality*				
		3 h	*24 h*	*48 h*	*72 h*	*96 h*
0	10	0	0	0	0	0
100	20	0	0	0	0	0

LC50	>100 mg/L at 96 hours.
NOEC (or LOEC)	100 mg/L at 96 hours.
Remarks – Results	The recovery of solutions was between 98-108% of the nominal concentration. No abnormal behaviour was observed in any of the test concentrations.

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

TEST FACILITY Safepharm (2000h)

C.2.2. Acute/chronic toxicity to aquatic invertebrates

TEST SUBSTANCE Bonjet Black 817-E

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

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	~250 mg $CaCO_3$/L
Analytical Monitoring	Visual Observation; HPLC
Remarks - Method	A preliminary range finding study was conducted using ten daphnia per test concentration of 0.010, 0.10, 1.0, 10 and 100 mg/L of test substance. A control was also run. Based on the findings of the range finding test a "Limit Test" was run by subjecting four replicates of ten daphnids to 100 mg/L of test substance. A duplicate control was also run. Temperature: 21.0°C pH: 7.7-8.0 Dissolved oxygen: 8.1-8.4 mg O_2/L Light 16 hours light, 8 hours dark with 20 minute transition.

RESULTS

Nominal Concentration (mg/L)	*Number of D. magna*	*Number Immobilised*	
		24 hour	*48 hour*
0	20	0	0
100	40	0	0

LC50	>100 mg/L at 48 hours *[acute]*
NOEC	100 mg/L at 48 hours *[acute]*
Remarks - Results	The recovery of solutions was between 100-103% of the nominal

concentration. No abnormal behaviour was observed in any test.

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

TEST FACILITY Safepharm (2000i)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Bonjet Black 817-E

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

Species	Green Alga (*Scenedesmus subspicatus*)
Exposure Period	7.2 hours
Concentration Range	Nominal: 0.10-10 mg/L Actual: 0.071-11 mg/L
Auxiliary Solvent	None
Water Hardness	~ 4 mg $CaCO_3$/L based on culture medium.
Analytical Monitoring	Particle Counter and haemocytometer
Remarks - Method	A range finding study was conducted by exposing algal cells (in duplicate) to 0.10, 1.0, 10 and 100 mg/L of test substance and a control. On the basis of the range finding test, three replicate cultures of nominal cell density of 1×10^4 per mL in direct contact with 0.10, 0.32, 1.0, 3.2 and 10 mg/L of test substance were prepared (Test A). Further single tests of the same concentrations were prepared but the alga was only shaded by the test substance but not in direct contact (Test B). A control was also run in triplicate. pH 7.3 – 7.4 Temperature 24 ± 1°C Light Intensity ~ 7,000 Lux

RESULTS

Test A (in direct contact and shaded)

Biomass *EbC50 (mg/L) at 72 h*	*Growth* *ErC50 (mg/L) at 72 h*
1.2	>11

Test B (shaded only)

Biomass *EbC50 (mg/L) at 72 h*	*Growth* *ErC50 (mg/L) at 72 h*
10	>11

Remarks - Results The results were based on the time-weighted average mean of the measured test concentrations. The percentage recovery of the nominal concentrations was between 71 and 113%. The 95% confidence interval (CI) was not calculated as the data did not fit the models available (Litchfield & Willcoxon, Probit Logistic and Weillbulls). Tests A and B show that there is a real toxic effect from the test substance and that the toxicity cannot be solely attributed to shading.

CONCLUSION The test substance is moderately toxic to algae.

TEST FACILITY Safepharm (2000j)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Bonjet Black 817-E
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test
Inoculum	Sewage Sludge from STP at Belper Derbyshire, UK treating predominantly domestic sewage.
Exposure Period	3 hours
Concentration Range	Nominal: 10- 1,000 mg/L
Remarks – Method	A range finding test was conducted by subjecting sewage sludge to 10, 100 and 1,000 mg/L of test substance. Control and reference substances (3,5-dichlorophenol) were used at 3.2 and 32 mg/L. On the basis of the range finding test a limit test was conducted by subjecting triplicate samples of sewage sludge to 1,000 mg/L of test substance. Control and reference substance concentrations of 3.2, 10 and 32 mg/L were used.
RESULTS	
IC50	>1,000 mg/L
NOEC	1,000 mg/L
Remarks – Results	The IC50 of 3,5-dichlorophenol was 8.3 mg/L. The test substance solutions appeared as a dark purple solution with no observed undissolved material. Less than 10% inhibition was observed in all test samples.
CONCLUSION	The test substance is practically non-inhibitory to sewage sludge microorganisms.
TEST FACILITY	Safepharm (2000k)