File No: STD/1166

September 2005

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

C-RB

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Director
NICNAS

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Canon Australia Pty Ltd (ABN66 005 02 951)
1 Thomas Holt Drive
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 Identity
Spectral data
Purity and identity of impurities

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows:
Dissociation constant
Flash point
Acute inhalation toxicity
Genotoxicity –*In vivo*
Acute/chronic toxicity to aquatic invertebrates

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
LVC/658

NOTIFICATION IN OTHER COUNTRIES
UK Annex VIIC notification 04-06-1760 (June 2004) and Annex VII notification (not yet completed)
US PMN notification P-04-0498 (July 2004)
Japan (Feb 2004)
Philippines Small quantity information clearance notification (Nov 2005)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
C-RB (preferred marketing name)
JPD Black C-RB Liquid

METHODS OF DETECTION AND DETERMINATION

METHOD	High Performance Liquid Chromatography (HPLC),
	Infrared Spectroscopy, ^{1}H Nuclear Magnetic Resonance (NMR) and UV/Vis Spectroscopy
Remarks	HPLC using UV/Vis detection allows the quantification of the notified chemical.
	Identity confirmed by ^{1}H NMR, UV/Vis and IR.

3. COMPOSITION

DEGREE OF PURITY
>75 %

4. 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 ink in sealed ink-jet printer cartridges at a concentration of 0.5 to 7%.

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

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

USE
The notified chemical acts as a dyesuff in printing ink.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, transport and storage

PORT OF ENTRY
Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
The ink cartridges will be stored at the notifier's warehouse prior to distribution to offices nationwide and office equipment retailers.

TRANSPORTATION AND PACKAGING
The size of the imported ink cartridge is 12mm x 20 mm x 15 mm – 70mm x 30mm x 120mm. Each cartridge contains 2 – 150 mL of ink. Transport in Australia will be by road, no special transport requirements are necessary.

5.2. Operation description

No processing such as reformulation, repackaging, filling or refilling of the cartridges containing the notified chemical, or any other handling of the notified chemical is carried out in Australia. Sealed ink cartridges containing the notified chemical will be handled by service technicians, office workers or the public, who will replace spent cartridges in the printers as necessary. Office workers and the public will use the printers for varied printing work.

5.3. Occupational exposure

Exposure Details
Waterside, storage and transport workers will only handle the sealed cartridges containing the notified chemical and therefore exposure is not expected unless the packaging is accidentally breached.

Service technicians may be exposed to the ink containing up to 7% notified chemical during repair and cleaning of ink jet printers. Dermal exposure is expected to be the main route of exposure.
As the notified chemical is a low volatility solid that is imported into Australia only as a component of a liquid preparation, inhalation exposure is considered to be an unlikely route of exposure. Due to the controlled nature of the ink release, ocular exposure is also expected to be unlikely.

Exposure while changing cartridges is expected to be limited to dermal exposure, occurring if the ink is inadvertently touched. Instructions on how to replace the cartridge safely are included with the cartridge. During the printing process, the ink turns into an extremely fine mist and is transferred to the paper, however mist emission of the non-volatile components of the ink from the printer is expected to be low. Occasional dermal exposure during use of the printer could occur if the printed pages were touched inadvertently before the ink had dried, or if ink-stained parts of the printer were touched. Once the ink dries, the chemical would be trapped in the printed paper, and therefore dermal exposure to the notified chemical from contact with the dried ink is not expected.

5.4. Release

RELEASE OF CHEMICAL AT SITE

Printer ink is imported in ready-to-use cartridges of 2 to 150 mL (containing <7% notified chemical). No release is expected as manufacturing and reformulation of the ink containing the notified chemical will not take place in Australia. Environmental release of the notified chemical is unlikely during importation, storage and transportation. However if there was a transport accident, the individual container capacity, container and packaging specifications would limit the extent of release to the environment.

RELEASE OF CHEMICAL FROM USE

The office staff and general public will change the cartridges. Release of the ink solution to the environment is not expected under normal use as ink cartridges are designed to prevent leakage. However, if leakage or spill does occur, the ink will be contained with absorbent material, which will presumably be disposed of in landfill.

Ultimately, the majority of the notified substance suffers the same fate as the paper to which it is bound. This will either be disposal to landfill, or incineration or recycling. Residues (up to 5%) left in empty cartridges which will be recycled or be disposed of to landfill. Cartridges are processed to recycle their component materials into new products. During the recycling process the residual ink will be washed from the cartridges and washings discharged to onsite wastewater treatment plants before release to sewer.

Recycling of treated paper may result in the release of a proportion of the notified chemical to the aquatic compartment.

5.5. Disposal

The total import volume of the notified polymer will ultimately be disposed as normal office/domestic waste that will either end up in landfill or be incinerated or recycled. The sludge from the deinking process will be disposed of to landfill.

While some of the empty cartridges will be recycled, the majority will be disposed of to landfill, accounting for up to 50 kg of notified substance annually.

5.6. Public exposure

The scenarios by which the public may be exposed to the notified chemical would involve home use of printers, and are similar to those for office workers (see section 5.3 above). However, it is expected that the public will be using the printer less often than workers.

6. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20ºC and 101.3 kPa Black powder

Melting Point >325ºC

METHOD	OECD TG 102 Melting Point/Melting Range EC Directive 92/69/EEC A.1 Melting/Freezing Temperature
Remarks	Determined by Differential Scanning Calorimetry. The notified chemical was determined decompose at approximately 325 – 329 ºC. Similar thermographic profiles were obtained using air and nitrogen atmospheres indicating that the decomposition observed is probably thermal and not oxidative.
TEST FACILITY	SafePharm Laboratories (2004a)

Density 1689 kg/m^3 at 21 $\pm$ 0.5ºC

METHOD	OECD TG 109 Density of Liquids and Solids. EC Directive 92/69/EEC A.3 Relative Density.

Remarks Determined by gas comparison pycnometer.
TEST FACILITY SafePharm Laboratories (2004a)

Vapour Pressure 2.0 x 10^{-12} kPa at 25°C

METHOD OECD TG 104 Vapour Pressure.
EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks Determined by vapour pressure balance system with measurements being made at several temperatures and linear regression analysis used to calculate the maximum vapour pressure at 25°C. The notified chemical is classified as slightly volatile (Mensink *et al.* 1995).The vapour pressure was calculated to be below the recommended range for this method
TEST FACILITY SafePharm Laboratories (2004b)

Water Solubility In the range 40.3 – 42% (w/w) at 20°C

METHOD OECD TG 105 Water Solubility.
EC Directive 92/69/EEC A.6 Water Solubility.
Remarks Determined by Flask Method. Analytical method: visual inspection (no analysis could be performed due to the viscous and gel-like mixtures formed).

The preliminary test indicated that the water solubility of the notified chemical was in the range 40.3 to 50% (w/w) with no excess test material observed in the 40.3% sample. In the main study six solutions from 32.1 to 44.1% (w/w) were made. Excess test material was observed in the 42% sample but not in the 38% sample.
TEST FACILITY SafePharm Laboratories (2004a)

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)*	$t_{½}$
4	25	> 1
7	25	> 1
9	25	> 1

Remarks The nominal concentration of the test solutions was 1.0 g/L. Aliquots (in duplicate) of sample solutions were taken at various times (0 and 120 hours) and the pH of the solution was recorded.

After 120 hours (5 days) at all pHs and at 50°C it was found that less than 10% of the test substance had hydrolysed, thus indicating a half-life of greater than 1 year at 25°C.

This indicates that the notified chemical is not likely to hydrolyse in the environment.
TEST FACILITY SafePharm Laboratories (2004a)

Partition Coefficient (n-octanol/water) log Pow = <-3.14at 20°C

METHOD OECD TG 117 Partition Coefficient (n-octanol/water).
EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks Shake Flask Method
Six measured amounts of the test substance and water saturated n-octanol were shaken by inversion at 21.0-22.0°C for 5 minutes. Aliquots of both the water and n-octanol phases were taken for analysis by HPLC.

This result indicates that the notified chemical is likely to favour the water phase.

TEST FACILITY SafePharm Laboratories (2004a)

Adsorption/Desorption log K_{oc} <1.25

METHOD OECD TG 121 Estimation of the Adsorption Coefficient (*Koc)* on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)

Remarks The HPLC screening method was used with the use of 12 reference standards with known adsorption coefficients. The retention time of the test substance was 1.63 minutes which was less than that for acetanilide (3.762 minutes) which has a known log K_{oc} of 1.25, therefore the log adsorption coefficient is less than 1.25.

The test was done at pH 7 and therefore reflects the ionised substance.

This result indicates that the notified chemical will be mobile in soils and sediments.

TEST FACILITY SafePharm Laboratories (2004a)

Dissociation Constant Various pKa's calculated to be between –1.92 and 11.52

Remarks The notified chemical contains multiple functional groups with a variety of pKa values. The various pKa values provided by the notifier were calculated by I-Lab Web Service version 8.02. Thirteen reference groups related to functional groups in the notified substance were entered into the model and the predicted pKa values obtained. The notified substance has strong acid functionalities, and will remain ionised throughout the environmental pH range of 4 to 9 as well as basic functionalities which are expected to display typical basicity.

Surface Tension 71.7 mN/m at 19.0 ± 0.5°C

METHOD OECD TG 115 Surface Tension of Aqueous Solutions.
EC Directive 92/69/EEC A.5 Surface Tension.

Remarks Concentration: 1.0 g/L
An interfacial tension balance was used to determine the surface tension. The test material is not surface active.

TEST FACILITY SafePharm Laboratories (2004a)

Particle Size

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

Range (μm)	*Mass (%)*
< 100 (respirable fraction)	30.4
<10 (inhalable fraction)	3.15

Remarks In a preliminary study the notified chemical was sieved for approximately 10 minutes using a 100 μm sieve. The main study was conducted using the cascade impactor method. Air containing the notified chemical enters the inlet port at a rate of 60 litres/minute. The impactor consists of five stages with cut-point aerodynamic diameters of 10, 5, 2.5, 1.25 and 0.625 μm, and a final glass fibre filter. Particles not deposited in the artificial throat are deposited according to size in collection cups.

Too few particles were of a size < 10 μm to allow accurate assessment of mass median aerodynamic diameter.

TEST FACILITY SafePharm Laboratories (2004a)

Flash Point Not determined

Remarks	The flash point in the lowest temperature at which a liquid evolves vapours in such an amount that a flammable vapour/air mixture is produced. The melting point of the notified chemical is >325°C and as such the flash point is also considered to be >325°C.

Flammability Limits Not highly flammable

METHOD	EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks	In the preliminary test the notified chemical failed to ignite and therefore the substance can be considered not highly flammable. The moisture content was calculated to be 2.7 %(w/w).
TEST FACILITY	SafePharm Laboratory (2004c)

Autoignition Temperature >400 °C

METHOD	92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
TEST FACILITY	SafePharm Laboratory (2004b)

Explosive Properties Not explosive

METHOD	EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks	The notified chemical was tested for thermal sensitivity, mechanical sensitivity with respect to shock and mechanical sensitivity with respect to friction.
TEST FACILITY	SafePharm Laboratory (2004b)

Oxidizing Properties Predicted not to have oxidising properties

METHOD	EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Remarks	There are no chemical groups that would imply oxidising properties, therefore the result has been predicted to be negative
TEST FACILITY	SafePharm Laboratory (2004b)

Reactivity

Remarks	The notified chemical is stable under normal conditions of use. The chemical is hydrolytically stable but decomposes at temperatures above 325 °C.

7. TOXICOLOGICAL INVESTIGATIONS

Endpoint	*Assessment Conclusion*
Rat, acute oral	low toxicity, LD50 >2000 mg/kg bw
Rat, acute dermal	low toxicity, LD50 >2000 mg/kg bw
Rat, acute inhalation	not provided
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	severely irritating
skin sensitisation – LLNA	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOEL 25mg/kg bw/day
Genotoxicity – bacterial reverse mutation (two independent studies)	non mutagenic
Genotoxicity – in vitro chromosome aberration test (two independent studies)	non genotoxic
Genotoxicity – in vivo	not provided

7.1. Acute toxicity – oral

TEST SUBSTANCE C-RB (85.5% purity)

METHOD OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain Rat/Sprague-Dawley
Vehicle Dimethylsulfoxide
Remarks - Method No significant protocol deviations. A correction factor was applied to account for the purity of the teat material.

Test conducted in accordance with GLP standards.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	3 female	2339*	0
II	3 female	2339*	0

*equivalent to 2000 mg/kg bw notified chemical.

LD50 >2000 mg/kg bw
Signs of Toxicity There were no signs of systemic toxicity. Purple colour staining of the bedding, urine and/or faeces was noted in all animals one and two days after dosing. All animals appeared normal three days after dosing. All animals showed expected gains in bodyweight over the study period.
Effects in Organs Dark liver was noted in all animals at necropsy
Remarks - Results

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

TEST FACILITY Safepharm Laboratories (2004d)

7.2. Acute toxicity – dermal

TEST SUBSTANCE C-RB (85.5% purity)

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
Vehicle Test substance moistened with arachis oil BP
Type of dressing Semi-occlusive.
Remarks - Method No significant protocol deviations. A correction factor was applied to

account for the purity of the teat material.

Test conducted in accordance with GLP standards.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5 male	2339*	
II	5 female	2339*	

*equivalent to 2000 mg/kg bw notified chemical.

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	Very dark purple staining was noted at the treatment sites of all animals one and two days after dosing and in nine animals three days after dosing, There were no signs of dermal irritation (the staining was reported not to affect evaluation of skin responses).
Signs of Toxicity - Systemic	There were no deaths or signs of systemic toxicity. All animals showed expected gains in bodyweight.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks - Results	

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

TEST FACILITY Safepharm Laboratories (2004e)

7.3. Acute toxicity – inhalation

Not provided. The notified chemical is a low volatility solid that is imported into Australia only as a component of a liquid preparation. Inhalation exposure is therefore considered to be an unlikely route of exposure.

7.4. Irritation – skin

TEST SUBSTANCE C-RB (85.5% purity)

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

Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	Test substance moistened with distilled water.
Observation Period	7 days
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations.

Test conducted in accordance with GLP standards.

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	1	0.33	0	1	72 hours	0
Oedema	0	0	0	0	N/A	0

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

Remarks - Results Dark purple-coloured staining was noted at all treated skin sites during the study which was resolved by day 9. This was reported not to affect

evaluation of skin reactions.

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY Safepharm Laboratories (2004f)

7.5. Irritation – eye

TEST SUBSTANCE C-RB (85.5% purity)

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 1
Observation Period 21 days
Remarks - Method No significant protocol deviations. Due to the ocular severity noted in the first treated animal, no additional animals were treated.

Test conducted in accordance with GLP standards.

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	0.67	-	-	2	48 hours	0
Conjunctiva: chemosis	0.33	-	-	2	24 hours	0
Conjunctiva: discharge	0.33	-	-	3	24 hours	0
Corneal opacity	0	-	-	0	N/A	0
Iridial inflammation	0	-	-	0	N/A	0

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

Remarks - Results Slight initial pain was noted after administration of the test substance. Black staining of the fur was noted around the treated eye throughout the study. Black stating of the cornea was noted in the treated eye throughout the study.

CONCLUSION Although based on the ocular lesions observed the notified chemical would only be classified as slightly irritating. However, as irreversible colouration of the eyes occurred, the notified chemical is considered to cause serious damage to the eye.

TEST FACILITY SafePharm Laboratories (2004g)

7.6. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE C-RB (85.5% purity)

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain Mouse/CBA Ca
Vehicle Dimethylsulfoxide
Remarks - Method Deviations from OECD TG 429:

The notified chemical was only tested at a maximum concentration of 5%. No explanation as to why higher concentrations were not tested although the report did state that the vehicle was chosen as it produced the highest concentration that was suitable for dosing indicating that solubility may have been an issue.

Test conducted in accordance with GLP standards.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	1133.63	
1	862.28	0.76
2.5	1145.39	1.01
5	1005.21	0.89
Positive Control (α-hexylcinnamaldehyde)		
5		1.76
10		2.78
25		5.06

Remarks - Results: There were no deaths, signs of systemic toxicity or remarkable bodyweight changes noted during the study. Dark blue coloured staining of the ears and fur was noted in all test animals one hour after dosing, on days 1, 2 and 3.

CONCLUSION: There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical tested up to a concentration of 5%.

TEST FACILITY: Safepharm Laboratories (2004h)

7.7. Repeat dose toxicity – 28 day oral toxicity study in the rat

TEST SUBSTANCE: C-RB (85.5% purity)

METHOD: OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).

Species/Strain: Rat/Sprague-Dawley

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. A correction factor was applied to account for the purity of the test material.

Test conducted in accordance with GLP standards.

RESULTS

Group	*Number and Sex of Animals*	*Dose* mg/kg bw/day*	*Mortality*
I (control)	5 per sex	0	0
II (low dose)	5 per sex	25	0
III (mid dose 1)	5 per sex	150	0
IV (mid dose 2)	5 per sex	300	0
V (high dose)	5 per sex	1000	0
VI (control recovery)	5 per sex	0	0
VII (high dose recovery)	5 per sex	1000	0

* dose level of notified chemical

Mortality and Time to Death

No mortality was observed during the treatment or recovery phases

Clinical Observations

Dark staining on cage-tray liners was noted for all group III, IV and V animals. Also in group V animals, blue fur staining was prevalent from day 12, dark eyes were observed by day 16 and blue extremities were evident from day 23. All findings other than fur staining were still evident in the high dose recovery group. These findings are considered simply to represent normal excretion of coloured test materials or their metabolites and the dispersal of the colouration during normal grooming behaviour.

There were considered to be no treatment related changes in behavioural parameters, functional performance parameters and sensory reactivity. No adverse effect on food consumption and bodyweight gain was observed during the study. High dose recovery group males showed a statistically significant decrease (42%, $p<0.01$) in body weight gain compared to control recovery animals during the first week of the treatment free period. In the absence of a similar reduction during the dosing period, this difference is considered to be incidental.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Clinical Chemistry

A slight but statistically significant reduction in chloride concentration occurred in group IV (1%, $P<0.05$) and group V (2%, $P<0.05$) males. In addition, a statistically significant increase in inorganic phosphorus levels (12%, $P<0.05$) occurred in group IV males. All individual values were within the respective normal ranges for rats of the strain and age used and therefore the differences were considered not to be treatment related.

A slight but statistically significant increase in plasma sodium concentration occurred in both high dose recovery males (1.4%, $P<0.05$) and females (1.4%, $P<0.01$). High dose recovery males also showed a statistically significant increase in plasma urea (14%, $P<0.05$). In the absence of similar changes detected for non-recovery animals at the end of the dosing period, these differences were considered to be incidental.

Haematology

Group V males showed a statistically significant increase in activated partial thromboplastin time (17%, $P<0.01$) whilst group V females showed a statistically significant increase in platelet count (24%, $P<0.05$). All individual values were within the respective normal ranges for rats of the strain and age used and therefore the differences were considered not to be treatment related.

High dose recovery females showed a statistically significant reduction in clotting time (6%, $P<0.05$), haemoglobin (4%, $P<0.05$), haemocrit (5%, $P<0.05$) and erythrocyte count (5%, $P<0.05$). In the absence of similar changes detected for non-recovery animals at the end of the dosing period, these differences were considered to be incidental.

Urinalysis

Group II, IV and V animals showed varying intensities of dark coloured urine. An increased concentration of haemoglobin may have been present in the urine of group IV, V and VII animals, however this may have been an observation due to discolouration of the test material and, in the absence of any evidence to suggest and anaemic condition is considered to be incidental. A positive ketone test occurred in 1 group V male, 2 group VII males, 3 group IV females and 3 group V females.

High dose recovery males showed a statistically significant increase in urine specific gravity (1.3%, $P<0.01$) and a statistically significant reduction in urine volume (60%, $P<0.01$). In the absence of similar changes detected for non-recovery animals at the end of the dosing period, these differences were considered to be incidental.

Effects in Organs

Organ Weights

High dose recovery males showed a statistically significant reduction in absolute brain (6%, $P<0.05$) and thymus weight (25%, $P<0.05$), whilst high dose recovery females showed a statistically significant increase (8%, $P<0.01$) in relative liver weight. In the absence of similar changes detected for non-recovery animals at the end of the dosing period, these differences were considered to be incidental.

Gross Pathology

Dark/blue discolouration of the majority of tissues in group V animals was detected. Dark staining was extended to the kidneys, lungs and gastrointestinal tract of group IV animals whilst dark kidneys and stained lungs were seen in group III animals. One group II male also showed stained lunges.

High dose recovery animals continued to show discolouration in the kidneys, liver and stomach following 14 days without treatment and males also showed dark testes.

Histopathology
The following treatment related changes were detected:

Liver: Hepatocyte enlargement was seen in some animals in all treated groups. The incidence of the condition was considered to be treatment related in group IV and V animals although there was no evidence of a well-defined dose response. One high dose recovery male also had hepatocyte enlargement. In view of the fact that hepatocyte enlargement is occasionally seen as a spontaneous condition among untreated animals, the appearance of the conditions in a few group II (2 female, 1 male) and group III (1 female, 1 male) animals cannot be reliably regarded as treatment related.

Kidneys: Proximal tubular basophilia and dilatation in the epithelium of proximal tubules were observed in 9/10 group V and to a lesser extent (5/10) group IV animals. Renal tubular dilatation was also seen in all group II males. There was evidence of significant regression of tubular dilation (observed in 1/10)_high dose recovery animals although tubular basophilia was observed following an additional 14 days without treatment.

Stomach: Agglomeration of gastric secretion, hyperplasia of mucous secreting cells and superficial mucosa; basophilia observed in group IV and group V animals were considered to be treatment related. Occasional instances of agglomeration seen among animals in group II (3/10) and group (III) (2/10) were not considered to be treatment related as the dose response was unconvincing and there were no associated degenerative stomach changes. There was evidence of significant regression of gastric changes among high dose recovery animals , although residual agglomeration of secretion in the gastric mucosa persisted following an additional 14 days without treatment.

Mesenteric Lymph Nodes: Foamy histiocytes were observed in group IV (8/10) and group V (10/10) animals. There was some evidence of regression of foamy histocytes (observed in 4/10) among high dose recovery animals.

Remarks – Results
Hepatocyte enlargement in commonly observed in the rodent liver following the administration of xenobiotics and, in the absence of associated inflammatory or degenerative changes is generally considered to be adaptive in nature.

CONCLUSION
The No Observed Adverse Effect Level (NO(A)EL) was established as 25 mg/kg bw/day in this study, based on the histopathological findings observed at the higher doses.

TEST FACILITY	Safepharm Laboratories (2004i)

7.8.1. Genotoxicity – bacteria

TEST SUBSTANCE	C-RB (85.5% purity)
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	S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver.
Concentration Range in Main Test	a) With metabolic activation: 50 – 5000 µg/plate b) Without metabolic activation: 50 - 5000 µg/plate
Vehicle	Distilled water

Remarks - Method: No significant protocol deviations. A correction factor was applied to account for the purity of the test material.

Test conducted in accordance with GLP standards.

RESULTS

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

Remarks - Results: No toxicity or precipitation was observed. A purple colour was observed at and above 150 µg/plate becoming darker with increasing concentration, although, this did not prevent the scoring if revertant colonies.

The test substance did not cause a marked increase in the number of revertants per plate of any of the bacterial strains either in the presence or absence of metabolic activation. The positive controls confirmed the activity of the activation system and the sensitivity of the test system.

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

TEST FACILITY: Safepharm Laboratories (2004j)

7.8.2 Genotoxicity – bacteria

TEST SUBSTANCE: C-RB (purity 100%)

METHOD: The test was carried out according to "Standards for Mutagenicity Test using Microorganisms" of "Occupational Safety and Health Law".
Pre incubation procedure

Species/Strain: *S. typhimurium*: TA1535, TA1537, TA98, TA100
E. coli: WP2uvrA (pKM101)

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

Concentration Range in Main Test: a) With metabolic activation: 312.5 - 5000 µg/plate
b) Without metabolic activation: 312.5 - 5000 µg/plate

Vehicle: Sterilized pure water

Remarks - Method: Deviations from OECD TG 471 Bacterial Reverse Mutation Test.

- Duplicate plating used instead of triplicate
- 2-Aminoanthracene used as the sole indicator of the efficacy of the S9-mix. In the absence of S9, 2-(-furyl)-3-(5-nitro-2-furyl) acrylamide was used as a positive control for strains TA100, WP2uvrA (pKM101) and TA98.

No other significant protocol deviations.

Test conducted in accordance with GLP standards.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*

Absent	5000 (TA1535, TA1537)*			
Test 1		5000 (TA1537)*	> 5000	negative
Test 2				
Present	5000 (TA1537)			
Test 1		> 5000	>5000	negative
Test 2				

* >50% decrease in the mean number of revertant colonies

Remarks - Results: The test substance did not cause a marked increase in the number of revertants per plate of any of the bacterial strains either in the presence or absence of metabolic activation. The positive controls confirmed the activity of the activation system and the sensitivity of the test system.

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

TEST FACILITY: Canon (2004a)

7.9. Genotoxicity – in vitro

TEST SUBSTANCE: C-RB (Purity 85.5%)

METHOD: OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.

Species/Cell Line: Chinese Hamster Lung cell
Metabolic Activation System: S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver.
Vehicle: Minimal Essential Media
Remarks - Method: No significant protocol deviations. A correction factor was applied to account for the purity of the test material in the main tests.

Test conducted in accordance with GLP standards.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 100, 200*, 400*, 800*, 1200, 1600	6 hours	24 hours
Test 2	0*, 5, 10, 20*, 40*, 60, 80*	24 hours	24 hours
Present			
Test 1	0*, 100, 200*, 400*, 600, 800*, 1200	6 hours	24 hours
Test 2	0*, 25, 50, 10*, 200*, 400*, 800	6 hours	24 hours

*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	312.5			
Test 1		400	>1600	negative
Test 2		>80	>80	Negative
Present	312.5			
Test 1		400	>1200	negative
Test 2		400	>800	negative

Remarks - Results: The test material did not induce any statistically significant increases in the frequency of cells with structural or numerical chromosome aberrations following either 24 hours continuous exposure in the absence

of activation or 6(18) hour pulse exposure in the presence or absence of activation. The test material did not induce any statistically significant increases in the number of polyploid cells at any dose level in either exposure group. The positive controls confirmed the sensitivity of the test system.

CONCLUSION The notified chemical was not clastogenic to Chinese Hamster Lung Cells treated in vitro under the conditions of the test.

TEST FACILITY Safepharm Laboratories (2004k)

7.9. Genotoxicity – in vitro

TEST SUBSTANCE C-RB (purity ~100%)

METHOD Chromosome Aberration Test – in house method
Species/Cell Line Chinese hamster lung cells
Metabolic Activation System S9 fraction from phenobarbital/benzoflavon induced rat liver.
Vehicle Sterile physiological saline
Remarks - Method No significant deviations from OECD TG 473.

Test conducted in accordance with GLP standards.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	39, 78, 156, 313, 625*, 1250*, 2500*, 5000	6 hour	24 hour
Test 2	625*, 1250*, 2500*	6 hour	24 hour
Test 3a	39, 78, 156*, 313*, 625*, 1250, 2500, 5000	24 hour	24 hour
Test 3b	39*, 78*, 156*, 313*, 625, 1250, 2500, 5000	48 hour	48 hour
Test 4a	221, 313*, 442*, 625*	24 hour	24 hour
Test 4b	110, 156*, 221*, 313*	48 hour	48 hour
Present			
Test 1	39, 78, 156, 313*, 625*, 1250*, 2500, 5000	6 hour	24 hour
Test 2	625, 880*, 1250*, 1770*, 2500	6 hour	24 hour

*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				
Test 1	2500	2500	> 2500	negative
Test 2	2500	2500	> 2500	negative
Test 3a	625	625	> 625	negative
Test 3b	313	313	> 313	negative
Test 4a	625	313	> 625	negative
Test 4b	313	221	> 313	negative
Present				
Test 1	2500	2500	> 1250	negative
Test 2	2500	1777	> 1777	negative

Remarks - Results In test 4b the number of metaphase cells scored at 313 µg/mL did not amount to two hundred. The lack of metaphase cells was due to cytotoxicity of the test substance.

The test material did not induce any statistically significant increases in the frequency of cells with structural or numerical chromosome

	aberrations following either short term treatment in the presence or absence of activation or continuous treatment in the absence of activation. The test material did not induce any statistically significant increases in the number of polyploid cells at any dose level in either exposure group. The positive controls confirmed the sensitivity of the test system.
CONCLUSION	The notified chemical was not clastogenic to chinese hamster lung cells treated in vitro under the conditions of the test.
TEST FACILITY	Canon (2004b)

7.10. Genotoxicity – in vivo

Not provided.

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

TEST SUBSTANCE: C-RB (purity 90.2%)

METHOD: OECD TG 301 C Ready Biodegradability: Modified MITI Test (I).

Method of testing the biodegradability of chemical substances by micro-organisms, in Testing methods for new chemicals substances, July 13, 1974, No 5 Planning and Coordination Bureau, Environment Agency.

Inoculum	Activated sludge – city plant
Exposure Period	28 days
Analytical Monitoring	BOD by Closed system oxygen consumption measurement – soda lime. TOC/DOC HPLC
Remarks - Method	Reference substance – aniline Concentration of suspended solids – 30 mg/L Treatments: water + test substance – 100 mg/L – vessel 1 sludge + test substance – 100 mg/L – vessel 2, 3 and 4 sludge + aniline – 100 mg/L – vessel 5 control blank – activated sludge only – vessel 6 Temperature measured daily – 25°C BOD was measured by data sampler and autorecorder. At termination of study the dissolved organic carbon, test substance concentration and pH were measured. Three peaks were detected in the HPLC analysis corresponding to three components of the test material. Test conducted in accordance with GLP standards.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	1	7	77
14	0	14	83
21	0	21	82
28	0	28	82

Percentage biodegradation via different methods – ONLY in test solutions (Vessels 2, 3 & 4)

Method	*% degradation*			
	Vessel 2	*Vessel 3*	*Vessel 4*	*Average*
BOD	0	0	0	0
TOC	4	0	3	2
HPLC*	0	0	0	0

*Based the combined area of the three peaks for the test material.

Remarks - Results: The HPLC analysis of one of the components indicated up to 51% loss of the compound from the solution. Repeating the study by acidifying the solution with HCl resulted no loss of the component from solution. The loss in the original test was attributed to salting out of the component rather than biodegradation. All test validation criteria were met. The reference substance (aniline) degraded by 82 % after 28 d confirming the suitability of the inoculum and test conditions.

CONCLUSION Under the study conditions the notified chemical was not readily biodegradable.

TEST FACILITY Kurume Laboratory (2004a)

8.1.2. Bioaccumulation

TEST SUBSTANCE C-RB (purity 90.2%)

METHOD OECD TG 305 Bioconcentration: Flow-through Fish Test.
EC Directive 98/73/EC C.13 Bioconcentration: Flow-Through Fish Test.

Method of testing the degree of accumulation of chemical substances in fish bodies, in Testing methods for new chemicals substances, July 13 1974 (Revised October 8 1998), No 5 Planning and Coordination Bureau, Environment Agency.

Species Carp (*Cyprinus carpio*)
Exposure Period Exposure: 28 days
Concentration Range
Nominal 2.0 mg/L (Level 1)
0.2 mg/L (Level 2)
Analytical Monitoring HPLC
Remarks - Method Continuous flow system. Test solutions were analysed once a week for a total of 8 times. Treated fish were analysed after 2, 4, 6 and 8 weeks of exposure (2 fish/analysis). There appears to have been no depuration phase. No abnormality in behaviour or appearance of the test fish was noted.

Test conducted in accordance with GLP standards.

RESULTS

Bioconcentration Factor

Component	*Level 1*	*Level 2*
1	≤ 4.0	≤ 39
2	≤ 2.1	≤ 21
3	≤ 0.77	≤ 7.7

CONCLUSION The above results indicate that none of the components of the notified chemical are likely to bioaccumulate.

TEST FACILITY Kurume Laboratory (2004b)

8.2. Ecotoxicological investigations

8.2.1. Acute toxicity to fish

TEST SUBSTANCE C-RB (85.5% purity)

METHOD OECD TG 203 Fish, Acute Toxicity Test – semi-static conditions.
EC Directive 92/69/EEC C.1 Acute Toxicity for Fish - semi-static conditions.

Species Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period 96 hours
Auxiliary Solvent None
Water Hardness 100 mg $CaCO_3$/L
Analytical Monitoring Spectrophotometry
Remarks – Method Based on range-finding tests it was determined that a limit test at 100 mg/L would be done. A measured amount of test substance was dissolved in water. The concentration and stability of the test solution was determined at 0, 24 and 96 hours.

The test vessels, each with 10 fish, were covered, maintained at 14ºC, exposed to a photoperiod of 16 dark/8 hours light and were aerated throughout the study. Temperature, pH and dissolved oxygen were recorded daily. Test solution was renewed daily. Observations were made at 3, 6, 24, 48, 72 and 96 hours with the fish being transferred to clean water for the observations.

Test conducted in accordance with GLP standards.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*6 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	-	10	0	0	0	0	0
100	90-97%	20	0	0	0	0	0

LC50 > 100 mg/L nominal at 96 hours.
NOEC 100 mg/L nominal at 96 hours.
Remarks – Results No sublethal effects were observed in the fish throughout the study. All environmental parameters stayed within acceptable ranges.

CONCLUSION Under the study conditions the notified chemical is very slightly toxic to fish (Mensink *et al.* 1995).

TEST FACILITY SafePharm Laboratories (2004l)

TEST SUBSTANCE C-RB (purity 90.2%)

METHOD Japanese Industrial Standard (JIS K 0102-1998-71.), "Testing Methods for industrial waste water, Acute toxicity test with fish"- Semi-static.
Species Orange–red killifish (*Oryzias laptipes*)
Exposure Period 96 h
Auxiliary Solvent None
Remarks – Method Water quality parameters of pH, water temperature, O_2 content remained within normal limits throughout the study. Test media was renewed every 24 h.

Test conducted in accordance with GLP standards.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*	
Nominal	*Actual*		*24 h*	*96 h*
Control		10	0	0
250		10	0	0
500		10	0	0
1000		10	0	3

LC50 > 1000 mg/L at 96 hours.
LOEC 1000 mg/L at 96 hours.
Remarks – Results

CONCLUSION Under the study conditions the notified chemical is very slightly toxic to fish (Mensink *et al.* 1995).

TEST FACILITY Kurume Laboratory (2004b)

8.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	C-RB (85.5% purity)
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test – static conditions. EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - static conditions.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	Spectrophotometry
Remarks - Method	Based on range-finding tests it was determined that a limit test at 100 mg/L would be done. The concentration and stability were verified by analysis at 0 and 48 hours. The solutions were clear throughout the study. The test vessels (4 replicates), each with 10 daphnia, were covered, maintained at 21°C, exposed to a photoperiod of 16 dark/8 hours light and were not aerated throughout the study. Temperature was recorded daily, while pH and dissolved oxygen were recorded at the start and end of the study. Observations were made at 24 and 48 hours. Two controls were done in parallel. Test conducted in accordance with GLP standards.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	-	20	0	0
100	98.5%	40	0	0

LC50	> 100 mg/L nominal at 48 hours
NOEC	100 mg/L nominal at 48 hours
Remarks - Results	No sublethal effects were observed in the daphnia throughout the study. All environmental parameters stayed within acceptable ranges.
CONCLUSION	Under the study conditions the notified chemical is very slightly toxic to aquatic invertebrates (Mensink *et al*. 1995).
TEST FACILITY	SafePharm Laboratories (2004m)

8.2.3. Algal growth inhibition test

TEST SUBSTANCE	C-RB (85.5% purity)
METHOD	OECD TG 201 Alga, Growth Inhibition Test. EC Directive 92/69/EEC C.3 Algal Inhibition Test.
Species	*Scenedesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 1.0, 3.2, 10, 32 and 100 mg/L Actual: 2.91, 8.91, 27.9, 94.4 and 294 mg/L at time 0 hours Actual: 3.26, 9.44, 28.8, 96.4 and 303 mg/L at time 72 hours
Auxiliary Solvent	None
Water Hardness	Not specified
Analytical Monitoring	Spectrophotometry
Remarks - Method	Two experimental methods were conducted in parallel to differentiate if the growth effects were due to toxicity or light intensity. Both used the

same test concentrations and a cell density of 2.11×10^4 cells/mL. Constant illumination and stirring, and temperature maintained at 24±1°C.

Experiment A: 3 replicates per concentration and 3 controls. Algae were exposed to test material in a flask enclosed from above by a petri dish containing culture medium only. Inhibition was due to a combination of toxicity and reduction in light intensity. The test solutions increased in pink colour to dark red intensity with increasing concentration.

Experiment B: 3 replicates per concentration and 3 controls. Algae were not exposed to the test material in the flasks, but the flasks were enclosed by petri dishes containing the culture media and the test material. Therefore, inhibition of algal growth was due to a reduction in light intensity alone.

Test solutions from experiment A at 0 and 72 hours were analysed to confirm concentration. It was found that the test concentrations ranged from 96 to 111% of the nominal concentration.

Test conducted in accordance with GLP standards.

RESULTS

Experiment A: Growth			*Experiment B: Growth*		
E_bC50 mg/L at 72 h	*E_rC50 mg/L at 72 h*	*NOEC mg/L*	*E_bC50 mg/L at 72 h*	*E_rC50 mg/L at 72 h*	*NOEC mg/L*
4.6	10	1.0	1.6	6.6	0.32

Remarks - Results

In experiment A, both the growth and biomass were affected by the presence of the test substance.
In experiment B, both the growth and biomass were affected by the reduction in light due to the presence of the test substance in the Petri dish.

In both experiments the cell concentration in the controls increased by a factor greater than 16 after 72 hours, which meets the validity criteria.

Since the inhibition of growth was slightly greater in Experiment B the growth inhibition is attributable to the reduction of light intensity due to the highly coloured nature of the test material rather than an intrinsic toxic properties of the test material.

CONCLUSION

Under the study conditions, the notified chemical is moderately toxic to algae (Mensink *et al.* 1995)

TEST FACILITY

SafePharm Laboratories (2004n)

8.2.4. Inhibition of microbial activity

TEST SUBSTANCE

C-RB (85.5% purity)

METHOD

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

Inoculum

Activated sewage sludge from a domestic STP

Exposure Period

3 hours

Concentration Range	Nominal: 1000 mg/L
Remarks – Method	From a range finding test, it was determined that only one test concentration needed to be used – 1000 mg/L. The study was conducted in triplicate. Vessels were aerated during the tests, and O_2 consumption rates were monitored. Temperature was maintained at 21°C. Duplicate controls were run in parallel. Reference substance – 3,5-dichlorophenol Rate of respiration was determined after 30 minutes and 3 hours contact. Total water hardness – 100 mg/L $CaCO_3$. Test conducted in accordance with GLP standards.
RESULTS	
EC50	> 1000 mg/L
NOEC	1000 mg/L
Remarks – Results	Reference substance 3 h EC50 = 13 mg/L The validity criteria for control respiration rates variation and reference material toxicity were satisfied. Environmental parameters were within acceptable ranges.
CONCLUSION	Under the study conditions the notified chemical is not toxic to micro-organisms.
TEST FACILITY	SafePharm Laboratories (2004o)

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

The environmental safety controls and use pattern for the notified chemical would indicate a limited potential for its release into the environment.

The notified chemical is readily soluble in water; however, aquatic release during use is considered unlikely and after drying the notified chemical is likely to be stable within an inert matrix on printed paper products. Waste paper may be disposed of directly to landfill with the notified chemical strongly bound to the paper. It is anticipated that prolonged residence in an active landfill environment would eventually degrade the compound. Incineration of waste paper will destroy the compound with the generation of water vapour and oxides of carbon, nitrogen and sulphur plus sodium salts.

Emptied ink cartridges containing a residue of notified chemical may be recycled or be sent to landfill for disposal. During recycling, the cartridges will be dismantled and the notified chemical will be washed off ultimately finding its way into onsite treatment works prior to discharge into the sewer. As a worst case, this would account for 50 kg of the notified chemical being discharged to sewer, assuming all cartridges were recycled and no removal occurs in onsite treatment works. In a landfill, the notified chemical is expected to be immobile, and eventually it will degrade through biotic and abiotic processes, and consequently, should not pose a significant exposure hazard to the environment.

Approximately 50% of the printed paper will enter the recycling process. During the recycling process, waste paper is repulped using a variety of alkaline, dispersing and wetting agents, water emulsifiable organic solvents and bleaches. These agents enhance fibre separation, toner detachment from the fibres, pulp brightness and the whiteness of the paper. Due to its high solubility, a predicted environmental concentration (PEC) can be estimated assuming 50% of the total imported notified chemical enters recycling, of which 50% (i.e. 25% of imported volume) will remain in the supernatant effluent discharged to sewer (assuming no WWTP attenuation).

Based on the releases to sewer from the recycling of cartridges and printed paper the predicted environmental concentration (PEC) of the notified chemical would be:

Amount in effluent entering sewer (from paper and cartridge recycling)	300 kg
Number of days	365
National population	20.1 million
Litres per person	200 L
PEC_{sewer}	0.30 μg/L.

A bioaccumulation study with carp found bioconcentration factors ≤ 39 for all components of the notified chemical indicating that the chemical is not likely to bioaccumulate.

9.1.2. Environment – effects assessment

The results of the ecotoxicological data indicate the notified chemical is harmful to algae, very slightly toxic to fish and daphnia and not toxic to micro-organisms. The most sensitive species are algae, where the E_bC50 of 4.6 mg/L. Acute results are available for 3 trophic levels, so it is applicable to apply an assessment factor of 100 to the most sensitive species (algae), thus the predicted no effect concentration (PNEC) is 46 μg/L.

9.1.3. Environment – risk characterisation

The worst case calculation indicates a PEC/PNEC ratio of 0.006 (0.3/46) for aquatic ecosystems via sewer discharge, indicating a low environmental risk.

The notified chemical is not likely to present a risk to the environment when it is stored, transported, used, recycled and disposed of in the proposed manner

9.2. Human health

9.2.1. Occupational health and safety – exposure assessment

The notified chemical will be imported in pre-packed sealed cartridges. During transport and storage, workers are unlikely to be exposed to the notified chemical except when cartridges are accidentally breached

Office workers and service technicians may be exposed to the notified chemical through dermal contact while changing spent cartridges, repairing printers or during normal printing processes. Ocular and inhalation exposure are not expected. Service technicians are expected to have the highest occupational exposure.

For all workers, due to the design of the cartridge dermal exposure is likely to occur only occasionally and to small quantities of the notified chemical at a concentration of <7% and as such dermal exposure is expected to be low. In addition, exposure is to be avoided because it would stain the skin and/or smudge the printed page. Exposure will be minimised by the use of disposable gloves by service personnel.

Exposure to the notified chemical on printed paper is low as the dye is bound to the paper matrix. Some intermittent exposure may occur if printing onto a non-absorbent substrate occurs and the ink does not dry for a time.

In the situation where a worker's entire palms of the hands are covered with the ink containing 7% notified chemical, exposure would be estimated as follows:

Product	*Concentration of notified polymer in product (mg/cm^3)[a]*	*Contact Area (cm^2)[b]*	*Thickness of Product Layer on Skin (cm)[b]*	*Dermal Absorption (%)[b]*	*Frequency of occurrence (per day)[c]*	*Exposure to notified polymer (mg/kg bw/day)[d]*
Ink	70	420	0.01	10	1	0.42

a) assuming ink has a specific gravity of 1.
b) data from European Chemical Bureau Technical Guidance Document on Risk Assessment (European Commission, 2003).
c) no frequency data is available. The occurrence of this scenario once per day is considered to be reasonable worst-case.
d) assuming 70kg body weight

9.2.2. Public health – exposure assessment

Similarly to office workers, the public may be intermittently exposed to the notified chemical when replacing spent cartridges, and during use of printers, however, as it is expected that the public will be using the printer less often than workers, exposure is also expected to be lower. Dermal exposure to ink containing the notified chemical could occur accidentally but would be avoided because skin staining and/or smudging of the printed page. Exposure to the notified chemical is considered to be limited to the dermal route.

Overall, exposure of the public is expected to be low, due to the small quantity of notified chemical in each cartridge, the design of the cartridge, the controlled release during printing, and intermittent nature of exposure.

9.2.3. Human health – effects assessment

Toxicokinetics, metabolism and distribution
The colouration observed in the urine and organs in the 28-day repeat dose study indicates that the notified chemical and/or its coloured metabolites are absorbed from the gastrointestinal tract

and distributed to the organs by blood. The notified chemical and/or its coloured metabolites are excreted via urine and faeces.

Due to the high molecular weight of the notified chemical dermal absorption of the notified chemical is expected to be negligible.

Acute toxicity
The notified chemical is considered to be of low acute oral and dermal toxicity. Although inhalation toxicity has not been established inhalation exposure is considered to be an unlikely route of exposure.

Irritation and Sensitisation
Based on the effects observed in a skin irritation study in rabbits, the notified chemical is considered to be slightly irritating to skin and it had caused discolouration of the skin during the first 3 days.

The notified chemical is not considered to be a sensitiser.

Based on the ocular lesions observed in an eye irritation study in rabbits the notified chemical would only be classified as slightly irritating. However, as irreversible colouration of the eyes occurred, the notified chemical is considered to cause serious damage to the eye.

Repeated Dose Toxicity
In a 28-day repeat dose oral toxicity study in rats, the following histopathological treatment related findings were observed in animals dosed at 300 mg/kg bw/day: hepatocyte enlargement (liver), proximal tubular basophilia and dilatation (kidneys), agglomeration of gastric secretion, hyperplasia of mucous secreting cells and superficial mucosal basophilia (stomach) and foamy histiocytes (mesenteric lymph nodes) with the renal changes also considered treatment related for animals dosed at 150 mg/kg bw/day. Based on these effects, the No Observed Effect Level (NOEL) was established as 25 mg/kg bw/day in this study.

Mutagenicity
The notified chemical was negative in two independent Ames tests and considered to be non-clastogenic in two independent *in vitro* chromosome aberration tests in chinese hamster lung cells.

Based on the available data, the notified chemical is classified as a hazardous substance in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC 2004).

9.2.4. Occupational health and safety – risk characterisation

The notified chemical as introduced (0.5 –7%) is unlikely to be a skin irritant but may be a slight to moderate eye irritant. However, as ocular exposure to the notified chemical is not expected during usual conditions of use, the risk of adverse local effects is considered to be low when cartridges are handled carefully.

A worst-case exposure of 0.42 mg/kg bw/day was estimated for workers. Based on a NOEL of 25 mg/kg bw/day, derived from a 28-day rat oral study the margin of exposure (MOE) is calculated as 59. Whilst this is less than the MOE considered acceptable to account for intra- and inter-species differences (100), this exposure is considered to be an overestimate (due to the large exposure area used and the negligible dermal absorption expected) and is unlikely to occur repeatedly. In addition, the estimated exposure is nearly 5000 times lower than the acute dermal toxicity LD50 (> 2000 mg/kg bw) and as such the risk of adverse systemic effects is considered to be low. As a precaution, service personnel are recommended to wear gloves during routine maintenance.

9.2.5. Public health – risk characterisation

As with office workers, ocular exposure to the notified chemical is not expected and hence the risk of eye irritation effects is considered to be low. Due the low and intermittent exposure

expected the risk of adverse systemic effects is also considered to be low.

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

10.1. Hazard classification

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

R41 Risk of serious eye damage

and

As a comparison only, the classification of 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***
Serious eye damage/eye irritation	1	Causes serious eye damage
Chronic hazards to the aquatic environment	2	Toxic to aquatic life with long lasting effects

10.2. 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.

10.3. Human health risk assessment

10.3.1. Occupational health and safety

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

10.3.2. Public health

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

11. MATERIAL SAFETY DATA SHEET

11.1. Material Safety Data Sheet

The MSDS of an ink product containing the notified chemical provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC 2003). It is published here as a matter of public record. The accuracy of the information on the MSDS remains the responsibility of the applicant.

11.2. Label

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

12. RECOMMENDATIONS

REGULATORY CONTROLS
Hazard Classification and Labelling

- The NOHSC Chemicals Standards Sub-committee should consider the following health hazard classification for the notified chemical:
  - R41 Risk of serious damage to eyes

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - Conc $\geq$ 10%: Xi; R41
  - 5% $\leq$ Conc < 10%: Xi; R36

- Use the following safety phrases for products/mixtures containing the notified chemical:
  - S25 Avoid contact with eyes

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced:
  - Avoid contact with eyes

- Service personnel should wear cotton or disposable gloves and ensure adequate ventilation is present when removing spent printer cartridges containing the notified polymer 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 NOHSC *Approved Criteria for Classifying Hazardous Substances*, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Environment

- The following control measures should be implemented by office staff and others to minimise environmental exposure during use of the notified chemical:
  - Use in controlled environment with no drains in the immediate area.

Disposal

- The notified chemical should be disposed of by incineration or to landfill in accordance with State/Territory waste disposal regulations. Paper products impregnated with ink containing the notified chemical may be incinerated, recycled or landfilled.

Emergency procedures

- Spills/release of the notified chemical should be handled by mechanically collecting spilled material (eg. sweeping dried material). Avoid raising dust. Do not allow material to contaminate ground, groundwater or waterways. Prevent product from entering drains or stormwater system.

12.1. Secondary notification

The Director of Chemicals Notification and Assessment must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(1) of the Act; if
- the importation volume of the notified chemical exceeds one tonne per annum
- if the concentration of notified chemical exceeds 10% in final product

or

(2) Under Section 64(2) of the Act:
- if any of the circumstances listed in the subsection arise.

The Director will then decide whether secondary notification is required.

No additional secondary notification conditions are stipulated.

13. BIBLIOGRAPHY

Canon (2004a) Report of Mutagenicity Test using Microorganisms (Report no. 697, 5 February 2004). Tokyo, Japan, Canon Inc. (Unpublished report provided by notifier).

Canon (2004b) C-RB Metaphase Analysis in CHL cells *in vitro* (Report no. C056, 9 February 2004). Tokyo, Japan, Canon Inc. (Unpublished report provided by notifier).

European Commission (2003) Technical Guidance Document on Risk Assessment in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No 1488/94 on Risk Assessment for Existing Substances and Directive 98/8/EC of the European Parliament and of the Council Concerning the Placing of Biocidal Products on the Market – Part I. Institute for Health and Consumer protection, European Chemicals Bureau, European Communities.

Kurume Laboratory (2004a) Biodegradation study of C-RB by microorganisms (Study number 14169, 19 March 2004). Japan, Kurume Laboratory Chemicals Evaluation and Research Institute. Sponsor: Nippon Kayaku Co. Ltd. (Unpublished report provided by the notifier).

Kurume Laboratory (2004b) Bioconcentration study of C-RB in carp (Study number 44195, 14 July 2004). Japan, Kurume Laboratory Chemicals Evaluation and Research Institute. Sponsor: Nippon Kayaku Co Ltd. (Unpublished report provided by the notifier).

Mensink BJWG, Montforts M, Wijkhuizen-Maslankiewicz L, Tibosch H and Linders JBHJ (1995) Manual for summarising and evaluating the environmental aspects of pesticides. National Institute of Public Health and Environmental Protection Bilthoven, The Netherlands.

NOHSC (1994) National Code of Practice for the Labelling of Workplace Substances [NOHSC:2012(1994)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

NOHSC (2003) National Code of Practice for the Preparation of Material Safety Data Sheets, 2nd edn [NOHSC:2011(2003)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

NOHSC (2004) Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

Safepharm Laboratories (2004a) C-RB: Determination of General Physico-chemical Properties (Project No. 345/672, 2 July 2004) Shardlow, UK, Safepharm Laboratories Ltd. Sponsor: Canon Inc, Japan. (Unpublished report provided by notifier).

Safepharm Laboratories (2004b) C-RB: Determination of Hazardous Physico-chemical Properties (Project No. 345/673, 12 July 2004) Shardlow, UK, Safepharm Laboratories Ltd. Sponsor: Canon Inc, Japan. (Unpublished report provided by notifier).

Safepharm Laboratories (2004c) C-RB: Determination of Flammability (solids) (Project No. 345/674, 24 February 2004) Shardlow, UK, Safepharm Laboratories Ltd. Sponsor: Canon Inc, Japan. (Unpublished report provided by notifier).

Safepharm Laboratories (2004d) C-RB: Acute Oral Toxicity in the Rat – Acute Toxic Class Method (Project No. 345/675, 2 April 2004) Shardlow, UK, Safepharm Laboratories Ltd. Sponsor: Canon Inc, Japan. (Unpublished report provided by notifier).

Safepharm Laboratories (2004e) C-RB: Acute Dermal Toxicity (Limit Test) in the Rat (Project No. 345/676, 16 June 2004) Shardlow, UK, Safepharm Laboratories Ltd. Sponsor: Canon Inc, Japan. (Unpublished report provided by notifier).

Safepharm Laboratories (2004f) C-RB: Acute Dermal Irritation in the Rabbit (Project No. 345/677, 2 April 2004) Shardlow, UK, Safepharm Laboratories Ltd. Sponsor: Canon Inc, Japan. (Unpublished report provided by notifier).

Safepharm Laboratories (2004g) C-RB: Acute Eye Irritation in the Rabbit (Project No. 345/678, 29 July 2004) Shardlow, UK, Safepharm Laboratories Ltd. Sponsor: Canon Inc, Japan. (Unpublished report provided by notifier).

Safepharm Laboratories (2004h) C-RB: Local Lymph Node Assay in the Mouse (Project No. 345/679, 2 April 2004) Shardlow, UK, Safepharm Laboratories Ltd. Sponsor: Canon Inc, Japan. (Unpublished report provided by notifier).

Safepharm Laboratories (2004i) C-RB: Twenty-eight day Repeated Dose Oral (Gavage) Toxicity Study in the Rat (Project No. 345/680, 9 September 2004) Shardlow, UK, Safepharm Laboratories Ltd. Sponsor: Canon Inc, Japan. (Unpublished report provided by notifier).

Safepharm Laboratories (2004j) C-RB: Reverse Mutation Assay "Ames Test" using *Salmonella Typhimurium* and *Escherichia Coli* (Project No. 345/682, 5 April 2004) Shardlow, UK, Safepharm Laboratories Ltd. Sponsor: Canon Inc, Japan. (Unpublished report provided by notifier).

Safepharm Laboratories (2004k) C-RB: Chromosome Aberration Test in CHL cells *in vitro* (Project No. 345/681, 8 July 2004) Shardlow, UK, Safepharm Laboratories Ltd. Sponsor: Canon Inc, Japan. (Unpublished report provided by notifier).

SafePharm Laboratories (2004l). C-RB: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) (Project No 345/683, 6 July 2004). Shardlow, UK, Safepharm Laboratories Ltd. Sponsor: Canon Inc, Japan. (Unpublished report provided by notifier).

SafePharm Laboratories (2004m). C-RB: Acute Toxicity to *Daphnia magna* (Project No 345/684, 5 July 2004). Shardlow, UK, Safepharm Laboratories Ltd. Sponsor: Canon Inc, Japan. (Unpublished report provided by notifier).

SafePharm Laboratories (2004n). C-RB: Algal Inhibition Test (Project No 345/686, 8 July 2004). Shardlow, UK, Safepharm Laboratories Ltd. Sponsor: Canon Inc, Japan. (Unpublished report provided by notifier).

SafePharm Laboratories (2004o). C-RB: Assessment of the inhibitory effect on the respiration of activated sewage sludge (Project No 345/683, 6 July 2004). Shardlow, UK, Safepharm Laboratories Ltd. Sponsor: Canon Inc, Japan. (Unpublished report provided by notifier).

United Nations (2003) Globally Harmonised System of Classification and Labelling of Chemicals (GHS). United Nations Economic Commission for Europe (UN/ECE), New York and Geneva.