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20 September 2005

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

C-193

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Director
NICNAS

TABLE OF CONTENTS

FULL PUBLIC REPORT

C-193

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Canon Australia Pty Ltd (ABN: 66 005 002 951)
1 Thomas Holt Drive
NORTH RYDE NSW 2113

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)

Data items and details claimed exempt from publication:
chemical name, other name, CAS number, molecular and structural formulae, molecular weight, spectral data, purity, non hazardous impurities, 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, flashpoint, explosive properties, reactivity, acute inhalation toxicity, induction of germ cell damage, daphnia acute immobilisation/reproduction, and bioaccumulation.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
LVC Permit No. 640

NOTIFICATION IN OTHER COUNTRIES
UK (2004), US (2004), Canada, Ontario state (2004), Japan (2005) and Philippines (Small Quantity Importation Clearance 2004)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
C-193,
JPD Magenta C-193,
JPD Magenta C-193 Liquid,
Substituted anthraquinone derivative.

METHODS OF DETECTION AND DETERMINATION

METHOD	Ultraviolet/Visible light (UV/VIS) spectroscopy, Infrared (IR) spectroscopy, ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy, and High Performance Liquid Chromatography (HPLC).
Remarks	Reference spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
<90%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None.

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (> 1% by weight)
There are three non hazardous impurities.

ADDITIVES/ADJUVANTS
None.

4. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported in ready to use sealed inkjet printing cartridges.

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

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

USE
The notified chemical is a component (<10%) of inkjet printer ink.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, transport and storage

PORT OF ENTRY
Sydney.

IDENTITY OF MANUFACTURER/RECIPIENTS
Canon Australia Pty Ltd
1 Thomas Holt Drive
NORTH RYDE NSW 2113

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a component of a ready to use sealed 2-150 mL inkjet cartridges. The cartridges will be transported by road.

5.2. Operation description

No reformulation or repackaging of the product occurs in Australia. The product is delivered to the end-user as it is imported into Australia. The sealed inkjet cartridges will be handled by service technicians, office workers, and members of the public when replacing the spent cartridges in the printer.

5.3. Occupational exposure

Number and Category of Workers

Category of Worker	*Number*	*Exposure Duration*	*Exposure Frequency*
Importation/Waterside	50	<8 hours/day	10-50 days/year
Storage and Transport	15	<8 hours/day	10-50 days/year
Office worker/consumer	2000000	10 seconds /day	2 days/year
Service Technicians	100	1 hour/day	170 days/year

Exposure Details
The notified chemical is contained in sealed cartridges. Any single cartridge would typically be approximately <10% notified chemical.

Office workers and customer service engineers will replace spent ink cartridges. Replacement of printing cartridges involves removal of the old printing cartridge from the printing machine and directly loading the new cartridge. These workers may have dermal contact with very small quantities of the notified chemical if they touch the print heads while replacing cartridges, or on handling printed paper or other inkjet printable substrates, particularly if the paper is handled before the ink is adequately dried or if printing to a non-absorbent substrate occurs by error. After the ink is dry the notified chemical is bound to the paper or other inkjet printable matrix and is not expected to be readily bioavailable. Dermal and possible ocular exposure could occur when handling faulty or ruptured cartridges.

5.4. Release

RELEASE OF CHEMICAL AT SITE

Printer ink is imported in ready-to-use cartridges of 2 to 150 mL (containing < 10% 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 is 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 cartridges will be changed by the office staff and general public. 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 chemical 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 will most likely be disposed of to landfill.

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

5.5. Disposal

The total import volume of the notified chemical will ultimately be disposed as normal office/domestic waste that will end up in either landfill or be incinerated or recycling. 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 150 kg of notified chemical annually.

5.6. Public exposure

Limited exposure may occur while changing inkjet cartridges, however this will be relatively infrequent and should only result in very limited exposure to small quantities of the notified chemical.

After the ink is dry the notified chemical is bound to the paper or other inkjet printable matrix and is not expected to be readily bioavailable. The public may be dermally exposed to the notified chemical, while handling printed paper or other substrates, where the ink is only partially dried. The manufacturer has estimated public exposure to the notified chemical via the printed substrate. One kilogram of pure dye would be expected to produce several million sheets of A4 coloured text or graphics in the case of a paper substrate. Under worst-case conditions, each piece of A4 paper can be assumed to incorporate 1 mg of notified chemical. Based on a 50% transfer on contact when handling printed paper (assuming only partially dry ink), and the relative areas of finger ends and paper size, it is estimated that potential removal is <1% of the applied ink in each event.

6. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20ºC and 101.3 kPa A dark red powder.

Melting Point >360ºC

METHOD OECD TG 102 Melting Point/Melting Range.
EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.

Remarks The test substance was examined using differential scanning calorimetry by heating from 30ºC to 400ºC at a heating rate of 5ºC/min. Loss of volatiles occurred at 108.5ºC and 109.2ºC and possible gradual decomposition occurred at 320.6 and 321.4ºC.

The melting temperature of the test substance was determined to be greater than 360ºC at 99.09 to 101.05 kPa. The melting temperature was calculated to be 349.85ºC using an adaptation of the Joback method.

TEST FACILITY SafePharm Laboratories (2004a)

Boiling Point Not determined.

Density 1680 kg/m^3 at 20ºC

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

Remarks Testing carried out using a gas comparison pycnometer.

TEST FACILITY SafePharm Laboratories (2004a)

Vapour Pressure 1.9 x 10^{-14} kPa at 25ºC

METHOD OECD TG 104 Vapour Pressure.
EC Directive 92/69/EEC A.4 Vapour Pressure.
US, EPA OPPTS Method 830.7950 Vapour Pressure

Remarks The vapour pressure balance method was used over an approximately 47 1/4-hour period in the temperature range of 230 to 240ºC.

TEST FACILITY SafePharm Laboratories (2004b)

Water Solubility 39.9-44.8% (w/w) at 20±0.5ºC

METHOD OECD TG 105 Water Solubility.
EC Directive 92/69/EEC A.6 Water Solubility.
USA, EPA OPPTS Method 830.7840 Water Solubility: Column Elution Method; Shake Flask Method

Remarks Flask Method.

Method variation: Due to high indeterminable saturation levels, it was not possible to prepare samples at five times the saturation level as recommended in the guidelines. Analysis could not be performed because of the unfilterable mixture produced at high solubility levels. Therefore the water solubility was based on visual inspection. Measured amounts of the test substance were added to double distilled water and shaken for 72 hours at 30ºC, allowed to stand for 24 hours at 20ºC and then visually assessed.

This result indicates that the notified chemical would be very readily soluble in water (Mensink, 1995).

TEST FACILITY SafePharm Laboratories (2004a)

Hydrolysis as a Function of pH Hydrolytically stable.

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

Remarks The nominal concentration of the test solutions was 0.2 g/L. Aliquots (in duplicate) of sample solutions were taken at various times (0. 2.4, 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.

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 <-3.69 at 20.5±0.5°C

METHOD OECD TG 107 Partition Coefficient (n-octanol/water).
EC Directive 92/69/EEC A.8 Partition Coefficient.

Remarks Shake Flask Method.

Measured amounts of the test substance and water saturated n-octanol were shaken by inversion at 22°C for 5 minutes. Aliquots of both the water and n-octanol phases were taken for analysis by HPLC. Methanol was used to dilute the organic samples by a factor of 2 and the aqueous phase was diluted using reverse osmosis water by a factor of 50.

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 (4.001 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 pKa range –2.13 to 5.61

Remarks Standard test methods are not applicable to substances with multiple pKas, so the computer modelling software ACD/pKa 8.03 was used. Nine reference groups related to functional groups in the notified chemical were entered into the model and the predicted pKa values obtained. The notified chemical has strong acid functionalities, and will remain ionised throughout the environmental pH range of 4 to 9.

Particle Size

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

Range (μm)	*Mass (%)*
<100	45.3 (sieve method)
<10	1.76 (cascade impactor)

Remarks There were too few particles of a size less than 10 μm to allow an accurate assessment of the mass median aerodynamic diameter. For the definitive test the sample container was rolled for approximately 10 minutes and samples were taken from the top, middle and bottom to ensure representative sampling.

The inspirable fraction was 45.3% and the respirable fraction was 1.76%.

TEST FACILITY SafePharm Laboratories (2004a)

Flash Point Not applicable.

Remarks Notified chemical is a high melting point solid.

Flammability Limits Not highly flammable.

METHOD EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks The flammability of the notified chemical was determined by measuring the burning rate of the notified chemical prepared as a pile of set dimensions. The pile failed to ignite during the two minutes the flame was applied. The notified chemical has been determined to be not highly flammable as it failed to ignite in the preliminary screening test.
TEST FACILITY SafePharm Laboratories (2004c)

Autoignition Temperature 324°C

METHOD 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks The test material was heated from ambient temperature to 400°C at rate of 0.5°C/min in an oven and the relative self ignition temperature was determined.
TEST FACILITY SafePharm (2004b)

Explosive Properties None.

Remarks The structure of the notified chemical as examined for groups that would imply that it could possess explosive properties. There are no chemical groups that would imply explosive properties, therefore the result has been predicted negative.
TEST FACILITY SafePharm Laboratories (2004b)

Oxidising Properties None.

Remarks The structure of the notified chemical was examined for groups that would imply that it could possess oxidising properties. There were no chemical groups that would imply oxidising properties, and therefore the result has been predicted negative.
TEST FACILITY SafePharm Laboratories (2004b)

Reactivity

Remarks The notified chemical is not expected to be reactive under normal environmental conditions.

Surface Tension	72.5 mN/m at 19.0±0.5ºC
METHOD	OECD TG 115 Surface Tension of Aqueous Solutions. EC Directive 92/69/EEC A.5 Surface Tension.
Remarks	Test concentration – 1.04 g/L. Measurements were taken at intervals until a constant reading was obtained using an interfacial tension balance. This result indicates that the notified chemical is not surface active.
TEST FACILITY	Safepharm Laboratories (2004b)

7. TOXICOLOGICAL INVESTIGATIONS

Endpoint and Result	*Assessment Conclusion*
Rat, acute oral LD50 > 2469 mg/kg bw (>2000 mg active ingredient/kg bw)	low toxicity
Rat, acute dermal LD50 > 2469 mg/kg bw (>2000 mg active ingredient/kg bw)	low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – local lymph node assay	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOEL = 150 mg/kg bw/day
Genotoxicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro chromosomal aberration in mammalian cells	non genotoxic

7.1. Acute toxicity – oral

TEST SUBSTANCE — C-193 (Batch Number MB310001)

METHOD
- Species/Strain: Rat/Sprague-Dawley CD
- Vehicle: Distilled water.
- Remarks - Method: No significant protocol deviation.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	3 females	2469*	0
II	3 females	2469*	0

*=Equivalent to 2000 mg active ingredient /kg bw

- LD50: >2469 mg/kg bw (> 2000 mg active ingredient/kg bw)
- Signs of Toxicity: There were no signs of systemic toxicity. All animals showed the expected bodyweight gains over the study period.
- Effects in Organs: No abnormalities were noted at necroscopy.
- Remarks - Results: Red-coloured staining of urine and faeces was noted in all animals during the study. Red coloured staining of the bedding was also noted. Animals appeared normal three to five days after dosing.

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-193 (Batch Number MB310001)

METHOD
- Species/Strain: Rat/Sprague-Dawley CD
- Vehicle: Arachis oil
- Type of dressing: Semi-occlusive.
- Remarks - Method: No significant protocol deviation.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*

I	5/sex	2469*	0

*=Equivalent to 2000 mg active ingredient /kg bw

LD50	>2469 mg/kg bw (>2000 mg active ingredient /kg bw)
Signs of Toxicity - Local	Purple coloured staining was noted at all treatment sites one day after dosing. No staining was observed in any animal two days after dosing. There were no signs of dermal irritation.
Signs of Toxicity - Systemic	There were no signs of systemic toxicity. All animals showed the expected body weight gains over the period of the study.
Effects in Organs	No abnormalities were noted at necroscopy.
Remarks - Results	None.

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

TEST FACILITY — Safepharm Laboratories (2004e)

7.3. Irritation – skin

TEST SUBSTANCE — C-193 (Batch Number MB310001)

METHOD — OECD TG 404 Acute Dermal Irritation/Corrosion.

Species/Strain	Rabbit/New Zealand White
Number of Animals	Three.
Vehicle	Distilled water.
Observation Period	7 days
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviation.

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	0	1	72 hrs	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

Pink coloured staining of the skin was noted at all treatment sites. However this did not affect the evaluation of the skin reaction.

Very slight erythema was noted at two treated sites one hour after patch removal. The very slight erythema persisted at the 24 hours observation at one treated site and at the 48 and 72 hours observation in the other treated site. All treated sites appeared normal at the 7-day observation. No erythema was noted at the third site during the study.

No oedema was observed at any treated sites during the study.

CONCLUSION — The notified chemical is slightly irritating to the skin.

TEST FACILITY — SafePharm Laboratories (2004f)

7.4. Irritation – eye

TEST SUBSTANCE C-193 (Batch MB310001)

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.
Observation Period 7 days
Remarks – Method No significant protocol deviation.

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	1	1	2	48 hrs	0
Conjunctiva: chemosis	0.33	0.33	0.33	2	24 hrs	0
Conjunctiva: discharge	0.33	0.67	0.67	2	48 hrs	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 Purple-coloured staining of the fur around the treated eye was noted in all treated eyes during the study.

Purple coloured staining of the conjunctival membrane and/or the cornea was noted in all treated eyes during the study.

No iridial effects were noted during the study.

Moderate conjunctival irritation was noted in all treated eyes one hour after treatment. Minimal to moderate conjunctival irritation was noted at the 24-hour observation. At the 48-hour observation minimal conjunctival irritation was noted.

CONCLUSION The notified chemical is slightly irritating to the eye

TEST FACILITY SafePharm Laboratories (2004g)

7.5 Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE C-193 (Batch Number MB310001)

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay.
Species/Strain Mouse/CBA/Ca
Vehicle Dimethyl sulphoxide.
Remarks - Method No significant protocol deviation.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	550.07	N/A

1	648.73	1.18
2.5	640.89	1.17
5	700.81	1.27
Positive Control		
5	-	1.76
10	-	2.78
25	-	5.06

Remarks - Results: The positive control substance exhibited a stimulation index in excess of 3, indicating the sensitivity of the study.

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

TEST FACILITY: SafePharm Laboratories (2004h)

7.6. Repeat dose toxicity

TEST SUBSTANCE: C193 (Lot Number MB310001)

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
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 deviation.

RESULTS

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

Mortality and Time to Death
No treatment related deaths were observed during the study. One male from the 25 mg/kg bw/day group was killed *in extremis* on day 25 following a physical injury to the eye.

Clinical Observations
No clinically observable signs of toxicity were detected.

Dark faeces and magenta coloured staining was detected on cage tray liners from animals of both sexes treated at doses of 150, 300, 1000 mg/kg bw/day from day 2 onwards and was evident in the high dose recovery animals for two days following the cessation of treatment. These findings were associated with the oral administration of a coloured material and not indicative of toxicity.

Functional observations
No treatment related changes were observed in the behavioural parameters and functional performance parameters measured. No treatment related changes in sensory reactivity were noted.

Body weight
A statistically significant ($p<0.05$) reduction in body weight gain was observed in 1000 mg/kg bw/day recovery males during the recovery period when compared to the control recovery males. The intergroup difference was considered incidental as similar effects were absent during the treatment period. No other effects on body weight and body weight gains were observed.

Food consumption
There was no adverse effect on food consumption during the study. Food efficiency in the treated animals was similar to that of the controls.

Water consumption
Base on visual inspections of water bottles revealed no intergroup differences in the water consumption.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Blood Chemistry
A statistically significant ($p<0.05$) reduction in plasma triglycerides was detected in all male treatment groups. This was considered to be attributable to the higher than expected control group values and hence not of toxicological significance. A statistically significant ($p<0.05$) increase in sodium levels was detected in 1000 mg/kg bw/day recovery females. In the absence of an increase in sodium levels during the treatment period, the increase was not of toxicological significance. No other effects on blood chemistry were observed.

Haematology
A statistically significant ($p<0.05$) intergroup increase in haemoglobin and haematocrit was detected in the 1000 mg/kg bw/day recovery males. Similar responses were not observed during the treatment period and hence the effect was not to be of toxicological significance. No other effects on haematological parameters were observed.

Urinalysis
Both male and female animals treated at 150, 300 or 1000 mg/kg bw/day displayed pink coloured urine, which resulted in difficulties in the analysis of some urine samples. No other statistically significant intergroup differences were observed.

Effects in Organs
Organ weight
Males and females in the 1000 mg/kg bw/day recovery groups showed reductions in the relative liver and heart weights, respectively. No other significant weights were observed.

Necropsy
Males and females treated at 1000 mg/kg bw/day showed red discolouration of the kidneys and the presence of red content in the large intestine. Complete regression was observed in the 1000 mg/kg bw/day recovery animals. These findings were observed as a consequence of the oral administration of coloured test material. One female treated at 25 mg/kg bw/day showed a hard and dark caudate lobe of the liver.

Histopathology
Kidneys
Two males treated at 1000 mg/kg bw/day demonstrated renal tubular basophilia/degeneration. An increase in the incidence of renal tubular basophilia/degeneration in the 1000 mg/kg bw/day males after the completion of the recovery period was also observed. Isolated groups of basophilic tubules are frequently encountered in the renal cortex of laboratory maintained rats and do not have pathological significance at the severities or frequencies reported in this study. Similarly, the focal corticomedullary mineralisation observed is a commonly observed background condition amongst female rats. Hydronephrosis, was reported in one rat, but this is widely considered a congenital condition and was not considered of toxicological significance.

An accumulation of pigment in the proximal tubular epithelium was observed in males and females treated at 300 mg/kg bw/day. No further degenerative changes were observed.

Stomach
Agglomeration of secretion, mucous cell hyperplasia, mucosal basophilia and acanthosis and hyperkeratosis of the limiting ridge were observed in the stomachs of males and females treated at 1000 mg/kg bw/day. There was evidence of complete regression of the treatment related gastric changes in the recovery 1000 mg/kg

bw/day animals. Agglomeration of secretion occasionally associated with mucous cell hyperplasia were seen in males and females treated at 300 mg/kg bw/day. Agglomeration of secretion was observed in the stomach tissue of animals at 25 and 150 mg/kg bw/day.

Heart
Focal myocarditis was observed in several control and treated animals. The severity of the condition was never greater than minimal, or one or two foci. Focal myocarditis is a common background entity in laboratory maintained rats.

Liver
Scattered mononuclear cell foci were observed in the majority of animals examined. Such observations are common in the rodent liver and at the severities observed are not considered indicative of any adverse condition.

Spleen
Minimal extramedullary haemopoiesis was observed in animals in the non recovery control and 1000 mg/kg bw/day treatment groups. Extramedullary haemopoiesis is a normal background condition in rats spleens and the severities observed were considered to be within normal limits.

Thyroid
Minimal follicular cell hypertrophy was observed in the control and 1000 mg/kg bw/day treatment groups.Follicular cell hypertrophy is a highly variable condition and frequently observed as a spontaneous entity in control rats. The distribution of the condition in this study was not indicative of a treatment related effect.

Lungs
A minimal severity of bronchus associated lymphoid tissue was reported for all animals examined in this study and was not an indicator of respiratory disease. Minor severity and low incidence of focal pneumonitis and accumulation of macrophages in the alveoli were observed in in one 1000 mg/kg bw/day female and one control male, respectively. These changes are commonly observed pulmonary changes in laboratory rats of the age of those in the study and not suggestive of significant respiratory disease.

Bone marrow
Minimal to slight adipose infiltration of marrow was observed in the control and 1000 mg/kg bw/day treatment group. No difference was observed between the control and treated groups.

Uterus
Dilation of the uterine horns was observed in the control and 1000 mg/kg bw/day groups. This is a commonly observed cyclical condition in laboratory maintained rats.

Remarks – Results
The principle clinical observations made during the study were the presence of dark faeces and magenta coloured staining on the cage tray-liners for animals treated at 150, 300 and 1000 mg/kg bw/day together with incidents of the test material staining fur. Such findings are commonly observed following the administration of a coloured material and represents normal excretion and grooming resulting in the dispersal of the test material on the external body surface. Postmortem investigation revealed discolouration of the kidneys and coloured content of the large intestine at 1000 mg/kg bw/day. Histopathological investigations revealed toxicologically significant renal tubular basophilia/degeneration in two males treated at 1000 mg/kg bw/day, with an increase incidence observed after the recovery period.

The changes observed in the stomach consisted of mucous cell hyperplasia, mucosal basophilia, acanthosis, hyperkeratosis, and agglomeration of secretion at 1000 and 300 mg/kg bw/day. Complete regression was observed for the recovery animals.

Agglomeration of secretion of the mucosa were observed at 150 and 25 mg/kg bw/day but these were not accompanied by degenerative changes and therefore considered adaptive rather than adverse effects.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 150 mg/kg bw/day in this study, based on histopathological changes in the kidney and stomach at higher doses.

TEST FACILITY Safepharm Laboratories (2004i)

7.7. Genotoxicity – bacteria

TEST SUBSTANCE C193 (Lot Number MB310001)

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*: WP2*uvr*A
Metabolic Activation System	Phenobarbitone/β-naphthoflavone induced rat liver S9 fraction.
Concentration Range in Main Test	a) With metabolic activation: 50, 150, 500, 1500, 5000 µg/plate b) Without metabolic activation: 50, 150, 500, 1500, 5000 µg/plate
Vehicle	Distilled water
Remarks – Method	No significant protocol deviation.

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	-
Test 2		>5000	>5000	-
Present				
Test 1	>5000	>5000	>5000	-
Test 2		>5000	>5000	-

Remarks – Results

To select appropriate dose levels for use in the main test, a preliminary assay was carried out to determine the toxicity of the test material to TA100 and WP2*uvr*A. The concentrations tested were 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500, 5000 µg/plate.

The test material caused no visible reduction in the growth of the bacterial background lawn at any dose level. Therefore the test material was tested up to the maximum recommended dose level of 5000 µg/plate. A pink colour was observed from 50 µg/plate, however this did not prevent the scoring of revertant colonies. No precipitation of the test material was observed on the plates at any of the doses tested in either the presence or absence of S9.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains at any dose level either with or without metabolic activation.

All of the positive control chemicals used in the test induced marked increase in the frequency of revertant colonies thus confirming the activity of the S9 mix and the utility of the bacterial strains to detect chemical mutagenesis. Negative controls were within historical limits.

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

TEST FACILITY	SafePharm Laboratories (2004j)

7.8. Genotoxicity – bacteria

TEST SUBSTANCE	C193 (Lot Number: MC-2)
METHOD	Standards for Mutagenicity Test using Microorganisms of Occupational Safety and Health Law (Japan) Pre-incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2*uvr*A (pKM101)
Metabolic Activation System	Phenobarbitone/5,6-Benzoflavone induced rat liver S9 fraction.
Concentration Range in Main Test	a) With metabolic activation: 0.1953, 0.7813, 3.125, 12.5, 50 µL/plate b) Without metabolic activation: 0.1953, 0.7813, 3.125, 12.5, 50 µL/plate
Vehicle	Sterilised pure water
Remarks – Method	No significant protocol deviation.

RESULTS

Metabolic Activation	*Test Substance Concentration (µL/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	>50	>50	>50	-
Present				
Test 1	>50	>50	>50	-

Remarks – Results

To select appropriate dose levels for use in the main test, a dose range finding study was carried out to determine the toxicity of the test material to TA1535, TA1537, TA98, TA100, WP2*uvr*A (pKM101). The concentrations tested were 0.1953, 0.7813, 3.125, 12.5, 50 µL/plate. The test material caused no visible reduction in the growth of the bacterial background lawn or precipitation at any dose level.

Therefore the test material was tested up to the maximum dose level of 50 µL/plate in the main test. No precipitation of the test material or inhibition of bacterial growth was observed on the plates at any of the doses tested in either the presence or absence of S9.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains at any dose level either with or without metabolic activation.

All of the positive control chemicals used in the test induced marked increase in the frequency of revertant colonies thus confirming, the activity of the S9 mix and the utility of the bacterial strains to detect chemical mutagenesis. Negative controls were within historical limits.

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-193 (Batch number MB310001)
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	Chinese Hamster Lung (CHL)
Metabolic Activation System	Phenobarbitone/β-naphthoflavone induced rat liver S9 fraction.
Vehicle	Minimal Essential Media
Remarks - Method	No significant protocol deviation.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 156.25, 312.5, 625, 1250*, 2500*, 5000*	6 hours	24 hours
Test 2	0*, 78.1, 156.25*, 312.5*, 625*, 1250, 1875	24 hours	24 hours
Present			
Test 1	0*, 156.25, 312.5, 625, 1250*, 2500*, 5000*	6 hours	24 hours
Test 2	0*, 156.25, 312.5, 625, 1250*, 2500*, 5000*	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				
Test 1	>5000	>5000	>5000	-
Test 2				
Present				
Test 1	>5000	>5000	>5000	-
Test 2	>1250			

Remarks - Results

A preliminary toxicity test was performed on cell cultures using 24-hour continuous exposure time without metabolic activation and a 6-hour exposure period (both with and without metabolic activation) followed by a 18-hour recovery period in treatment-free media. The dose range used in the Cell Growth Inhibition Test was 19.53 to 5000 μg/mL. The 6-hour exposure induced a cell-cycle synchronisation, as demonstrated by dose related increases in mitotic index which was taken to be evidence of toxicity. No precipitation of the test material occurred at any dose level. There was a small dose related reduction in cell counts in each of the exposure groups. Microscopic assessments of the slides showed that metaphase cell were present up to 5000 μg/mL. In the 24 hour continuous exposure group the maximum dose with metaphases present was found to be 1250 μg/mL.

Experiment 1.
There were small dose related reductions in cell counts observed in both groups. The test material induced cell-cycle synchronisation as had been observed in the Cell Growth Inhibition Test. The data showed a dose related increase in the mitotic index, however no mitotic inhibition was observed. It was considered that toxicity related effects were seen up to the maximum recommended dose level of 5000 μg/mL. No precipitation of the test material was observed at any dose for both exposure groups. The maximum dose selected for metaphase analysis was therefore the maximum recommended dose level 5000 μg/mL for both exposure groups. The vehicle control groups had frequencies of cells with chromosome aberrations within the expected range. The positive control induced statistically significant increases in frequency in cells with aberrations. The metabolic activation system was therefore satisfactory

and the test method was operating as expected.

The test material did not induce any statistically significant increases in frequency of cells with aberrations in either the presence or absence of metabolic activation.

The test material did not induce any significant increase in the number of polypoid cells at any dose level in either exposure group.

Experiment 2.
The measured cell counts and mitotic index were similar to those observed in the Cell Growth Inhibition Test and therefore it was considered the test material had induced the expected level of toxicity. No reduction in cell count was observed in the 24 hour group but a small dose related reduction in cell counts was observed in the 6 hours exposure with 2% S9. The mitotic index data indicated that at 312.5 μg/mL, there was a 50% inhibition of growth and a toxicity related cell cycle synchronisation, as evidenced by an increase in mitotic index, at 625 μg/mL in the 24 hours exposure group. The toxicity observed in the S9 exposure group was similar to that observed in Experiment 1, with a dose related increase in mitotic index up to 5000 μg/mL. No precipitation of the test material was observed at the end of the exposure period. The maximum dose selected for metaphase analysis was the maximum recommended dose level 5000 μg/mL for the pulse exposure group and 625 μg/mL based on toxicity on the 24 hours exposure group. The vehicle control groups had frequencies of cells with chromosome aberrations within the expected range. The positive control induced statistically significant increases in frequency in cells with aberrations. The metabolic activation system was therefore satisfactory and the test method was operating as expected.

The test material did not induce any statistically significant increases in frequency of cells with chromosome aberrations in either the 24-hour continuous exposure group or in the 6-hour pulse exposure group dosing at 2% final S9 concentration.

The test material did not induce any significant increase in the number of polypoid cells at any dose level in either exposure group.

CONCLUSION — The notified chemical was not clastogenic to CHL treated in vitro under the conditions of the test.

TEST FACILITY — SafePharm Laboratories (2004k)

7.10. Genotoxicity – in vitro

TEST SUBSTANCE — C-193 (Lot Number MC-3KT)

METHOD — Guidelines of "Occupational Safety and Health Law" (Japan) Low Concerning the Examination and Regulation of Manufacture etc., of Chemical Substance (Japan).

Cell Type/Cell Line	Chinese Hamster Lung (CHL) cells
Metabolic Activation System	Phenobarbitone/β-naphthoflavone induced rat liver S9 fraction.
Vehicle	Minimal Essential Media
Remarks – Method	No significant protocol deviation.

Metabolic Activation	*Test Substance Concentration (mg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Experiment 1	1.25*, 2.5*, 5.0*,	6	24
Experiment 1	0.039, 0.078, 0.156, 0.313, 0.625*, 1.25*, 2.5*, 5.0	24	24

Experiment 1	0.039, 0.078, 0.156, 0.313*, 0.625*, 1.25*, 2.5, 5.0	48	48
Experiment 2	1.25*, 2.50*, 5.0*	6	24
Experiment 2	0.884, 1.25*, 1.77, 2.50*	24	24
Experiment 2	0.625*, 0.884, 1.25*, 1.77	48	48
Present			
Experiment 1	0.039, 0.078, 0.156, 0.313, 0.625, 1.25*, 2.5*, 5.0*	6	24
Experiment 2	1.25*, 2.5*, 5.0*	6	24

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (mg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Experiment 1	>5.0	>5.0	Not provided.	-
Experiment 1	>2.5	>2.5	Not provided.	-
Experiment 1	>1.25	>1.25	Not provided.	-
Experiment 2		>5.0	Not provided.	-
Experiment 2		>2.5	Not provided.	-
Experiment 2		>1.25	Not provided.	-
Present				
Experiment 1	>5.0	>5.0	Not provided.	-
Experiment 2		>5.0	Not provided.	-

Remarks - Results

A cell growth inhibition test was performed to determine the cytotoxicity of the test substance. Following short term treatment with and without metabolic activation, growth inhibition did not exceed 50% at the maximum concentration of 5.0 mg/mL. Following continuous treatment for 24 hours and 48 hours, more than 50% growth inhibition was noted at and above 2.5 mg/mL and 1.25 mg/mL, respectively.

Experiment 1
In the short term treatment experiments no significant increases in the frequency of chromosomal aberrations were observed with and without S9. No dose related increases were observed.

In the continuous treatment experiments, due to cytotoxicity, less than 100 metaphase cells could be scored at 2.5 mg/mL. However since more the fifty metaphase cells could be analysed it was considered suitable for evaluation. No significant increases in the frequency of chromosomal aberrations were observed following 24 and 48-hour treatments. No dose related increases were observed.

The vehicle control groups had frequencies of cells with chromosomal aberrations within the expected range. The positive control induced statistically significant increases in frequency in cells with aberrations. The metabolic activation system was therefore satisfactory and the test method was operating as expected.

Experiment Two
In the short term treatment experiments no significant increases in the frequency of chromosomal aberrations were observed with and without S9. No dose related increases were observed.

In the continuous treatment experiments, due to cytotoxicity, less than 100 metaphase cells could be scored at 2.5 mg/mL. No significant increases in the frequency of chromosomal aberration were observed following 24 and 48-hour treatments. No dose related increases were observed.

The vehicle control groups had frequencies of cells with chromosomal aberrations within the expected range. The positive control induced statistically significant increases in frequency of cells with aberrations. The metabolic activation system was therefore satisfactory and the test method was operating as expected.

CONCLUSION

The notified chemical was not clastogenic to CHL treated in vitro under the conditions of the test.

TEST FACILITY

Canon (2004b)

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

TEST SUBSTANCE

C-193

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
Auxiliary Solvent	None.
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^{o}C.

BOD was measured by data sampler and autorecorder.

At termination of study the dissolved organic carbon, test substance concentration and pH were measured.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	0	7	54
14	0	14	70
21	0	21	72
28	0	28	74

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	3	1	3	2
HPLC	2	0	1	1

Remarks - Results

CONCLUSION — Under the study conditions the test substance was not readily biodegradable.

TEST FACILITY — Kurume (2004)

8.1.2. Bioaccumulation

Not determined. However, the notified chemical has a low bioaccumulation potential based on its very low log P_{ow}.

8.2. Ecotoxicological investigations

8.2.1. Acute toxicity to fish

TEST SUBSTANCE — C-193

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 by ultrasonication for 10 minutes. 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 14°C, exposed to a photoperiod of 16 hours light/8 hours dark 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.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*6 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	-	20	0	0	0	0	0
100	94-101%	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 test substance is very slightly toxic to fish (Mensink, 1995).

TEST FACILITY — SafePharm Laboratories (2004l)

8.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	C-193
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 21ºC, exposed to a photoperiod of 16 hours light/8 hours dark 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.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	-	20	0	0
100	96%	40	0	20

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 test substance is very slightly toxic to aquatic invertebrates (Mensink, 1995).
TEST FACILITY	SafePharm Laboratories (2004m)

8.2.3. Algal growth inhibition test

TEST SUBSTANCE	C-193
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: 3.2, 10, 32, 100 and 320 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 x 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.

RESULTS

Experiment A: Growth			*Experiment B: Growth*		
E_bC_{50} mg/L at 72 h	*E_rC_{50} mg/L at 72 h*	*NOEC mg/L*	*E_bC_{50} mg/L at 72 h*	*ErC50 mg/L at 72 h*	*NOEC mg/L*
50	440	3.2	83	240	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 greater than 10%, the effect was determined to be due to the toxicity of the test substance in combination with light intensity. In which case the results from Experiment A should be used in any conclusions and risk assessments.

CONCLUSION

Under the study conditions, the test substance is harmful to algae (United Nations 2003).

TEST FACILITY

SafePharm Laboratories (2004n)

8.2.4. Inhibition of microbial activity

TEST SUBSTANCE

C-193

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 $CaCO_3$.
RESULTS	
EC50	>1000 mg/L
NOEC	1000 mg/L
Remarks – Results	Reference substance 3 h EC_{50} = 11 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 test substance 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 release into the environment.

The notified chemical is readily soluble in water; however, aquatic release 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 will generally be sent to landfill for disposal. 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 lead to significant exposure in 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% (ie 25% of imported volume) will remain in the supernatant effluent discharged to sewer (assuming no WWTP attenuation). The predicted environmental concentration (PEC) of the notified chemical would be:

Amount in effluent entering sewer	750 kg
Number of days	365
National population	20 million
Litres per person	200 L
PEC_{sewer}	0.5 μg/L.

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_bC_{50} of 50 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 500 μg/L.

The notified chemical is not readily biodegradable or hydrolysable and may persist in the in the aquatic environment until degraded by abiotic and biotic processes. Due to its low log P_{ow} and high molecular weight it is not likely to bioaccumulate.

9.1.3. Environment – risk characterisation

The worst case calculation indicates a PEC/PNEC ratio of 0.001 (0.5/500) for aquatic ecosystems via sewer discharge, indicating a low environmental risk.

The notified chemical is not likely to present a hazard 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

There is low potential for worker exposure to the notified chemical when replacing spent cartridges as the notified chemical is at low concentration (<10%) in the ink formulations which are sealed within the cartridge. Service technicians may occasionally experience dermal contact

with the notified chemical during maintenance; however, the notified chemical is at low concentrations in the ink formulations. Exposure to the notified chemical on printed paper is low as the dye is bound to the paper matrix.

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.

9.2.2. Public health – exposure assessment

From the point of importation to the end use of the ink preparation containing the notified chemical, the ink preparation is either enclosed in a cartridge made for insertion in ink jet printers or is present on printed paper in a cured state. Public exposure through importation, transportation or storage is assessed as negligible. There is little potential for exposure during cartridge changes. Any exposure to the ink preparation that does occur is most likely to be dermal and of a minimal and transient nature. Ink containing the notified chemical on the printed page is bound to the paper and is not biologically available. Public exposure is assessed as low.

9.2.3. Human health – effects assessment

The notified chemical has low acute oral and dermal toxicity to rats. The notified chemical is slightly irritating to the skin and eyes of rabbits and was not a skin sensitiser mouse local lymph node assay.

In a 28-day repeat dose oral toxicity study in rats, a NOAEL of 150 mg/kg bw/day was established based on the on changes in the gastric mucosa at higher doses.

The notified chemical was neither mutagenic nor genotoxic in a bacterial reverse mutation test or a mammalian chromosomal aberration test, respectively.

Based on the available data, the notified chemical is not 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 OHS risk presented by the notified chemical is expected to be low given that the notified chemical is present in the ink at <10%, the ink is contained in enclosed cartridges, and printer maintenance personnel wear gloves.

9.2.5. Public health – risk characterisation

Members of the public are not likely to make contact with the notified chemical during cartridge changes unless the cartridge is ruptured or otherwise tampered with. Additionally the notified chemical is present at low concentrations in a formulation that is not classified as hazardous. Ink containing the notified chemical on the printed pages is bound to the paper and is not readily bioavailable.

Consumer exposure to the notified chemical via the printed paper has been estimated by the manufacturer. One kilogram of pure dye would be expected to produce several million sheets of A4 coloured text or graphics. Under worst-case conditions, each piece of A4 paper can be assumed to incorporate 1mg of notified chemical. Based on a 50% transfer on contact when handling printed - paper (assuming only partially dry ink), and the relative areas of finger ends and paper size, it is estimated that potential removal is <1% of the applied ink in each event.

Estimated Exposure
Area of contact with finger ends (four fingers on one hand) = 8 cm^2
A4 sized paper substrate = ca. 600 cm^2

% Removal = (8/600) × 0.5 × 100 = < 1%
Therefore total removal to finger ends at point of contact would be < 1% of 1 mg notified chemical per event = < 0.01 mg

For extensive contact (i.e. > 10 events per day) the daily body burden, assuming no washing between events, 70 kg person and 100% absorption, would be < 0.01 × 10/70 = ca. 0.0014 mg/kg bw/day.

The NOAEL from the 28-day repeat dose study for the analogue C-193 was 150 mg/kg bw/day.

The Margin of Exposure (NOAEL/Estimated Exposure) is 107142. As the MOE exceeds 100, the notified chemical does not pose a public health concern..

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

10.1. Hazard classification

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

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.

The notified chemical is not classified according to the GHS criteria for human health end points. For environmental end points the notified chemical would have the classification of Chronic 3.

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 Negligible Concern to public health when used as described in this notification.

11. MATERIAL SAFETY DATA SHEET

11.1. Material Safety Data Sheet

The MSDS of the products 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 the products 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

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 ocular and dermal exposure
- Service personnel should wear cotton or disposable gloves and ensure adequate ventilation is present when removing spent printer cartridges containing the notified chemical and during maintenance and repair.
- 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(2) of the Act:
  - if any of the circumstances listed in the subsection arise.

The Director will then decide whether secondary notification is required.

13. BIBLIOGRAPHY

Canon (2004a) Report of Mutagenicity Test Using Microorganism Report No.695, Canon Inc. (5 February 2004) Ohta-ku, Japan (Unpublished report submitted by notifier).

Canon (2004b) C-193: Metaphase Analysis in CHL cells in vitro Report No.C054, Canon Inc. (9 February 2004) Ohta-ku, Japan (Unpublished report submitted by notifier).

Kurume Laboratory (2004) Biodegradation study of C-193 by micro-organisms. Study number 14166. Chemicals Evaluation and Research Institute, Japan.

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

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.

SafePharm Laboratories (2004a) C-193: Determination of General Physico-Chemical Properties (SPL Project Number 345/717 15 March 2004), SafePharm Laboratories Limited Derbyshire UK. (Unpublished report submitted by notifier).

SafePharm Laboratories (2004b) C-193: Determination of Hazardous Physico-Chemical Properties (SPL Project Number 345/718 12 July 2004), SafePharm Laboratories Limited Derbyshire UK. (Unpublished report submitted by notifier).

SafePharm Laboratories (2004c) C-193: Determination of Flammability (Solids) (SPL Project Number 345/719 24 February 2004), SafePharm Laboratories Limited Derbyshire UK. (Unpublished report submitted by notifier).

SafePharm Laboratories (2004d) C-193: Acute Oral Toxicity in the Rat –Acute Toxic Class Method (SPL Project Number 345/720 2 April 2004), SafePharm Laboratories Limited Derbyshire UK. (Unpublished report submitted by notifier).

SafePharm Laboratories (2004e) C-193: Acute Dermal Toxicity (Limit Test) in the Rat (SPL Project Number 345/721 16 June 2004), SafePharm Laboratories Limited Derbyshire UK. (Unpublished report submitted by notifier).

SafePharm Laboratories (2004f) C-193: Acute Dermal Irritation in the Rabbit (SPL Project Number 345/722 2 April 2004), SafePharm Laboratories Limited Derbyshire UK. (Unpublished report submitted by notifier).

SafePharm Laboratories (2004g) C-193: Acute Eye Irritation in the Rabbit (SPL Project Number 345/723 29 July 2004), SafePharm Laboratories Limited Derbyshire UK. (Unpublished report submitted by notifier).

SafePharm Laboratories (2004h) C-193: Local Lymph Node Assay in the Mouse (SPL Project Number 345/724 2 April 2004), SafePharm Laboratories Limited Derbyshire UK. (Unpublished report submitted by notifier).

SafePharm Laboratories (2004i) C-193: Twenty-Eight Day Repeated Dose Oral (Gavage) Toxicity Study in Rats (SPL Project Number 345/725, 2 April 2004), SafePharm Laboratories Limited Derbyshire UK. (Unpublished report submitted by notifier).

SafePharm Laboratories (2004j) C-193: Reverse Mutation Assay "Ames Test" Using *Salmonella typhimurium* and *Escherichia coli* (SPL Project Number 345/727, 5 April 2004), SafePharm Laboratories Limited Derbyshire UK. (Unpublished report submitted by notifier).

SafePharm Laboratories (2004k) C-193: Chromosome Aberration Test in CHL Cells *in vitro* (SPL Project Number 345/726, 9 August 2004), SafePharm Laboratories Limited Derbyshire UK. (Unpublished report submitted by notifier).

SafePharm Laboratories (2004l) C-193: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*), (SPL Project Number 345/728, 19 July 2004), SafePharm Laboratories Limited, Derbyshire, UK (Unpublished report submitted by the notifier).

SafePharm Laboratories (2004m) C-193: Acute Toxicity to *Daphnia magna*, (SPL Project Number 345/729, 20 July 2004), Safepharm Laboratories Limited, Derbyshire, UK (Unpublished report submitted by the notifier).

SafePharm Laboratories (2004n) C-193: Inhibition of Algal Growth Caused By Coloured Test Substances, (SPL Project Number 345/730, 20 July 2004), Safepharm Laboratories Limited, Derbyshire, UK (Unpublished report submitted by the notifier).

SafePharm Laboratories (2004o) C-193: Assessment of the Inhibitory Effect on the Respiration of Activated Sewage Sludge, (SPL Project Number 345/731, 8 July 2004), Safepharm Laboratories Limited, Derbyshire, UK (Unpublished report submitted by the 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.