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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

CIM-21

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

For the purposes of subsection 78(1) of the Act, this Public Report may be inspected at our NICNAS office by appointment only at Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

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

Street Address:	Level 7, 260 Elizabeth Street, SURRY HILLS NSW 2010, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX	+ 61 2 8577 8888
Website:	www.nicnas.gov.au

Director
NICNAS

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SUMMARY

The following details will be published in the NICNAS *Chemical Gazette:*

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/1623	Canon Australia Pty Ltd	CIM-21	ND*	≤ 1 tonne per annum	Component of inkjet printer ink

*ND = not determined

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals* (GHS), as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

Human health risk assessment
Under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

When used in the proposed manner, the notified chemical is not considered to pose an unreasonable risk to public health.

Environmental risk assessment
On the basis of the PEC/PNEC ratio and the assessed use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations
CONTROL MEASURES
Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced:
  - Avoid skin and eye contact
  - Do not generate aerosols
  - Clean up spills promptly

- Service personnel should wear impervious gloves and ensure adequate ventilation is present when removing spent printer cartridges containing the notified chemical and during routine maintenance and repairs.

- A copy of the (M)SDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by physical containment, collection and subsequent safe disposal.

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if
- the importation volume exceeds one tonne per annum notified chemical;
- the notified chemical is imported in any form other than as a component of sealed ink-jet cartridges;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from component of inkjet printer ink or is likely to change significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

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

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

ASSESSMENT DETAILS

This notification has been conducted under the cooperative arrangement with the United States Environmental Protection Agency (US EPA). Information pertaining to the assessment of the notified chemical by the US EPA was provided to NICNAS and, where appropriate, used in this assessment report. The other elements of the risk assessment and recommendations on the safe use of the notified chemical were carried out by NICNAS.

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
Limited-small volume: Chemical other than polymer (1 tonne or less per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: chemical name, other names, molecular and structural formulae, molecular weight, analytical data, impurities and identity of manufacturer.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: dissociation constant, and flash point.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
LVC/846.

NOTIFICATION IN OTHER COUNTRIES
China (2011)
USA (2011)
Japan (2012)
Korea (2012)
Philippines (2012)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
CIM-21

MOLECULAR WEIGHT
> 500 Da

ANALYTICAL DATA
Reference NMR, IR, HPLC, LC-MS spectra were provided.

3. COMPOSITION

DEGREE OF PURITY > 85%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Magenta coloured crystalline solid

Property	Value	Data Source/Justification
Melting Point	Decomposes at > 320°C	Measured
Density	1380 kg/m^3 at 20°C	Measured
Vapour Pressure	< 4.7 x 10^{-8} kPa at 25°C	Measured
Water Solubility	450-500 g/L at 20°C	Measured (EC 440/2008, A6; OECD 105, shake flask

		method).Water solubility was estimated based on visual inspection.
Hydrolysis as a Function of pH	$t_{1/2}$ > 1 years (pH 4, 7 and 9) at 25°C	Measured (EC 440/2008, C7; OECD 111).
Partition Coefficient (n-octanol/water)	log Pow = -3.08 at 20°C	Measured (EC 440/2008, A8; OECD 107). Test was carried out at approximately neutral pH with the notified chemical in its ionised form.
Adsorption/Desorption	log K_{oc} < 1.25	Measured (EC 440/2008, C19; OECD 121, HPLC screening method). Test was carried out at approximately neutral pH with the notified chemical in its ionised form.
Dissociation Constant	Estimated pKa < 0 - 14.6	Calculated for the free acid form. The notified chemical is a salt which is expected to be ionised under environmental conditions.
Particle Size	Inhalable fraction (< 100 μm): 0.98%	Measured
Flammability	Not highly flammable	Measured
Autoignition Temperature	333°C	Measured
Explosive Properties	Not expected to be explosive	Contains no functional groups that would imply explosive properties
Oxidising Properties	Not expected to oxidise	Contains no functional groups that would imply oxidative properties

DISCUSSION OF PROPERTIES
For full details of tests on physical and chemical properties that have not been assessed in the US EPA report, refer to Appendix A.

Reactivity
The notified chemical is expected to be stable under normal conditions of use.

Physical hazard classification
Based on the submitted physico-chemical data depicted in the above table, the notified chemical is not recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as a component (≤ 7%) of inkjet printer ink.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤ 0.1	≤ 1	≤ 1	≤ 1	≤ 1

PORT OF ENTRY
Sydney (by air and sea)

IDENTITY OF RECIPIENTS
Canon Australia Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a component of inkjet printer ink in sealed cartridges. The cartridges will vary in size between 2.5-2600 mL and will be packaged in sealed foil bags. The printer cartridges will be transported by road to the Canon Australia Pty Ltd warehouse and then distributed to retail outlets/end-users.

USE
The notified chemical will be used as a component (≤ 7%) of inkjet printer ink for commercial and household printers.

OPERATION DESCRIPTION
The notified chemical will be imported as a component of ink in sealed cartridges. Reformulation will not take place in Australia.

End-users (including service technicians, office workers and the general public) will remove the cartridge from the packaging and place the cartridge into the printer. The cartridge will be disposed of when empty.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Import/waterside	< 8	10-50
Storage and transport	< 8	10-50
Office workers	10 seconds	2
Service technicians	1	170

EXPOSURE DETAILS
Waterside, storage and transport workers may come into contact with the notified chemical, as a component of ink (≤ 7%), only in the unlikely event of an accident.

Service technicians may be exposed to the ink containing 7% or less notified chemical during repair and cleaning of ink jet printers. Due to the low volatility of the notified chemical, dermal exposure is expected to be the main potential route of exposure. Exposure to the notified chemical may occur while changing cartridges if the ink is inadvertently handled.

Office workers and home users may be exposed to the ink when replacing the cartridge but the amount of exposure is predicted to be very low. Instructions on how to replace the cartridges safely are included with the cartridge. Occasional dermal exposure during use of the printer may occur if the printed pages were handled inadvertently before the ink had dried, or if ink-stained parts of the printer were touched. Once the ink dries, the chemical would be bonded to the printed-paper, and therefore dermal exposure to the notified chemical from contact with dried ink is not expected.

6.1.2. Public Exposure
Dermal exposure of the public to inks containing the notified chemical (at ≤ 7%) is expected to be similar, though less frequent, than that described above for office workers.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical are summarised in the following table. For full details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw low toxicity
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* mammalian chromosomal aberration	non genotoxic

Toxicokinetics.
Given the relatively high molecular weight (> 500 Da) and low partition coefficient (log Pow = -3.08 at 20 °C) of the notified chemical, dermal absorption is not expected. However, absorption across the gastrointestinal tract,

although expected to be limited, cannot be ruled out.

Acute toxicity.
The notified chemical was found to be of low acute oral toxicity in a study conducted in rats (LD50 > 2000 mg/kg bw). There were no signs of systemic toxicity.

Irritation/Sensitisation.
The notified chemical contains functional groups that have been associated with skin and eye irritation. The potential for the effect may be limited by the relatively high molecular weight (> 500 Da) of the notified chemical.

Mutagenicity.
The notified chemical was not mutagenic in a bacterial reverse mutation study and was not clastogenic to human lymphocytes in an *in vitro* mammalian chromosome aberration test.

Health hazard classification
Based on the available information, the notified chemical cannot be classified according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The notified chemical is of low acute oral toxicity and is not expected to be genotoxic. Based on structural alerts the notified chemical may be irritating to the eye and skin, however this is expected to be limited given the high molecular weight of the notified chemical. Based on its physico-chemical properties, the chemical is likely to have limited potential for dermal absorption. Toxicity from repeat exposure is not known but is expected to be limited by the dermal route.

The notified chemical may be handled by workers at ≤7% concentration. Dermal exposure to the notified chemical may occur when replacing spent cartridges (and/or as incidental exposure when touching wet ink on printed pages). At these low concentrations, skin and eye irritation is not expected. Toxicity arising from repeated exposure to the notified chemical cannot be ruled out but is not expected at the low proposed use concentrations (≤ 7%) and use in contained cartridges.

While significant dermal exposure of technicians to the notified chemical is not expected given its containment within cartridges, performing printer maintenance operations, in an industrial setting, may occur on a frequent basis. Therefore, measures should be taken to avoid exposure to the notified chemical (e.g. use of impervious gloves).

Dermal exposure of office workers to the notified chemical is expected to be infrequent and of a low level, given the containment of the chemical within cartridges and the provision of instructions for replacing the cartridges. There may be frequent exposure to dried ink containing the notified chemical, however, the chemical will be cured in the ink matrix and not be available for exposure.

Therefore, provided that measures to protect technicians are being adhered to (i.e., use of impervious gloves and adequate ventilation when performing printer maintenance operations), and based on the expected low exposure of office workers to the notified chemical, the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

6.3.2. Public Health
Public exposure to the notified chemical is expected to be similar, though less frequent than that experienced by office workers. Therefore, the risk to the health of the public from use of the notified chemical is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported into Australia as a component of inkjet printer ink sealed in cartridges. No release of the notified chemical to the environment is expected from manufacturing or reformulation since these activities are not expected to occur in Australia. Release of the notified chemical to the environment is unlikely during importation, storage and transportation as printer cartridges are designed to minimise release.

RELEASE OF CHEMICAL FROM USE

Most of the notified chemical will be fixed within an inert ink matrix adhering to paper and is not expected to be released to the environment once cured. The release of the notified chemical may occur from leakage of ink during use, installation or replacement of cartridges. Any releases are expected to be contained with absorbent material and disposed of to landfill.

RELEASE OF CHEMICAL FROM DISPOSAL

Following its use as printer ink, the majority of the notified chemical is anticipated to share the fate of printed paper and be disposed of to landfill or subjected to paper recycling processes. Up to half of the printed paper is expected to be recycled, and the notified chemical may be released to sewage treatment plants (STPs) during these processes. Residual notified chemical (approximately 5% of the total annual import volume) in used ink cartridges is expected to be disposed of to landfill.

7.1.2. Environmental Fate

Most of the notified chemical is expected to be disposed of to landfill along with printed paper or released to sewer in recycling wastewaters when used paper is recycled. During paper recycling processes, waste paper is repulped using a variety of chemical agents which, amongst other things, enhance detachment of ink from the fibres. Given that it is highly water soluble, the notified chemical has the potential to partition to the supernatant water and be released to sewer during paper recycling processes. During waste water treatment processes in STPs, the notified chemical is not expected to be efficiently removed from influent due to its water solubility and low soil adsorption coefficient. Thus, the notified polymer may be released to surface water. The notified chemical is hydrolytically stable under environmental conditions and is not readily biodegradable. In landfill and soil, the notified chemical is likely to be mobile based on its high water solubility and low soil adsorption coefficient. However, the notified chemical is not expected to bioaccumulate due to its high water solubility, low n-octanol/water partition coefficient and low measured bioconcentration factor (BCF < 8.8). It is expected to eventually degrade by biotic and abiotic processes to form water, oxides of carbon, nitrogen and sulphur, and inorganic salts. For the details of the environmental fate studies please refer to Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) can be estimated as outlined below assuming that 50% of the annual import volume of the notified chemical will be released to sewer during recycling of the used paper. For the worst case scenario, it is assumed that the notified chemical is not removed from influent during STPs processes. It was assumed that release of the notified chemical occurs over 260 days per annum corresponding to release only on working days.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1,000	kg/year
Proportion expected to be released to sewer	50%	
Annual quantity of chemical released to sewer	500	kg/year
Days per year where release occurs	260	days/year
Daily chemical release:	1.92	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	0%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1	
Dilution Factor - Ocean	10	
PEC - River:	0.43	µg/L
PEC - Ocean:	0.04	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.43 µg/L may potentially result in a soil concentration of approximately 2.84 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 14.17 µg/kg and 28.35 µg/kg, respectively.

7.2. Environmental Effects Assessment

The result of ecotoxicological investigation conducted on the notified chemical is summarised in the table below. Details of the study for fish acute toxicity can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity (96 hours)	LC50 > 200 mg/L	Not harmful

Based on the acute toxicity endpoint for fish, the notified chemical can be formally classified as "not classified for acute hazard" under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009).

7.2.1. Predicted No-Effect Concentration

The endpoint for fish acute toxicity is used to calculate the predicted no-effect concentration (PNEC). An assessment factor of 1000 was used as only one endpoint was available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50 (Fish).	> 200	mg/L
Assessment Factor	1,000	
PNEC:	> 200	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River:	0.43	> 200	< 0.002
Q - Ocean:	0.04	> 200	< 0.001

The Risk Quotient (Q = PEC/PNEC) for the worst case scenario have been calculated to be < 1 for the river and ocean compartments. Although the notified chemical may be released into waterways, it is unlikely to pose a risk to the aquatic environment given that it is not expected to bioaccumulate nor is it released at ecotoxicologically relevant concentrations. Therefore, on the basis of the PEC/PNEC ratio and the assessed use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point Decomposes without melting at > 320 °C

Method OECD TG 102 Melting Point/Melting Range.
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks Determined by Differential Scanning Calorimetry
Test Facility Harlan (2011a)

Density 1380 kg/m^3 at 20°C

Method OECD TG 109 Density of Liquids and Solids.
EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks Determined by Gas Comparison Pycnometer
Test Facility Harlan (2011a)

Vapour Pressure < 4.7 x 10^{-8} kPa at 25°C

Method OECD TG 104 Vapour Pressure.
EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks Determined by Vapour Pressure Balance
Test Facility Harlan (2011b)

Dissociation Constant Expected to be ionised under environmental conditions

Method The notified chemical contains functionalities with overlapping dissociation constants, making direct measurement of its dissociation constants impractical. The notifier provided pKa values for the free acid form of the notified chemical. The estimation was performed using Advanced Chemistry Development I-lab Web Service, in lieu of measured values.
Remarks The notified chemical contains basic groups with calculated pKa values of 14.5, 14.6, 6.5, 2.5, 0.8 and less than 0, one acidic group with a calculated pKa value of less than 0.
DSEWPac notes that the notified chemical is a salt which is expected to be ionised under environmental conditions.
Test Facility Harlan (2011a)

Particle Size

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

Range (μm)	*Mass (%)*
< 100	0.984

Remarks The notified chemical is considered to be essentially non-inhalable.
Test Facility Harlan (2011 a)

Flammability Not highly flammable

Method EC Council Regulation No 440/2008 A.10 Flammability (Solids).
Remarks In a preliminary screening test the test substance did not ignite when a flame was applied for 2 minutes.
Test Facility Harlan (2011b)

Autoignition Temperature 333°C

Method EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids.
Remarks The temperature of the test item reached 400ºC at an oven temperature of 333°C.
Test Facility Harlan (2011b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure. EC Council Regulation No 440/2008 B.1 bis Acute toxicity (oral) fixed dose method.
Species/Strain	Rat/Wistar
Vehicle	Distilled water
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	1 Female	2000	0/1
2	4 Female	2000	0/4

LD50	> 2000 mg/kg bw
Signs of Toxicity	There were no signs of systemic toxicity. Pink coloured staining of the fur was noted in four animals.
Effects in Organs	No abnormalities were detected in the organs.
Remarks - Results	All animals showed expected gains in bodyweight.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Harlan (2011c)

B.2. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Council Regulation No 440/2008 B.13/14 Mutagenicity- Reverse Mutation Test using Bacteria Plate incorporation procedure (Test 1) and Pre incubation procedure (Test 2)
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 fraction from rat liver induced with phenobarbitone/β-napthoflavone
Concentration Range in Main Test	a) With metabolic activation: 50-5000 µg/plate b) Without metabolic activation: 50-5000 µg/plate
Vehicle	Sterile distilled water, test substance administered as solution
Remarks - Method	No significant protocol deviations

RESULTS

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

Remarks - Results	The test item did not cause any visible reduction in the growth of the

bacterial background lawn at any dosage levels and was, therefore, tested up to the maximum recommended dose level of 5000 µg/plate. A pink test item induced colouration was observed at and above 50 µg/plate. Although this observation did not prevent scoring, manual counts were performed at 5000 µg/plate because of the intense colouration. No test item precipitate was observed on the plates at any of the doses tested in either the presence or absence of metabolic activation.

There were no significant increases in the frequency of revertant colonies for any bacterial strains, at any dose level either with or without metabolic activation or exposure method.

All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9- mix and the sensitivity of the bacterial strains.

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

TEST FACILITY Harlan (2011d)

B.3. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
EC Council Regulation No 440/2008 B.13/14 Mutagenicity- Reverse Mutation Test using Bacteria

Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 fraction from rat liver induced with phenobarbitone/β-napthoflavone
Vehicle	Eagle's minimal essential medium (MEM)
Remarks - Method	No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*,156.25, 312.5 ,625, 1250*, 2500*, 5000*	4h	24h
Test 2	0*, 156.25, 312.5, 625*, 1250*, 2500*, 5000*	24h	24h
Present			
Test 1	0*,156.25, 312.5, 625, 1250*, 2500*, 5000*	4h	24h
Test 2	0*,156.25, 312.5, 625*, 1250*, 2500*, 5000*	4h	24h

*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	negative
Test 2	≥ 2500	≥ 2500	> 5000	negative
Present				
Test 1	> 5000	> 5000	> 5000	negative
Test 2		> 5000	> 5000	negative

Remarks - Results A precipitate of the test substance was not observed in any dose group, however magenta colouration was observed at all dose levels in all exposure groups. In the main test, growth inhibition was achieved at 2500

(53%) and 5000 (55%) µg/mL in the 24-hour exposure without metabolic activation only. This was comparable to that seen in the preliminary toxicity test.

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

All of the vehicle control cultures had frequencies of cells with chromosome aberrations within the expected range. The positive control items induced statistically significant increases in the frequency of cells with aberrations. The metabolic activation system was therefore shown to be functional and the test method itself was operating as expected.

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

TEST FACILITY Harlan (2011e)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I).
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Closed system oxygen consumption measuring apparatus to determine Biochemical Oxygen Demand (BOD) Total organic carbon analysis method for the measurement of Dissolved Organic Carbon measurement (DOC) HPLC for the measurement of test substance
Remarks - Method	Conducted according to the guidelines above with no significant deviations to the protocol.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation(BOD)*	*Day*	*% Degradation (BOD)*
7	1.7	7	59
14	4	14	70
21	3	21	71
28	5	28	74

Remarks - Results	All validity criteria for the test were satisfied. Degradability results based on BOD, DOC and residual test substance amount measurements were 5%, 3% and 0% after 28 days, respectively. No transformation product was generated under the conditions of test.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	CERI Kurume (2011)

C.1.2. Bioaccumulation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 305 Bioconcentration: Flow-through Fish Test.
Species	Carp (*Cyprinus carpio*)
Exposure Period	Exposure: 28 days
Auxiliary Solvent	None
Concentration Range	Nominal: 1 and 0.1 mg/L Actual: 0.96 and 0.098 mg/L
Analytical Monitoring	Liquid chromatography-mass spectrometry
Remarks - Method	Conducted according to the guidelines above with no significant deviations to the protocol.
RESULTS	
Bioconcentration Factor	At high exposure level of 1 mg/L: BCF < 0.67-2.6 At low exposure level of 0.1 mg/L: BCF < 8.8
Remarks - Results	The change in fish lipid contents (33%) was higher than the criteria value of 25% after the completion of experiment. However, it is not expected to have significant influence on the bioaccumulation assessment since no abnormal appearance or behaviour was observed for the tested fishes. All

other validity criteria for the test were satisfied.

CONCLUSION The notified chemical has low potential for bioaccumulation.

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C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE Notified chemical

METHOD Japanese Industrial Standard , JIS K 0102-2010-71, –Semi static

Species	Orange-red killifish (*Oryzias latipes*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	Not measured
Analytical Monitoring	Liquid chromatography-mass spectrometry
Remarks – Method	A limit test was conducted according to the guidelines above with no significant deviations from the protocol. Test solutions were changed every 24 hours.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality (%)*			
Nominal	*Actual*		*24 h*	*48 h*	*72 h*	*96 h*
0	-	10	0	0	0	0
200	-	10	0	0	0	0

LC50	> 200 mg/L at 96 hours.
NOEC (or LOEC)	Not determined
Remarks – Results	All validity criteria for the test were satisfied.

CONCLUSION The notified chemical is not harmful to fish

TEST FACILITY CERI Kurume (2012)

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