File No: LTD/1626

November 2012

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

CIM-26

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:

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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/1626	Canon Australia Pty Ltd	CIM-26	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

REGULATORY CONTROLS
Hazard Classification and Labelling

- 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 printer 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, CAS number, 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/849.

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

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
CIM-26

MOLECULAR WEIGHT
> 1000 Da

ANALYTICAL DATA
Reference NMR, IR, HPLC, LC-Mass, UV-Vis spectra were provided.

3. COMPOSITION

DEGREE OF PURITY > 85%

4. PHYSICAL AND CHEMICAL PROPERTIES

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

Property	Value	Data Source/Justification
Melting Point	Decomposes at 321 °C	Measured
Density	1550 kg/m^3 at 20 °C	Measured
Vapour Pressure	8.4 x 10^{-16} kPa at 25 °C	Measured
Water Solubility	320–340 g/L at 20 °C	Measured (EC 440/2008, A6; OECD 105; flask method)
Hydrolysis as a Function of pH	$t_{½}$ > 1 yr at 25 °C; pH 4, 7 and 9	Measured (EC 440/2008, C7; OECD

		111)
Partition Coefficient (n-octanol/water)	log Pow < -3.78 at 20 °C	Measured (EC 440/2008, A8; OECD 107; shake-flask method). Test conducted at approximately pH 7 with the notified chemical in its ionised form.
Adsorption/Desorption	log K_{oc} < 1.25 at 30 °C	Measured (EC 440/2008, C19; OECD 121; HPLC screening method). Test conducted at approximately pH 7 with the notified chemical in its ionised form.
Dissociation Constant	Estimated pKa < 1.6; 5.7 to 6.8; > 13.5	The notified chemical is a salt which is expected to be ionised under environmental conditions.
Particle Size	Inhalable fraction (< 100 μm): 22.3% Respirable fraction (< 10 μm): 4.34 %	Measured
Flammability	Not highly flammable	Measured
Autoignition Temperature	> 400 °C	Measured
Explosive Properties	Not expected to be explosive	Measured
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/polymer 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 MANUFACTURER/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 printer 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; however, the amount of exposure is predicted to be very small. Instructions on how to replace the cartridges safely are included with the cartridge. During the printing process, the ink turns into an extremely fine mist and is transferred to the paper; however, mist emission of the non-volatile components of the ink from the printer is expected to be very low. 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 (Ames)	non mutagenic
Mutagenicity- bacterial reverse mutation (incorporating Prival and Mitchell modification for azo colourants)	non mutagenic
Genotoxicity – *in vitro* mammalian chromosomal aberration	non genotoxic

Toxicokinetics.

Given the relatively high molecular weight (> 1000 Da), high water solubility (320-340 g/L) and low partition coefficient (log Pow < -3.78 at 20 °C) of the notified chemical, absorption is not expected by any route. However, bacterial skin microflora have been reported to be able to break down azo dyes into smaller species, which may be more readily absorbed (SCCNFP, 2002).

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 and 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 high molecular weight (> 1000 Da) of the notified chemical.

Mutagenicity.
Azo dyes are a concern for their potential induction of mutagenicity and carcinogenicity. Liver azo reductase enzymes reductively cleave the molecule into component amines. This reduction may contribute to the carcinogenicity of many azo dyes. The notified chemical is not expected to be reductively cleaved to release any of the aromatic amines with carcinogenic potential listed in EU SCCNFP/0495/01 (SCCNFP, 2002). However, the notified chemical can be broken by azo reduction into a number of arylamine species, some of which could potentially be mutagenic.

The notified chemical was not mutagenic in a standard bacterial reverse mutation study and was not clastogenic in an *in vitro* mammalian chromosome aberration test under the conditions employed. Furthermore, the notified chemical was found to be not mutagenic using the modified Ames test for azo dyes (Prival and Mitchell, 1982). This modified test is thought to yield a greater detection of mutagenic azo dyes as it utilises a reductive pre-incubation step (during which the azo dye is reduced to amine species) before the test is carried out.

Overall, based on the weight of evidence the notified chemical is not expected to be genotoxic.

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 notified chemical is likely to have limited potential for dermal absorption; however metabolism to smaller species could occur on the skin. Given the low proposed use concentration (≤ 7%) and use in contained cartridges, systemic toxicity is not expected.

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.

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 in ready-to-use cartridges. Release of the ink solution to the environment is not 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.

RELEASE OF CHEMICAL FROM USE

The ink cartridges are designed to prevent leakage and will not be opened during transport, use, installation or replacement. Therefore, release of ink containing the notified chemical to the environment is not expected under normal conditions. However, if leakage or spillage does occur, the ink will be contained with absorbent material, which is expected to be disposed of to landfill along with empty cartridges and printer heads.

The sealed cartridges are contained in the printer until they are removed for disposal. Residual ink (< 5% of the total annual import of the notified chemical) left in empty cartridges will most likely be disposed of to landfill. The majority of the ink will be bound to printed paper that will be disposed of to landfill or recycled.

RELEASE OF CHEMICAL FROM DISPOSAL

Half of the paper that the notified chemical is bound to is expected to be recycled, which may result in the release of a proportion of the notified chemical to the aquatic compartment. Waste paper is pulped using a variety of chemical treatments that result in fibre separation and ink detachment from the fibres. The effluent is expected to be released to sewer.

7.1.2. Environmental Fate

The majority of the notified chemical is expected to enter the environment from disposal of printed paper products that ink containing the notified chemical will be used on. Approximately 50% of the notified chemical will be disposed of to landfill by binding on the printed waste paper. Notified chemical that is not bound to paper in landfill may leach due to the low adsorption/desorption (K_{OC}) value and high water solubility where it may enter surface waters. The remaining 50% of the notified chemical has the potential to be released to sewer, after the de-inking of paper during recycling. The notified chemical is not expected to be removed during sewage treatment plant (STP) processes due to its high water solubility and low potential to sorb to sludge. Therefore, the notified chemical from paper recycling may be released from STPs into surface waters. Notified chemical that enters surface waters from landfill leachate and STPs is expected to disperse and eventually degrade. The notified chemical is not readily biodegradable. For the details of the biodegradability study please refer to Appendix C. The notified chemical is not expected to bioaccumulate due to its very low n-octanol partition coefficient (log Pow) and high solubility in water. The notified chemical is expected to eventually degrade *in-situ* by abiotic and biotic processes into water, inorganic salts and oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

Using a worst-case scenario, it is assumed that 50% of the paper products containing the notified chemical will be recycled and the notified chemical will be released into sewers with no removal of the notified chemical during recycling or STP processes. As the notified chemical is to be processed at paper recycling facilities located throughout Australia, it is anticipated that such releases will occur on 260 days into the Australian effluent volume. The resultant estimate for the predicted environmental concentration (PEC) in sewage effluent nationwide is presented below.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1000	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:	0.043	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 0.425 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 14.2 µg/kg and 28.4 µg/kg, respectively.

7.2. Environmental Effects Assessment

No ecotoxicity data for the notified chemical were submitted. Similar inkjet dyes are generally not harmful to fish and aquatic invertebrates (L(E)C50 > 100 mg/L), but can be moderately toxic to green algae. Effects on algae are mostly related to the colour of dyes, which can reduce the light needed for the algae's growth, rather than from direct toxic effects. Based on the algal toxicity found for similar chemicals, the acute toxicity for algae is estimated to be greater than 1 mg/L for the notified chemical.

The estimation procedure used here is based on data for similar chemicals and is considered acceptable for the purpose of risk assessment. However, this toxicity estimation is not considered sufficient to formally classify the acute and long term hazard of the notified chemical to aquatic life under the Globally Harmonised System for the Classification and Labelling of Chemicals (United Nations, 2009).

7.2.1. Predicted No-Effect Concentration

The endpoint for the most sensitive species (Algae) is used to calculate the predicted no-effect concentration (PNEC). An assessment factor of 100 was used as the endpoint for the most sensitive species is conservatively estimated.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Algae)	> 1	mg/L
Assessment Factor	100	
PNEC:	> 10	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.43	> 10	< 0.043
Q - Ocean	0.043	>10	< 0.004

The Risk Quotients (Q = PEC/PNEC) for the worst case discharge scenario have been calculated to be much less than 1 for the river and ocean compartments. This indicates that the notified chemical is present in the environment at much lower concentrations than the concentration expected to cause adverse effects to aquatic organisms. Therefore, the notified chemical is not expected to pose an unreasonable risk to the aquatic environment based on its reported use pattern.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point Decomposes at 321 °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 (DSC).
Test Facility Harlan (2011a)

Density 1550 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 8.4 x 10^{-16} 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 functional groups with calculated pKa values of less than 1.6, 5.7 to 6.8 and greater than 13.5.
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	22.3
< 10	4.34
< 5.5	1.76

Remarks Too few particles were of a size less than 10.0 μm to allow accurate assessment of the mass median aerodynamic diameter.
Test Facility Harlan (2011a)

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 two minutes.
Test Facility Harlan (2011b)

Autoignition Temperature > 400 °C

Method EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids.

Remarks	The test substance self-heated to a peak at an oven temperature of ~ 285 °C. However the temperature of this peak did not reach 400 °C and therefore was not regarded as self-ignition. The temperature of the test substance reached 400 °C at an oven temperature of ~378 °C; however as this was regarded by the study authors to be as a result of the possible slow decomposition of the test substance rather than self-ignition. Hence, the test substance was determined not to have a relative self-ignition temperature below 400 °C.
Test Facility	Harlan (2011b)

Explosive Properties

Method	EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks	The explosive properties could not be predicted as negative based on the chemical structure, hence thermal analysis by differential scanning calorimetry (DSC) was conducted. The thermogram showed a broad exotherm between 250 °C and 350 °C, due to decomposition. The decomposition energy was calculated to be ~ 410 J/g. As this value is below 500 J/g, the explosive properties of the test substance were predicted negative.
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. Grey coloured staining of the fur as well as black coloured staining of the faeces were noted in 4/5 animals on day 1 only.
Effects in Organs	No abnormalities were detected in individual 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 (AMES)

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	Water
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 blue/black test item induced colouration was observed at and above 50 µg/plate. This observation did not prevent the scoring of revertant colonies. 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 – bacteria (Modified AMES: Prival and Mitchell method for azo dyes)

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.
Pre-incubation procedure incorporating the Prival and Mitchell modification for azo dyes.

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA 102, TA98, TA100
Metabolic Activation System	S9 fraction from hamster 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	Water
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 blue/black test item induced colouration was observed at and above 50 µg/plate which became intense at and above 1500 µg/plate. This observation did not prevent scoring of revertant colonies. 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 (2011e)

B.4. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
EC Council Regulation No. 440/2008 B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.

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. The recommended dose level of 5000 µg/plate was not achieved as the purity of the test substance was not known on commencing the study.

Metabolic Activation	*Test Substance Concentration (µg/mL)#*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 36.45,72.89, 145.78, 291.56, 583.13, 1166.25*, 2332.5*, 4665*, MMC 0.4*	4h	24h
Test 2	0*, 145.78,291.56, 583.13, 1166.25*, 2332.5*, 4665*, MMC 0.2*	24h	24h
Present			
Test 1	0*, 36.45,72.89, 145.78, 291.56, 583.13, 1166.25*, 2332.5*, 4665*, CP 5*	4h	24h
Test 2	0*, 145.78, 291.56, 583.13, 1166.25*, 2332.5*, 4665*, CP 5*	4h	24h

Adjusted for concentration of test substance
*Cultures selected for metaphase analysis.
MMC = Mitomycin C, CP = Cyclophosphamide

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 4665	> 4665	≥ 36.45	negative
Test 2	≥ 1166.25	≥ 4665	?	negative
Present				
Test 1	> 4665	> 4665	≥ 36.45	negative
Test 2		> 4665	?	negative

? The study authors stated it was difficult to assess precipitation due to the nature of the test substance.

Remarks - Results In Test 1, a precipitate of the test item was observed at the end of the treatment period in both exposure groups at all dose levels. In Test 2, a precipitate of the test item was difficult to assess due to the black colouration of the test item. However, black colouration was observed in the blood cultures at the end of treatment period at all doses in each

	exposure group. In the main test, marked growth inhibition (69%) was achieved at 4665 µg/mL, in the continuous exposure group without metabolic activation. 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.
CONCLUSION	The notified chemical was not clastogenic to human lymphocytes treated *in vitro* under the conditions of the test.
TEST FACILITY	Harlan (2011f)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE

METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I).
Inoculum	Activated, cultivated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biochemical oxygen demand (BOD): Closed-system oxygen consumption measuring apparatus Dissolved organic carbon (DOC): TOC analyser Residual test substance: HPLC
Remarks - Method	The test was conducted according to test guidelines using good laboratory practice (GLP) with no significant deviations.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation (BOD)*	*Day*	*% Degradation (BOD)*
7	0	7	57
14	0.3	14	71
21	0	21	73
28	0	28	73

Remarks - Results	All relevant test validity criteria were met. The average percentage biodegradation was calculated using BOD, DOC and the test item concentration; 0%, 8% and 4% respectively.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	CERI Kurume (2011)

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