File No: LTD/1939

November 2016

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

CIM-47

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (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 conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment and Energy.

This Public Report is 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/1939	Canon Australia Pty Ltd	CIM-47	ND*	≤ 1 tonne per annum	Component of printing 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 of 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 reported 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

  Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

- Service personnel should wear disposable 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 of 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

- Where reuse or recycling are not appropriate, dispose of the notified chemical in an environmentally sound manner in accordance with relevant Commonwealth, state, territory and local government legislation.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by containment with adsorbent material, 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;

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from component of printing ink or is likely to change significantly;
  - the amount of chemical being introduced has increased, or is likely to increase, 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 and product containing the notified chemical provided by the notifier were reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Canon Australia Pty Ltd (ABN: 66 005 002 951)
Building A, The Park Estate, 5 Talavera Road
MACQUARIE PARK 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, degree of purity, residual impurities, and import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: density, vapour pressure, hydrolysis as a function of pH, absorption/desorption, dissociation constant, flash point, flammability, autoignition temperature, explosive properties, and oxidising properties.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
China (2016), Japan (2016), Korea (2016), Philippines (2016), USA (2016)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
CIM-47

CAS NUMBER
Not assigned

MOLECULAR WEIGHT
> 1,000 Da

ANALYTICAL DATA
Reference HPLC/MS, IR, UV and NMR spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 99%

4. PHYSICAL AND CHEMICAL PROPERTIES

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

Property	Value	Data Source/Justification
Melting Point	Decomposes from 252 °C without melting	Measured
Boiling Point	Not determined	Decomposition expected prior to boiling
Density	Not determined	Expected to be > 1000 kg/m^3
Vapour Pressure	Not determined	Expected to be low based on high molecular weight
Water Solubility	40 – 42% w/w at 20 °C	Measured

Property	Value	Data Source/Justification
Hydrolysis as a Function of pH	Not determined	The notified chemical is expected to be hydrolytically stable under the environmental pH range of 4 – 9 as it does not contain readily hydrolysable functionalities
Partition Coefficient (n-octanol/water)	log Pow = < -2.99	Measured
Adsorption/Desorption	Not determined	Due to its high water solubility and expected low partition co-efficient the notified chemical is not expected to adsorb to soils/sediment to any great extent
Dissociation Constant	Not determined	The notified chemical contains functionality that is expected to be ionised in the environment
Particle Size	Inhalable fraction (< 100 μm): 27.5% Respirable fraction (< 10 μm): ≤ 0.644%	Measured
Flash Point	Not determined	Based on its relatively high molecular weight, it is expected that no significant level of flammable vapour will occur
Flammability	Not determined	Based on its relatively high molecular weight, it is expected that no significant level of flammable vapour will occur
Autoignition Temperature	Not determined	Not expected to undergo autoignition.
Explosive Properties	Not determined	Contains explosive functional groups but not expected to have explosive properties
Oxidising Properties	Not determined	Contains an oxidising functional group but not expected to have oxidising properties

DISCUSSION OF PROPERTIES
For full details of tests on physical and chemical properties, 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 of 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 not be manufactured or reformulated in Australia. It will be imported as a component of printer ink at < 7% concentration in cartridges and bottles.

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

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

PORT OF ENTRY
Sydney (by sea and air)

TRANSPORTATION AND PACKAGING
The printer ink containing the notified chemical will be imported into Australia in cartridges and bottles sealed in plastic bags. The cartridges and bottles will vary in size between 2.5-2,600 mL (cartridges) and 50-300 mL (bottles).

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 cartridges and bottles. No reformulation of the notified chemical will take place in Australia.

The printer ink will be used for varied printing work in workplace office printers and home office printers. Ink cartridges containing the notified chemical will be manually fitted into printers and replaced with new ink cartridges as necessary. Printer ink (containing the notified chemical) inside the ink bottles will be manually transferred into the printer ink tank as required.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration*	*Exposure Frequency (days/year)*
Transport and warehousing	< 8	10-50
Service technicians	1	170
Retail workers	< 8	10-50
Office workers	< 0.5	2

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

Service technicians and office workers may be exposed to the ink containing the notified chemical at < 7% concentration when replacing spent cartridges, during transfer of the ink from ink bottles to printers, and during repair and cleaning of ink jet printers. Dermal exposure is expected to be the main route of exposure, although incidental ocular exposure is possible. However, given the design of the ink cartridges and ink bottles, exposure to the notified chemical is expected to be limited if workers follow the safety instructions provided with the ink cartridges and ink bottles.

Occasional dermal exposure during use of printers could also occur if the printed pages were touched before the ink had dried. Once the ink dries, the notified chemical will be bound to the paper and is not expected to be bioavailable, thus further dermal contact should not lead to exposure. Inhalation exposure to the notified chemical is not expected under the proposed use scenario.

6.1.2. Public Exposure
The ink cartridges and ink bottles containing the notified chemical at < 7% concentration will be made available to the public for home use. Exposure of the public to the notified chemical 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
Mutagenicity – bacterial reverse mutation (incorporating Prival and Mitchell modification for azo dyes)	non mutagenic
Genotoxicity – *in vitro* mammalian micronucleus test	non-genotoxic

Toxicokinetics, metabolism and distribution
Given the high molecular weight (> 1,000 Da), high water solubility and low partition coefficient (log Pow < -2.99) of the notified chemical, dermal absorption is expected to be limited. However, bacterial skin microflora has been reported to be able to break down azo compounds into smaller species, which may be more readily absorbed through azo reduction (SCCNFP, 2002).

Absorption through GI tract is also expected to be limited, based on the above physical/chemical properties. However, azo compound reduction in the small intestine with possible absorption of the reduction products through the GI tract cannot be ruled out.

Acute toxicity
The notified chemical is of low acute oral toxicity based on a study conducted in rats.

Irritation
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.

Repeated dose toxicity
No repeated dose toxicity data were submitted on the notified chemical. Given the low potential for dermal absorption, systemic toxicity by the dermal route is not expected.

Mutagenicity/genotoxicity
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 classified as carcinogens in the EU and identified in the REACH list of 22 aromatic amines in Annex XVII Appendix 8 (European Commission, 2006). 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 micronucleus test. 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 is not recommended for classification according to the *Globally Harmonised System of 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 design feature of the ink cartridges and ink bottles limiting exposure, systemic toxicity is not expected.

Dermal or possibly incidental ocular exposure to the notified chemical at < 7% concentration may occur during operations including replacing spent ink cartridges, during transfer of the ink from ink bottles to printers, and during printer maintenance and cleaning. Dermal exposure is also possible when handling printed substrates before the ink dries. However, the exposure is expected to be infrequent or only incidental in nature, given the containment of the notified chemical within purposely designed ink cartridges and ink bottles at a relatively low concentration (up to 7%), and the provision of instructions for safe use of the ink cartridges and ink bottles. Once the ink dries, the notified chemical will be bound to the matrix of the substrates and is not expected to be bioavailable.

Given the low proposed end use concentration, irritation effects are not expected.

Overall, based on the limited expected exposure and dermal absorption potential, the risk to workers is not considered to be unreasonable.

6.3.2. Public Health

The types of public exposure to the notified chemical during the use of inkjet printers is expected to be similar to that experienced by workers, but the exposure is expected to be much less frequent. The public may also come into contact with printed substrates containing the notified chemical. However, once dried the notified chemical is bound into the substrates and will not be bioavailable. Therefore, based on very low exposure potential, the risk of the notified chemical to the public 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 ink bottles and sealed ready-to-use ink cartridges. The notified chemical will not be manufactured, reformulated or repackaged in Australia; therefore, release of the notified chemical from these activities is not expected.

RELEASE OF CHEMICAL FROM USE

The ink cartridges and bottles 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 will presumably be disposed of to landfill along with empty cartridges and printer heads in accordance with local government regulations.

The sealed cartridges are contained in the printer until they are removed for disposal. Residual ink (5 %) 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

The notified chemical will be used in printer ink for printing onto paper substrates. The majority of the notified chemical is expected to share the fate of the printed articles to which it is bound. It is assumed that 50% of the printed paper will be disposed of to landfill, and the rest will undergo paper recycling processes. Empty ink cartridges and ink bottles containing residues of the notified chemical are expected to be recycled or disposed of to landfill. The ink remaining in the ink cartridges and ink bottles during the recycling process is not expected to be reused but disposed of to landfill. Hence, the majority of the notified chemical is expected to be disposed of to landfill, with a potential for some release to sewer through paper recycling processes. During paper recycling processes, waste paper is pulped using a variety of chemical treatments that results in ink detachment from the fibres. Waste water containing the notified chemical will be released to sewer.

7.1.2. Environmental Fate

No environmental fate studies were submitted. Based on its high molecular weight, high water solubility and low partition coefficient (log Kow < -2.99), the notified chemical is not expected to bioaccumulate. The majority of the notified chemical is expected to enter the environment from disposal of printed paper products to which the printer ink containing the notified chemical is bound. Approximately 50% of the notified chemical

is expected to be disposed of to landfill as part of printed waste paper. Notified chemical that is not cured and bound to paper in landfill may leach due to its 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 printed paper during recycling processes. The notified chemical is not expected to be removed during sewage treatment plant (STP) processes due to its high water solubility and low adsorption coefficient. Therefore, the notified chemical from paper recycling may be released from STPs to surface waters. Notified chemical released to surface waters from STPs and landfill leachate is expected to disperse and eventually degrade. In landfill and in surface waters, the notified chemical is expected to degrade through biotic and abiotic processes to form water and oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has been calculated to assume a worst case scenario, with 50% of the paper products containing the notified chemical undergoing recycling, and the notified chemical to be released into sewers with no removal during recycling or STP processes. As the notified chemical bound to paper substrates is to be processed at paper recycling facilities located throughout Australia, it is anticipated that such releases will occur over 260 working days per annum into the Australian effluent volume.

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	200.0	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	0%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.42	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/m2/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/m3). Using these assumptions, irrigation with a concentration of 0.42 µg/L may potentially result in a soil concentration of approximately 2.83 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of the 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

No ecotoxicity data were submitted. The calculated PEC is well below the EC50 for algae (the most sensitive species) of the most toxic anionic polymers (EC50 > 1 mg/L).

7.2.1. Predicted No-Effect Concentration

A predicted no-effect concentration (PNEC) has not been calculated for the notified chemical as no ecotoxicity data were submitted. The release of the notified chemical to the aquatic environment will be very limited based on its reported use pattern.

7.3. Environmental Risk Assessment

A risk quotient (PEC/PNEC) for the notified chemical was not calculated, as a PNEC was not derived. The notified chemical is unlikely to reach ecotoxicologically significant concentrations in the environment based on its annual importation quantity and use pattern. The notified chemical is not expected to bioaccumulate based on its high molecular weight. Therefore based on its annual importation quantity and assessed use pattern the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point Decomposes from 252 °C without melting

Method OECD TG 102 Melting Point/Melting Range, 27 July 1995.
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature, 30 May 2008.
Remarks Differential scanning calorimetry method. The notified chemical decomposed without melting.
Test Facility Envigo (2016a)

Water Solubility 40 – 42% w/w at 20 ± 0.5 °C

Method OECD TG 105 Water Solubility, 27 July 1995.
EC Council Regulation No 440/2008 A.6 Water Solubility, 30 May 2008.
Remarks Modified Flask Method. The standard method was not considered applicable to the test item due to the expected high saturation level. The intense blue/black colouration of the test solutions made it difficult to see the presence of any undissolved test item. Hence, the upper limit of the test item solubility range was considered as the point when the solution became significantly viscous.
Test Facility Envigo (2016a)

Partition Coefficient (n-octanol/water) log Pow = < -2.99 at 24 ± 1 °C

Method OECD TG 107 Partition Coefficient (n-octanol/water), 27 July 1995.
EC Council Regulation No 440/2008 A.8 Partition Coefficient, 30 May 2008.
Remarks Shake Flask Method.
Test Facility Envigo (2016a)

Particle Size

Method EC Technical Guidance Document EUR20268 Determination of Particle Size Distribution, Fibre Length and Diameter Distribution of Chemical Substances, 2002.

Range (µm)	*Mass (%)*
< 100	27.5
< 10	≤ 0.644
< 5.5	≤ 0.032

Remarks Sieve Screening Test was used to determine mass of test item particles < 100µm. A cascade impactor was used to determine mass of test item particles < 10 and < 5µm.
Test Facility Envigo (2016a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure (2001). EC Council Regulation No 440/2008 B.1 bis Acute toxicity (oral) fixed dose method.
Species/Strain	Rats/Wistar (RccHan:WIST)
Vehicle	Distilled water
Remarks - Method	No significant deviation of protocol was noted. A sighting test with the test substance was conducted on one rat. In the absence of toxicity at a dose of 2000 mg/kg, a further four rats were tested with this dose.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity	No mortality occurred throughout the study. There were no signs of systemic toxicity. Black staining of the faeces was noted in all animals up to 4 days after dosing.
Effects in Organs	No macroscopic abnormalities were noted at necropsy.
Remarks - Results	Animals displayed expected weight gains except for one animal which showed expected body weight gain in the first week after dosing but body weight loss during the second week.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Envigo (2016b)

B.2. Genotoxicity – bacteria reverse mutation test (Prival and Mitchell modification)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test, 21 July 1997. Pre incubation procedure - Prival and Mitchell modification of azo dyes (Prival & Mitchell, 1982; Prival et al. 1984).
Species/Strain	<u>*Standard Ames test*</u> *S. typhimurium*: TA1535, TA1537, TA98, TA100; *E. coli*: WP2uvrA <u>*Modified azo compound test*</u> *S. typhimurium*: TA98, TA100
Metabolic Activation System	<u>*Standard test*</u> S9 mix (no further details provided) <u>*Modified azo compound test*</u> Hamster liver homogenate metabolising system (containing no enzyme inducers)
Concentration Range in Main Test	a) With metabolic activation: 313 - 5000 µg/plate b) Without metabolic activation: 313 - 5000 µg/plate
Vehicle	Water
Remarks - Method	Full details of the tests were not provided (summary only). There were no deviations from the study plan. The dose range for the main test was determined from the preliminary test.

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
Present				
Test 1*	> 5000	> 5000	> 5000	Negative
Test 1**	> 5000	> 5000	> 5000	Negative

* Standard test
** Modified azo compound test

REMARKS - RESULTS No significant increases in the frequency of revertant colonies were recorded for any of the strains of bacteria, at any dose level either with or without metabolic activation. The positive controls performed as expected, confirming the validity of the test system.

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

Test Facility Canon (2015a)

B.3. Genotoxicity – bacterial reverse mutation test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test, 21 July 1997.
Pre incubation procedure.

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 fraction from phenobarbital/5,6-benzoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 313 - 5000 µg/plate b) Without metabolic activation: 313 - 5000 µg/plate
Vehicle	Water
Remarks - Method	

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test (all replicates)*	*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 No significant increases in the frequency of revertant colonies were recorded for any of the strains of bacteria, at any dose level either with or without metabolic activation.

The positive controls performed as expected, confirming the validity of the test system.

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

TEST FACILITY BML (2016)

B.4. Genotoxicity – in vitro micronucleus test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 487 *In vitro* Mammalian Cell Micronucleus Test, 29 September 2014.
Species/Strain	Human
Cell Type/Cell Line	Lymphoblastoid TK6 cells
Metabolic Activation System	S9 mix (no further details provided)
Vehicle	10% HS-RPMI cell culture media supplemented with sodium pyruvate and 10% donor horse serum.
Remarks - Method	No details of the study provided (summary only)

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	39.1*, 78.1*, 156*, 313*, 625*, 1250, 2500, 5000	3 h	24 h
Test 2	39.1*, 78.1*, 156*, 313*, 625, 1250, 2500, 5000	24 h	24 h
Present			
Test 1	39.1*, 78.1*, 156*, 313*, 625*, 1250*, 2500, 5000	3 h	24 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*		
	Cytotoxicity in Main Test	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	> 625	> 5,000	Negative
Test 2	> 313	> 5,000	Negative
Present			
Test 1	> 1250	> 5,000	Negative

Remarks - Results	The test substance did not increase the number of cells with micronuclei.
CONCLUSION	The notified chemical was not clastogenic to mammalian cells under the conditions of the test.
TEST FACILITY	Canon (2015b)

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