File No: LTD/1853

October 2015

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Chemical in ESI-Y001

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.

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/1853	Epson Australia Pty Ltd	Chemical in ESI-Y001	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 recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

Hazard classification	*Hazard statement*
Serious eye damage/eye irritation (Category 2A)	H319 – Causes serious eye injury

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase:
R36: Irritating to eyes

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

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Serious eye damage/eye irritation (Category 2A): H319 – Causes serious eye injury

The above should be used for products/mixtures containing the notified chemical, if applicable, based on the concentration of the notified chemical present and the intended use/exposure scenario.

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:
  - Avoid contact with eyes

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical when replacing printer cartridges:
  - Eye protection if ocular exposure to the ink may occur.

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

- 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, physical 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 inkjet printer 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 product containing 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

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Epson Australia Pty Ltd (ABN: 91 002 625 783)
3 Talavera Road
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, degree of purity, impurities, additives/adjuvants, use details and import volume

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
USA (2011)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Chemical in ESI-Y001

MOLECULAR WEIGHT
> 1,000 Da

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

3. COMPOSITION

DEGREE OF PURITY
> 85%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: red-brown solid (granules)

Property	Value	Data Source/Justification
Melting Point/Freezing Point	Decomposition at 293 °C	Measured
Density	1,650 kg/m^3 at 20 °C	Measured
Vapour Pressure	Not determined	Based on the high molecular weight, vapour pressure is expected to be low.
Water Solubility	> 300 g/L at 25 °C	Measured
Hydrolysis as a Function of pH	t½ > 1 year at pH 7, 9	Measured
Partition Coefficient (n-octanol/water)	log Pow < -2.7at 25 °C	Measured
Adsorption/Desorption	log Koc < 1.5	Estimated based on water solubility and partition coefficient.
Dissociation Constant	pKa = 5.48 at 22 °C	Measured
Particle Size	Not determined	Introduced as a component of formulated products
Flammability	Not highly flammable	Measured

Autoignition Temperature	> 285 °C	Measured
Explosive Properties	Predicted negative	Measured (no significant exotherms when heated to 500 ºC)
Oxidising Properties	Predicted negative	Contains no functional groups that would imply oxidative 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 be imported as a component (< 10%) of inkjet printer ink.

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
Melbourne and Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
Epson 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 5-900 mL and will be packaged in sealed foil bags. The printer cartridges will be transported by road to the notifier's warehouse and then distributed to retail outlets/end-users.

USE
The notified chemical will be used as a component (< 10%) 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 hours/day	10-50

Storage and transport	< 8 hours/day	10-50
Office workers	10 seconds/day	2
Service technicians	1 hour/day	230

EXPOSURE DETAILS
Waterside, storage and transport workers may come into contact with the notified chemical, as a component of ink (< 10%), only in the unlikely event of an accident.

Service technicians and office workers may be exposed to the ink containing <10% of the notified chemical when replacing spent cartridges and during the repair and cleaning of ink jet printers. Dermal exposure is expected to be the main route of exposure, and it is expected to be minimised by users following instructions for replacing spent cartridges, which will be included with the cartridges.

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
Dermal exposure of the public to inks containing the notified chemical (at < 10%) 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 > 2,500 mg/kg bw; low toxicity
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	irritating
Mouse, skin sensitisation – local lymph node assay	no evidence of sensitisation
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* mammalian chromosome aberration test	non genotoxic

Toxicokinetics, metabolism and distribution
No data on toxicokinetics for the notified chemical was provided. For dermal absorption, molecular weights below 100 Da. are favourable for absorption and molecular weights above 500 Da. do not favour absorption (ECHA, 2014). Substances with log P values < –1 are not likely to be sufficiently lipophilic to cross the stratum corneum, and dermal absorption is likely to be low (ECHA, 2014). Therefore, absorption of the notified chemical across the skin is expected to be limited by the high molecular weight (> 500 Da) and the low partition coefficient (log POW < -2.7) of the notified chemical. However, the notified chemical contains a number of identified impurities (< 5%), that are similarly structured but with a lower molecular weight, that may be absorbed. In addition, the notified chemical contains functional groups that are expected to allow the notified chemical to be metabolised into lower molecular weight components, which may be more readily absorbed.

Acute toxicity
The notified chemical was of low acute oral toxicity in rats.

Irritation and sensitisation
The notified chemical was non-irritating to the skin and irritating to the eyes. The notified chemical was not a skin sensitiser in a local lymph node assay with mice.

Mutagenicity/Genotoxicity/Carcinogenicity
The notified chemical was negative in a modified bacterial reverse mutation assay for azo dyes (Prival and Mitchell, 1982). The modified test is thought to yield a greater detection of mutagenic azo dyes as it utilises a reductive pre-incubation (during which the azo dye is reduced to amine species) before the test is carried out. The notified chemical was not clastogenic in an in vitro mammalian chromosome aberration test using human lymphocytes.

Additionally the notified chemical is an azo compound and may break down to its component amines. Azo bond reduction and cleavage occurs by an enzyme-mediated metabolism in the liver, skin and intestines. In the liver, metabolism is facilitated by cytosolic and microsomal enzymes (Platzek *et al*., 1999), including NADH cytochrome P450 reductase, NAD(P)H quinone oxidoreductase, and cytochrome P450s (OEHHA, 2012). Bacterial strains in human faeces have been shown to cleave azo dyes, suggesting the important role of the intestinal microflora in azo reduction (Platzek *et al*., 1999).

Although azo reduction occurs favourably in anaerobic conditions, several in vitro and in vivo studies indicated that this process could also occur aerobically when azo dyes are applied to the skin (SCCP, 2005). In vitro, the skin microflora of mouse, guinea pig and human caused reductive cleavage of the azo dyes, followed by percutaneous absorption (SCCNFP, 2002). In addition, non-biological processes, such thermal and photochemical degradation, have also been reported to break azo linkages (Engel *et al*., 2009).

None of the component amines of the notified chemical are on the European Union (EU) Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) list of 22 carcinogenic aromatic amines in Annex XVII Appendix 8 (European Commission, 2006).

Health hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

Hazard classification	*Hazard statement*
Serious eye damage/eye irritation (Category 2A)	H319 – Causes serious eye injury

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase(s):
R36: Irritating to eyes

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The notified chemical is classified as an eye irritant but is otherwise of low toxicity, with low potential for systemic effects due to the predicted low dermal absorption. The notified chemical will be handled by workers at < 10% concentration, and at such concentrations, eye irritation is not expected.

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, once dried the notified chemical will be bound to the paper and is not expected to be available for exposure.

Therefore, based on the expected low exposure of 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 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 sealed ready-to-use ink 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, transport and storage, and is likely to be limited to accidental spills and leaks.

RELEASE OF CHEMICAL FROM USE
During use, the majority of the notified chemical will be cured within an inert ink matrix and bound to paper substrates, and will not be released from printed paper substrates. It is estimated by the notifier approximately 5% of the ink containing the notified chemical will remain in spent cartridges. The ink remaining in the ink cartridges during the cartridge recycling process will not be reused, and will be disposed of to landfill with the packaging in accordance with local government regulations. Environmental release of the notified chemical is possible during paper recycling and from the disposal of used print cartridges.

RELEASE OF CHEMICAL FROM DISPOSAL
Following use, spent ink cartridges containing residues of the notified chemical will be collected for recycling in collection boxes at general merchandising stores and post offices, etc., or be disposed of to landfill. The spent cartridges collected for recycling will be sent for disassembly and recycled into raw materials such as plastics. Ink residues containing the notified chemical separated from the spent cartridges will be disposed of in accordance with local government regulations, most likely to landfill.

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 remainder will undergo paper recycling processes. During paper recycling processes, waste paper is repulped using a variety of chemical treatments which, amongst other things, will enhance ink detachment from the fibres. Waste water containing the notified chemical will be released to sewer.

7.1.2. Environmental Fate
The notified chemical is not readily biodegradable (< 5% in 28 days). For details of the environmental fate study, please refer to Appendix C. 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 and low adsorption coefficient (log K_{OC} < 1.5), 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 log K_{OC}. 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. Based on its high water solubility and low partition coefficient (log P_{OW} < -2.7), the notified chemical is not expected to bioaccumulate. In landfill and in surface waters, the notified chemical is expected to eventually degrade through biotic and abiotic processes to form water and oxides of carbon, nitrogen and sulphur.

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:	0.425	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 2.835 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

The results from ecotoxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 > 120 mg/L	Not harmful to fish
Daphnia Toxicity	48 h EC50 > 54 mg/L	Potentially harmful to *Daphnia*
Lemna Toxicity*	7 d E_rC50 > 28 mg/L (dry wt)	Potentially harmful to *Lemna*

*Non-standard test

Based on the above acute ecotoxicological endpoints, the notified chemical is not expected to be harmful to fish, and was not harmful to daphnids at a geometric mean measured concentration of 54 mg/L, although the toxicity to Daphnia at higher concentrations is unknown.

7.2.1. Predicted No-Effect Concentration

The predicted no-effects concentration (PNEC) has been calculated from the most sensitive endpoint for *Daphnia*. A safety factor of 1000 was used given acute endpoints for two trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (*Daphnia*, 48 h)	> 54	mg/L
Assessment Factor	1000	
Mitigation Factor	1.00	
PNEC:	> 54	µg/L

7.3. Environmental Risk Assessment

The Risk Quotient (Q = PEC/PNEC) has been calculated based on the predicted PEC and PNEC.

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.425	> 54	< 0.008
Q - Ocean	0.043	> 54	< 0.001

The Risk Quotients for discharge of treated effluents containing the notified chemical to the aquatic environment indicates that the notified chemical is unlikely to reach ecotoxicologically significant concentrations in surface waters, based on its maximum annual importation quantity. Whilst the notified chemical is not readily biodegradable, it is expected to have a low potential for bioaccumulation. On the basis of the PEC/PNEC ratio, maximum annual importation volume, and assessed use pattern in printing ink, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point Decomposition at 293 °C

Method	EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks	Determined using a Buchi Melting Point Apparatus B-545. A change in form was observed at 293 °C, which was likely due to decomposition.
Test Facility	Intertek (2011)

Density 1,650 kg/m^3 at 20 ºC

Method	EC Directive 92/69/EEC A.3 Relative Density.
Remarks	Determined using a Micromeritics Pycnometer 1330 TC.
Test Facility	Intertek (2011)

Water Solubility > 300 g/L at 25 °C

Method	EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks	The solubility was determined by a visual assessment of the test substance.
Test Facility	Intertek (2011)

Hydrolysis as a Function of pH $t_{½}$ > 1 year at pH 7 and 9

Method EC Council Regulation No 440/2008 C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$ (years)*
7	25	> 1
9	25	> 1

Remarks	The test substance was not soluble at pH 4 and therefore not measured. After 5 days under the accelerated conditions of 50 ± 1 °C, the hydrolysis of the notified chemical was < 10% at pH 7 and 9. Therefore, it can be concluded that under the conditions of the test, the notified chemical is expected to be stable under neutral and basic conditions.
Test Facility	Intertek (2011)

Partition Coefficient (n-octanol/water) log Pow < -2.7 at 25 °C

Method	EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	Shake Flask Method
Test Facility	Intertek (2011)

Dissociation Constant pKa = 5.48 at 22 °C

Method	In-house method
Remarks	Determined by titration with 0.1M HCl using a potentiometric titrator fitted with a pH electrode; performed at three different sample weights, with pKa estimated by extrapolation to infinite dilution.
Test Facility	Intertek (2011)

Flammability Not highly flammable

Method	EC Council Regulation No 440/2008 A.10 Flammability (Solids).
Remarks	Determined by measuring the burning rate of the test material. The test material failed to ignite following application of a Bunsen burner flame for a period of two minutes.
Test Facility	Harlan (2011a)

Autoignition Temperature > 285 °C

Method	EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature (Solids)
Remarks	Determined by heating aliquots of the test substance in an oven (up to 285 °C) and observing for any signs of ignition.
Test Facility	Harlan (2011a)

Explosive Properties Predicted negative

Method	EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks	Following the observation of functional groups in the notified chemical that imply explosive properties, thermal analysis was conducted using differential scanning calorimetry (DSC) (25-500 °C temperature program at a rate of 5 °C/min, in an air atmosphere). Under the conditions of the test, there were no significant exotherms.
Test Facility	Harlan (2011a)

Oxidizing Properties Predicted negative

Method	EC Council Regulation No 440/2008 A.17 Oxidizing Properties (Solids).
Remarks	The structure of the notified chemical was assessed for chemical groups that imply oxidising properties.
Test Facility	Harlan (2011a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method. EC Council Regulation No 440/2008 B.1 tris Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Wistar (RccHan™:WIST)
Vehicle	Water
Remarks - Method	No protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 F	2,000	0/3
2	3 F	2,000	0/3

LD50	> 2,000 mg/kg bw
Signs of Toxicity	No signs of systemic toxicity were noted.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks - Results	All animals showed expected bodyweight gain.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Harlan (2011b)

B.2. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Council Regulation No 440/2008 B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White (Hsdlf:NZW)
Number of Animals	2 M
Vehicle	Water
Observation Period	72 hours
Type of Dressing	Semi-occlusive.
Remarks - Method	No protocol deviations

RESULTS

Remarks - Results	There was no indication of skin irritation noted in either animal during the study. Both animals showed expected bodyweight gain. Yellow coloured staining, not preventing evaluation of skin responses, was noted at both treated skin sites during the study.
CONCLUSION	The notified chemical is non-irritating to the skin.
TEST FACILITY	Harlan (2011c)

B.3. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Council Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 M
Observation Period	14 days
Remarks - Method	No protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	2	2	2	2	< 14 days	0
Conjunctiva: chemosis	2	1	1	2	< 14 days	0
Conjunctiva: discharge	1.3	1.3	1.3	2	< 7 days	0
Corneal opacity	1	0.7	1	1	< 7 days	0
Iridial inflammation	1	0.7	1	1	< 7 days	0

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

Remarks - Results	During the study, scattered or diffuse corneal opacity, iridal inflammation and moderate conjunctival irritation were noted. All treated eyes were normal at the 14-day observation. Orange coloured staining of the fur was also noted around all treated eyes. All animals showed expected bodyweight gains.

CONCLUSION The notified chemical is irritating to the eye.

TEST FACILITY Harlan (2011d)

B.4. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
EC Council Regulation No 440/2008 B.42 Skin Sensitisation (Local Lymph Node Assay)

Species/Strain	Mouse/CBA/Ca
Vehicle	Water
Preliminary study	Yes
Positive control	Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using α-hexylcinnamaldehyde.
Remarks - Method	No protocol deviations

RESULTS

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	4 F	758.86	-
5	4 F	743.73	0.98
10	4 F	820.82	1.08
25	4 F	730.81	0.96
Positive Control			
25	5 (sex unknown)	not reported	6.77

Remarks - Results	There were no deaths. No signs of systemic toxicity were noted in the test or control animals during the study. All test and control animals showed comparable bodyweight gain.

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

TEST FACILITY Harlan (2011e)

B.5. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test incorporating the Prival and Mitchell modification for azo dyes (Prival and Mitchell, 1982)
Pre incubation procedure

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, TA102
Metabolic Activation System	S9 fraction from Phenobarbitone/β-Naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 0, 50, 150, 500, 1,500, 5,000 µg/plate b) Without metabolic activation: 0, 50, 150, 500, 1,500, 5,000 µg/plate
Vehicle	Water
Remarks - Method	*E. coli* was not used. 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	>5,000			
Test 1		> 5,000	> 5,000	negative
Test 2		> 5,000	> 5,000	negative
Present	> 5,000			
Test 1		> 5,000	> 5,000	negative
Test 2		≥ 5,000	> 5,000	negative

Remarks - Results

No visible reduction in the growth of the bacterial background lawn was caused by the test substance at any dose level except for TA1537 at 5,000 µg/plate with metabolic activation in test 2. A yellow test substance colouration observed at ≥ 150 µg/plate but did not prevent the scoring of revertant colonies.

No significant increases in the frequency of revertant colonies were noted for any of the bacterial strains, with any dose of the test substance, either with or without metabolic activation.

The positive controls produced satisfactory responses, thus confirming the activity of 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 (2011f)

B.6. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

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

Cell Type/Cell Line	Human lymphocytes
Metabolic Activation System	S9 fraction from Phenobarbitone/β-Naphthoflavone induced rat liver
Vehicle	Eagle's minimal essential medium with HEPES buffer (MEM)
Remarks - Method	No protocol deviations

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 19.53, 39.06*. 78.13*, 156.25, 312.5*, 625, 937.5*, 1250	4	24
Test 2	0*, 15, 30, 60*, 120*, 240*, 360*	24	24
Present			
Test 1	0*, 19.53, 39.06*. 78.13*, 156.25, 312.5*, 625, 937.5*, 1250	4	24
Test 2	0*, 30, 60*, 120, 240*, 480*, 960*	4	24

*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	≥ 1,250	> 937.5	≥ 156.25	negative
Test 2	≥ 312.5	> 360	≥ 120	negative
Present				
Test 1	≥ 1,250	> 937.5	≥ 156.25	negative
Test 2	≥ 1,250	> 960	≥ 120	negative

Remarks - Results

In both tests 1 and 2, the test substance did not induce a significant increase in the numbers of polyploid cells or in the frequency of cells with chromosome aberrations at any dose level in either of the exposure groups.

The vehicle control and positive control cultures gave values of chromosome aberrations within the expected range, indicating that the metabolic activation system and test method were satisfactory.

CONCLUSION

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

TEST FACILITY

Harlan (2012)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Theoretical Oxygen Demand (ThOD)
Remarks - Method	No significant deviation in protocol.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
5	< 5	5	55
10	< 5	10	63
15	< 5	15	66
20	< 5	20	65
28	< 5	28	67

Remarks - Results	All validity criteria for the test were satisfied. The percentage degradation of the reference compound, sodium benzoate, surpassed the threshold level of 60% by 10 days (63%), and attained 67% degradation by 28 days. Therefore, the test indicates the suitability of the inoculums. The notified chemical attained < 5% degradation by 28 days. Therefore, the notified chemical cannot be classified as readily biodegradable according to the OECD (301F) guideline.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	Brixham (2011b)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-static.
Species	*Cyprinus carpio* (carp)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	47 mg $CaCO_3$/L
Analytical Monitoring	HPLC-PDA
Remarks – Method	No significant deviation in protocol.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality (%)*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	Control	10	0	0	0	0	0
120	120-140	10	0	0	0	0	0

LC50	> 120 mg/L at 96 hours.
NOEC	120 mg/L at 96 hours.

Remarks – Results	All validity criteria for the test were satisfied. The test solutions were renewed every 48 hours during the 96 h test period. The 96 h LC50 and NOEC for fish were determined to be > 120 mg/L and 120 mg/L, respectively, based on measured concentrations.
CONCLUSION	The notified chemical is not harmful to fish.
TEST FACILITY	Brixham (2013)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	50 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	No significant deviation in protocol. Based on a preliminary study, the test solutions were prepared in dechlorinated water.

RESULTS

Concentration mg/L		*Number of D. magna*	*Cumulative Immobilised (%)*	
Nominal	*Actual*		*24 h*	*48 h*
Control	Control	20	0	0
120	54	20	0	0

EC50	> 54 mg/L at 48 hours
NOEC	54 mg/L at 48 hours
Remarks - Results	All validity criteria for the test were satisfied. The test solutions were not renewed during the 48 h test period. The 48 h EC50 for daphnids was determined to be > 54 mg/L, based on measured concentrations. However, as the measured concentration falls below 100 mg/L, the results are inconclusive in determining the acute toxicity of the notified chemical to aquatic invertebrates.
CONCLUSION	The notified chemical may potentially be harmful to daphnids.
TEST FACILITY	Brixham (2011a)

C.2.3. Acute toxicity to *Lemna*

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 221 *Lemna sp*. Growth Inhibition Test – Static.
Species	*Lemna minor* (duckweed)
Exposure Period	7 days
Concentration Range	Nominal: 0.36-120 mg/L Actual: 0.14-28 mg/L
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	Not specified
Remarks - Method	No significant deviation in protocol.

RESULTS

Concentration (mg/L dry wt)		*Increase in Plant Dry Weight Compared to Control (%)*
Nominal	*Actual*	

Control	Control	—
0.36	0.14	100
1.1	0.52	92
3.7	2.2	79
12	5.3	89
38	7.5	98
120	28	97

E_rC50	> 28 mg/L (dry wt) at 7 days
NOE_rC	28 mg/L (dry wt) at 7 days
Remarks - Results	The pH of the test solutions ranged from 6.8 to 8.6, and exceeded the maximum expected increase of 1.5 units; however, this was not deemed to have had a significant impact on the validity or integrity of the study. All other validity criteria for the test were met and satisfied. The test solutions were not renewed during the 7 d test period. The 7 d E_rC50 and NOE_rC for *Lemna* were determined to be > 28 mg/L (dry wt) and 28 mg/L (dry wt), respectively, based on measured concentrations.
CONCLUSION	The notified chemical may potentially be harmful to duckweed.
TEST FACILITY	Brixham (2012)

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