File No: LTD/1612

November 2012

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

E-C104

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/1612	Epson Australia Pty Ltd	E-C104	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).

The environmental hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS)* is presented below. Environmental classification under the GHS is not mandated in Australia and carries no legal status but is presented for information purposes.

Hazard classification	*Hazard statement*
Acute Category 3	Harmful to aquatic life
Chronic Category 3	Harmful to aquatic life with long lasting effects

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

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

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

Recommendations

CONTROL MEASURES
Occupational Health and Safety

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

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

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

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

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if
- the importation volume exceeds one tonne per annum notified chemical;
- the notified chemical is imported in any form other than as a component of sealed ink-jet cartridges of capacity 100 g or less;
- further information becomes available on the genotoxicity potential of the notified chemical;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a colourant in inkjet printing 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

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, CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, impurities, additives/adjuvants 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
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
E-C104

MOLECULAR WEIGHT
> 500 Da

ANALYTICAL DATA
Reference HPLC-UV, UV-Vis and FTIR spectra were provided

3. COMPOSITION

DEGREE OF PURITY > 85%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: blue powder

Property	Value	Data Source/Justification
Melting Point/Boiling Point	Decomposition observed from 300 °C	Measured
Density	1720 kg/m^3 at 20 °C	Measured
Vapour Pressure	Not determined	As the notified chemical is a solid and has a high molecular weight, the vapour pressure is expected to be low.
Water Solubility	> 403 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	$t_{1/2}$ >1 year at 25 °C (pH 4, 7, 9)	Measured
Partition Coefficient (n-octanol/water)	log Pow < -4.6 at 20 °C	Measured
Surface Tension	73.7 mN/m at 20 °C	Measured
Adsorption/Desorption	log K_{oc} < 1.3 at 35 °C	Measured
Dissociation Constant	Estimated pKa = 1.7-11.4	Calculated for the free acid form. The notified chemical is a salt which is expected to be ionised under environmental conditions.
Particle Size	Inhalable fraction (< 100 µm): 53.72% Respirable fraction (< 10 µm): 11.66% MMAD* = 116.98 µm	Measured
Flammability	Not highly flammable	Measured
Autoignition Temperature	Not auto-ignitable	Measured

Explosive Properties	Not explosive	Measured
Oxidising Properties	Predicted negative	Contains no functional groups that would imply oxidising properties

* MMAD = Mass Median Aerodynamic Diameter

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. The notifier has advised that the notified chemical is not considered to be a self-reactive substance.

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 (up to 3%) 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
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. Printer cartridges containing the notified chemical (at up to 3% concentration) will be transported within Australia (to/from warehousing facilities and retail outlets/end-users) by road.

USE
The notified chemical will be used as a component (up to 3%) of inkjet printing ink for commercial and household printers.

OPERATION DESCRIPTION
The notified chemical will not be manufactured, reformulated or repackaged in Australia.

Following distribution of the imported ink containing the notified chemical (in the sealed cartridges) to end-use sites, printer service technicians, office workers and home users will open the packaging and insert the cartridges into the printers. When empty, the spent cartridges will be removed from the printer and disposed of.

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)*
Transport workers	2	50
Warehouse workers	2-6	260
Printer technicians	8	260
Office workers	8	260

EXPOSURE DETAILS

During transport and storage, workers are unlikely to be exposed to the notified chemical (unless the packaging is breached).

Printer technicians and office workers may be exposed to the ink containing the notified chemical (up to 3% concentration) when replacing used ink cartridges, clearing paper jams from the printer and during printer maintenance. Dermal exposure is expected to be the most likely route of exposure. However, given the design of the cartridges, exposure to the notified chemical is expected to be limited if users follow the instructions for replacing spent 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 matrix and is not expected to be bioavailable. Inhalation exposure to the notified chemical is not expected under the proposed use scenario.

6.1.2. Public Exposure

The public may use inkjet printer cartridges containing the notified chemical (at ≤3% concentration) for printing purposes at home. Exposure of these users to the notified chemical is expected to be of a similar or lesser extent than the exposure experienced by office workers.

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay (LLNA)	no evidence of sensitisation
Rat, combined repeat dose and reproductive /developmental oral toxicity – 42 days.	NOAEL = 250 mg/kg bw/day (repeated dose) = 1000 mg/kg bw/day (reproductive /developmental)
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* Mammalian Cell Mutation Test	genotoxic
Genotoxicity – *in vitro* Mammalian Chromosomal aberration test	non genotoxic
Genotoxicity – *in vivo* Mammalian Erythrocyte Micronucleus Test.	Equivocal

Toxicokinetics, metabolism and distribution.

Given the relatively high molecular weight (> 500 Da) and low partition coefficient (log Pow < -4.6 at 20 °C) of

the notified chemical, dermal absorption is not expected.

Given the coloured urine seen in the acute oral toxicity study and coloured organs in the repeated dose oral toxicity study, it is likely that the notified chemical can be absorbed from the gastrointestinal tract following oral exposure.

Acute toxicity.
The notified chemical was found to be of low acute oral and dermal toxicity in rats (LD50 > 2000 mg/kg bw). In the acute oral toxicity study, bluish coloured urine was noted in 4/5 animals on days 1-4 indicating absorption of the blue coloured notified chemical.

Irritation and Sensitisation.
The notified chemical was found to be non-irritating to the skin of rabbits and slightly irritating to the eyes of rabbits. In the eye irritation study, only slight conjunctival effects were observed that cleared within 24 hours. Blue pigmentation of the conjunctiva and partial cornea was noted in the treated eye of all animals. Pigmentation of the conjunctiva was still observed at the end of the 96-hour observation period.

The notified chemical was non-sensitising in a Local Lymph Node Assay (LLNA) and no dose-related increases in stimulation index were noted at the concentrations tested. However it is noted that the chemical was only tested up to 50% concentration.

Repeated Dose Toxicity
In a combined repeated dose and reproductive oral toxicity study, blue colouration of the stool and organs was noted at all doses. In addition, there was an increase in globule leukocytes in the glandular stomach in males receiving 1000 mg/kg bw/day, which persisted till the end of the recovery period.

Based on an increase of globule leukocytes in the glandular stomach in males at 1000 mg/kg bw/day, the NOAEL was determined to be 250 mg/kg bw/day.

Genotoxicity
The notified chemical was found to be non-mutagenic in the bacterial reverse mutation test. However, the notified chemical was shown to have the potential to induce gene mutation in cultured mammalian cells in an *in vitro* Mammalian Cell Gene Mutation Test. The notified chemical induced a significant increase in total and small mutant colonies at 2500 µg/mL and above in the 24 hour exposure group. There was no corresponding significant increase in the large mutant colonies at any dose. According to the study authors, this increase in small but not large colonies is considered to show the substance has a potential to induce chromosomal aberration rather than point gene mutations. The study authors note that the positive result in the Mammalian Cell Gene Mutation Test may be due to the chemical's copper component. In addition, the authors note that mutations due to copper may have the potential to be recognised in the *in vitro* test system and not recognised in an *in vivo* test system.

The notified chemical was found to be non clastogenic to Chinese Hamster cells in a Mammalian Chromosomal aberration test and negative under the conditions of the test in an *in vivo* Mammalian Erythrocyte Micronucleus Test. However, it is not clear from the *in vivo* study, whether the test substance reached the target organ.

Overall, these results do not rule out the genotoxic potential of notified chemical, as some studies showed positive or equivocal results.

Toxicity for reproduction.
During the combined repeated dose and reproductive/developmental toxicity study, no adverse reproductive or developmental outcomes were noted. The NOAEL for these endpoints was set at 1000 mg/kg bw/day.

Health 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).

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on available information, the potential for the notified chemical to cause genotoxic effects cannot be

ruled out. The notified chemical is not expected to be irritating or sensitising at the concentrations of use, and has low acute oral toxicity. Based on its physico-chemical properties, the chemical is likely to have limited potential for dermal absorption.

Dermal or possibly ocular exposure to workers may occur during printing, changing cartridges, printer repair and contact with printed substrates before they have dried. This exposure is expected to be infrequent and only incidental in nature, given the containment of the notified chemical within very small ink cartridges and its concentration in the ink (< 3%). Inhalation exposure is not expected to occur.

Where more frequent contact may occur, e.g. to printer technicians, any exposure potential would be further controlled through use of personal protective equipment (PPE).

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

6.3.2. Public Health

The potential of the notified chemical to have genotoxic effects cannot be ruled out. The type of public exposure to the notified chemical during the use of inkjet printers is expected to be similar to that experienced by workers, but less frequent. Therefore, based on very low potential exposure, the risk 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 as a component of ink sealed in printer cartridges. No release of the notified chemical to the environment is expected due to manufacture, reformulation or repackaging as these activities will not occur in Australia.

RELEASE OF CHEMICAL FROM USE

During use, the notified chemical will be fixed within an inert ink matrix adhering to paper and is not expected to be released to the environment once cured. The spillage or leakage of ink during transport, use, installation or replacement will be contained with absorbent material and disposed of to landfill.

RELEASE OF CHEMICAL FROM DISPOSAL

Following its use, the notified chemical is anticipated to share the fate of printed paper to be disposed of to landfill or subjected to paper recycling processes. Up to half the amount of the total import volume of the notified chemical may be released to sewage treatment plants when recycling waste water is disposed of to sewer. Residues of the notified chemical in empty cartridges (up to 3% of the total annual import volume) are expected to be disposed of to landfill along with the empty cartridges.

7.1.2. Environmental Fate

The notified chemical as a component of ink is expected to remain fixed to paper for its useful life. The notified chemical is expected to be disposed of to landfill along with printed paper or released to sewer in recycling wastewaters when paper is recycled. During paper recycling processes, waste paper is repulped using a variety of chemical agents which, amongst other things, enhance detachment of ink from the fibres. The notified chemical will partition to the supernatant water based on its high water solubility (> 403 g/L), which is expected to be released to the sewer. During waste water treatment processes in sewage treatment plants (STPs), the notified chemical is not expected to be removed from waste water due to its water solubility and low soil adsorption coefficient (log Koc < 1.3) and may be released to surface waters. The notified chemical is not readily nor inherently biodegradable (0% over 28 days for both) and hydrolysis is negligible at environmental conditions (> 1 year at pH 4, 7 and 9). In landfill, the notified chemical is likely to be mobile based on its high water solubility and low soil adsorption coefficient (log Koc < 1.3). However, the notified chemical is not expected to bioaccumulate due to the low n-octanol/water partition coefficient (log Pow < -4.6), high water solubility and molecular weight (> 500 Da). It is expected to eventually degrade by biotic and abiotic processes to form water, oxides of carbon, nitrogen and sulphur, and inorganic salts. For the details of the environmental fate studies please refer to Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)

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

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

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.425 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 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 hours)	LC50 > 97.9 mg/L (Medaka)	Not harmful
	LC50 > 106 mg/L (Zebra fish)	Not harmful
Daphnia Toxicity (48 hours)	EC50 = 60.7 mg/L	Harmful
Algal Toxicity (72 hours)	E_rC50 > 103 mg/L	Not harmful
	NOEC = 16.3 mg/L	
Inhibition of Bacterial Respiration	EC50 > 100 mg/L	Not inhibitory to microorganism respiration

Based on the acute toxicity endpoint for *daphnia*, the notified chemical is formally classified as "Acute Category 3: Harmful to aquatic life" under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009). One chronic toxicity endpoint for algae was available. Therefore, the long-term classification for the notified chemical was determined based on the most stringent outcome by comparing the long-term hazard classification using either the acute or chronic data. The most stringent outcome resulted from classification based on the acute endpoint for *daphnia*. The notified chemical is therefore formally classified under GHS as "Chronic Category 3: Harmful to aquatic life with long lasting effects".

7.2.1. Predicted No-Effect Concentration

The endpoint for the most sensitive species (*Daphnia*) from the reported results is used to calculate the predicted no-effect concentration (PNEC). An assessment factor of 100 was used as the endpoints for three tropic levels are available. The acute toxicity endpoint for *daphnia* was used because it provides the lowest, most conservative PNEC value.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Invertebrates).	60.7	mg/L
Assessment Factor	100	
PNEC:	607	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	*PEC µg/L*	*PNEC µg/L*	*Q*
Q - River:	0.43	607	< 0.001
Q - Ocean:	0.04	607	< 0.001

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

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting/Boiling Point Decomposition observed from 300 °C

Method OECD TG 102 Melting Point/Melting Range
OECD TG 103 Boiling Point
Remarks Determined using differential scanning calorimetry. An exothermic effect was detected between 300 °C and 400 °C, which was determined to be due to reaction and/or decomposition of the test substance.
Test Facility NOTOX (2011c)

Density 1720 kg/m^3 at 20 ºC

Method OECD TG 109 Density of Liquids and Solids
Remarks Determined using a gas comparison stereopycnometer.
Test Facility NOTOX (2011c)

Water Solubility > 403 g/L at 20 ºC

Method OECD TG 105 Water Solubility.
EC Directive 440/2008, A.6 Water Solubility.
Remarks Flask Method. Following two preliminary tests, one test sample was used for the water solubility determination of the notified chemical. The solution pH was 7.4.
Test Facility NOTOX (2011c)

Hydrolysis as a Function of pH $t_{1/2}$ > 1 year at 25 ºC (pH 4, 7, 9)

Method OECD TG 111 Hydrolysis as a Function of pH
EC Directive 440/2008, C.7 Degradation-Abiotic Degradation: Hydrolysis as a Function of pH

pH	*T (°C)*	*t½ (years)*
4	25	> 1
7	25	> 1
9	25	> 1

Remarks Preliminary tests were performed at pH 4, 7 and 9. At each pH value, less than 10% hydrolysis was observed after 5 days. This is equivalent to a half-life of > 1 year at 25 ºC.
Test Facility NOTOX (2011c)

Partition Coefficient (n-octanol/water) log Pow < -4.6 at 20 ºC

Method OECD TG 117 Partition Coefficient (n-octanol/water)
EC Directive 440/2008, A.8 Partition Coefficient (n-octonal/water)
Remarks Estimation method. The test substance is a complex mixture of organic salts and an estimation method was used according to the guideline above. A n-octonal/water partition coefficient (Pow) was calculated from individual solubilities of the notified chemical in water (> 403 g/L) and n-octanol (< 9.6 mg/L).
Test Facility NOTOX (2011c)

Surface Tension 73.7 mN/m at 20 °C

Method OECD TG 115 Surface Tension of Aqueous Solutions
Remarks Determined using the OECD harmonised ring method (concentration: 1 g/L).
The test substance was considered not to be surface active.
Test Facility NOTOX (2011c)

Adsorption/Desorption – screening test log K_{oc} < 1.3 at 35 °C

Method OECD TG 121 Estimation of the adsorption Coefficient (K_{oc}) on Soil and on Sewage sludge using HPLC
EC Directive 440/2008,C.19 Estimation of the Adsorption Coefficient (K_{oc}) on Soil and on Sewage Sludge using HPLC

Remarks The notified chemical eluted before the reference substance (log K_{oc} = 1.26). The log K_{oc} was concluded to be < 1.3.

Test Facility NOTOX (2011c)

Dissociation Constant Expected to be ionised under environmental conditions

Method The notified chemical is a mixture of components, making direct measurement of its dissociation constants impractical. The notifier had provided pKa values for the free acid form of the notified chemical, calculated by the Perrin method, in lieu of measured values.

Remarks The notified chemical contains two acidic groups with calculated pKa values of 9.64 to 11.4. Calculated pKa values for two basic groups are 1.7 and 9.47.
The notified chemical is a salt which is expected to be ionised under environmental conditions.

Test Facility NOTOX (2011c)

Particle Size

Method ISO 13320:2009 Particle Size Analysis – Laser Diffraction Methods

Range (μm)	*Mass (%)*
< 259.92	90
< 100.00	53.72
< 89.20	50
< 10.00	11.66
< 8.40	10

Remarks The test substance was dispersed in silicone oil and analysed (over the range 0.02 μm to 2000 μm) 5 times using laser diffraction. The MMAD was 116.98 μm.

Test Facility Chilworth (2011)

Flammability Not highly flammable

Method EC Council Regulation No 440/2008 A.10 Flammability (Solids)

Remarks In the preliminary test, no propagation of combustion (200 mm length within 4 minutes) was observed.

Test Facility NOTOX (2011c)

Autoignition Temperature Not auto-ignitable

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

Remarks The test substance was heated in an oven at 0.5 °C/min and the temperature of the sample/oven measured using thermocouples. An exothermic event was noted at an oven temperature starting at 319 °C (sample temperature < 400 °C). During further heating the temperature of the test substance continued to remain above the temperature of the oven, with the temperature of the test substance reaching 400 °C at an oven temperature of 362 ° C. As no sharp temperature rise was observed, the test substance was not considered to be auto-ignitable.

Test Facility NOTOX (2011c)

Explosive Properties Not explosive

Method EC Council Regulation No 440/2008 A.14 Explosive Properties

Remarks	Determined using differential scanning calorimetry (25-550 °C temperature program at a rate of 100 °C/min, under a flow of nitrogen). Exothermic decomposition was observed at 386 °C, with an exothermic decomposition energy of 173 J/g. Under the conditions of the test, substances were considered to have explosive properties if the exothermic decomposition energy was greater than 500 J/g with an onset of decomposition below 500 °C. Therefore, the test substance was not considered to have explosive properties. The study authors noted that the sample chamber was swollen following the experiment.
Test Facility	NOTOX (2011c)

Oxidizing Properties Predicted negative

Method	EC Council Regulation No 440/2008 A.17 Oxidizing Properties (Solids)
Remarks	The structure of the test substance was not considered to contain functional groups that would imply oxidising properties.
Test Facility	NOTOX (2011c)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
- Species/Strain: Rat/Crl:CD(SD)
- Vehicle: Water
- Remarks - Method: No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	3F	2000	0/3
II	3F	2000	0/3

- LD50: > 2000 mg/kg bw
- Signs of Toxicity: No animals died during the course of the study. The test substance was noted in the faeces of all animals (up to day 4) and chromaturia (bluish) was noted in all animals on days 2-3. These effects were attributed to the test substance.
- Effects in Organs: None

CONCLUSION: The notified chemical is of low toxicity via the oral route.

TEST FACILITY: MCMC (2010c)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 402 Acute Dermal Toxicity – Limit Test.
- Species/Strain: Rat/Crl:CD(SD)
- Vehicle: None
- Type of dressing: Occlusive
- Remarks - Method: No significant protocol deviations
  The test substance was applied to a lint cloth (lined with an impermeable sheet) that was moistened with water and the cloth applied to the skin.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5M	2000	0/5
II	5F	2000	0/5

- LD50: > 2000 mg/kg bw
- Signs of Toxicity: None
- Effects in Organs: None
- Remarks - Results: Decreased body weight was noted in one female at day 4, with the animal noted to have gained weight by day 8.

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

TEST FACILITY: MCMC (2011a)

B.3. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	Water
Observation Period	72 hours
Type of Dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations The test substance was moistened with water and applied to a patch, which was then applied to the skin.
RESULTS	
Remarks - Results	No signs of irritation were noted.
CONCLUSION	The notified chemical is non-irritating to the skin.
TEST FACILITY	MCMC (2010a)

B.4. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	6
Observation Period	96 hours
Remarks - Method	All animals received 0.1 g test substance in one eye. In 3/6 animals, the treated eyes were washed with 20 mL distilled water for 30 seconds, from 30 seconds after the test substance administration. In the remaining 3/6 animals, the treated eyes remained unwashed.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0	0	0	1	< 24 hours	0
Conjunctiva: chemosis	0	0	0	2	< 24 hours	0
Conjunctiva: discharge	0	0	0	1	< 24 hours	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

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

Remarks - Results	Slight conjunctival effects were noted in all 3 rabbits (eyes unwashed) at the 1 hour observation, which had reversed by the 24 hour observation. From the 24 hour observation period blue pigmentation of conjunctiva and partial cornea was noted in the treated eye of all animals. At the end of the observation period, pigmentation of the conjunctiva (but not the cornea) was still observed in all animals. Similar effects were noted in the 3 rabbits which had their eye washed following administration of the test substance.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	MCMC (2010b)

B.5. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain	Mouse/CBA/JNCrlj
Vehicle	Acetone/olive oil (4:1)
Remarks - Method	No significant protocol deviations. The maximum concentration tested (50%) was based on the absence of effects at 50% concentration (reported as the maximum feasible dose) in a preliminary study. Negative (vehicle) and positive (α-hexylcinnamaldehyde; 25%) controls were run in parallel with the test substance.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	1396.8	-
5	1076.8	0.77
15	616.0	0.44
50	746.5	0.53
Positive Control		
25	3979.8	2.85

Remarks - Results	No signs of systemic toxicity were noted. Stimulation indices of < 3 were recorded for the test substance, indicating the absence of skin sensitisation potential. However, it is noted that the stimulation index was also < 3 for the positive control. The study authors indicated that the proliferative response value that was obtained for the positive control was within the historical range for the test facility, and was therefore acceptable, although it appears that the value obtained for the vehicle is outside the historical range of the facility (≤968.2). Considering the proliferative responses obtained for the test substance (including the absence of a dose-response relationship), the high vehicle control value does not appear to have impacted on the study conclusion.
CONCLUSION	Under the conditions of the test, there was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	MCMC (2010f)

B.6. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test.
Species/Strain	Rat/Crl:CD(SD)
Route of Administration	Oral – gavage
Exposure Information	Males: From 14 days before mating (42 days) Females: From 14 days before mating until day 4 of lactation. Recovery females: For 42 days without mating. Dose regimen: 7 days per week Post-exposure observation period: 14 days for recovery groups

Vehicle	Water
Remarks - Method	No significant protocol deviations. Dose levels were set on the basis of a preliminary 14-day study (not provided) in which the only observed effects were blue colouration of the stomach or intestinal contents, lymph nodes, kidneys, lungs and faeces at the highest dose of 1000 mg/kg bw/day.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	7M/12F	0	0/19
low dose	12M/12F	50	1/24
mid dose	12M/12F	250	0/24
high dose	7M/12F	1000	0/19
control recovery	5M/5F	0	0/10
high dose recovery	5M/5F	1000	0/10

	Necropsy: end dosing period								*Necropsy: end recovery period*			
Sex	*Male*				*Female*				*Male*		*Female*	
Dose mg/kg	0	50	250	1000	0	50	250	1000	0	1000	0	1000
Organ Findings *No. animals*	5	5	5	5	5	5	5	5	5	5	5	5
Glandular stomach												
Globule leukocyte, increased	0	0	0	4	0	0	0	0	0	1	0	0
Appearance, macrophage, pigment-laden	0	0	0	3	0	0	0	0	0	4	0	0
Duodenum Appearance, macrophage, LP, pigment-laden	0	0	0	4	0	0	0	0	0	5	0	0
Jejunum Appearance, macrophage, LP, pigment-laden	0	0	4	5	0	0	4	5	0	5	0	5
Ileum												
Appearance, macrophage, LP, pigment-laden	0	0	0	5	0	0	0	2	0	4	0	5
Appearance, macrophage, PP, pigment-laden	0	0	2	5	0	0	1	5	0	5	0	5
Cecum Appearance, macrophage, LP, pigment-laden	0	0	0	5	0	0	0	3	0	5	0	5
Colon Appearance, macrophage, LP, pigment-laden	0	0	0	5	0	0	0	3	0	5	0	5
Lung Accumulation, macrophage, alveolus, pigment-laden	0	1	3	1	0	0	3	1	0	1	0	0
Mesenteric lymph node Appearance, macrophage, pigment-laden	0	0	5	5	0	0	5	5	0	5	0	5
Cervical lymph node Appearance, macrophage, pigment-laden			2/2	3/3			5/5	2/2		2/2		4/4
Bronchial lymph node Appearance, macrophage, pigment-laden			1/1				1/1	1/1		1/1		1/1

All results were grade 1 (mild)
LP: lamina propria
PP: Peyer's patches
Numbers as fractions are: number of animals with tissues examined/number of animals with adverse effects

Mortality and Time to Death

There were no treatment related mortalities during the study. Incidental deaths (that were considered by the study authors to be unrelated to treatment) included one female treated at 50 mg/kg bw/day which died during delivery on day 22 of gestation. The animal was noted to have dystocia and delivered no offspring.

Clinical Observations

Test substance was noted in the faeces (blue colouration) from day 2 of dosing until recovery day 4 and 5 of males and females respectively, dosed at 50 mg/kg and above.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No treatment related changes were noted in urinalysis or haematology outcomes tested. There was an increase in monocyte count in males dosed at 50 and 250 mg/kg, however this was considered to be incidental as there were no finding in animals dosed at 1000 mg/kg.

At the end of the recovery period only it was noted there was a significantly high reticulocyte ratio in males, and prothrombin time prolongation and low lymphocyte ratio in females dosed at 1000 mg/kg bw/day. However, these results were considered by the study authors to be not toxicologically significant due to a lack of related expected physiological changes or lack of response at the end of the dosing period.

Effects in Organs

There were no toxicologically significant organ weight changes noted. There was a statistically significant increase in the seminal vesicle weight in males dosed at 50 mg/kg bw/day, but was considered to be incidental due to lack of response in the 250 and 1000 mg/kg bw/day group.

There were a number of effects noted at necropsy (see table above). Blue colouration was observed in the gastrointestinal contents (stomach, small intestine, large intestine), lung, lymph nodes (mesenteric, submaxillary, cervical and bronchial), and kidneys in both sexes with no reversibility confirmed in the lymph nodes, lung and kidney. Pigment laden macrophages were seen in the entire digestive tract, lung and lymph in males receiving 1000 mg/kg bw/day which was still evident in the animals after the recovery period. Macrophages were noted in the small and large intestine of females receiving 250 and 1000 mg/kg bw/day, which persisted until the end of the recovery period.

A selection of animals were assessed for lymph node effects with all tissues sampled exhibiting increased presence of macrophages.

In addition, at the end of the dosing period an increase in globule leukocytes in the glandular stomach was noted in males receiving 1000 mg/kg. However, this finding decreased in the animals subjected to necropsy at the end of the recovery period, indicating reversibility.

Effects on reproduction

There were no test related substance effects in any index tested, including copulation, fertility or gestation effects.

Effects on F1 pups

There were no test related substance effects in any index tested, including sex ratio and viability. There were no gross external examination changes or changes in body weight compared to negative controls.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) for adults was established by the study authors as 250 mg/kg bw/day in this study, based on an increase of globule leukocyte in the glandular stomach in males at 1000 mg/kg bw/day. The NOEL for reproductive toxicity was determined to be 1000 mg/kg bw/day in the absence of any treatment related effects.

TEST FACILITY MCMC (2011e)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. 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	No significant protocol deviations

A preliminary toxicity study was performed at concentrations 1.22-5000 µg/mL. There was no significant increase in any revertant colonies with or without metabolic activation or any microbial growth inhibition evident. Precipitation was observed with metabolic activation in all strains at 313 µg/plate and above.

Vehicle and positive controls (2-(2-Fury)-3-(5-nitro-2-furyl) acrylamide (AF-2), Sodium azide (NaN3), and 9-Aminoacridine hydrate (9-AA) without metabolic activation and 2-Aminoanthracene (2-AA) with metabolic activation) were used in parallel with the test material.

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	≥ 313	Negative
Test 2	-	> 5000	≥ 313	Negative

Remarks - Results	No significant increases in the frequency of revertant colonies were noted for any of the bacterial strains, either with or without metabolic activation. The positive controls gave satisfactory responses, confirming the validity of the test system.

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

TEST FACILITY GTRI (2010)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE Test substance

METHOD OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.

Cell Type/Cell Line	Mouse lymphoma/L5178Y
Metabolic Activation System	S9 fraction from phenobarbital and 5,6 – benzoflavone induced rat liver
Vehicle	Water
Remarks - Method	No significant protocol deviations A preliminary toxicity test was conducted (3 hour exposure period with and without metabolic activation, and 24 hour exposure period without activation) at concentrations of 78.1-5000 µg/mL. In the preliminary cytotoxicity tests there was no precipitation noted at any dose level. Relative survival rate was >20% for each dose group. Vehicle and positive controls (Methyl methanesulfonate (MMS) without metabolic activation and Cyclophosamide monohydrate (CP) with metabolic activation) were run in parallel with the test material.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	313, 625, 1250, 2500, 5000	3 hours	10 - 11 days
Test 2	156, 313, 625, 1250, 2500, 5000	24 hours	10 - 11 days
Present			
Test 1	313, 625, 1250, 2500, 5000	3 hours	10 - 11 days
Test 2	313, 625, 1250, 2500, 5000	3 hours	10 - 11 days

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5000	> 5000	> 5000	Negative
Test 2	> 5000	≥ 2500	> 5000	Positive
Present				
Test 1	> 5000	> 5000	> 5000	Negative
Test 2	-	> 5000	> 5000	Negative

Remarks - Results: The notified chemical induced a significant and dose dependent increase in total mutant and small mutant colonies at 2500 µg/mL and above in the 24 hr exposure group. There was no corresponding significant increase in large mutant colonies at any dose. There was no precipitation at any dose level tested.

CONCLUSION: The notified chemical was clastogenic to mouse lymphocyte L5178Y ($tk^{+/-}$ -3.7.2C) cells treated in vitro under the conditions of the test.

TEST FACILITY: MCMC (2010e)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Species/Strain: Chinese hamsters
Cell Type/Cell Line: Chinese hamster lung (CHL/IU)
Metabolic Activation System: S9 fraction from phenobarbital and 5,6 – benzoflavone induced rat liver
Vehicle: Saline
Remarks - Method: A preliminary toxicity study was performed (6 hour exposure, with and without activation and 24 hour exposure without activation) at concentrations 9.77-5000 µg/mL, with cytotoxicity evident from 214.4 µg/mL in the 24-hour exposure assay (based on the cell growth index).

Vehicle and positive controls (Mitomycin C without metabolic activation and Benzo [a] pyrene with metabolic activation) were used in parallel with the test material.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	500, 1000*, 1250*, 1500*, 1750, 2000	6 hours	24 hours
Test 2	62.5*, 125*, 250*, 500, 750, 1000	24 hours	24 hours
Present			
Test 1	500*, 1000*, 1250*, 1500, 1750, 2000	6 hours	24 hours
Test 2	500*, 1000*, 1250*, 1500, 1750, 2000	6 hours	24 hours

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 1362.4	≥ 1500	> 2000	Negative
Test 2	≥ 214.5	≥ 250	> 1000	Negative
Present				
Test 1	≥ 1310.5	≥ 1250	> 2000	Negative
Test 2		≥ 1250	> 2000	Negative

Remarks - Results

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.

The positive controls gave satisfactory responses, confirming the validity of the test system.

CONCLUSION

The notified chemical was not clastogenic to Chinese hamster lung (CHL/IU) cells treated in vitro under the conditions of the test.

TEST FACILITY

MCMC (2011d)

B.10. Genotoxicity – in vivo

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 474 Mammalian Erythrocyte Micronucleus Test.

Species/Strain: Rat/Crl:CD(SD)

Route of Administration: Oral – gavage

Vehicle: Water

Remarks - Method: Vehicle and positive control (CP) were used in parallel with the test material.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	5M	0	24
II (low dose)	5M	500	24
III (mid dose)	5M	1000	24
IV (high dose)	5M	2000	24
V (positive control, CP)	5M	20	24

CP=cyclophosphamide.

RESULTS

Doses Producing Toxicity

There were no abnormal clinical signs in any group. Compound coloured stool was noted in 1/5, 3/5 and all animals dosed at 500, 1000 and 2000 mg/kg bw/day respectively. Compound coloured stool was found in the cages of all the dose groups before the second dosing or thereafter. The study authors did not note any chromaturia as observed in the acute oral toxicity study. There was no reduction in the number of immature erythrocytes (IMEs) in the bone marrow to indicate some level of toxicity.

Genotoxic Effects

There was no statistically significant increase in the incidence of micronucleated immature erythrocytes (MNIMEs) in any dose group. There were no statistically significant differences in the percentage of IME's in any dose group compared to negative control.

There was a statistically significant increase in the number of MNIMEs

	per 10000 IMEs and a significant reduction of IME's compared to negative control in the positive control group, confirming the validity of the test system.
Remarks - Results	As there were no signs of systemic toxicity (such as chromaturia) or decrease in the number of IME's in the bone marrow, it cannot be confirmed that the test substance reached the bone marrow.
CONCLUSION	The notified chemical was not clastogenic under the conditions of this *in vivo* Mammalian Erythrocyte Micronucleus Test; however it is not clear whether the test substance reached the target organ.
TEST FACILITY	MCMC (2011f)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

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

RESULTS

Test substance		*Aniline*	
Day	*% Degradation (BOD)*	*Day*	*% Degradation (BOD)*
7	0	7	55
14	0	14	74
21	0	21	74
28	0	28	74

Remarks - Results	All validity criteria for the test were satisfied. Degradability results based on BOD, DOC and residual test substance amount measurements were 0%, 8% and 3% after 28 days, respectively. No transformation product was generated under the conditions of test.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	MCMC (2011g)

C.1.2. Inherent biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD Guideline for the Testing of Chemicals, 302B Inherent Biodegradability Zahn-Wellens EMPA Test
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Dissolved Organic Carbon (DOC) Dissolved oxygen meter for the measurement of Chemical Oxygen Demand (COD)
Remarks – Method	Conducted according to the guidelines above with no significant deviations from the protocol.

RESULTS

Test substance		*Ethylene glycol*	
Day	*% Degradation (COD)*	*Day*	*% Degradation (COD)*
7	1.3	7	99.9
14	0	14	100.2
21	0		
28	0		

Remarks – Results: All validity criteria for the test were satisfied. The toxicity control attained 52.6% on the 14th day, thereby confirming that the test material was non-toxic to sewage sludge micro-organisms.

CONCLUSION: The notified chemical is not inherently biodegradable

TEST FACILITY: Laboratory of Ecotoxicity & Environmental Safety (2011b)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Notified chemical

METHOD
- Species: Medaka (*Oryzias latipes*)
- Exposure Period: 96 hours
- Auxiliary Solvent: None
- Water Hardness: 48 mg $CaCO_3$/L
- Analytical Monitoring: HPLC
- Remarks – Method: Following a range finding test, a limit test was conducted according to the guidelines above with no significant deviations from the protocol. Test solutions were changed every 48 hours.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality (%)*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
0		10	0	0	0	0	0
100	97.9	10	0	0	0	0	0

- LC50: > 97.9 mg/L at 96 hours
- NOEC: Not determined
- Remarks – Results: No abnormal symptoms were observed for living fish. All validity criteria for the test were satisfied.

CONCLUSION: The notified chemical is not harmful to fish

TEST FACILITY: MCMC (2011c)

C.2.2. Acute toxicity to fish

TEST SUBSTANCE: Notified chemical

METHOD
- Species: Zebra fish (*Brachydanio rerio*)
- Exposure Period: 96 hours
- Auxiliary Solvent: None
- Water Hardness: 112.16 mg $CaCO_3$/L
- Analytical Monitoring: Oxygen meter

	HPLC
Remarks – Method	A limit test was conducted according to the guidelines above with no significant deviations from the protocol. Test solutions were changed every 48 hours.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality (%)*			
Nominal	*Actual*		*24 h*	*48 h*	*72 h*	*96 h*
Blank control		10	0	0	0	0
106	103	10	0	0	0	0

LC50	> 106 mg/L at 96 hours
NOEC	106 mg/L at 96 hours
Remarks – Results	No abnormal symptoms were observed for living fish. All validity criteria for the test were satisfied.

CONCLUSION The notified chemical is not harmful to fish

TEST FACILITY Laboratory of Ecotoxicity & Environmental Safety (2011a)

C.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test-Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	240 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	Two range finding tests were conducted under semi-static conditions. Definitive tests were conducted under static conditions since the concentration in the test solution was maintained during the exposure period predicted from the range finding tests. Definitive tests were conducted according to the guidelines above with no significant deviations from the protocol.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	-	20	0	0
5.0	4.07	20	0	2
11	10.0	20	0	4
22	20.9	20	0	7
47	46.4	20	0	7
100	103	20	0	13

EC50	60.7 mg/L at 48 hours (95% confidence limits: 33.8-211 mg/L)
NOEC	Not observed
Remarks - Results	All validity criteria for the test were satisfied.

CONCLUSION The notified chemical is harmful to aquatic invertebrates

TEST FACILITY MCMC (2011b)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (2006)-Static
Species	Unicellular green algae (*Pseudokirchneriella subcapitata*)
Exposure Period	72 hours
Concentration Range	Nominal: 10, 18, 32, 56, 100 mg/L Actual: 8.55, 16.3, 30.9, 56.0, 103 mg/L
Auxiliary Solvent	None
Water Hardness	~4.9 mg $CaCO_3$/L
Analytical Monitoring	Electric particle counter Microscope with hemacytometer HPLC
Remarks - Method	Tests were conducted in an open system with shaking of the test vessels. Following a range finding test, the definitive tests were conducted according to the guidelines above with no significant deviations from the protocol.

RESULTS

Biomass		*Growth*	
E_bC50 *mg/L at 24 h*	*NOEC* *mg/L*	*E_rC50* *mg/L at 72 h*	*NOEC* *mg/L*
Not reported	Not reported	> 103	16.3

Remarks - Results	All validity criteria for the test were satisfied. Experiments with liquid light transmission and with reduced light path were conducted to investigate the effect of the limitation of photosynthetic activity on the growth inhibition. The test was conducted under the conditions that reduced the effect of light attenuation to the extent possible.
CONCLUSION	The notified chemical is not harmful to algae
TEST FACILITY	MCMC (2010d)

C.2.5. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test (1984)
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 100 mg/L Actual: Not reported
Remarks – Method	Conducted according to the guidelines above with no significant deviations from the protocol.
RESULTS	
EC50	> 100 mg/L at 3 hours
NOEC	Not determined
Remarks – Results	All validity criteria for the test were satisfied. No significant inhibition of respiration rate of the sludge was recorded at 100 mg/L for the test substance.
CONCLUSION	The notified chemical is not expected to inhibit microbial respiration
TEST FACILITY	NOTOX (2011a)

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