File No: LTD/1606

December 2012

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

E-Y110

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/1606	Epson Australia Pty Ltd	E-Y110	Not determined	≤1 tonne per annum	Colourant in inkjet printing ink

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

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

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

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

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

Recommendations

REGULATORY CONTROLS
(Material) Safety Data Sheet

- The (M)SDS provided by the notifier for the ink containing the notified chemical should be amended as follows:
  - In Section 8.2.1.2 the following text should be added: "Use impervious gloves if dermal contact is expected"

CONTROL MEASURES
Occupational Health and Safety

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

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

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

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Globally Harmonised System for the Classification and Labelling of Chemicals*

(GHS) as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if
- the importation volume exceeds one tonne per annum notified chemical;
- the notified chemical is imported in any form other than as a component of sealed ink-jet cartridges 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 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, 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-Y110

MOLECULAR WEIGHT
> 1,000 Da

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

3. COMPOSITION

DEGREE OF PURITY > 85%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: orange powder

Property	Value	Data Source/Justification
Melting Point/Boiling Point	Decomposition observed from 350 °C	Measured
Density	1710 kg/m^3 at 20 °C	Measured
Vapour Pressure	Not determined	As the notified chemical is solid and has a high molecular weight, the vapour pressure is expected to be low.
Water Solubility	≥ 405 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	$t_{1/2}$ > 1 year at 25 °C (pH 4,7 and 9)	Measured
Partition Coefficient (n-octanol/water)	Log K_{ow} < -3.3 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 pK_a = 7.97	Calculated for the free acid form. The notified chemical is a salt and is expected to be ionised under environmental conditions.
Particle Size	Inhalable fraction (< 100 µm): 70.43% Respirable fraction (< 10 µm): 15.15% MMAD* = 64.033 µm	Measured
Flammability	Not highly flammable	Measured
Autoignition Temperature	300 °C	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.

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 2.5%) 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 of 5 g capacity containing the notified chemical (at up to 2.5% concentration) will be transported within Australia by road.

USE
The notified chemical will be used as a component (up to 2.5%) in inkjet printing ink, intended for use in 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 retail centres and/or end-use sites, predominantly 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).

Although the ink cartridges containing the notified chemical are primarily intended for household use, some

office/commercial use may also occur. Printer technicians and office workers may be exposed to the ink containing the notified chemical (at up to 2.5%) when replacing used ink cartridges, clearing paper jams from the printer and during printer repair. Dermal exposure is expected to be the most likely route of exposure; however accidental ocular exposure could also occur. Inhalation exposure is not expected. However, given the design and small capacity of the cartridges and the fact that workers would be aware of any exposure to the coloured ink, exposure to the notified chemical is expected to be limited if users follow the instructions for replacing used 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 will use inkjet printer cartridges containing the notified chemical (at up to 2.5%) 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 that would be 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
Rat, acute inhalation toxicity	LC50 > 2.3 mg/L/4 hour;
Rabbit, skin irritation	Non-irritating
Rabbit, eye irritation	Slightly irritating
Mouse, skin sensitisation – Local lymph node assay	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 chromosome aberration test	Equivocal
Genotoxicity - *in vitro* mammalian cell gene mutation test	Potentially genotoxic
Genotoxicity - *in vivo* unscheduled DNA synthesis (UDS) test	Non genotoxic
Genotoxicity - *in vivo* mouse micronucleus test	Non genotoxic

Toxicokinetics, metabolism and distribution:
Absorption of the notified chemical through the skin is not expected to be significant, given its relatively high molecular weight (> 1000 Da), high water solubility (> 100 g/mL), and low partition coefficient (log P < -3). These parameters demonstrate its low lipophilicity, indicating that it is not likely to cross the stratum corneum (European Commission, 2003). However, bacterial skin microflora have been reported to be able to break down azo dyes into smaller species, which may be more readily absorbed, through azo reduction (SCCNFP, 2002).

Given the coloured urine seen in the acute oral toxicity study and 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 LD50 of the notified chemical was > 2000 mg/kg bw by the oral and dermal routes, with no deaths or significant adverse effects seen in the treated animals. In an acute inhalation study the LC50 was > 2.3 mg/L. This was the only level tested, and was not high enough to determine hazard classification for this endpoint.

Irritation and Sensitisation.
The notified chemical was non-irritating to the skin and only slightly irritating to eyes in rabbit studies to OECD guidelines. At the introduced concentration (< 2.5%), it is likely to be non-irritating.

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 (sub acute, sub chronic, chronic).
No deaths were observed during the combined repeated dose and reproductive oral toxicity study. However, some clinical effects were noted in the animals treated with high dose (1000 mg/kg bw/day) of the notified chemical, including chromaturia, increase in motor activity in females and increase in eosinophil ratio in males. Based on the increased motor activity in females at 1000 mg/kg bw/day, the NOAEL was determined to be 250 mg/kg bw/day.

Mutagenicity.
Azo dyes are a concern for their potential induction of mutagenicity and carcinogenicity. The azo linkage is the most labile portion of an azo dye molecule, and it is readily enzymatically metabolised in mammals, including man (SCCNFP, 2002). Liver azo reductase enzymes reductively cleave the molecule into component amines. Some metabolism may also occur in the cells of the bladder wall, and during percutaneous absorption. Skin and anaerobic intestinal bacteria are also capable of reductive cleavage of the azo linkage.

Azo reduction is thought to contribute to the carcinogenicity of many azo dyes. The notified chemical is not expected to be reductively cleaved to release any of the aromatic amines with carcinogenic potential listed in EU SCCNFP/0495/01 (SCCNFP, 2002). However, the notified chemical can be broken by azo reduction into a number of arylamine species, some of which could potentially be mutagenic.

Several in vitro and in vivo genotoxicity studies were carried out on the notified chemical. The results of a bacterial reverse mutagen test performed according to OECD Guideline 471 were negative in the presence and absence of metabolic activation. However, the test guideline strongly recommends the use of alternative procedures for the detection of mutagenicity of azo dyes, as it is recognised that the standard procedure may not be sufficiently sensitive for these chemicals. According to the OECD Guideline 471, modified tests, such as that of Prival and Mitchell (1982), utilise a reductive pre-incubation step (during which the azo dye is reduced to amine species) before the test is carried out. This modified test is thought to yield a greater detection of mutagenic azo dyes. As such, bacterial mutagenicity potential of the notified chemical cannot be ruled out on the basis of the study performed.

Significant and dose-dependent increase of mutant frequency was observed in an in vitro mammalian cell gene mutation test when the cells were treated with 625 µg/mL or above of the chemical for 24 hours. The results suggested that the notified chemical has a potential to induce point gene mutations. A third in vitro study performed was a chromosome aberration study in Chinese Hamster lung cells. The results of this study were equivocal, with an increase in aberrant cells only at the highest dose level tested, with a 24 h incubation time.

The notified chemical had negative results in two in vivo genotoxicity studies to OECD guidelines: a Mouse Micronucleus test and an Unscheduled DNA Synthesis test. However, in neither case could it be demonstrated that the test substance reached the target organ.

In addition, azo dyes are known to have impurities, including the presence of component arylamines (SCCNFP, 2002; Øllgaard, 1998). Such impurities are thought to contribute to their carcinogenicity, as these species may be more readily absorbed and activated as carcinogens. As such, these impurities may possibly contribute to the carcinogenicity potential of the notified chemical.

Overall, these results do not rule out the genotoxic potential of the notified chemical, as some studies showed positive or equivocal results and reductive metabolism may be significant *in vivo*.

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; however metabolism to smaller species could occur in the skin.

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 (< 2.5%). 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 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 cartridges. Release of the ink solution to the environment is not expected as manufacturing and reformulation of the ink containing the notified chemical will not take place in Australia. Environmental release of the notified chemical is unlikely during importation, storage and transportation.

RELEASE OF CHEMICAL FROM USE

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

The sealed cartridges are contained in the printer until they are removed for disposal. Residual ink (< 2.5%) left in empty cartridges will most likely be disposed of to landfill. The majority of the ink will be bound to printed paper that will be disposed of to landfill or recycled.

RELEASE OF CHEMICAL FROM DISPOSAL

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

7.1.2. Environmental Fate

The notified chemical is not readily biodegradable (0% biodegradation after 28 days) nor inherently biodegradable (7.5% biodegradation after 28 days). For the details of the environmental fate studies please refer to Appendix C. The majority of the notified chemical is expected to enter the environment from disposal of printed paper products that ink containing the notified chemical will be used on. Approximately 50% of the notified chemical will be disposed of to landfill by binding on the printed waste paper, and eventually degrade *in-situ* by abiotic and biotic processes into water, inorganic salts and oxides of carbon and nitrogen. Notified

chemical that is not bound to paper in landfill may leach due to the low adsorption/desorption (K_{OC}) value and high water solubility.
The remaining 50% of the notified chemical is expected to be released to sewer, after the de-inking of paper during recycling. The notified chemical is not expected to be removed during sewage treatment plant (STP) processes due to its high water solubility and low potential to sorb to sludge. Therefore, the notified chemical from paper recycling may be released from sewage treatment plants into surface waters where the notified chemical is expected to disperse and eventually degrade. However, the notified chemical is not expected to bioaccumulate due to its very low n-octanol partition coefficient (log K_{OW}) and high solubility in water.

7.1.3. Predicted Environmental Concentration (PEC)

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

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:	0.43	µg/L
PEC - Ocean:	0.043	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.425 µg/L may potentially result in a soil concentration of approximately 2.835 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*
Acute Toxicity		
Fish Toxicity (96 hours)	LC50 > 100 mg/L	Not harmful
Daphnia Toxicity (48 hours)	EC50 > 100 mg/L	Not harmful
Algal Toxicity (72 hours)	E_rC50 > 100 mg/L	Not harmful
	NOEC > 100 mg/L	Not harmful
Inhibition of Bacterial Respiration	EC50 > 100 mg/L	Not expected to be significantly inhibitory to microbial activity

The notified chemical is not expected to be harmful to aquatic life on an acute basis. Therefore, the notified chemical is not formally classified for acute or long-term hazard under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009).

7.2.1. Predicted No-Effect Concentration

The endpoint for the most sensitive species from the reported results is used to calculate the predicted no-effect concentration (PNEC). All trophic levels had the same results for this assessment. An assessment factor of 100 was used as full study reports were available on the acute toxicity endpoints for all three trophic levels.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Algae, 72 h)	> 100	mg/L
Assessment Factor	100	
PNEC	> 1000	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.43	> 1000	< 0.001
Q - Ocean	0.043	> 1000	< 0.0001

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

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Boiling Point Decomposition observed from 350 °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 350 °C and 400 °C, which was determined to be due to reaction and/or decomposition of the test substance.
Test Facility NOTOX (2011)

Density 1710 kg/m^3 at 20 °C

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

Water Solubility ≥ 405 g/L at 20 °C

Method OECD TG 105 – Water Solubility (1995)
EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks Flask method
Test Facility NOTOX (2011)

Hydrolysis as a Function of pH $t_{½}$ > 1 year at 25 °C (pH 4, 7 and 9)

Method OECD TG 111 – Hydrolysis as a function of pH (2004)
EC Council Regulation No 440/2008 C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$ (years)*
4	25	> 1
7	25	> 1
9	25	> 1

Remarks Less than 10% hydrolysis was observed after 5 days during the preliminary test (Tier 1). Therefore, the test substance is considered hydrolytically stable with a half-life greater than 1 year.
Test Facility NOTOX (2011)

Partition Coefficient (n-octanol/water) log K_{OW} < -3.3 at 20 °C

Method OECD TG 107 - Partition Coefficient (n-octanol/water): Shake Flask Method (1995)
EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks Measurements should be conducted on ionisable substances in their non-ionised form. The test substance is a salt and it is expected to be dissociated in solution at pH 4 - 9. Therefore, tests were conducted on the test substance in its ionised form at pH 7.
Test Facility NOTOX (2011)

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 (2011)

Adsorption/Desorption – main 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 high performance liquid chromatography (HPLC) (2001)
EC Council Regulation No 440/2008 C.19 Estimation of the adsorption coefficient (K_{oc}) on soil and on sewage sludge using high performance liquid chromatography (HPLC).

Remarks Measurements should be conducted on ionisable substances in their non-ionised form. The test substance is a salt and it is expected to be dissociated in solution at pH 4 - 9. Therefore, tests were conducted on the test substance in its ionised form at pH 7.

Test Facility NOTOX (2011)

Dissociation Constant Expected to be ionised under environmental conditions

Method The notifier provided calculated values for the free acid form of the notified chemical using the Perrin calculation method (pKalc 5.0, module in Pallas 3.0, CompuDrug International, San Francisco, CA, USA)

Remarks The notified chemical contains basic groups with a calculated pKa of 7.97. The acid groups were found to have pKas outside the logarithmic range of 1-14 and were therefore not reported. The test substance is a salt and is expected to be ionised at the environmental pH range 4 to 9.

Test Facility NOTOX (2011a)

Particle Size MMAD - 64.033 µm

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

Range (µm)	*Mass (%)*
< 226.512	90
< 100	70.43
< 48.967	50
< 10	15.15
< 6.757	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 64.033 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 (2011)

Autoignition Temperature 300 °C

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 of 300 °C (temperature of the test substance reached 400 °C) and this was considered to be the autoignition temperature.

Test Facility NOTOX (2011)

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 405 °C, with an exothermic decomposition energy of 273 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 (2011)

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 (2011)

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*
1	3F	2000	0/3
2	3F	2000	0/3

LD50	> 2000 mg/kg bw
Signs of Toxicity	Test substance-mixed feces and chromaturia (thick and yellowish) were observed during the test. No deaths, effects on body weight or adverse signs at necropsy were noted.
Effects in Organs	Not observed
Remarks - Results	The clinical signs noted are likely to be due to the coloured nature of the test substance.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Mitsubishi Chemical Medience Corporation (2010)

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	Water
Type of dressing	Occlusive
Remarks - Method	No significant protocol deviations. The test substance was applied via moistened lint.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5M	2000	0/5
2	5F	2000	0/5

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Not observed
Signs of Toxicity - Systemic	Not observed
Effects in Organs	Not observed
Remarks - Results	Decreased body weight in one animal at Day 4 only was attributed to the use of the occlusive dressing or neck collar.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	Mitsubishi Chemical Medience Corporation (2011)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 403 Acute Inhalation Toxicity.
Species/Strain	Rat/Crl:CD(SD)
Vehicle	None
Method of Exposure	Nose only.
Exposure Period	4 hours
Physical Form	Solid aerosol (particulate).
Particle Size	MMAD 4.8 - 5.3 μm (33 - 39% particles < 4 μm)
Remarks - Method	Dust was generated from the test substance via a fluidized-bed dust generator. It was unclear from the study report whether the target concentration was 2 or 5 mg/L.

RESULTS

Group	*Number and Sex of Animals*	*Concentration <mg/L>*		*Mortality*
		Nominal	*Actual*	
1	6M/6F	2	2.3	0/12

LC50	> 2.3 mg/L/4 hours
Signs of Toxicity	Not observed
Effects in Organs	Not observed
Remarks - Results	Soiled fur derived from the test substance was observed during the test. Reversible decreases of body weight or suppression of body weight gain were noted in the test animals.
CONCLUSION	The LC50 of the notified chemical via inhalation was > 2.3 mg/L under the conditions of the test.
TEST FACILITY	Mitsubishi Chemical Medience Corporation (2011a)

B.4. 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 material was moistened with water prior to application

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0	0	0	0	0	0
Oedema	0	0	0	0	0	0

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

Remarks - Results

CONCLUSION The notified chemical is non-irritating to the skin.

TEST FACILITY Mitsubishi Chemical Medience Corporation (2010a)

B.5. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD
Species/Strain Rabbit/New Zealand White
Number of Animals 6
Observation Period 96 hours
Remarks - Method In 3 of the 6 tested animals, the eyes were left unwashed following the administration of the notified chemical. In the other 3 rabbits, treated eyes were washed with water 30 seconds after the administration.
The treated eyes were examined with 2% fluorescein solution at the 24 h observation.

RESULTS

Eyes unwashed after administration

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	1	< 24 hours	0
Conjunctiva: discharge	0	0	0	1	< 24 hours	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

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

Eyes washed with water after administration

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	1	< 24 hours	0
Conjunctiva: discharge	0	0	0	0	0	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

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

Remarks – Results Effects in both washed and unwashed eyes had resolved by 24 h.

CONCLUSION The notified chemical has slight irritation potential to the rabbit eye under the conditions of the test.

TEST FACILITY Mitsubishi Chemical Medience Corporation (2010b)

B.6. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Notified chemical

METHOD
Species/Strain Mouse/CBA/JNCrlj) female
Vehicle Acetone/olive oil (4:1 v/v)
Remarks - Method α-Hexylcinnamaldehyde (HCA) was used as positive control.

Concentrations > 50% were not tested due to difficulty suspending the test substance in the solvent.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	444.5	1
5	504.2	1.13
15	401.0	0.90
50	370.7	0.83
Positive Control		
25	2969.2	6.68

Remarks - Results

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 Mitsubishi Chemical Medience Corporation (2010e)

B.7. 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
Frequency of exposure: once daily
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 yellowish chromaturia and feces 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	0/24
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

Mortality and Time to Death
No deaths were observed during the study.

Clinical Observations
Bright yellow chromaturia was noted in all males and 11 females in the high dose group. Females with an increase in motor activity were also noted in the same group.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
A high value of the eosinophil ratio was noted in high dose males, both at the end of dosing and in the

recovery group. The significance of this change is not clear, as it was not accompanied by changes in the eosinophil or leucocyte count, and the study authors considered it to be not toxicologically significant. Other sporadic changes in haematology or blood chemistry parameters were not considered to be toxicologically significant..

Effects in Organs
In some high dose animals, histopathological examination showed orange pigment in granulocytes of villi in the small intestine that was considered to be derived from the colour of the test substance. Pigment deposit disappeared during the post-exposure observation period and was considered a transient change related to the absorption of the test substance, and not toxicologically significant.

Reproductive/developmental Toxicity
No significant changes were noted in reproductive performance, pregnancy or offspring. One female in the mid dose group did not become pregnant, however the cause was not determined and was considered to not be toxicologically significant. An irregular estrous cycle in one mid dose and one control animal was considered incidental. A statistically significant change in the sex ratio of the offspring of the high dose group was attributed to a lower than usual sex ratio of the control group.

Remarks – Results
Chromaturia was considered to be related to the colour of the notified chemical, and not toxicologically significant.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) for repeated dose effects was established as 250 mg/kg bw/day in this study, based on increased motor activity observed in high dose (1000 mg/kg bw/day) females. The NOAEL for reproductive and developmental outcomes was considered to be 1000 mg/kg bw/day, the highest dose tested.

TEST FACILITY Mitsubishi Chemical Medience Corporation (2011b)

B.8. 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: WP2*uvrA*$^-$
Metabolic Activation System S9 mix 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 were noted.

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.

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

Remarks - Results

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

TEST FACILITY Japan Oilstuff Inspector's Corporation (2010)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE Notified Chemical

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Species/Strain Chinese hamster
Cell Type/Cell Line Chinese hamster lung (CHL/IU)
Metabolic Activation System S9 fraction from phenobarbital/5,6-benzoflavone induced rat liver
Vehicle Saline
Remarks - Method Test concentrations were chosen on the basis of a preliminary cell growth inhibition test. Positive controls used were mitomycin C (MMC) without S9 mix or Benzo[a]pyrene (BP) with S9.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	625, 1250*, 2500* and 5000*	6 hours	24 hours
Test 2	9.77, 19.5, 39.1*, 78.1*, 156*, 313, 625, 1250, 2500 and 5000	24 hours	24 hours
Present			
Test 1	625, 1250*, 2500* and 5000*	6 hours	24 hours
Test 2	625, 1250*, 2500* and 5000*	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	> 5000	> 5000	> 5000	negative
Test 2	> 78.1	> 78.1	> 156	equivocal
Present				
Test 1	> 5000	> 5000	> 5000	negative
Test 2		> 5000	> 5000	negative

Remarks - Results

The study authors considered that a negative result had been obtained if both structural and numerical aberrant cells were observed at < 5%. While none of the test systems had aberrations > 5%, the highest dose tested (156 μg/mL) in the 24h exposure test (without metabolic activation) had 4.0% aberrant cells, suggesting a possible clastogenic effect.

The positive controls gave satisfactory responses, confirming the validity of the test system. The mitotic index was 38.0% at this concentration.

CONCLUSION The notified chemical produced equivocal results for clastogenicity to CHL/IU cells treated in vitro under the conditions of the test.

TEST FACILITY Mitsubishi Chemical Medience Corporation (2010c)

B.10. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
Species/Strain	Mouse
Cell Type/Cell Line	Lymphoma cell/L5178Y ($tk^{+/-}$-3.7.2C)
Metabolic Activation System	S9 mix from phenobarbital /5,6-benzoflavone induced rat liver
Vehicle	Water
Remarks - Method	Test concentrations were chosen on the basis of a preliminary test. Methyl methanesulfonate (MMS) and cyclophosphamide were used as positive controls in the study.

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

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5000	> 5000	> 5000	negative
Test 2	> 5000	> 5000	> 5000	negative
Test 3	> 5000	> 5000	> 5000	positive
Present				
Test 1	> 5000	> 5000	> 5000	negative
Test 2	> 5000	> 5000	> 5000	negative

Remarks - Results	Significant and dose-dependent increase of total mutant and large colony mutant frequency was observed in Test 2 in the absence of metabolic activation when the cells were treated for 24 hours with the notified chemical at concentrations of 625 µg/mL and above. Positive and negative controls were within historical limits. Based on the effects observed, it was considered that the notified chemical has a potential to induce point gene mutations.
CONCLUSION	The notified chemical was potentially mutagenic to mouse lymphoma cells treated in vitro under the conditions of the test.
TEST FACILITY	Mitsubishi Chemical Medience Corporation (2010d)

B.11. Genotoxicity – in vivo

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 486 Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo.
Species/Strain	Rat/Crl:CD(SD)
Route of Administration	Oral – gavage
Vehicle	Water
Remarks - Method	Positive controls dimethylnitrosamine (DMN) and 2-acetylamino-fluorene (2-AAF) were used in the study. Dose levels were set based on the results of a separate acute oral toxicity study.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	3M	0	2
II (low dose)	3M	1000	2
IV (high dose)	3M	2000	2
V (positive control DMN)	3M	10	2

DMN = dimethylnitrosamine.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	3M	0	16
II (low dose)	3M	1000	16
IV (high dose)	3M	2000	16
V (positive control 2-AAF)	3M	50	16

2-AAF = 2-acetylaminofluorene

RESULTS

Doses Producing Toxicity	Signs of toxicity were not observed. In the 16 h post-treatment groups, compound-coloured stool was found in the cages. In the absence of toxicity it is not clear whether systemic exposure to the test substance occurred.
Genotoxic Effects	The mean net grains in all test groups were < 1 and the mean percentages of cells in repair ranged from 1.7 to 2.3%. Based on the test laboratory's criteria (mean net grains in test group > 5 and mean percentage of cells > 20%, no genotoxic effects were observed. Significant increases in both these parameters were seen in the positive controls.
Remarks - Results	

CONCLUSION	The notified chemical was not clastogenic under the conditions of this in vivo unscheduled DNA synthesis (UDS) test with mammalian liver cells.
TEST FACILITY	Mitsubishi Chemical Medience Corporation (2011c)

B.12. 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	Cyclophosphamide monohydrate (CP) was used as a positive control. Dosing was carried out twice, at a 24 h interval. The sacrifice time was 24 h after the final dosing.

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

CP=cyclophosphamide.

RESULTS

Doses Producing Toxicity	Test substance coloured stool was observed in animals in Groups III (mid dose) and IV (high dose). No other clinical signs were observed.
Genotoxic Effects	The numbers of micronucleated immature erythrocytes was not significantly increased in the treatment groups, and no-dose related

	increase was seen. The positive controls showed significantly increased numbers as expected
Remarks - Results	The ratio of immature erythrocytes to total erythrocytes was not altered in the test groups. Because of this and the lack of systemic clinical signs, 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.
TEST FACILITY	Mitsubishi Chemical Medience Corporation (2011d)

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) (1992)
Inoculum	Activated sludge, non-adapted
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biochemical oxygen demand (BOD): Closed-system oxygen consumption measuring apparatus Dissolved organic carbon (DOC): TOC analyser Residual test substance: HPLC
Remarks - Method	The test was conducted according to test guidelines using good laboratory practice (GLP) with no significant deviations.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation (BOD)*	*Day*	*% Degradation (BOD)*
28	0	7	51.5
		14	69.4

Remarks - Results	All relevant test validity criteria were met. At the end of biochemical oxygen demand (BOD) measurement, pH values were 7.3, 7.2 and 7.2 for test suspensions 1, 2 and 3 respectively and 7.5 for the abiotic control. Growth of the sludge was observed in the activity control in contrast to inoculum blank. No growth of the sludge was observed in the test suspensions.
CONCLUSION	The notified chemical is not readily biodegradable
TEST FACILITY	Mitsubishi Chemical Medience Corporation (2010f)

C.1.2. Inherent biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 302 B Inherent Biodegradability: Zahn-Wellens/EMPA Test (1992)
Inoculum	Activated sludge, non-adapted
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Dissolved oxygen (DO); Oxi 330i dissolved oxygen meter
Remarks – Method	The test was conducted according to test guidelines using good laboratory practice (GLP) with no significant deviations.

RESULTS

Test substance		*Ethylene Glycol*	
Day	*% Degradation*	*Day*	*% Degradation*
28	7.5	14	100

Remarks – Results	All relevant test validity criteria were met. The percentage degradation of the toxicity control was 52% on Day 14 indicating that the test substance was not considered to have a toxic effect on the sewage sludge

microorganisms used in the study.

CONCLUSION The notified chemical is not inherently biodegradable

TEST FACILITY Guangdong Detection Center of Microbiology (2011)

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	*Oryzias latipes* (Ricefish)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	48 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	Following a range-finding test, the limit test was conducted according to test guidelines using good laboratory practice (GLP) with no significant deviations.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	0	10	0	0	0	0	0
100	102	10	0	0	0	0	0

LC50	> 100 mg/L at 96 hours.
NOEC	100 mg/L at 96 hours.
Remarks – Results	All relevant test validity criteria were met. A range-finding test indicated no mortalities or toxicological symptoms at a test substance concentration of 100 mg/L. No mortalities or toxicological symptoms were observed for fish at a test substance concentration of 102 mg/L.

CONCLUSION The notified chemical is not harmful to fish

TEST FACILITY Mitsubishi Chemical Medience Corporation (2011e)

C.2.2. 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	248 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	The limit test was conducted according to test guidelines using good laboratory practice (GLP) with no significant deviations.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	0	2 × 5	0	0
100	102	2 × 5	0	0

LC50	> 100 mg/L at 48 hours
NOEC	100 mg/L at 48 hours
Remarks - Results	All relevant test validity criteria were met.
CONCLUSION	The notified chemical is not harmful to aquatic invertebrates
TEST FACILITY	Mitsubishi Chemical Medience Corporation (2011f)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test – Static
Species	*Pseudokirchneriella subcapitata* (Green Algae)
Exposure Period	72 hours
Concentration Range	Nominal: 100 mg/L Actual: 95.6 mg/L
Auxiliary Solvent	None
Water Hardness	50 mg $CaCO_3$/L
Analytical Monitoring	Cell densities were determined by a CDA-500 electronic particle counter
Remarks - Method	The limit test was conducted according to test guidelines using good laboratory practice (GLP) with no significant deviations. Because of the colour of the test substance, a separate study on the light absorption or interception caused by the coloured test substance was carried out. The growth of algae cultured under light filtered through containing the test substance versus light filtered through a petri dish containing the control test medium was compared. Another test was carried out to test the effect of the light absorption or interception by reducing the test volume, therefore reducing the light path.

RESULTS

Biomass		*Growth*	
E_bC50	*NOEC*	*E_rC50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
> 100	100	> 100	100

Remarks - Results	All relevant test validity criteria were met. In regards to the light absorption or interception caused by the coloured test substance, the light transmission filter test showed an inhibition rate of 19.9% of the test substance group compared to the control group. The reduced light path test showed that a reduction in test solution volume reduced the inhibition of algal growth. Final algal growth inhibition tests were conducted under conditions that reduced the effect of light attenuation to the extent possible.
CONCLUSION	The notified chemical is not harmful to algae
TEST FACILITY	Mitsubishi Chemical Medience Corporation (2010g)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 - Activated Sludge, Respiration Inhibition Test - Static EC Directive 88/302/EEC C.11 - Biodegradation: Activated Sludge Respiration Inhibition Test
Inoculum	Activated sludge of a predominantly domestic sewage
Exposure Period	3 hours
Concentration Range	Nominal: 100 mg/L
Remarks – Method	The test was conducted according to test guidelines using good laboratory practice (GLP) with no significant deviations.
RESULTS	
IC50	> 100 mg/L
IC10	100 mg/L
Remarks – Results	All relevant test validity criteria were met. There was a 7 and 11% inhibition of microbial respiration rate at a test substance concentration of approximately 100 mg/L.
CONCLUSION	The notified chemical is not expected to significantly inhibit microbial respiration.
TEST FACILITY	NOTOX (2011b)

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