File No: LTD/1607

December 2012

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

E-BW102

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/1607	Epson Australia Pty Ltd	E-BW102	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-BW102

MOLECULAR WEIGHT
> 1,000 Da

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

3. COMPOSITION

DEGREE OF PURITY > 80%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: reddish brown powder

Property	Value	Data Source/Justification
Melting Point/Boiling Point	Decomposition observed from 300 °C	Measured
Density	1560 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	142 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} < -3.2 at 20 °C	Measured
Surface Tension	74.9 mN/m at 20 °C	Measured
Adsorption/Desorption	log K_{oc} < 1.3 at 35 °C	Measured
Dissociation Constant	Estimated pK_a = 5.60 and 9.11	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): 73.97% Respirable fraction (< 10 µm): 15.83% MMAD* = 59.807 µm	Measured
Flammability	Not highly flammable	Measured
Autoignition Temperature	276 °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. 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 2%) 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% concentration) will be transported within Australia by road.

USE
The notified chemical will be used as component (up to 2%) of 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) retail centres and/or to 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 predominantly 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%) 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 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 up to 2%) 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 > 5 mg/L/4 hour; low toxicity
Rabbit, skin irritation	Non-irritating
Rabbit, eye irritation	Slightly irritating
Mouse, skin sensitisation – Local lymph node assay	No evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL = 50 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	Non-mutagenic
Genotoxicity – *in vitro* mammalian chromosome aberration test	Non-clastogenic

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 (but not reported in the repeated dose oral toxicity study), it is likely that the notified chemical can be absorbed to some extent 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 > 5 mg/L, also indicating low acute toxicity.

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%), 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).
In a 28 day oral gavage study, 5 of 10 male rats died during the study when tested at the highest dose of 1000 mg/kg bw/day and this dose was reduced for the remainder of the study. The high doses were associated with effects in the gastrointestinal tract, the spleen and adrenal cortex. Pigment laden macrophages that may be related to the colour of the test substance were noted in several organs, and occurred even at the lowest dose in the jejunum and mesenteric lymph node. These were considered by the study authors to be biological and not adverse. Coloured feces were also noted in the mid and high dose groups. It was not clear whether some changes noted in haematology and blood chemistry were related to the test substance. The NOAEL was determined by the study authors to be 50 mg/kg bw/day, based on histopathological effects at 250 mg/kg bw/day, including hyperplasia of the squamous epithelium in the forestomach, increases of the globular leukocyte in the glandular stomach, and hypertrophy of some renal cells.

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.

The notified chemical was negative in the presence and absence of metabolic activation in a bacterial reverse mutagen test performed according to OECD Guideline 471. 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. The notified chemical was also considered non-clastogenic in an *in vitro* chromosome aberration study, however in this study polyploidy was observed after short-term treatment in the absence of metabolic activation. The significance of the polyploidy for human health is not known.

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, the results of these *in vitro* studies do not rule out the notified chemical as genotoxic and/or a possible carcinogen, as one study showed polyploidy, and the other study did not take into account reductive metabolism that may be significant *in vivo*.

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 but a relatively low NOAEL of 50 mg/kg bw/day in a 28-day repeated dose study by oral gavage. 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%). 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%) 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 (3.1% 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*
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 = 9.81 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 acute toxicity 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 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 (2011)

Density 1560 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 142 g/L at 20 ºC

Method OECD TG 105 Water Solubility.
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.
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} < -3.2 at 20 ºC

Method OECD TG 117 Partition Coefficient (n-octanol/water).
EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks Shake Flask Method.
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 74.9 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 log K_{OC} < 1.3 at 35 °C
– main test

Method OECD TG 121 Estimation of the adsorption coefficient (K_{oc}) on soil and on sewage sludge using high performance liquid chromatography (HPLC)
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 both their ionised and non-ionised forms. Because the test substance is a salt, it is expected to be dissociated in solution at pH 4-9. Therefore, tests were only 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 Calculated using the Perrin calculation method (pKalc 5.0, module in Pallas 3.0, CompuDrug International, San Francisco, CA, USA)

Remarks The molecular structure of the main component of the notified chemical was too large for the applied calculations. A smaller component of the notified chemical contains functional groups with a calculated pKas of 5.60 and 9.11. It is noted that 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 - 59.807 µm

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

Range (µm)	*Mass (%)*
< 170.119	90
< 100.00	73.97
< 47.884	50
< 10	15.83
< 6.689	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 59.807 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 276 °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 observed at an oven temperature of 276 °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 366 °C, with an exothermic decomposition energy of 356 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 (reddish brown) were observed during the test. One animal was observed with soiled periproctal area and the other with a decrease of body weight at Day 4.
Effects in Organs	No adverse effects were observed at necropsy.
Remarks - Results	The reddish brown colouration is likely to be related to the colour 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

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	Decrease of body weight was observed in 1 female on Day 4.
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	Oro-nasal exposure.
Exposure Period	4 hours
Physical Form	Solid aerosol (particulate).
Particle Size	MMAD* = 4.96 μm
Remarks - Method	MMAD was slightly larger than 4 μm, the recommended upper limit in the OECD test guideline.

RESULTS
*MMAD: Mass Median Aerodynamic Diameter

Group	*Number and Sex of Animals*	*Concentration <mg/L>*		*Mortality*
		Nominal	*Actual*	
1	5M/5F	14.05	5.01	0/10

LC50	> 5 mg/L/4 hours
Signs of Toxicity	None observed
Effects in Organs	None observed
Remarks - Results	Reversible decreases of body weight were observed in the test animals.

CONCLUSION The notified chemical is of low toxicity via inhalation.

TEST FACILITY IET Japan (2011)

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

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 OECD TG 405 Acute Eye Irritation/Corrosion.

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.

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	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.

Remarks – Results	In both washed and unwashed eyes, slight irritation was seen at 1 hour, but all effects had resolved by the 24 hour observation.

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 OECD TG 429 Skin Sensitisation: Local Lymph Node Assay

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

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	265.2	1
5	240.8	0.91
15	283.0	1.07
50	166.0	0.63
Positive Control		
25	1598.7	6.03

Remarks - Results

CONCLUSION	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 (2010d)

B.7. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain	Rat/Crl:CD(SD)
Route of Administration	Oral – gavage/diet/drinking water
Exposure Information	Total exposure days: 28 days Dose regimen: once daily, 7 days per week Post-exposure observation period: 14 days
Vehicle	Water
Remarks - Method	The protocol was altered during the study, due to the death of 5 males of the high dose (1000 mg/kg bw day) group between days 8 and 25. Consequent to this, the high dose for males from day 26 was changed to 500 mg/kg bw/day, and this male group was referred to as the 1000 [500] mg/kg bw/day group. The male 1000 mg/kg bw/day group was abandoned, with all surviving male 1000/500 animals necropsied after the 28-day exposure period. A new 500 mg/kg bw/day recovery group was established, along with a second control group.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	10M/10F	0	0/20
Low dose	5M/5F	50	0/10
Mid dose	5M/5F	250	0/10
High dose	10M/10F	500M/1000F	0/20
Control recovery	5F	0	0/5
High dose recovery	5F	1000	0/5

Mortality and Time to Death
Five of ten males in the original high dose 1000 mg/kg bw/day group died from day 8 to day 25. The dying animals showed significant body weight loss and food consumption reduction before death. No unscheduled deaths occurred from day 26 onwards, when the high dose for males was changed from 1000 to 500 mg/kg bw/day.

Clinical Observations
Emaciation was noted in one of the dying animals treated with 1000 mg/kg bw/day of the notified chemical. Test substance mixed feces were noted from day 2 in animals treated with the notified chemical at doses ≥ 250 mg/kg bw/day.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
In haematology tests, high values of the reticulocytes count were noted in males and females in high dose group (500 mg/kg bw/day for males and 1000 mg/kg bw/day for females) and high values of reticulocyte ratio were noted in females in the same group. In blood chemistry, low values of sodium, potassium and chloride were observed in both males and females in groups with doses ≥ 250 mg/kg bw/day. In the high dose group, significant urine volume increase was observed in urinalysis. Low values of osmolality and specific gravity and colour change (pale yellow) were noted in high dose group with females having high values of potassium and chloride. Low values of osmolality and specific gravity were also noted in mid dose group (250 mg/kg bw/day).

Effects in Organs
Males treated with doses ≥ 250 mg/kg bw/day were observed with adrenal gland enlargement. Low values of prostate weight and high values of liver weight were noted in males in the high dose group. Brain weight increases in females were observed in dose groups ≥ 250 mg/kg bw/day. Low values of spleen weight in males were also noted in high dose group (≥ 500 mg/kg bw/day). Necropsy of the dead males treated with 1000 mg/kg bw/day showed dilatation of stomach and reddish-brown or jelly-like contents were found in the stomach, ileum, cecum and colon. Histopathological changes including hyperplasia, necrosis, hypertrophy and appearance of pigment-laden macrophages attributable to the treatment of the notified chemical were noted in the stomachs, jejunums, ileums, mesenteric lymph nodes, kidneys and adrenal glands of both the males and females, the duodenums of the males and the spleens of the females. Pigment-laden macrophages occurred even at the lowest dose in the jejunum and mesenteric lymph node. Atrophy of thymus and splenic white pulp, and decrease of vacuolation of the zona fasciculate cell of the adrenal cortex were also noted in the dead males treated with 1000 mg/kg bw/day of the notified chemical.

Remarks – Results
Significant histopathological effects of the notified chemical at dose ≥ 250 mg/kg bw/day were observed, including hyperplasia of the squamous epithelium of the limiting ridge of the forestomach, increases of the global leucocyte in the glandular stomach and hypertrophy of the epithelial cells in the distal renal tubule and collecting tubule.

CONCLUSION
The No Observed Effect Level (NOEL) was established as 50 mg/kg bw/day in this study, based on histopathological findings in the animals treated with the notified chemical at 250 mg/kg bw/day.

TEST FACILITY Mitsubishi Chemical Medience Corporation (2011a)

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 livers.
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	Pre-incubation method with and without S9 mix was used. Positive controls used were sodium azide, 9-aminoacridine, AF-2 [2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide] and 2-aminoanthracene

RESULTS

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

Remarks - Results The 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 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 CHL/IU from the lungs of female Chinese hamsters
Cell Type/Cell Line Mammalian cell line
Metabolic Activation System S9 mix from phenobarbital/5,6-benzoflavone induced rat livers
Vehicle Saline
Remarks - Method 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	9.77, 19.5, 39.1, 78.1, 156, 313, 625, 1250*, 2500*, 5000*	6 hours	24 hours
Test 2	9.77, 19.5*, 39.1*, 78.1*, 156, 313, 625, 1250, 2500, 5000	24 hours	24 hours
Test 3	1250*, 2500*	6 hours	24 hours
Present			
Test 1	9.77, 19.5, 39.1, 78.1, 156, 313*, 625*, 1250*, 2500, 5000	6 hours	24 hours
Test 3	200*, 300*, 400*	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	> 2500	> 2500	> 5000	Negative
Test 2	> 39.1	> 39.1	> 5000	Negative
Test 3	-	NR*	NR*	Negative
Present				
Test 1	> 1250	> 625	> 5000	Equivocal
Test 3	-	NR*	NR*	Negative

*NR: Not reported

Remarks - Results Dose-dependent cell growth inhibition was observed with notified chemical. The incidence of structural aberrant cells was 6.0% in Test 1 with metabolic activation at one dose only. This was not seen in a repeat study (Test 3).
In addition, an increased incidence of numerically aberrant cells (polyploidy) was seen in Tests 1 and 3 without metabolic activation. Therefore the potential for aneugenicity cannot be ruled out. The UK Committee on Mutagenicity (2011) notes that polyploidy may not be a reliable indicator for aneugenicity and may result from a number of different genetic changes.

CONCLUSION Based on available information, the notified chemical was not concluded as not clastogenic to CHL/IU cells treated in vitro under the conditions of the test.

TEST FACILITY Mitsubishi Chemical Medience Corporation (2010c)

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	51.9
		14	66.9

Remarks - Results	All relevant test validity criteria were met. At the end of biochemical oxygen demand (BOD) measurement, pH values were 7.1, 7.2 and 7.1 for test suspensions 1, 2 and 3 respectively and 7.0 for the abiotic control. At Day 28, 20% degradation was observed using the analysis of dissolved organic carbon (DOC). The test substance is likely to have formed a hardly-water soluble salt which could account for higher degradation determined by DOC analysis.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	Mitsubishi Chemical Medience Corporation (2011b)

C.1.2. Inherent biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 302 B Inherent Biodegradability: Zahn-Wellens/EMPA Test (1992)
Inoculum	Activated sludge
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	1.25	14	100

Remarks – Results	All relevant test validity criteria were met. The percentage degradation of the toxicity control was 49.8% on Day 14 indicating that the test substance was not considered to have a toxic effect on the sewage sludge micro-

organisms 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	99.8	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 99.8 mg/L.

CONCLUSION The notified chemical is not harmful to fish

TEST FACILITY Mitsubishi Chemical Medience Corporation (2011c)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Static test (2004)
Species *Daphnia magna*
Exposure Period 48 hours
Auxiliary Solvent None
Water Hardness 54 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 [acute]*	*48 h [acute]*
Control	0	2 × 5	0	0
100	96.4	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 (2011d)

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: > 99.6 mg/L
Auxiliary Solvent	None
Water Hardness	Not reported
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 72 h*	*mg/L*
Not determined	Not determined	> 100	9.81

Remarks - Results	All relevant test validity criteria were met. 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. The no observed effect concentration on the growth rate (NOEC) was determined by an analysis of variance (ANOVA), Williams test, subsequent to Barlett test for homogeneity of variances. The inhibition of growth is very likely to have been influenced by the light absorbing properties of the notified chemical but it cannot be concluded that algal growth has been inhibited solely as a reduction in light intensity. However, the test substance is not considered harmful to algae.

CONCLUSION The notified chemical is not harmful to algae.

TEST FACILITY Mitsubishi Chemical Medience Corporation (2010e)

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 3 and 10% 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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