File No: LTD/1742

July 2014

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Chemical in FASTOGEN Blue Products

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment.

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:

Street Address:	Level 7, 260 Elizabeth Street, SURRY HILLS NSW 2010, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX:	+ 61 2 8577 8888
Website:	www.nicnas.gov.au

Director
NICNAS

TABLE OF CONTENTS

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/1742	DIC Australia Pty Ltd	Chemical in FASTOGEN Blue Products	Yes	≤ 1 tonne per annum	Additive in pigments and printing inks

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard classification	*Hazard statement*
Skin Sensitisation Category 1	H317 – May cause an allergic skin reaction.

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

R43: May cause sensitisation by skin contact

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

Hazard classification	*Hazard statement*
Acute (Category 1)	H400 - Very toxic to aquatic life
Chronic (Category 1)	H410 - Very toxic to aquatic life with long lasting effects

Human health risk assessment
Provided that the recommended controls are being adhered to, under the conditions of the occupational settings, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

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

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

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Skin Sensitisation 1- H317: May cause an allergic skin reaction.

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

- Due to the environmental properties of the notified chemical, the notifier should consider their obligations under the Australian Dangerous Goods Code.

Health Surveillance

- As the notified chemical is a skin sensitiser employers should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of skin sensitisation.

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical:
  - Use of enclosed automated processes, if possible
  - Ventilation system including local exhaust ventilation when the chemical in powder form is transferred or weighed

- 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 and inks:
  - Avoid contact with the skin and eyes
  - Avoid inhalation of powder
  - Clean up spills promptly

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced in powdered pigment:
  - Gloves
  - Safety glasses
  - Coveralls
  - Respiratory protection sufficient for respirable particulates during processes where exposure to dust may occur

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical in printing inks:
  - Gloves
  - Safety glasses
  - Coveralls

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

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

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Globally Harmonised System 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.

Storage

- The handling and storage of the notified chemical should be in accordance with the Safe Work Australia Code of Practice for *Managing Risks of Hazardous Chemicals in the Workplace* (SWA, 2012) or relevant State or Territory Code of Practice.

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.

or

(2) Under Section 64(2) of the Act; if
  – the function or use of the chemical has changed from additive in pigments and printing inks, or is likely to change significantly;
  – the amount of chemical being introduced has increased, or is likely to increase, significantly;
  – the chemical has begun to be manufactured in Australia;
  – additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

(Material) Safety Data Sheet
The (M)SDS of the product containing the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
DIC Australia Pty Ltd (ABN: 000 079 550)
323 Chisholm Rd
Auburn NSW 2144

NOTIFICATION CATEGORY
Limited-small volume: Chemical other than polymer (1 tonne or less per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: chemical name, other names, CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, impurities, use details and import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: hydrolysis as a function of pH, partition co-efficient, absorption/desorption, dissociation constant and flashpoint.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
EU, Japan.

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
FASTOGEN Blue (pigments containing the notified chemical)
B508

MOLECULAR WEIGHT
600-1600 Da (components of the UVCB)

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

3. COMPOSITION

DEGREE OF PURITY
UVCB substance

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: blue crystals (agglomerated crystalline particles)

Property	Value	Data Source/Justification
Melting Point/Freezing Point	Not observed below decomposition temperature	Measured
Boiling Point	Not observed below the decomposition temperature	Measured
Density	1550 kg/m^3 at 20°C	Measured
Vapour Pressure	< 8.4 x 10^{-10} kPa at 20 °C	Measured
Water Solubility	< 0.967 x 10^{-3} g/L at 20 °C	Measured
Hydrolysis as a Function of	Not determined	Does not contain hydrolysable

pH		functionality and is not expected to hydrolyse under environmental conditions (pH 4-9)
Partition Coefficient (n-octanol/water)	Not determined	Expected to partition to n-octanol based on its low water solubility
Adsorption/Desorption	Not determined	Expected to partition to sediment/sludge based on its low water solubility
Dissociation Constant	Not determined	The notified chemical is a salt and is expected to ionise in the environment
Particle Size	Inhalable fraction (< 100 μm): 50.2% Respirable fraction (< 10 μm): 10.6% MMAD* = 78.398	Measured
Flash Point	Not determined	The notified chemical is solid at room temperature
Solid Flammability	Not highly flammable	Measured
Autoignition Temperature	232°C	Measured
Explosive Properties	Not explosive	Not expected to be explosive based on structure
Oxidising Properties	Not oxidising	Not expected to be oxidising based on structure

* MMAD = Mass Median Aerodynamic Diameter for < 500 μm fraction

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.

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 in pigment for reformulation (≤ 20% concentration) and as a component of finished inks (≤ 5% concentration). More than 90% of the import quantity will be in pigments.

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

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

PORT OF ENTRY
Melbourne

TRANSPORTATION AND PACKAGING
The pigments containing the notified chemical will be imported in 10 kg paper bags (3-ply kraft paper with laminated liner) and 300 kg flexible containers. Inks containing the notified chemical will be imported and transported by road in 18 L cans and 200 L drums.

USE
The notified chemical will be imported as a component in pigments at 10 - 20% for reformulation in Australia or as a component of flexographic or gravure ink at 0.2 - 5%. The inks will be used to print packaging and publications, and for industrial coating.

OPERATION DESCRIPTION
Reformulation

Pigment containing up to 20% of the notified chemical will be transported to the ink formulation site where paper bags containing the pigment in powder form will be opened by an operator for weighing and transfer. The pigment will be mixed together with other ink components (varnishes, solvents) in tanks which range between 1- to 10-tonne in capacity. The liquid mix will be transferred to a mill to produce ink or ink intermediate. The milling process is a closed system.

The liquid ink formulations, containing up to 5% of the notified chemical, will be packaged into ink containers prior to distribution to industrial end users for printing. In some cases, ink concentrates (base liquid colour inks) will be produced and transferred to other sites for formulation into the finished inks.

After use, the mixing tank and mill are washed out with organic solvents.

End-use
The liquid ink formulations containing up to 5% of the notified chemical will be used in industrial printing. Inks are transferred to the application equipment using pump lines and, if necessary, through a filter. The inks are applied onto two types of printing substrate: paper or plastic film.

Application of ink onto printing substrate (e.g. flexible packaging, newspaper, publication gravures) will be by roller, spreader, flow coating and printing. Printing is an automatic process.

Cleaning of printing equipment will involve removal of the filters used for loading and washing them with organic solvents. Any ink remaining on the printing machine will be wiped off with a waste cloth soaked in organic solvents. Any ink remaining in the ink containers after use will be washed out with organic solvents.

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)*
Pigments		
Charging of pigments into the pre-mixing tank	0.5	60
Sampling and quality control during milling process	0.5	60
Packaging	2	60
Cleaning of equipment	2	60
Ink		
Loading of inks into equipment	0.5	unknown
Print operators	> 4	unknown
Cleaning of equipment	1	unknown

EXPOSURE DETAILS
Reformulation
Dermal, inhalation and ocular exposure of workers to the notified chemical (at up to 20% concentration) may occur when opening paper bags containing pigments in powder form, adding the powder into a mixing tank, sampling and quality control of products. Direct exposure to the notified chemical is negligible during the milling process.

Exposure during transfer, mixing, sampling and quality control is expected to be limited by the use of engineering controls such as local exhaust ventilation and the use of personal protective equipment (PPE) including gloves, protective clothing, goggles and, where necessary, a respirator or filtered mask.

End-use
Dermal, inhalation and ocular exposure to the notified chemical (at up to 5% concentration) may occur during ink application (roller, spreader, flow coating and printing), cleaning and maintenance of printers. Direct

exposure to the printing inks containing the notified chemical during printing is expected to be very low since printing is an automated process. Worker exposure during handling of ink concentrates may also occur.

Worker contact with printed products may occur. However, once the printing ink is cured, the notified chemical is expected to remain bound within the ink matrix and will not be bioavailable.

6.1.2. Public Exposure

The pigments and inks containing the notified chemical will not be sold to the public. Contact with printed products may occur. However, once the printing ink is cured, the notified chemical is expected to remain bound within the ink matrix and will not be bioavailable.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical are summarised in the following table. For full details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute inhalation toxicity	LC50 > 4 mg/L/4 hour; low toxicity
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – local lymph node assay	evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL = 1000 mg/gk bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro chromosome aberration test	equivocal
Genotoxicity – in vitro mammalian cell gene mutation test	non genotoxic
Rat, reproductive and developmental toxicity	NOAEL (parental toxicity) = 250 mg/kg bw/day; NOAEL (embryotoxicity) = 1000 mg/kg bw/day

Toxicokinetics, metabolism and distribution.
A toxicokinetic assessment was provided for the notified chemical, which is a UVCB with varying chemical species. Due to the relatively high molecular weight (> 500 Da) and low water solubility (< 1 mg/L) of the notified chemical, passive diffusion across the gastrointestinal tract is not expected. At least 50% of the notified chemical is inhalable and more than 10% is expected to reach the alveolar region of the respiratory tract. Accumulation in the lungs may occur as demonstrated by the blue foci in the lungs of surviving animals in the acute inhalation study. Dermal absorption of the notified chemical is likely to be low due to its high molecular weight.

The oral, inhalation and dermal absorption rates for the notified chemical is estimated by the notifier to be 50%, 100% and 10%, respectively (NOTOX B.V., 2012).

Acute toxicity.
The notified chemical was of low acute oral, dermal and inhalation toxicity in the rat. No adverse effects were seen when the notified chemical was administered orally and dermally at a maximum concentration of 2000 mg/kg bw. However in the acute inhalation toxicity study where an attainable concentration of 4 mg/L / 4 hours was administered in the rat, one male animal out of ten animals died. Other adverse effects observed included laboured respiration and irregular breathing in all surviving animals and rales in the majority of females.

Irritation and sensitisation.
The notified chemical was non-irritating to the rabbit skin and slightly irritating to the rabbit eye. Effects on the conjunctiva and cornea were observed up to the 72 h and 24 h observations, respectively, in all animals.

The notified chemical was a skin sensitiser in a mouse local lymph node assay (LLNA). The stimulation index (SI) exceeded the threshold of 3 for sensitisation at 11.6% (EC3 value). No information is available on the respiratory sensitisation potential of the chemical.

Repeated dose toxicity.

In a 28-day repeated dose oral toxicity study in the rat, the No Observed (Adverse) Effect Level (NOAEL) was established as 1000 mg/kg bw/day, the highest dose tested. The significance of some changes in clinical chemistry and organ weights was not clear.

Mutagenicity/Genotoxicity.
The notified chemical was non-mutagenic in a bacterial reverse mutation assay and non-genotoxic in an *in vitro* mammalian cell gene mutation test. Equivocal results were seen in an *in vitro* chromosome aberration study in the presence of metabolic activation. Overall the studies do not raise a strong concern for genotoxicity.

Toxicity for reproduction.
In a reproductive/developmental toxicity screening study, rats were administered orally with the notified chemical at up to 1000 mg/kg bw/day.

A NOAEL for parental toxicity was established at 1000 mg/kg bw/day for females based on no significant toxicity observed at 1000 mg/kg bw/day and 250 mg/kg bw/day in males based on the increased incidence of sperm granulomas in the high dose group during microscopic examination, associated with the macroscopic observation of unilateral yellowish-green soft nodules on the tail of the epididymides in one low dose and one high dose animal. It is noted that similar changes were not seen the 28-day repeated dose study.

A NOAEL of 1000 mg/kg bw/day for embryotoxicity was established based on no significant toxicity observed in offspring at 1000 mg/kg bw/day.

Health hazard classification
Based on the available information, the notified chemical is 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. The recommended hazard classification is presented in the following table.

Hazard classification	***Hazard statement***
Skin Sensitisation Category 1	H317 – May cause an allergic skin reaction.

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

R43: May cause sensitisation by skin contact

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The notified chemical is classified as a skin sensitiser. It is of low acute dermal and oral toxicity. Effects seen in an acute inhalation study suggest that it has potential adverse effects on the respiratory system. It is non-irritant to the skin but is slightly irritating to the eyes. Up to 50% of particles are in the inhalable range and over 10% in the respirable range. The highest potential for exposure is for reformulation workers who may be exposed to up to 20% of the notified chemical in powder form via dermal, inhalation and ocular routes. The use of enclosed, automated processes where possible, local exhaust ventilation and safe work practices would minimise the potential for exposure to the notified chemical. Use of PPE (impervious gloves, goggles, coveralls and respiratory protection, if significant inhalation exposure is expected) would further reduce the exposure and risk.

Print operators may also have dermal and ocular exposure to the notified chemical at up to 5% in ink formulations. The highest potential for exposure is during cleaning and maintenance of printing equipment, as well as handling ink concentrates. Adequate ventilation, safe work practices and appropriate PPE (impervious gloves, goggles and coveralls) would minimise exposure.

Provided that control measures are in place to minimise worker exposure, the risk to the health of workers from the use of the notified chemical is not considered to be unreasonable.

6.3.2. Public Health
The pigments and ink containing the notified chemical will not be sold to the public.

The public may have dermal contact with printed products containing the notified chemical. Exposure will be minimal as the notified chemical will be bound to the paper or other printing substrates in the ink matrix.

ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will be imported to Australia as a raw material for local reformulation of inks. During reformulation, spills and leaks are expected to be collected and disposed of via a licensed waste contractor.

Empty containers are likely to be disposed of to landfill. Pigment dust that is collected from the mixing tank is expected to be disposed of to landfill. The notified chemical in tank wash-outs (mixing tank, mill) is expected to be released to sewer or sent to an external waste treatment company. Release of notified chemical in vapours is not expected due to the very low vapour pressure of the notified chemical.

RELEASE OF CHEMICAL FROM USE
The majority of the release of the notified chemical from use to the environment will be spills, washings of printing equipment and residual ink. Washings from ink containers are expected to be sent to sewer or as collected material to an external waste treatment company. The notified chemical will be physically bound on printed substrate. Hence it is expected to be stable within an inert matrix on printed substrates once it is cured.

RELEASE OF CHEMICAL FROM DISPOSAL
The majority of the notified chemical is expected to be used in ink for printing on various papers and plastic films. The notified chemical is expected to share the fate of the printed articles which are expected to be disposed of to landfill at the end of their useful life, or be recycled after use. Hence, up to 50% of the total import volume of the notified chemical may be released to sewers as residues in recycling waste waters. Empty containers containing residues of the notified chemical are expected to be disposed of to landfill.

7.1.2. Environmental Fate
The notified chemical is not readily biodegradable according to the biodegradation study provided. However, the notified chemical has a potential for bioconcentration based on the study provided. However, a depuration process was not conducted in the bioconcentration study provided. Depuration is the process of eliminating of chemicals from live test organisms (e.g. fish). If the bioconcentration of notified chemical in test organisms is fully eliminated during the depuration process, it cannot be regarded that the notified chemical has potential for bioaccumulation. Therefore, the conclusion made from the provided bioconcentration study should be treated with caution. Additionally, only some components of the notified chemical have potential for bioconcentration. Since the bioconcentration factor (BCF) of the notified chemical is less than 2000, the notified chemical can be confidently regarded as a non persistent, bioaccumulative and toxic (PBT) substance. For the details of the environmental fate studies please refer to Appendix C.

Approximately half of the paper to which the ink containing the notified chemical is applied to is likely to be recycled. During recycling processes, waste paper is repulped using a variety of chemical agents which, amongst other things, enhance detachment of ink from the fibres. The notified chemical is anticipated to partially partition to sludge and/or sediment based on its low water solubility. Sludge from treatment plants may be collected for disposal to landfill or used in soil remediation. The majority of the notified chemical in sludge is expected to be disposed of to landfill where it is anticipated to degrade by biotic and abiotic processes to form water and oxides of carbon, nitrogen, copper and strontium.

7.1.3. Predicted Environmental Concentration (PEC)
It was indicated by the notifier that the notified chemical will be used as ink to print packaging, publications, and for industrial coating. It was conservatively assumed that 100% of the total import volume of the notified chemical will be used as ink for printing on papers. Of this, it is assumed that 50% of the total import volume of notified polymer may be released to sewer from recycling processes. A Predicted Environmental Concentration (PEC) for the worst case scenario has been calculated on the assumption the recycling processes occurs only on working days, which is 260 days per annum. It is conservatively assumed that 0% of the notified polymer will be removed at Sewage Treatment Plants (STPs).

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:	0.04	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m3). Using these assumptions, irrigation with a concentration of 0.425 µg/L may potentially result in a soil concentration of approximately 2.8 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 14.2 µg/kg and 28.4 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	LL50 (96 hours) = 0.76 mg/L*	Very toxic to fish
Daphnia Toxicity	EL50 (48 hours) = 0.54 mg/L*	Very toxic to aquatic invertebrates
Algal Toxicity	E_rL50 (72 hours) = 2 mg/L*	Toxic to algae
Inhibition of Bacterial Respiration	EL50 > 100 mg/L	Not inhibitory to microbial respiration

* Filtered Water Accommodated Fraction (WAF)

Based on the endpoint for the notified chemical, it is expected to be very toxic to fish and aquatic invertebrates and, toxic to algae. Under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009), the notified chemical is formally classified as Acute Category 1; Very toxic to aquatic life. Based on the acute toxicity of the notified chemical and lack of ready biodegradability, it has been formally classified under GHS as Chronic Category 1; Very toxic to aquatic life with long lasting effects.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) for the notified chemical has been calculated and is presented in the table below. The PNEC is calculated based on the endpoint for the most sensitive species for the notified chemical (daphnia, EC50). An assessment factor of 100 has been used as acute toxicity endpoints for three trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Invertebrates)	0.54	mg/L
Assessment Factor	100	
PNEC:	5.4	µg/L

7.3. Environmental Risk Assessment

The risk quotients for the notified chemical are presented in the table below.

Risk Assessment	*PEC µg/L*	*PNEC µg/L*	*Q*
Q - River:	0.43	5.4	0.079
Q - Ocean:	0.04	5.4	0.008

The Risk Quotients (Q = PEC/PNEC) for a worst case discharge scenario have been calculated to be < 1 for the river and ocean compartments. The notified chemical is not readily biodegradable. Whilst components of the notified chemical may have potential to bioconcentrate (BCF ≥ 500), the notified chemical is not regarded as a persistent, bioaccumulative and toxic (PBT) substance. The notified chemical is unlikely to result in ecotoxicologically significant concentrations in aquatic environment for the assessed use pattern. Therefore, the notified chemical is not considered to pose an unreasonable risk to the environment from the assessed use scenario.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point Not observed below decomposition temperature

Method OECD TG 102 Melting Point/Melting Range.
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks Determined using a Q100 differential scanning calorimeter (DSC) with a preliminary test using a Q50 thermogravimetric analyser (TGA). Weight loss of the sample occurred during heating, and no melting or boiling was observed. In the preliminary test, weight loss occurred from 325ºC.
Test Facility NOTOX B.V. (2009f)

Boiling Point Not observed below decomposition temperature

Method OECD TG 103 Boiling Point.
EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks Determined using a Q100 differential scanning calorimeter (DSC) with a preliminary test using a Q50 thermogravimetric analyser (TGA).
Test Facility NOTOX B.V. (2009f)

Density 1550 kg/m^3 at 20°C

Method OECD TG 109 Density of Liquids and Solids.
EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks Determined using a gas comparison stereopycnometer.
Test Facility NOTOX B.V. (2009f)

Vapour Pressure < 8.4 x 10^{-10} kPa at 20 °C

Method OECD TG 104 Vapour Pressure.
EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks Determined using the isothermal thermogravimetric effusion method.
Test Facility NOTOX B.V. (2009f)

Water Solubility < 0.967 x 10^{-3} g/L at 20 °C

Method OECD TG 105 Water Solubility.
EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks Flask Method/Column Elution Method
Test Facility NOTOX B.V. (2009f)

Particle Size

Method OECD TG 110 Particle Size Distribution/Fibre Length and Diameter Distributions.

Range (µm)	*Volume (%)*
< 1	0.49
< 5	7.4
< 10	14.1
< 25	27.8
< 100	66.8
< 200	92.0

Remarks A manual sieve analysis was initially performed on the test substance to remove large agglomerated lumps. The analysis indicated that 75.1% by weight of the test substance had a particle size of ≤ 500 µm. The results reported above are from the subsequent laser diffraction analysis on the ≤ 500 µm fraction. The particle size is reported in volume (%) as it was necessary to disperse the test substance in silicone oil.
The Mass Median Aerodynamic Diameter (MMAD) of the fraction ≤ 500 was 78.4 µm.
Test Facility Chilworth (2009)

Solid Flammability Not highly flammable

Method	EC Council Regulation No 440/2008 A.10 Flammability (Solids)
Remarks	A preliminary screening test was conducted; the distance of smouldering caused by the test substance was 10 mm after 4 minutes.
Test Facility	NOTOX B.V. (2009f)

Autoignition Temperature 232°C

Method	EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids.
Remarks	Determined using a 2L laboratory oven. The temperature of the oven at which the test substance reached > 400°C by self-heating was used to define the autoignition temperature.
Test Facility	NOTOX B.V. (2009f)

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/WI (Han), outbred (SPF quality)
Vehicle: Propylene glycol

RESULTS

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

LD50: > 2000 mg/kg bw
Signs of Toxicity: Hunched posture was observed in all animals which was resolved within 2 or 4 hours post-treatment.
Effects in Organs: None
Remarks - Results: All animals showed expected gains in body weight during the study except for one individual that showed slight loss in body weight in the second week post-treatment.

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

TEST FACILITY: NOTOX B.V. (2009d)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 402 Acute Dermal Toxicity.
Species/Strain: Rat/Crl:CD(SD) (SPF quality)
Vehicle: Propylene glycol
Type of dressing: Semi-occlusive.
Remarks - Method: The test substance was dispersed in the vehicle to give a 20% suspension.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
2000 mg/kg	5F/5M	2000	none

LD50: > 2000 mg/kg bw
Signs of Toxicity - Local: There were no signs of local toxicity.
Signs of Toxicity - Systemic: There were no signs of systemic toxicity.
Effects in Organs: No abnormalities were observed in organs during necropsy.
Remarks - Results: The bluish staining on the application sites and bluish faeces observed in all animals during patch removal day and the next day after patch removal were not considered of toxicological significance. The reduction in body weight in all animals during patch removal day (compared to during pre-treatment) was followed by a satisfactory increase in body weight in all animals during the observation period.

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

TEST FACILITY	DSTC (2009)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 403 Acute Inhalation Toxicity.
Species/Strain	Rat: Crl:WI(Han), outbred (SPF quality)
Vehicle	Pressurised air
Method of Exposure	Nose-only inhalation chamber.
Exposure Period	4 hours
Physical Form	Solid aerosol (particulate).
Particle Size	2.6-2.9 µm
Remarks - Method	Traditional protocol was used with only one concentration level (limit test). Thirteen-week old animals were used. Animals were not housed individually.

RESULTS

Group	*Number and Sex of Animals*	*Concentration mg/L*		*Mortality*
		Nominal	*Actual*	
5 mg/L	5F/5M	42.2	4.0	0F/1M

LC50	> 4 mg/L / 4 hours
Signs of Toxicity	One male died approximate 2 ½ hours after the initiation of exposure. Laboured respiration was noted among all survivors. After exposure, hunched posture, lethargy, laboured respiration and irregular breathing were noted among surviving animals. Majority (4/5) of females experienced rales. Piloerection was noted in all animals. The animals recovered from all the above symptoms by Day 10. Blue staining of fur was noted in all surviving animals until Day 15. In all survivors, body weight loss was noted at Day 8 and body weight gain was noted at Day 15. The latter is considered by the study authors as normal of untreated animals of the same age and strain during the second week of exposure. Alopecia was noted in 4/5 females between Days 7 and 15.
Effects in Organs	No abnormalities were found during macroscopic post-mortem examination of the animal that died. Macroscopic post-mortem examinations of surviving animals at termination revealed bluish foci in lungs and bluish discolouration of the tail skin of all animals.
Remarks - Results	The geometric standard deviation (3.4) for MMAD was outside the recommended range. Blue faeces noted in all animals at Day 4 may be due to ingestion of the test substance from grooming. Piloerection and blue staining of fur were considered by the study authors not to be due to systemic toxicity; alopecia was also considered of no toxicological significance as it is common in group housed rats. Although one mortality was seen during the study, and other animals experienced respiratory difficulty, the study authors considered that the LC50 (4h) was likely to exceed 5 mg/L.
CONCLUSION	The notified chemical is of low toxicity via inhalation.
TEST FACILITY	NOTOX B.V. (2009c)

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	3M
Vehicle	50% ethanol solution
Observation Period	72 hours
Type of Dressing	Semi-occlusive.
Remarks - Results	No skin irritation was observed after 4 hours of exposure. Blue staining of the treated skin was observed throughout the observation period which did not influence the scoring of the skin reactions.
CONCLUSION	The notified chemical is non-irritating to the skin.
TEST FACILITY	NOTOX B.V. (2009h)

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	3M
Observation Period	7 days

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1.33	1.33	1.33	2	< 7 days	0
Conjunctiva: chemosis	1.0	0.33	0.33	2	< 72 h	0
Conjunctiva: discharge	0.67	0.33	0.33	1	< 72 h	0
Corneal opacity	0.33	0.33	0.33	1	< 48 h	0
Iridial inflammation	0.33	0.0	0.0	1	< 48 h	0

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

Remarks - Results	The corneal injury consisted of epithelial damage as well as opacity. All effects had reversed by the 7 day observation. Blue staining of the fur on the head and paws was noted throughout the observation period. Remnants of the test substance remained in the eyes up until the 72 h observation.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	NOTOX B.V. (2009a)

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 strain, inbred (SPF quality)
Vehicle	Dimethyl formamide
Remarks - Method	The positive control study was carried out as part of a 6-monthly reliability check.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	300	1.0
10	797	2.7
25	1674	5.5
50	1299	4.3
Positive Control(hexylcinnamicaldehyde)		
0 (acetone:olive oil(4:1))	359	1.0
5	628	1.7
10	1018	2.8
25	1302	3.6

Remarks - Results: One animal from mid dose group showed an extremely enlarged node and the result from this animal was rejected and not used for interpretation.
No oedema was observed in any of the animals. No erythema was observed in control animals; blue staining prevented the scoring for erythema in all treated animals. Alopecia was noted in all animals from the high dose group. An EC3 value of 11.6% was derived.

CONCLUSION: There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY: NOTOX B.V. (2009e)

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
Exposure Information: Total exposure days: 28 days
Dose regimen: 7 days per week
Post-exposure observation period: 14 days
Vehicle: Methylcellulose solution (0.5 w/v%)
Remarks - Method: Dosage for the main study was chosen on the basis of a preliminary 14-day repeated dose toxicity study.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control (vehicle)	5F/5M	0	0
low dose	5F/5M	50	0
mid dose	5F/5M	250	0
high dose	5F/5M	1000	0
control recovery (vehicle)	5F/5M	0	0
high dose recovery	5F/5M	1000	0

No treatment-related effects were observed in the following parameters: detailed clinical observations, body weights, food intakes and urinalyses.

Significant locomotor activity changes at were seen in high dose (10-20 mins) and low dose (50-60 mins) males and in low dose females (0-10 mins) but were not evident over the total 60 minute observation time and were not considered to be test substance related.

Clinical Observations
Dark indigo stool was continuously observed in both sexes treated at all doses including the recovery group up

until the fourth day post dosing.

Laboratory Findings – Clinical Chemistry and Haematology
Some significant changes in blood chemistry and haematology parameters were seen in both sexes but were mostly not dose-related. A significant decrease in blood glucose was seen in males at mid and high doses and during the recovery period of high dose males. The cause of the decrease is not known.

Effects in Organs
Relative heart weight increased significantly in mid and high dose males. A significant increase in relative kidney weight was seen in high dose males. Absolute organ weights were not affected. The cause of these changes is not known, and they were not associated with histopathological changes. A recessed region of the kidney was found in one high dose male during recovery period.

The digestive organs of some males and females at mid and high doses displayed dark blue contents.

Remarks – Results
The study authors established a No Observed (Adverse) Effect Level (NOAEL) of 1000 mg/kg bw/day for the notified chemical.
Although changes in relative organ weights and clinical chemistry were noted at this dose, it is not clear whether these effects are adverse.

Conclusion

The (NO(A)EL) was established as 1000 mg/kg bw/day in this study, the highest dose tested. .

TEST FACILITY CERI (2009)

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 and TA100
E. coli: WP2uvrA
Metabolic Activation System S9 fraction from phenobarbital/5,6-benzoflavone induced rat liver
Concentration Range in Main Test a) With metabolic activation: 156, 313, 625, 1250, 2500 and 5000 µg/plate
b) Without metabolic activation: 156, 313, 625, 1250, 2500 and 5000 µg/plate
Vehicle Dimethyl sulfoxide
Remarks - Method A dose-finding test (preliminary test) was performed.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Preliminary Test	> 5000		≥ 1250	Negative
Test 1		> 5000	≥ 1250	Negative
Test 2		> 5000	≥ 1250	Negative
Present				
Preliminary Test	> 5000		≥ 1250	Negative
Test 1		> 5000	≥ 1250	Negative
Test 2		> 5000	≥ 1250	Negative

Remarks – Results The positive controls showed the expected increase in revertant cells.

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

TEST FACILITY CERI (2008)

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 Lung fibroblasts (CHL/IU cells)
Metabolic Activation System S9 fraction from phenobarbital/5,6-benzoflavone induced rat liver
Vehicle Carboxymethyl cellulose sodium salt solution (0.5% [w/v])
Remarks - Method Numerical aberration (increase in ploidy) was also measured. The positive controls used were Mitomycin C (MMC) without metabolic activation and Cyclophosphamide monohydrate (CPA) with metabolic activation.
The study authors considered structural aberration frequencies less than 5% to represent a negative result.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Preliminary Test 1	19.5, 39.1, 78.1, 156, 313, 626, 1250 and 5000	6 hr	24 hr
Preliminary Test 2	19.5, 39.1, 78.1, 156, 313, 626, 1250 and 5000	24 hr	24 hr
Test 1	210, 262*, 328*, 410*, 512, 640, and 800	6 hr	24 hr
Test 2	134, 168*, 210*, 262*, 328, 410, 512 and 640	24 hr	24 hr
Present			
Preliminary Test 1	19.5, 39.1, 78.1, 156, 313, 626, 1250 and 5000	6 hr	24 hr
Test 1	262, 328, 410*, 512*, 640*, 800, 1000 and 1250	6 hr	24 hr

*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				
Preliminary Test 1			≥ 39.1	Negative
Preliminary Test 2			≥ 19.5	Negative
Test 1		≥ 410	≥ 210	Negative
Test 2		≥ 262	≥ 134	Negative
Present				
Preliminary Test 1			≥ 39.1	Negative
Test 1		≥ 640	≥ 262	Equivocal

Remarks - Results In general, aberration frequencies for the test substance were higher than the negative controls but less than 5%. A confirmation test was conducted to verify a 6% structural aberration frequency seen in the short-term treatment (with metabolic activation) at the highest dose level (640 µg/mL), which raised a suspicion of clastogenicity. This was not reproduced in the confirmation test, however the frequency seen in the confirmation test was 4%, and was higher than expected, based on historical negative controls. The clastogenicity result in the presence of metabolic activation is considered equivocal. No increase in numerical aberrations was seen in the test substance, compared to the controls. All positive controls showed high increases in structural aberrations, confirming the validity of the test system

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

TEST FACILITY CERI (2007)

B.10. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
EC Council Regulation 440/2008 B.17 Mutagenicity - In vitro Mammalian Cell Gene Mutation Test.

Species/Strain Mouse
Cell Type/Cell Line L5178Y/TK$^{+/-}$-3.7.2C mouse lymphoma cells
Metabolic Activation System S9 fraction from phenobarbital/β-naphthoflavone induced rat liver
Vehicle Dimethyl sulfoxide
Remarks - Method A dose range-finding test was conducted. The notified chemical was poorly soluble in aqueous solution therefore the highest tested concentration in the range-finding test was 100 µg/mL of exposure medium.
In all tests, initial culture counts for 3and 24 hr exposure periods were 8 x 10^6 and 5 x 10^6 cells respectively.
The positive controls used were methyl methane sulfonate (MMS) in the absence of metabolic activation, and cyclophosphamide (CP) in the presence of metabolic activation.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Range-finding test 1	1, 3, 10, 33, 66, 100	3 hrs		
Range-finding test 2	1, 3, 10, 33, 66, 100	24 hrs		
Test 1	0.1*, 0.3, 1*, 3, 10*, 33*, 100*, 110, 125*, 140*, 160*	3 hrs	2 days	11-12 days
Test 2	0.1, 0.3, 1*, 3*, 10*, 33*, 100*, 125*, 140*, 160*	24 hrs	2 days	11-12 days
Present				
Range-finding test 1	1, 3, 10, 33, 66, 100	3 hrs		
Test 1	0.03*, 0.1*, 0.3*, 1*, 3*, 10*, 33*, 100*	3 hrs	2 days	11-12 days
Test 2	0.03*, 0.1*, 0.3*, 1*, 3*, 10*, 33*, 100*	3 hrs	2 days	11-12 days

*Dose levels selected to measure mutation frequencies at the TK-locus

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Range-finding test 1		≥ 100	≥ 66	
Range-finding test 2		> 100	≥ 66	
Test 1		≥ 140	≥ 100	negative
Test 2		≥ 140	≥ 100	negative
Present				
Range-finding test 1		≥ 100	≥ 66	
Test 1		> 100	≥ 100	negative
Test 2		> 100	≥ 100	negative

Remarks - Results No significant increase in mutation frequency at the TK locus was observed, in the presence and absence of metabolic activation. The mutation frequencies of both solvent control and test cultures in test 1 (without S9) were above the limit of historical data range, however this

was not considered to affect the acceptability of the test.

CONCLUSION The notified chemical was not clastogenic to mouse lymphoma cells treated in vitro under the conditions of the test.

TEST FACILITY NOTOX B.V. (2009g)

B.11. Reproduction/developmental toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 421 Reproduction/Developmental Toxicity Screening Test
Species/Strain Rat/Crl:WI(Han), outbred (SPF quality)
Route of Administration Oral – gavage
Exposure Information Exposure days and observation period:
Continuous daily exposure and observation until necropsy;
male - 28 days; female - 40 or 49 days:
Vehicle Methylcellulose (0.5% [w/v] solution)
Remarks - Method No significant protocol deviations.
An analytical report was also included to determine the accuracy of preparation (acceptable mean accuracy is between 85% and 115% and coefficient of variation ≤ 10%) and homogeneity between stored and freshly taken samples of formulated test substance.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
1	10 M/10 F	0	0/12
2	10 M/10 F	50	0/12
3	10 M/10 F	250	0/12
4	10 M/10 F	1,000	0/12

Mortality and Time to Death
There was no mortality noted at any dose level.

Effects on Parental generation
Salivation (slight and intermittent) was noted for most of the animals in the high dose group. Blue faeces was observed in male and female treated animals.

In males, sperm granulomas were observed in most groups during microscopic examination – one from control dose (slight), two from low dose (slight or moderate) and three from high dose (slight or moderate). In the high dose males, two out of three cases of sperm granulomas were bilateral. Macroscopic findings include discoloration of tail skin in all male animals in the high dose group.

In females, significantly decreased relative food consumption was observed in the high dose group during pre-mating. No adverse effects were noted in pregnancy duration or any delivery outcomes of dams in any dose group. No significant test substance related differences were observed between the control and treatment groups on the number of corpora lutea and implantation sites, implantation index and delivery index.

No significant test substance related differences were observed between the control and treatment groups for mating performance, fertility parameters, duration of gestation, number of dead and living pups at first litter check, postnatal loss and viability index.

Effects on F1 generation
No significant adverse effects of the test substance on mortality or development of offspring were noted during the observation period and up until sacrifice.

Remarks - Results

Soft yellowish-green nodules in the epididymides were observed in one low dose male (unilateral) and two high dose males (unilateral and bilateral). The occurrence of sperm granulomas observed at 50 and 1000 mg/kg bw/day was also observed in one animal of the control group.

A dose-related though not statistically significant decrease in sex ratio (percentage of live males at first litter check / percentage of live females at first litter check) was observed in F1.

CONCLUSION
The No Observed (Adverse) Effect Level (NOAEL) for female parental toxicity was established as 1000 mg/kg bw/day in this study based on no significant toxicity observed at the high dose. The NOAEL for male parental toxicity was established as 250 mg/kg bw/day based on a higher incidence and severity of sperm granulomas in the high dose group compared to the low dose and control groups. The NOAEL for developmental toxicity wasestablished as 1000 mg/kg bw/day based on no observed significant adverse effects on offspring.

TEST FACILITY NOTOX B.V. (2009b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I).
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Shimadzu TOC-V_{CPH} total organic carbon (TOC) analyser for dissolved organic carbon (DOC) and TOC, and HPLC for residual substance concentration.
Remarks - Method	The test was conducted according to the guidelines above using good laboratory practice (GLP). No significant deviations from the test guidelines were reported.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	1	7	68
14	24	14	72
21	23	21	70
28	21	28	69

Remarks - Results	All validity criteria for the test were satisfied. The reference compound, aniline, reached the 65% pass level by day 7 indicating the suitability of the inoculum. The degree of degradation of the notified chemical after the cultivation period was 21%. Therefore, the test substance is classified as not readily biodegradable according to the OECD (301 C) guideline.
CONCLUSION	The notified chemical is not readily biodegradable
TEST FACILITY	CERI (2010)

C.1.2. Bioaccumulation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 305 Bioconcentration: Flow-through Test
Species	Carp (*Cyprinus carpio*)
Exposure Period	Exposure: 60 days
Auxiliary Solvent	HCO-40, dimethyl sulfoxide
Concentration Range	Level 1: 15 µg/L Level 2: 1.5 µg/L
Analytical Monitoring	HPLC, LCMS
Remarks - Method	The test was conducted according to the guidelines above using good laboratory practice (GLP). However, the only deviation from the guideline above was that no depuration period was reported.

RESULTS

Bioconcentration Factor (BCF)	Level 1:	22 to 38 (Peak 1- steady state was reached at 33) < 12 to 31 (Peak 2- steady state was reached at 25) 6.2 to 12 (Peak 3- steady state was reached at 9) < 0.81 to 6.9 (Peak 4- steady state was not reached)

	470 to 680 (Peak 5- steady state was reached at 540)
	Level 2: < 110 (Peak 1- steady state was not reached) < 130 (Peak 2- steady state was not reached) 17 to 35 (Peak 3- steady state was reached at 22) < 8.2 (Peak 4- steady state was not reached) 460 to 730 (Peak 5- steady state was reached at 600)
Remarks - Results	All validity criteria for the test were satisfied. No significant differences among the BCFs were observed at the two levels. There are two groups of components in the test substance. HPLC was used to quantify one group of components, and four peaks (Peaks 1 - 4) were detected by this method. Of these peaks, none of the components of this group were considered to have potential for bioconcentration as all the bioconcentration factors (BCFs) were found to be < 500.The other group of components were quantified by LCMS. Only two peaks on the LCMS chromatogram were suitable for evaluation, and these peaks were considered to be 'Peak 5'. Other components detected by this method were excluded from analysis as they had short retention times and were considered to be polar compounds and thus not bioconcentrating. Components of Peak 5 were found to exhibit BCF values > 500, and therefore these components may be considered to have potential for bioconcentration (EPHC, 2009). A depuration period was not conducted during the study to determine whether concentrations of the test substance were reduced in the test organism when it was no longer exposed to the test substance. Therefore, the results of this study should be treated with caution.
CONCLUSION	Under the conditions of this study, components of the notified chemical have potential for bioconcentration. However, due to the absence of a depuration period in the study, these results should be treated with caution.
TEST FACILITY	CERI (2011a)

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 test
Species	Medaka
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	44 mg $CaCO_3$/L
Analytical Monitoring	SHIMADZU TOC-V_{CPH}
Remarks – Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported. Water Accommodated Fractions (WAFs) of six different loading rates were prepared by stirring the mixtures with a magnetic stirrer for 48 hours. The mixtures were then filtered by suction filtration using glass fibre filters. The filtrates were stirred with a stirrer to recover the dissolved oxygen decreased in the process of suction filtration.
RESULTS	

Concentration (mg/L)		*Number of Fish*	*Cumulative mortality (%)*				
Nominal	*Measured TOC (Geometric mean)*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	Control	7	0	0	0	0	0
1.0	0.13	7	0	0	0	0	0
2.0	0.26	7	0	0	0	0	0
4.0	0.53	7	0	0	0	0	14
8.0	1.1	7	0	0	57	71	86
16.0	2.1	7	0	100	100	100	100
32.0	3.3	7	43	100	100	100	100

LL50	0.76 (0.53 – 1.1) mg/L (filtered WAF) at 96 hours
NOEL	0.26 mg/L (filtered WAF) at 96 hours
Remarks – Results	All validity criteria for the test were satisfied. The 96-hour LL_{50} was calculated based on measured Total Organic Carbon (TOC) concentrations.

CONCLUSION The notified chemical is very toxic to fish

TEST FACILITY CERI (2011b)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Semi-static test

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	44 mg $CaCO_3$/L
Analytical Monitoring	SHIMADZU TOC-V_{CPH}
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported. Water Accommodated Fractions (WAFs) of seven different loading rates were prepared by stirring the mixtures with a magnetic stirrer for 48 hours. The mixtures were then filtered by suction filtration using glass fibre filters. The filtrates were stirred with a stirrer to recover the dissolved oxygen decreased in the process of suction filtration.

RESULTS

Concentration (mg/L)		*Number of D.magna*	*Immobility (%)*
Nominal	*Measured TOC (Geometric mean)*		
Control	Control	*20*	0
0.63	0.088	20	0
1.3	0.18	20	5
2.5	0.35	20	5
5.0	0.7	20	75
10	1.4	20	100
20	2.8	20	100
40	3.7	20	100

EL50	0.54 (0.45 – 0.65) mg/L at 48 hours (filtered WAF)
NOEL	0.088 mg/L at 48 hours (filtered WAF)
Remarks - Results	All validity criteria for the test were satisfied. The 48 hour EL50 was

	calculated based on measured Total Organic Carbon (TOC) concentrations. The Probit analysis was used to calculate the EL50 value.
CONCLUSION	The notified chemical is very toxic to aquatic invertebrates
TEST FACILITY	CERI (2011c)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 0.1, 0.32, 1.0, 3.2, 10, 32, and 100 mg/L Measured Total Organic Carbon (TOC): 0.021, 0.066, 0.21, 0.66, 2.1, 6.6, 8.4 mg/L
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	SHIMADZU TOC-V_{CPH}
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported. Water Accommodated Fractions (WAFs) were prepared by stirring the mixtures with a magnetic stirrer for 48 hours. The mixtures were then filtered by suction filtration using a membrane filter. Since weighing the test substance was difficult at concentrations such as 1.0, 0.32, and 0.10 mg/L, these concentrations were prepared by diluting the treatment solution of 3.2 mg/L (nominal concentration).

RESULTS

Growth (72 h)	
E_rL50 (mg/L)	*NOE_rL*
2.0	0.21

Remarks - Results	All validity criteria for the test were satisfied. The 48 hour EL50 was calculated based on measured Total Organic Carbon (TOC) concentrations. Bartlett's test and Dunnett's multiple comparison tests were used to conduct statistical analyses.
CONCLUSION	The notified chemical is toxic to algae
TEST FACILITY	CERI (2011d)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 100 mg/L
Remarks – Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported.

RESULTS

EL50	> 100 mg/L (loading rate)
NOEL	≥ 100 mg/L (loading rate)
Remarks – Results	All validity criteria for the test were satisfied.
CONCLUSION	The notified chemical is not expected to inhibit microbial respiration up to the concentration of 100 mg/L
TEST FACILITY	NOTOX B.V. (2008)

BIBLIOGRAPHY

CERI (2007) Chromosomal aberration test of [notified chemical] using cultured mammalian cells (Study No.: K06-121, December, 2007). Oita, Japan, Hita Laboratory, Chemicals Evaluation and Research Institute (CERI). (Unpublished report submitted by notifier).

CERI (2008) Mutagenicity test of [notified chemical] using microorganisms (Study No.: K01-4082, May, 2008). Oita, Japan, Hita Laboratory, Chemicals Evaluation and Research Institute (CERI). (Unpublished report submitted by notifier).

CERI (2009) Twenty-eight day repeated-dose oral toxicity study of [notified chemical] in rats (Study No.: B11-0899, March, 2009). Oita, Japan, Hita Laboratory, Chemicals Evaluation and Research Institute (CERI). (Unpublished report submitted by notifier).

CERI (2010) Biodegradation study of [notified chemical] by microorganisms (Study No.: 15106, December, 2010). Kurume, Japan, Evaluation and Research Institute (CERI). (Unpublished report submitted by the notifier).

CERI (2011a) Bioconcentration study of [notified chemical] in carp (Study No.: 45107, March, 2011). Kurume, Japan, Evaluation and Research Institute (CERI). (Unpublished report submitted by the notifier).

CERI (2011b) A 96-hour acute toxicity study of [notified chemical] in Medaka (Study No.: 94611, June, 2011). Kurume, Japan, Evaluation and Research Institute (CERI). (Unpublished report submitted by the notifier).

CERI (2011c) A 48-hour acute immobilisation study of [notified chemical] in *Daphnia magna* (Study No.: 94610, June, 2011). Kurume, Japan, Evaluation and Research Institute (CERI). (Unpublished report submitted by the notifier).

CERI (2011d) Algae Growth Inhibition Study of [notified chemical] in *Pseudokirchneriella subcapitata* (Study No.: 94609, June, 2011). Kurume, Japan, Evaluation and Research Institute (CERI). (Unpublished report submitted by the notifier).

Chilworth (2009) Particle size analysis on a sample of [notified chemical] (Report No.: GLP101772R1V1/09, July, 2009). Southampton, United Kingdom, Chilworth Technology Limited. (Unpublished report submitted by notifier).

DSTC (2009) Acute dermal toxicity study with [notified chemical] in rats (Study No.: 0871, March, 2009). Saitama, Japan, Saitama Laboratory, Drug Safety Training Center Co Ltd (DSTC). (Unpublished report submitted by notifier).

EPHC (2009) Environmental Risk Assessment Guidance Manual for Industrial Chemicals. Environment Protection and Heritage Council, Australia, 109 pp.

NOHSC (2004) Approved Criteria for Classifying Hazardous Substances, 3rd edition [NOHSC:1008(2004)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

NOTOX B.V. (2008) Activated sludge respiration inhibition test with [notified chemical] (Project No.: 487785, November, 2008). 's-Hertogenbosch, The Netherlands, NOTOX B.V. (Unpublished report submitted by notifier).

NOTOX B.V. (2009a) Acute eye irritation/corrosion study with [notified chemical] in the rabbit (Study No.: 487782. July, 2009). 's-Hertogenbosch, The Netherlands, NOTOX B.V. (Unpublished report submitted by notifier).

NOTOX B.V. (2009b) A reproduction/developmental toxicity screening test of [notified chemical] in rats by oral gavage (Study no.: 487764, November, 2009). 's-Hertogenbosch, The Netherlands, NOTOX B.V. (Unpublished report submitted by notifier).

NOTOX B.V. (2009c) Assessment of acute inhalatory toxicity with [notified chemical] in the rat (Study No.: 488482, June, 2009). 's-Hertogenbosch, The Netherlands, NOTOX B.V. (Unpublished report submitted by notifier).

NOTOX B.V. (2009d) Assessment of acute oral toxicity with [notified chemical] in the rat (acute toxic class method) (Study No.: 487763, July, 2009). 's-Hertogenbosch, The Netherlands, NOTOX B.V. (Unpublished report submitted by notifier).

NOTOX B.V. (2009e) Assessment of contact hypersensitivity to [notified chemical] in the mouse (Study No.: 487783, July, 2009). 's-Hertogenbosch, The Netherlands, NOTOX B.V. (Unpublished report submitted by notifier).

NOTOX B.V. (2009f) Determination of physico-chemical properties of [notified chemical] (Project No.: 487758, August, 2009). 's-Hertogenbosch, The Netherlands, NOTOX B.V. (Unpublished report submitted by notifier).

NOTOX B.V. (2009g) Evaluation of the mutagenic activity of [notified chemical] in an *in vitro* mammalian cell gene mutation test with L5178Y mouse lymphoma cells (with independent repeat) (Study No.: 491125, August, 2009). 's-Hertogenbosch, The Netherlands, NOTOX B.V. (Unpublished report submitted by notifier).

NOTOX B.V. (2009h) Primary skin irritation/corrosion study with [notified chemical] in the rabbit (Study no.: 487781, July, 2009). 's-Hertogenbosch, The Netherlands, NOTOX B.V. (Unpublished report submitted by notifier).

NOTOX B.V. (2012) Toxicokinetic assessment of [notified chemical] (Study No.: 487784, July, 2012). 's-Hertogenbosch, The Netherlands, NOTOX B.V. (Unpublished report submitted by notifier).

SWA (2012) Code of Practice: Managing Risks of Hazardous Chemicals in the Workplace, Safe Work Australia, http://www.safeworkaustralia.gov.au/sites/swa/about/publications/pages/managing-risks-of-hazardous-chemicals-in-the-workplace.

United Nations (2009) Globally Harmonised System of Classification and Labelling of Chemicals (GHS), 3rd revised edition. United Nations Economic Commission for Europe (UN/ECE), <http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html >.