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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

NT-62

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:

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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
STD/1517	Canon Australia Pty Ltd	NT-62	No	≤ 10 tonnes per annum	Component of printer toner

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

CONTROL MEASURES
Occupational Health and Safety

- Service personnel should wear impervious gloves and ensure adequate ventilation is present when removing spent printer toner containers 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

- Disposal should be in accordance with Australian, state, territory and local government laws. Landfilling is a disposal option frequently used for industrial chemicals.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by physical containment, solidification, 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 notified chemical is imported in any form other than in sealed printer toner containers;
- information on the mutagenicity/carcinogenicity potential of the notified chemical becomes available;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component of printer toner, 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 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

This notification has been conducted under the cooperative arrangement with the United States Environmental Protection Agency (US EPA). Information pertaining to the assessment of the notified chemical by the US EPA was provided to NICNAS and, where appropriate, used in this assessment report. The other elements of the risk assessment and recommendations on the safe use of the notified chemical were carried out by NICNAS.

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT
Canon Australia Pty Ltd (ABN: 66 005 002 951)
Building A
The Park Estate
5 Talavera Road
MACQUARIE PARK NSW 2113

NOTIFICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne 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 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, adsorption/desorption and dissociation constant.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
EPA (USA) 2006

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
NT-62

MOLECULAR WEIGHT
> 500 Da

3. COMPOSITION

DEGREE OF PURITY
> 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Black powder

Property	Value	Data Source/Justification
Melting Point	Decomposes without melting at > 320 °C	Measured
Boiling Point	Decomposes without boiling at > 320 °C	Measured
Density	1609 kg/m^3 at 24.3 °C	Measured
Vapour Pressure	< 1.0 x 10^{-8} kPa at 25 °C	Measured
Water Solubility	< 5 x 10^{-6} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Hydrolysis is not expected to occur in the environmental pH range (4-9) under

Property	Value	Data Source/Justification
		ambient conditions due to its low solubility in water
Partition Coefficient (n-octanol/water)	log Pow > 4.9	Measured
Adsorption/Desorption	Not determined	Expected to adsorb on sludge/sediment based on its low water solubility
Dissociation Constant	Not determined	Not expected to be ionised due to its low solubility in water
Particle Size	Inhalable fraction (< 100 μm): 100% Respirable fraction (< 10 μm): 60% MMAD* = 3.6 μm	Measured
Flammability	Not highly flammable	Measured
Autoignition Temperature	240 °C	Measured
Explosive Properties	Not considered explosive	Measured
Stability Testing	Decomposition range: 300 – 370 °C	Measured
Oxidising Properties	Not considered oxidative	Measured

* MMAD = Mass Median Aerodynamic Diameter

DISCUSSION OF PROPERTIES
For full details of tests on physical and chemical properties not evaluated in the US report, refer to Appendix A.

Reactivity
The notified chemical is expected to be stable under normal conditions of use.

Physical hazard classification
Based on the submitted physico-chemical data depicted in the above table, the notified chemical is not recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported into Australia as a component (< 10% concentration) of printer toner.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤ 3	≤ 10	≤ 10	≤ 10	≤ 10

PORT OF ENTRY
Nationwide

IDENTITY OF MANUFACTURER/RECIPIENTS
Canon Australia Pty. Ltd.

TRANSPORTATION AND PACKAGING
The notified chemical will be manufactured outside Australia and imported as a component of toner powder in sealed plastic containers. The containers will vary in size between 200-4000 mL and will be packaged in plastic bags before being boxed and fixed with size packaging. The toners will be transported by road or rail throughout Australia.

USE
The notified chemical will be used as a component (≤ 10% concentration) of toner for electro-photocopying and electro-photographic printer machines.

OPERATION DESCRIPTION
The notified chemical will be imported as a component (≤ 10% concentration) of printer toner in sealed containers. Reformulation will not take place in Australia.

End-users (including service technicians and the general public) will remove the container from the packaging and remove the sealing tape from the container before placing the container into the printer. The container will be disposed of when empty.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

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

The notified chemical will be contained within purpose-built plastic toner containers; hence significant dermal and inhalation exposure is not expected during printing operations in industrial settings. Maintenance workers may be exposed to the toner containing 10% or less of the notified chemical during repairs and cleaning of printing equipment. Given the notified chemical is of inhalable particle size, there is potential for both dermal and inhalation exposure.

Office workers may be exposed to the toner when replacing the container but the amount of exposure is predicted to be very low. The toner container is designed not to release toner until the sealing tape has been removed. No specific handling instructions are provided. Occasional dermal exposure during use of the printer may occur if the printed pages are handled before the toner has dried, or if toner stained parts of the printer are touched. During the copy or printing operation, the toner is transferred onto paper and fixed by heat. Once dried, the notified chemical is bonded to the printed paper, and as such dermal exposure to the notified chemical from contact with dried toner is not expected.

Service technicians may be exposed to the toner containing 10% or less of the notified chemical during repairs and cleaning of printing equipment.

6.1.2. Public Exposure
Exposure of the public to toner containing the notified chemical (at ≤ 10%) is expected to be similar, though less frequent, than that described above for office workers

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute inhalation toxicity	LC50 > 3 mg/L; low toxicity
Skin irritation (*in vitro*) – Human Skin Model Test	non-corrosive
Eye irritation (*in vitro*) – Human Tissue Models	non-irritating
Mouse, skin sensitisation – Modified Local Lymph Node Assay	no evidence of sensitisation
Rat, repeat dose toxicity – 28 days	NOAEL = 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* chromosome aberration	non genotoxic

Toxicokinetics, metabolism and distribution.
Given the relatively high molecular weight (> 500 Da), low partition coefficient (log Pow > 4.9 at 20 ^{0}C) and low water solubility (< 5 x 10^{-6} g/L) of the notified chemical, absorption across biological membranes is not expected. This is supported by the low acute toxicity observed via the oral, dermal and inhalation routes in

studies conducted in rats and the absence of treatment related effects in a 28-day repeated dose oral toxicity study.

The notified chemical is an azo compound. Azo compounds may break down to their component amines. The azo linkage is the most labile portion of an azo colourant molecule, and it is readily enzymatically metabolised in mammals, including man (SCCNFP, 2002). Liver azo reductase enzymes reductively cleave the molecules into component amines. Some metabolism of azo colourants may also occur in the cells of the bladder wall, and during percutaneous absorption. Intestinal bacteria are also capable of catalysing reductive cleavage of the azo bond. The notified chemical may be broken down to an aromatic amine which is a suspected mutagen and carcinogen. Given the low bioavailability of the notified chemical reductive cleavage is not expected to occur via the dermal route. However there is potential for formation of the aromatic amine in the GI tract, although this is expected to be limited given the low water solubility of the notified chemical, which is expected to be strongly absorbed.

The notified chemical is of inhalable (< 100 μm) particle size and could be inhaled into the upper respiratory tract. A significant portion (~60%) is also of small enough particle size (<10 μm) to reach the lower respiratory tract (tracheobronchial and pulmonary regions). Larger particles of inhalable size are expected to deposit in the nasopharyngeal region and be cleared by coughing/sneezing or be swallowed. Due to the low water solubility of the notified chemical smaller particles lodging in the tracheobronchial region are likely to be cleared by the mucociliary mechanism and swallowed. However, inhaled respirable particulates of the notified chemical lodging in the pulmonary region may not be readily cleared due to their low water solubility. Absorption across the respiratory tract epithelium is not expected as supported by the low toxicity observed in acute inhalation toxicity in rats; however higher exposure concentrations may be expected to result in increased impairment of clearance mechanisms.

Acute toxicity.
The notified chemical was found to have low acute oral, dermal and inhalation toxicity in rats. No signs of systemic toxicity were noted.

Irritation and sensitisation.
The notified chemical was found to be non-corrosive to skin in an *in vitro* skin irritation test using the EpiDerm™ Reconstructed Human Epidermis model and non-irritating to eyes in an *in vitro* eye irritation test using the EpiOcular™ Reconstructed Human Corneal Epithelium model.

The notified chemical at concentrations up to 50% in a modified Local Lymph Node Assay in mouse showed no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation. In addition the notified chemical did not induce any non-specific (irritant) stimulation response indicative of skin irritation.

Repeated dose toxicity.
A 28 day repeat dose study by oral gavage was conducted in rats with the notified chemical at dose levels of 62.5, 250 and 1000 mg/kg bw/day. No statistically significant treatment related changes were noted in the study. Based on the results of this study the No Observed Adverse Effect Level (NOAEL) was established as 1000 mg/kg bw/day for systemic toxicity.

Mutagenicity/Genotoxicity.
The notified chemical was not mutagenic in bacteria (under the conditions of the Ames test used), nor did it induce chromosomal aberrations in mammalian cells *in vitro*. An *in vivo* genotoxicity study was not provided.

The Ames test provided was not performed in accordance with the modified procedure suggested for azo compounds (Prival and Mitchell, 1982). Modified tests, such as that of Prival and Mitchell, utilise a reductive pre-incubation step (during which the azo colourant may be reduced to amine species) before the test is carried out to yield a greater detection of mutagenic azo species. It is recognised that the standard procedure is not sufficiently sensitive for azo compounds, likely due to their complex metabolism *in vivo* (Brown and DeVito 1993, referenced in Øllgaard *et al* 1998). Given this deficiency in the study performed on the notified chemical and the potential for formation of an aromatic amine suspected of being a mutagen and carcinogen from reductive cleavage of the azo bond, the test result may not be strongly predictive of the mutagenicity of the notified chemical *in vivo*. Hence, the mutagenicity potential of the notified chemical cannot be totally ruled out.

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 the available studies the notified chemical is of low hazard. However, the potential for mutagenicity/carcinogenicity via the oral route cannot be totally ruled out based on the potential formation of an arylamine metabolite from reductive cleavage of the azo bond in the GI tract, although this may be limited by the low water solubility of the notified chemical. The notified chemical is of low acute inhalation toxicity; however, lung overloading effects may occur if large amounts are inhaled.

The notified chemical will be contained within purpose-built plastic containers; hence, significant dermal and inhalation exposure is not expected during printing operations. Maintenance workers/service technicians may be exposed to the notified chemical at ≤ 10% concentration during repairs and cleaning of printing equipment. The use of impervious gloves and adequate ventilation during these operations are therefore recommended to reduce exposure. There may be frequent exposure to dried toner containing the notified chemical. However, once dried the notified chemical will be bound to the paper and is not expected to be available for exposure.

Therefore, provided that measures to protect maintenance workers and service technicians are in place to reduce exposure (i.e. use of impervious gloves and adequate ventilation when performing maintenance operations), and based on the expected low exposure of office workers to the notified chemical, the risk to health of workers from use of the notified chemical is not considered to be unreasonable.

6.3.2. Public Health

Public exposure to the notified chemical is expected to be similar, though less frequent than that experienced by office workers. Therefore, the risk to the health of the public from use of the notified chemical is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will not be manufactured, reformulated or repackaged in Australia. Therefore, no environmental release is expected from these activities. The notified chemical will be imported into Australia as a component of printer toner in sealed containers.

RELEASE OF CHEMICAL FROM USE

Under normal use conditions, environmental release of toner containing the notified chemical from sealed plastic containers is not expected. Once the notified chemical is applied to paper, the majority of the notified chemical is expected to remain fused to the paper or trapped within the print. Approximately half of the paper to which the notified chemical will be bound within the print will eventually be disposed of to landfill with the other half expected to be recycled. In the case of recycling, the notified chemical may be released in effluent from the de-inking process.

RELEASE OF CHEMICAL FROM DISPOSAL

Empty sealed toner containers containing some residual toner are expected to be disposed of to landfill. Notified chemical may also be disposed of to landfill indirectly from waste printed paper.

7.1.2 Environmental fate

The notified chemical is not readily biodegradable or bioaccumulative based on the environmental fate studies for the notified chemical. For the details of the environmental fate studies, please refer to Appendix C. Notified chemical applied to papers as a component of toner will be bound within the print matrix. Half of the printed papers containing the notified chemical are expected to be disposed of to landfill where the notified chemical will slowly degrade by biotic and abiotic processes to form water and oxides of carbon and nitrogen, and low

molecular weight inorganic compounds.

Approximately half of the paper to which the toner 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 toner from the fibres. Due to its very low solubility in water and high partition coefficient, very little of the notified chemical is expected to partition to the supernatant water which is released to the sewer. Notified chemical associated with sludge generated through the recycling process 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 and nitrogen, and low molecular weight inorganic compounds.

7.1.3 Predicted Environmental Concentration (PEC)

The calculation for the predicted environmental concentration (PEC) is summarised in the table below. Based on the reported use in printing, it is conservatively assumed that 50 % of the total import volume of the notified chemical is released to sewer over 260 days per annum corresponding to release only on working days. It was also assumed for the worst case scenario that there is no removal of the notified chemical from effluent during sewage treatment plant (STP) processes.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	10,000	kg/year
Proportion expected to be released to sewer	50%	
Annual quantity of chemical released to sewer	5,000	kg/year
Days per year where release occurs	260	days/year
Daily chemical release:	19.23	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	0%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	4.25	µg/L
PEC - Ocean:	0.43	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 4.25 µg/L may potentially result in a soil concentration of approximately 28.4 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 141.7 µg/kg and 283.5 µg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity (96 h)	LL50 > 100 mg/L* (filtered WAF)	Not harmful to fish
Daphnia Toxicity (48 h)	EL50 > 100 mg/L* (filtered WAF)	Not harmful to aquatic invertebrates
Algal Toxicity (96 h)	E_rL50 > 100 mg/L* (filtered WAF)	Not harmful to algae
Inhibition of Bacterial Respiration	EC50 (3 h) > 1000 mg/L	Not inhibitory to microbial respiration

* Water accommodated fraction

The notified chemical is not considered to be harmful to fish, aquatic invertebrates and algae based on its acute toxicity. Therefore, the notified chemical has not been formally classified under either the acute or chronic

category under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009).

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 common endpoint for the test species (fish, daphnia and algae) of the notified chemical. Acute ecotoxicity endpoints for aquatic species from three trophic levels are available. Therefore, an assessment factor of 100 has been used.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Fish).	100	mg/L
Assessment Factor	100	
PNEC:	1,000	µg/L

7.3. Environmental Risk Assessment

Based on the above PEC and PNEC values, the following Risk Quotient (Q) has been calculated:

Risk Assessment	***PEC µg/L***	***PNEC µg/L***	***Q***
Q - River:	4.25	1000	0.004
Q - Ocean:	0.43	1000	0.0004

The risk quotient (Q = PEC/PNEC) for aquatic exposure is calculated to be < 1 based on the above calculated PEC and PNEC values. Therefore, the notified chemical is unlikely to result in ecotoxicologically significant concentrations in the aquatic environment. The notified chemical is considered to be not readily biodegradable and is not expected to be bioaccumulative. Therefore, the notified chemical is not expected to pose an unreasonable risk to the aquatic environment from the assessed use pattern.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point Decomposes without melting at > 320 °C

Method	OECD TG 102 Melting Point/Melting Range.
Remarks	Determined using differential scanning calorimetry (DSC).
Test Facility	Siemens (2008a)

Boiling Point Decomposes without boiling at > 320 °C

Method	OECD TG 103 Boiling Point.
Remarks	Determined using DSC.
Test Facility	Siemens (2008a)

Density 1609 kg/m^3 at 24.3 °C

Method	OECD TG 109 Density of Liquids and Solids.
Remarks	Determined using a gas comparison pycnometer.
Test Facility	Siemens (2008b)

Vapour Pressure < 1.0 x 10^{-8} kPa at 25 °C

Method	OECD TG 104 Vapour Pressure.
Remarks	Determined using a vapour pressure balance (effusion method).
Test Facility	Siemens (2008a)

Partition Coefficient (n-octanol/water) log Pow > 4.89

Method	OECD TG 107 Partition Coefficient (n-octanol/water). EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	Flask Method. The partition coefficient Pow was estimated from the saturation concentrations in n-octanol (3.3 x 10^{-1} g/L) and water (< 4.2 x 10^{-6} g/L).
Test Facility	Siemens (2008f)

Particle Size

Method	Determined using scanning electron microscopy (SEM) analysis
Remarks	The calculated mass median aerodynamic diameter (MMAD), derived from analysis of 310 particles by SEM, was determined to be 3.6 µm. Based on the MMAD approximately 60% of the test substance was estimated to be in the respirable range based on the American Conference of Governmental Industrial Hygienists (ACGIH) definition for airborne particulate matter.
Test Facility	Fraunhofer (2008a)

Flammability Not highly flammable

Method	EC Council Regulation No 440/2008 A.10 Flammability (Solids).
Remarks	In a preliminary screening test, the test substance burned slower than 200 mm within 4 minutes.
Test Facility	Siemens (2008c)

Autoignition Temperature 240 °C

Method	EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids.
Test Facility	Siemens (2008c)

Explosive Properties No explosive properties

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

Remarks	No explosions were observed when testing with respect to friction, shock, or thermal sensitivity.
Test Facility	Siemens (2008d)

Stability Testing Decomposition range: 300 – 370 °C

Method	OECD TG 113 Screening Test for Thermal Stability and Stability in Air.
Remarks	Determined using differential scanning calibration (DSC). Two measurements were taken in closed glass crucibles. Energy of 532J /g and 617 J/g.
Test Facility	Siemens (2008a)

Oxidizing Properties Not oxidising

Method	EC Council Regulation No 440/2008 A.17 Oxidizing Properties (Solids).
Remarks	The maximum burning rate of the mixtures of test substance and cellulose was less than the maximum burning rate of the reference mixture.
Test Facility	Siemens (2008e)

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/Wistar
Vehicle	Corn oil
Remarks - Method	No significant protocol deviations were recorded. The animals were treated in two steps. Three animals were treated first, and then the three remaining animals were treated 7 days later.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1a	3 F	2000	0/3
1b	3 F	2000	0/3

LD50	> 2000 mg/kg bw
Signs of Toxicity	No signs of systemic toxicity were recorded.
Effects in Organs	No abnormalities were detected in the organs.
Remarks - Results	With the exception of low body weight gains in the first 3 days of the study, no other treatment-related effects were observed.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Fraunhofer (2008b)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test.
Species/Strain	Rat/Wistar
Vehicle	Corn oil
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviation.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	No signs of local toxicity were recorded.
Signs of Toxicity - Systemic	No signs of systemic toxicity were recorded.
Effects in Organs	No abnormalities were detected in the organs.
Remarks - Results	All animals showed continuous increase in body weight. After treatment, the animals showed slight signs of discomfort, but no toxicity effects.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	Fraunhofer (2008c)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 433 Acute Inhalation Toxicity – Limit Test.
Species/Strain	Rat/Wistar
Vehicle	Air
Method of Exposure	Nose-only exposure.
Exposure Period	4 hours
Physical Form	Solid aerosol (particulate).
Particle Size	2.92 ± 1.78 μm (average MMAD)
Remarks - Method	No significant protocol deviations were recorded. The limit test concentration of 2969 mg/m^3, was the highest, technically feasible concentration of test substance with an acceptable MMAD (< 4 μm). Animals were tested in two steps, with the second half of the group tested two days after the first to allow for any delayed toxicity to be observed.

RESULTS

Group	*Number and Sex of Animals*	*Concentration mg/m^3*		*Mortality*
		Nominal	*Actual*	
1a	5 F	2000 - 3000	2969	0/5
1b	5 F	2000 - 3000	2969	0/5

LC50	> 2969 mg/m^3/4 hours (i.e. > 3 mg/L/4 hours)
Signs of Toxicity	Signs of toxicity such as general bad condition, abdominal breathing and diarrhoea were observed during the first 4 hours after exposure. These signs were not observed 20 hours after exposure. Three animals showed affected reflexes 1 hour after treatment including reduced mobility (3/3), poor fur condition (2/3) and lower body temperature compared to other animals in the group (2/3). All animals appeared normal after 24 hours.
Effects in Organs	Grey-black areas in the lung were observed in all animals. No other exposure related observations were seen at necropsy
Remarks - Results	A decrease in bodyweight was seen in 5/10 animals in the first three days of the study. However all animals showed satisfactory body weight gains at the end of the study.
CONCLUSION	The notified chemical is of low toxicity via inhalation.
TEST FACILITY	Fraunhofer (2008d)

B.4. Irritation – skin (in vitro)

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 431 In vitro Skin Corrosion - Human Skin Model Test EpiDerm™ Reconstructed Human Epidermis Model.
Vehicle	Distilled water
Remarks - Method	The test substance was weighed (25 mg) onto paper one day before being directly applied to tissue models moistened with 25 μL water. While not all of the test substance could be removed from the paper, the surface was completely covered meeting the criteria of the OECD guideline. A preliminary test was not conducted to determine if the test substance

was able to directly reduce MTT.

RESULTS

Test material	*Test 1 (3 minute exposure period)*		*Test 2 (1 hour exposure period)*	
	Mean OD_{570} of duplicate tissues	*Relative mean viability (%)*	*Mean OD_{570} of duplicate tissues*	*Relative mean viability (%)*
Negative control	2.264	100	2.531	100
Test substance	2.359	104	2.280	90
Positive control	0.857	38	0.223	9

OD = optical density.

Remarks - Results: The positive (8N potassium hydroxide) and negative controls gave satisfactory results, confirming the validities of the test systems.

CONCLUSION: The notified chemical was non-corrosive to the skin under the conditions of the test.

The test method used does not allow for the evaluation of skin irritation. The result does not exclude an irritation potential of the test substance. For final assignment of a risk phase for skin irritation, results from an in vivo study or an additional in vitro assay conducted according to OECD 439 would be needed.

TEST FACILITY: FREY-TOX (2009a)

B.5. Irritation – eye (in vitro)

TEST SUBSTANCE: Notified chemical

METHOD: Determination of Ocular Irritation Potential Using the EpiOcular™ Reconstructed Human Corneal Epithelium Model

Vehicle: None

Remarks - Method: The test substance was weighed (100 mg) onto paper one day before being directly applied to tissue models moistened with 100 µL water. While not all of the test substance could be removed from the paper, a complete covering of the tissue surface was achieved.

Tissue models were pre-incubated in assay medium for 1 hour at 37 °C and 5% CO_2.

Exposure times were 3, 30 and 60 minutes for the test substance, 60 minutes for the negative control (distilled water), and 4, 15 and 45 minutes for the positive control (0.3% Triton X-100). After termination of the chemical treatment, the residues of the test substance were rinsed form the tissues with PBS and then submerged in medium for 10 minutes. Tissues were then incubated at 37 °C and 5% CO_2 in MTT for 3 hours. Following overnight extraction, optical density of the remaining solution was measured (570 nm).

The exposure time required for the test item to reduce the viability of the treated tissues to 50% of control tissues (ET_{50}) was determined.
The test substance tests were performed in duplicate for each exposure period, the negative control was tested in triplicate, and a single positive control was used for each exposure period.

RESULTS

Test material	*Exposure Period (minutes)*	*Mean OD_{570} of tissues*	*Relative mean viability (%)*

Negative control	60	2.154	100
Test substance	3	2.105	98
	30	1.899	88
	60	2.188	98
Positive control	4	1.982	92
	15	1.246	58
	45	0.300	14

OD = optical density

Remarks - Results

The ET_{50} of the test substance was calculated as > 60 minutes.

The positive control decreased tissue viability in accordance with expectations.

CONCLUSION

The notified chemical was considered to be non-irritating to the eye under the conditions of the test.

TEST FACILITY

FREY-TOX (2009b)

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

TEST SUBSTANCE

Notified chemical

METHOD

Modified Local Lymph Node Assay
EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)

Species/Strain: Mouse/ Crl:NMRI

Vehicle: DMF

Remarks - Method

The authors recorded that the positive control (α-hexyl cinnamaldehyde) was tested in June 2008 in concentrations of 21.25%, 42.5% and 85% and sensitising potential was demonstrated, verifying the experimental technique.

Lymph node pairs were weighed and cell suspensions prepared. Cell counts were determined manually by trypan blue exclusion using a NEUBAUER-chamber.

RESULTS

Concentration (% w/w)	*Index for Ear Thickness*	*Index for Ear Weight*	*Proliferative response (lymph node cell count)*	*Stimulation Index (Test/Control Ratio)*
Test Substance				
0 (vehicle control)	1	1.00	14,020,000	1.00
10	1.07	1.09	10,900,000	0.78
25	1.07	1.18	14,126,667	1.01
50	1.17	1.29	13,546,667	0.97

Remarks - Results

Animals showed a normal body weight gain during the study. A black colouration of the ears of the animals of the three test groups and a yellowish-black discoloration of the whole fur were observed.

Ear thickness and ear weight results indicated that the test substance has an irritant effect. The effect increased with increasing test substance concentration. The positive threshold values concerning the index of ear thickness was exceeded only in the case of animals treated with the highest concentration of test substance (50%). A differentiation index of 0.00 was calculated indicating no signs for an irritant and/or allergic reaction were identified.

	An increase of lymph node weights was recorded for the two test groups with the highest concentrations of test substance. In the three test groups there were no signs shown of an increase of proliferation by cell counting. Animals treated with the test substance (at 10%, 25% and 50%) did not cause a transgression of the positive threshold value concerning the lymph node cell count.
CONCLUSION	There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	FREY-TOX (2009c)

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/ Wistar (Crl:WU)
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	Corn oil
Remarks - Method	Histopathology was completed on animals in the high dose group only.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	12 M, 12 F	0	0/24
low dose	6 M, 6 F	62.5	0/12
mid dose	6 M, 6 F	250	0/12
high dose	12 M, 12 F	1000	0/24

Mortality and Time to Death
There were no treatment-related unscheduled deaths during the study.

Clinical Observations
No significant treatment related effects on body weight development, food and water consumption and functional performance were observed.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
No significant treatment related changes in haematological, urinalysis and clinical chemistry parameters were observed.

Effects in Organs
No significant treatment related effects in gross pathology and absolute and relative organ weights were observed.

Remarks – Results
Clinical observations did not indicate dose-related signs of intolerance during the experimental procedure. No statistically significant test substance related changes were observed. Any differences observed were considered to be incidental and unrelated to the test-substance.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1000 mg/kg bw/day in this study, based on the absence of adverse effects on the tested animals.

TEST FACILITY Fraunhofer (2008e)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Pre incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 fraction from rat liver induced with phenobarbital and 5,6-benzoflavone
Concentration Range in Main Test	a) With metabolic activation: 2.4-5000 µg/plate b) Without metabolic activation: 0.61-20 µg/plate
Vehicle	Dimethyl sulfoxide
Remarks - Method	Negative control plates were treated with DMSO. Positive controls: with S9 activation: Benzo[a]pyrene (TA100, TA98, TA1537), 2-Aminoanthracene (TA1535, WP2*uvrA*) and without S9 activation: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (TA100, WP2*uvrA,* TA98), Sodium azide (TA1535), 2-Methoxy-6-chloro-9-[3-(2-chloroethyl)aminopropylamino]acridine·2HC (TA1537) Dose finding test: growth inhibition by test substance was observed at ≥ 20 µg/plate in all test strains without metabolic activation and ≥ 78 µg/plate in TA1537 with metabolic activation. Precipitate of test substance was observed at ≥ 1250 µg/plate with and without metabolic activation. Test 2 with metabolic activation was only performed on strain TA1537 with a dose range of 2.4-78 µg/plate; all five strains described above were tested at a dose range of 0.61-20 µg/plate without metabolic activation. No significant protocol deviations.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 20	≥ 10	> 20	negative
Test 2		≥ 10	> 20	negative
Present				
Test 1	≥ 78	≥ 39	≥ 625	negative
Test 2		≥ 39	> 78	negative

Remarks - Results	In the dose finding tests and the main tests, increases in the number of revertant colonies or a dose-related response was not observed for any dose with or without metabolic activation. The positive controls used in the test induced marked increases in the frequency of revertant colonies confirming the activity of the S9 mix and sensitivity of bacterial strains.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	BML (2005)

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	V79 Chinese hamster lung fibroblast cells
Metabolic Activation System	S9 fraction from rat liver induced with phenobarbitone/β-napthoflavone
Vehicle	Dulbecco's-minimum essential medium (DMEM)
Remarks - Method	Vehicle was supplemented with high glucose, GlutaMax™, sodium pyruvate and 10% foetal bovine serum (without S9-mix) or 2% foetal bovine serum (with S9-mix) to act as negative controls. Positive control (without s9-mix): ethyl methanesulfonate. Positive control (with S9-mix): cyclophosphamide monohydrate Notified chemical was pre-dissolved in ethanol which increased the osmolality of the incubation media. Pre-experiment testing demonstrated concentration-dependant cytotoxicity at concentrations of test substance between 0.5 µg/mL and 50 µg/mL after 4h incubation (without metabolic activation). Concentrations > 50 µg/ml exhibited reduced cytotoxicity with strong precipitation given as a possible cause. Cytotoxicity was less intense at 0.5 µg/mL and 5 µg/mL after 24h incubation (without metabolic activation).

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0.1*, 1*, 3*, 10*, 30	4 h	24 h
Test 2	0.5*, 1*, 3*, 6*	24 h	24 h
Present			
Test 1	0.1*, 1*, 3*, 6*, 10, 30	4 h	24 h

*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	≥ 0.5	≥ 10		negative
Test 2	≥ 0.5	≥ 6		negative
Present				
Test 1	-	≥ 3		negative

Remarks - Results	The study authors suggest that the observed cytotoxicity of the test substance may be substance specific, but might also reflect pre-dissolution in ethanol and increase in osmolality. In the main experiments the solvent controls exhibited lower cell viability than the negative controls under all treatment modalities, especially in the 24 hour exposure test. In addition, the study authors suggest that the cytotoxicity may be based on particle-like effects due to the low solubility of the test substance in aqueous solutions (0.20 mgL). Under the tests conditions used, the test substance did not significantly increase the frequency of cells with structural chromosomal aberrations (gaps excluded) irrespective of the treatment.

	Only 1 endoreduplication (1 µg/mL, 4 hours, with S9 mix) and 1 chromatid break (25 µg/ml, 24 hours, without S9 mix) were found. These aberration types are more frequently observed aberrations which can also occur spontaneously in negative controls. In addition, aberration frequencies for the test substance all fell in the range of historical negative control data. Percentage of cells with structural aberrations in negative/solvent control cultures fell in the normal range of historical data and the positive control items induced biologically relevant increases in the proportion of cells with structural aberrations (minimum 5% aberrant metaphases)
CONCLUSION	The notified chemical was not clastogenic to hamster lung fibroblast cells treated in vitro under the conditions of the test.
TEST FACILITY	Fraunhofer (2008f)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	Ready Biodegradability Study. The test was conducted in accordance with the "Standards for Testing Facilities Conducting the Tests Concerning Newly Registered Chemical Substances (Japan)".
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	Not reported
Analytical Monitoring	Measured of biochemical oxygen demand (BOD). The amount of the test substance was analysed by HPLC
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. The test guideline used was very similar to OECD TG 301 C: Ready Biodegradability.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation (BOD)*	*Day*	*% Degradation (BOD)*
28	2	14	70

Remarks - Results	All validity criteria for the test were satisfied. The reference compound, aniline, reached the 70% pass level after 14 days indicating the suitability of the inoculum. It was not mentioned in the study that a toxicity control was included. The degree of degradation of the test substance after the cultivation period was 2%. The biodegradation on day 28 determined by HPLC was 0%.Therefore, the test substance cannot be classified as readily biodegradable.
CONCLUSION	The notified chemical is not readily biodegradable
TEST FACILITY	Hodogaya (2006a)

C.1.2. Bioaccumulation

TEST SUBSTANCE	Notified chemical
METHOD	Bioconcentration: Flow-through Fish Test - Continuous flow. The test was conducted in accordance with "Bioaccumulation test of chemical substance in the body of fish and shellfish" stipulated in "the Order Prescribing the Items of the Test Relating to the New Chemical Substance (Japan)".
Species	Carp (*Cyprinus carpio*)
Exposure Period	Exposure: 28 days
Auxiliary Solvent	None reported
Concentration Range	Level 1: 0.0125 mg/L Level 2: 0.00125 mg/L
Analytical Monitoring	HPLC Method
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. The test guideline used was very similar to OECD TG 305. No significant deviations from the test guidelines were reported.

RESULTS

Bioconcentration Factor (BCF)	Level 1:	< 2.0
	Level:2:	< 18.0

Remarks - Results: All validity criteria for the test were satisfied. BCFs of the test substance were considered to have reached equilibrium within 28 days. No significant differences among the BCFs were observed at the two levels. A depuration study was not conducted in this bioaccumulation study.

CONCLUSION: The notified chemical is not expected to bioaccumulate in fish.

TEST FACILITY: Hodogaya (2006b)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Notified chemical

METHOD

Species	Zebra fish (*Danio rerio*)
Exposure Period	96 hours
Auxiliary Solvent	Not reported
Water Hardness	Not reported
Analytical Monitoring	The actual test concentration was measured, but the analytical method was not reported
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. The fish ecotoxicity test was conducted in a water accommodated fraction (WAF) of the notified chemical as it is a complex mixture and has low water solubility. A WAF of the nominal loading rate of 100 mg/L was prepared by stirring the test substance in dilution water for 48 hours at room temperature. WAF treatment solutions were filtered using a 0.22 µm filter paper (Millipore MCE-MF). The treatment solutions were renewed every 48 hours.

RESULTS

Concentration		*Number of Fish*	*Mortality (%)*				
Nominal (filtered (WAF;mg/L)	*Geometric mean measured (µg/L)*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	Control	10	0	0	0	0	0
100	41	10	0	0	0	0	0

LL50	> 100 mg/L at 96 hours (filtered WAF); > 41µg/L (geometric mean measured concentration)
NOEL	100 mg/L at 96 hours (filtered WAF)
Remarks – Results	The toxicity test was conducted as a limit test. The actual concentrations of the test substance in WAFs were measured at 0 and 48 hours within the 96-h test period. However, median lethal loading rate (LL50) and no observed effect loading rate (NOEL) values were calculated based on the nominal loading rates as the toxicity cannot be attributed to a single component or mixture of components but to the test substance as a whole. All validity criteria for the test were satisfied. The end points were

determined by visual observations.

CONCLUSION The notified chemical is not harmful to fish.

TEST FACILITY Fraunhofer (2008g)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Static Test

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Not reported
Water Hardness	Not reported
Analytical Monitoring	The actual test concentration was measured, but the analytical method was not reported
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. The daphnia ecotoxicity test was conducted in a water accommodated fraction (WAF) of the notified chemical as it is a complex mixture and has low water solubility. A WAF of the nominal loading rate of 100 mg/L was prepared by stirring the test substance in dilution water for 48 hours at room temperature. WAF treatment solutions were filtered using a 0.22 µm filter paper (Millipore MCE-MF).

RESULTS

Concentration		*Number of D. magna*	*Cumulative % Immobilised*	
Nominal (filtered (WAF;mg/L)	*Geometric mean measured (µg/L)*		*24 h*	*48 h*
Control	Control	20	0	0
100	56	20	0	0

EL50	> 100 mg/L at 48 hours (filtered WAF); > 56 µg/L (geometric mean measured concentration)
NOEL	56 mg/L at 48 hours (filtered WAF)
Remarks - Results	The toxicity test was conducted as a limit test. The actual concentrations of the test substance in WAFs were measured at 0 and 48 hours within the 48-h test period. However, median lethal loading rate (LL50) and no observed effect loading rate (NOEL) values were determined based on the nominal loading rates as the toxicity cannot be attributed to a single component or mixture of components but to the test substance as a whole. All validity criteria for the test were satisfied. The end points were determined by visual observations.

CONCLUSION The notified chemical is not harmful to aquatic invertebrates.

TEST FACILITY Fraunhofer (2008h)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Desmodesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 6.25, 12.5, 25, 50, and 100 mg/L
Auxiliary Solvent	Not reported
Water Hardness	Not reported
Analytical Monitoring	The actual test concentration was measured, but the analytical method was not reported
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. The algae ecotoxicity test was conducted in a water accommodated fraction (WAF) of the notified chemical as it is a complex mixture and has low water solubility. A WAF of the nominal loading rate of 100 mg/L was prepared by stirring the test substance in dilution water for 48 hours at room temperature. WAF treatment solutions were filtered using a 0.22 µm filter paper (Millipore MCE-MF). The filtered test solution was diluted with growth medium to prepare the lower test treatments.

RESULTS

Biomass (72 h)		*Growth (72 h)*	
E_yL50 (filtered WAF) (mg/L)	*NOE_yL (mg/L)*	*E_rL50 (filtered WAF) (mg/L)*	*NOE_rL (mg/L)*
> 100	100	> 100	100

Remarks - Results	The actual concentrations of the test substance in WAFs were measured at 0 and 72 hours within the 72-h test period. Measured concentration of the treatments cannot be determined as the concentration of the test substance in all treatments was below the limit of quantification (LOQ) of 1.5 µg/L. However, E_rL50 and NOE_rL values were calculated based on the nominal loading rates as the toxicity cannot be attributed to a single component or mixture of components but to the test substance as a whole. All validity criteria for the test were satisfied. The E_rL50 and E_yL50 were calculated using one way analysis of variance (ANOVA) statistical method.
CONCLUSION	The notified chemical is not harmful to algae.
TEST FACILITY	Fraunhofer (2010)

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: 197.5, 296.3, 444.4, 666.6, and 1000 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	
EC20	Not reported
EC50	> 1000 mg/L
Remarks – Results	All validity criteria for the test were satisfied. The EC50 was out of the tested concentration range (> 1000 mg/L).
CONCLUSION	The notified chemical is not expected to inhibit microbial respiration.
TEST FACILITY	Fraunhofer (2008i)

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