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August 2012

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Chemical in Cablelite 9D9-464

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

For the purposes of subsection 78(1) of the Act, this Public Report may be inspected at our NICNAS office by appointment only at Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

This Public Report is also available for viewing and downloading from the NICNAS website or available on request, free of charge, by contacting NICNAS. For requests and enquiries please contact the NICNAS Administration Coordinator at:

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Director
NICNAS

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SUMMARY

The following details will be published in the NICNAS *Chemical Gazette:*

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS SUBSTANCE	INTRODUCTION VOLUME	USE
STD/1423	Prysmian Telecom Cables & Systems Australia Pty Ltd The J Vlaeminck Family Trust	Chemical in Cablelite 9D9-464	Yes	≤ 200 tonnes per annum	Coating component for cables

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 Irritation (Category 2)	H315 – Causes skin irritation
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:
Xi: R43 May cause sensitisation by skin contact
Xi: R38 Irritating to skin

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 category	*Hazard statement*
Aquatic Environment	Acute 3	Harmful to aquatic life
	Chronic 3	Harmful to aquatic life with long lasting effects

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

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

Environmental risk assessment
On the basis of the 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 Irritation (Category 2): H315 – Causes skin irritation
- Skin Sensitisation (Category 1): H317 – May cause an allergic skin reaction

- The following classifications should be used for products/mixtures containing the notified chemical:
  - Conc. ≥ 10%: H315; H317
  - ≥ 1% Conc. < 10%: H317

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 sensitisation.

CONTROL MEASURES
Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following isolation and engineering controls to minimise occupational exposure to the notified chemical as introduced:
  - Automated processes whenever possible
  - Exhaust ventilation during coating processes

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced:
  - Avoid contact with skin and eyes
  - 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:
  - Gloves
  - Protective clothing
  - Safety glasses/goggles

  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.

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(2) of the Act; if
- the function or use of the chemical has changed from a component of plastic coatings, or is likely to change significantly;
- the amount of chemical being introduced has increased from 200 tonnes per annum, 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.

No additional secondary notification conditions are stipulated.

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Prysmian Telecom Cables & Systems Australia Pty Ltd (ABN 14 001 313 551)
1 Thew Parade
DEE WHY NSW 2099

The J Vlaeminck Family Trust (ABN 88 891 187 886)
10 Woodview Court
WHEELERS HILL VIC 3150

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, additives/adjuvants, use details and import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Cablelite 9D9-464 (product containing the notified chemical at a concentration of < 40%)

MOLECULAR WEIGHT
< 1,000 Da

ANALYTICAL DATA
Reference NMR, IR, HPLC-MS and UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY > 99%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Clear liquid to milky paste

Property	Value	Data Source/Justification
Melting Point/Freezing Point	-11 °C	Measured, glass transition temperature
Boiling Point	> 125°C at 101.3 kPa	Measured, the test substance was observed to decompose at this temperature.
Density	1,160 kg/m^3 at 20 °C	Measured
Vapour Pressure	< 1.5 × 10^{-6} kPa at 20 °C < 4.2 × 10^{-6} kPa at 25 °C	Measured
Water Solubility	47.1×10^{-3} g/L at 20°C and pH 7.5	Measured
Hydrolysis as a Function of pH	$t_{1/2}$ at 25°C: > 1 year, 193 days, and 48 hours at pH 4, 7 and 9, respectively.	Measured
Partition Coefficient (n-octanol/water)	log P_{OW} = 2.7/2.8	Measured
Adsorption/Desorption	log K_{oc} = 3.2	Measured
Dissociation Constant	Not determined	The notified chemical does not contain any dissociable functionality.
Surface Tension	52.3 mN/m at 20°C	Measured
Particle Size	Not determined	The notified chemical is a liquid at room temperature
Flash Point	> 100°C	Measured
Flammability	Not highly flammable	Estimated
Pyrophoric Properties	Does not ignite in contact with air	Estimated
Autoignition Temperature	440°C	Measured
Explosive Properties	Not expected to be explosive	Estimated
Oxidising Properties	Not expected to be oxidising	Estimated

DISCUSSION OF PROPERTIES
For full details of tests on physical and chemical properties, refer to Appendix A.

Reactivity
The notified chemical will undergo UV initiated polymerisation to form the finished composite articles; however, it is expected to be stable under normal environmental conditions.

Dangerous Goods 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 not be manufactured within Australia. The notified chemical will be imported into Australia at a concentration of < 40% in the product Cablelite 9D9-464.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	1-50	50-200	50-200	50-200	50-200

PORT OF ENTRY
Sydney

TRANSPORTATION AND PACKAGING
The notified chemical will be imported in closed 200 kg lined metal drums. Transportation of the notified chemical within Australia is expected to be by road.

USE
The notified chemical will be used as a coating component for cables.

OPERATION DESCRIPTION
The notified chemical will not be manufactured or reformulated within Australia. It will be imported in the product formulation Cablelite 9D9-464 at a concentration of < 40%.

After importation the drums will be transported to the notifier's site where the outlet port of the drum will be opened by the operator and the hose adaptor will be screwed in to the outlet port on top of the drum. The product containing the notified chemical will then be pumped through hoses into the coating machine. The coating of the cables is a closed process with the coating machine fitted with exhaust lines to reduce the possibility of any fumes escaping. After the cables have been coated with the product containing the notified chemical they will pass through a UV light source within the coating machine where the coating is cured. At the end of the production run the application die within the coating machine is cleaned with acetone while under vacuum. At the end of a shift the die is removed for cleaning in an ultrasonic bath containing acetone.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

EXPOSURE DETAILS
It is anticipated that transport and warehouse/stores personnel would only be exposed to the notified chemical in the event of an accident.

Dermal and ocular exposure to the imported product Cablelite 9D9-464 containing the notified chemical at a concentration of < 40% is possible during the connection and disconnection of hoses to the imported drums, and during cleaning processes. Dermal and ocular exposure would be limited by the use of personal protective equipment (PPE) including gloves, protective clothing and safety glasses. Inhalation exposure is not expected as the notified chemical has a low vapour pressure (< 1.5×10^{-6} kPa at 20 °C) and the coating machine is fitted with exhaust lines to reduce the possibility of any fumes escaping.

Workers will be exposed to cables that have been coated with products containing the notified chemical; however after curing the notified chemical will be bound within a polymer matrix and hence will not be bioavailable.

6.1.2. Public Exposure
The notified chemical is intended for industrial use only, and will not be available to the public. Direct exposure would therefore not be expected. Indirect exposure from accidental spills or environmental sources may be possible, but are unlikely for the proposed use.

Members of the public are unlikely to experience dermal exposure to cables coated with products containing the notified chemical, as once cured the notified chemical will be bound within a polymer matrix and hence will not be bioavailable.

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Evaluation of Skin Irritation Potential using the EPISKIN Reconstituted Human Epidermis Model	moderately irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days	NOAEL > 1,000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro Mammalian Chromosome Aberration Test	non genotoxic

Toxicokinetics, metabolism and distribution.
Based on the low molecular weight (< 500 Da) and the lipophilicity of the notified chemical (water solubility 47.1×10^{-3} g/L at 20°C and pH 7.5; log Pow = 2.7/2.8) dermal absorption may occur, but the transfer from the stratum corneum into the epidermis is expected to be slow.

Acute toxicity.
The notified chemical is considered to be of low acute toxicity via the oral and dermal routes based on tests conducted in rats.

Irritation and Sensitisation.
Based on a test conducted in rabbits the notified chemical is considered to be irritating to the eye, however the effects were not sufficiently severe for classification. The notified chemical was shown to have skin irritation potential using the EPISKIN Reconstituted Human Epidermis Model.

There was evidence of induction of a significant lymphocyte proliferative response indicative of skin sensitisation to the notified chemical. The Stimulation Index (SI) is estimated to be < 10%.

Repeated Dose Toxicity (sub chronic).
The test material was administered orally to rats for a period of 28 consecutive days at dose levels of 1,000, 300 and 100 mg/kg bw/day. No adverse treatment related effects were seen at any dose level and hence the No Observed Adverse Effect Level (NOAEL) can be regarded as the highest dose level tested, which was 1,000 mg/kg bw/day.

Mutagenicity.
The notified chemical was found to not be mutagenic using a bacterial reverse mutation test, and is not clastogenic to human peripheral lymphocytes *in vitro*.

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 table below.

Hazard classification	***Hazard statement***
Skin Irritation (Category 2)	H315 – Causes skin irritation
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:

Xi: R43 May cause sensitisation by skin contact
Xi: R38 Irritating to skin

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Dermal, ocular and inhalation exposure to the notified chemical, at concentrations < 40%, by workers may occur during the connection and disconnection of hoses to the imported drums and during cleaning processes. After the coatings containing the notified chemical have been cured it will be bound within a polymer matrix and hence will not be bioavailable.

Toxicological studies on the notified chemical indicate that it is a skin sensitiser and irritant and hence its use at concentrations > 1% is only considered to be reasonable when sufficient engineering controls, safe work practices and PPE are used to greatly reduce the potential for exposure. Dermal and ocular exposure is expected to be limited with the use of personal protective equipment (gloves, protective clothing and safety glasses/goggles). Inhalation exposure to the notified chemical is unlikely as the coating machine is fitted with exhaust lines to reduce the possibility of any fumes or aerosols escaping.

Therefore, although the notified chemical is classified as a skin sensitiser, given the use of appropriate workplace controls to minimise exposure, it is not expected to pose an unreasonable health risk to workers.

6.3.2. Public Health

The notified chemical and articles incorporating it will not be available to the public; when present in articles it will be bound within a polymer matrix and hence will not be bioavailable. Therefore the risk to the public is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported as a component of a formulation for end-use in Australia and will not be reformulated in Australia. Therefore, no environmental release is expected from the manufacture or reformulation of the notified chemical in Australia. No potential for exposure of the notified chemical to the aquatic environment during transport, storage is expected. Accidental spills of the product are not expected to be significant and are expected to be absorbed by inert absorbent material, swept up and placed into containers and disposed of to landfill.

RELEASE OF CHEMICAL FROM USE

The majority of the imported notified chemical will be incorporated into the cured coating layer on cable. No environmental exposure is expected from the coating process. The coating of the cables is a closed process. After the cables have been coated with the product containing the notified chemical they will pass through a UV light source within the coating machine where the coating is cured. At the end of the production run the application die within the coating machine is cleaned with acetone while under vacuum. At the end of a shift the die will then be removed for cleaning in an ultrasonic bath containing acetone. The residual notified chemical from the die washing waste is expected to be captured as solid wastes during the solvent recovery process, and be collected by a licensed contractor for disposal to landfill.

Release from residues in import drums is estimated to be < 1%. The residues are expected to be either disposed of to landfill with empty containers or treated in accordance with local regulations as part of the drum recycling process.

RELEASE OF CHEMICAL FROM DISPOSAL

Most of the notified chemical will be cured in a solid matrix after the coating application and will share the fate of the cable. At the end of the cable's useful life, the notified chemical will be sent to landfill with the used cable. Small amounts of the notified chemical are expected to be disposed to landfill as residues with empty drums or as collected die washing wastes. Any accidental spills are expected to be collected and disposed of to landfill.

7.1.2. Environmental Fate

The notified chemical is not expected to be readily biodegradable according to the provided study report. For the details of the environmental fate studies please refer to Appendix C. It is not expected to have high potential to bioaccumulate in aquatic organisms based on its expected low P_{OW} (2.7/2.8), despite of its low molecular weight (< 1000 Da). The notified chemical is expected to be predominantly disposed of to landfill, in forms of either used cable or collected wastes from application. In landfill, the notified chemical is expected to be slowly degraded via biotic and abiotic pathways, forming water and oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

The calculation of predicted environmental concentration (PEC) is not necessary due to the limited release of the notified chemical to the aquatic component from the proposed use pattern.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h EC50 = 20 mg/L	Harmful to fish
Daphnia Toxicity	48 h EC50 = 44 mg/L	Harmful to daphnids
Algal Toxicity	72 h EC50 = 37 mg/L 72 h NOEC = 2 mg/L	Harmful to algae

The notified chemical is considered acutely harmful to the aquatic organisms.

Under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009) the notified chemical is classified as acutely harmful to aquatic organisms. The algal 72 h NOEC is available as a chronic endpoint for aquatic organisms. Therefore, the long-term classification for the notified chemical was determined based on the most stringent outcome by comparing the long-term hazard classification using either the acute or chronic data. Based on the acute endpoints for fish, daphnids and algae, and considering it is not readily biodegradable, the notified chemical is formally classified as Chronic Category 3; Harmful to aquatic life with long lasting effects.

7.2.1. Predicted No-Effect Concentration

The calculation of predicted no-effect concentration (PNEC) is not considered necessary considering that limited release of the notified chemical to the aquatic environment is expected based on the proposed use pattern.

7.3. Environmental Risk Assessment

The Risk Quotient (PEC/PNEC) was not calculated since no significant release to the aquatic environment is expected. The notified chemical is not expected to pose an unreasonable risk to aquatic environment based on the assessed use pattern.

For PBT consideration, the notified chemical may meet the criteria for persistence based on its stability under acidic conditions and lack of potential for rapid biodegradability. It is not considered to meet the criteria for bioaccumulation given its expected low log P_{OW} (2.7/2.8), despite of its low molecular weight (< 1000 Da). It is not considered to meet the criterion for toxicity based on the provided 72 h NOEL of 2 mg/L for algae.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point -11 ºC

Method	OECD TG 102 Melting Point/Melting Range. EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks	The melting point/freezing point and the boiling point were measured in the same experiments using differential scanning calorimetry (DSC). The experiment was done in duplicate. During cooling a glass transition was observed between -10°C and -35°Cad between -50°C and 25°C during heating. The extrapolated onset temperature of the glass transitions in the two experiments were -11.56°C and -10.12°C. After the experiment, an orange residue remained in the sample container.
Test Facility	NOTOX (2012a)

Boiling Point > 125 ºC at 101.3 kPa

Method	OECD TG 103 Boiling Point. EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks	The notified chemical showed decomposition or reaction from 125°C onwards. A thermogravimetric analysis experiment showed a sample weight decrease of 25% at 365°C and 70% at 453°C. A brown residue remained in the sample container indicating reaction/decomposition of the chemical. Boiling was not observed.
Test Facility	NOTOX (2012a)

Density 1,160 kg/m^3 at 20 ºC

Method	OECD TG 109 Density of Liquids and Solids. EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks	The density was determined using a gas comparison stereopycnometer. To decrease viscosity, the notified chemical was heated in an oven at 70°C.
Test Facility	NOTOX (2012a)

Vapour Pressure $< 1.5 \times 10^{-6}$ kPa at 20 °C
$< 4.2 \times 10^{-6}$ kPa at 25 °C

Method	OECD TG 104 Vapour Pressure (2006) EC Directive 761/2009, A.4 Vapour Pressure (2009)
Remarks	The vapour pressure of the notified chemical was determined using the isothermal thermogravimetric effusion method. The weight loss of the notified chemical at 110°C – 140°C were lower than that of the reference substance hexachlorobenzene at the same temperatures. Therefore, the vapour pressure for the notified chemical is lower than that for hexachlorobenzene, i.e. $< 1.5 \times 10^{-6}$ kPa at 20 °C or $< 4.2 \times 10^{-6}$ kPa at 25 ºC. The notified chemical is considered to be slightly volatile based on the test results.
Test Facility	NOTOX (2012a)

Surface Tension 52.3 mN/m at 20 ºC

Method	OECD TG 115 Surface Tension of Aqueous Solutions. EC Council Regulation No 440/2008 A.5 Surface Tension.
Remarks	The test was performed at a concentration of 90% saturation solubility of the notified chemical in water at 20 ± 0.5ºC. The notified chemical is considered to be surface active based on the test result.
Test Facility	NOTOX (2012a)

Water Solubility 47.1×10^{-3} g/L at 20 ºC

Method	OECD TG 105 Water Solubility (1995) EC Directive 440/2008, A.6 Water solubility (2008)
Remarks	Following a preliminary test, the Flask Method was used at pH 7.5 with the concentration determined by HPLC-MS/MS. The chromatogram of the test substance solution showed

two components of the notified chemical. The sum of the peak area was used as response in the calculations. Concentrations were detected to be 48.0 mg/L, 55.2 mg/L, and 46.2 mg/L after 24, 48 and 72 hours, respectively. As the 48-hour measurement was significantly higher than the 24- and 72-hour measurements, the water solubility of the notified chemical was calculated as the mean value of the 24- and 72-hour measurements by the study author.

Test Facility NOTOX (2012a)

Hydrolysis as a Function of pH

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

pH	*T (°C)*	*$t_{½}$ (days)*
4	25	> 1 year
7	20	403 days
	25	193 days
9	20	3.6 days
	25	2 days

Remarks The hydrolysis test was conducted in buffer solutions of pH 4, 7 and 9 at a nominal concentration of 1000 µg/L and 50.0 ± 0.1°C. A spiking solution in acetonitrile was used with the volume being < 1% of the sample volume. HPLC-MS/MS was used for sample analysis.
The test results show that > 90% of the notified chemical remained non-hydrolysed after 5 days at 50 °C at pH 4, and the notified chemical is considered hydrolytically stable.
The half-life of the notified chemical at pH 7 was determined to be 403 days, 193 days, 166 hours and 50 hours at 20°C, 25°C, 50°C and 60°C, respectively.
The half-life at pH 9 was determined to be 87 hours, 48 hours, 9.8 hours and 3.0 hours at 20°C, 25°C, 40°C and 50°C, respectively. The hydrolysis occurred by breakdown of the ester groups.
Based on the above results, the notified chemical is considered stable at acidic conditions, and unstable at basic conditions.

Test Facility NOTOX (2012a)

Partition Coefficient (n-octanol/water) log Pow = 2.7/2.8

Method OECD TG 117 Partition Coefficient (n-octanol/water) (2004).
EC Council Regulation No 440/2008 A.8 Partition Coefficient (2008).

Remarks HPLC Method at neutral pH was sued. Solutions of reference substances with known log Koc values based on soil adsorption data and the test substance were analysed. The dead time was determined by Thiourea. In the chromatogram of the test solution (in 60/40 (v/v) methanol/water mobile phase), two major peaks (69% and 23% of the total area) and several smaller peaks (each < 6% of the total area) were observed. The log P_{OW} values for the two major components were determined as 2.7 and 2.8.
Because the notified chemical is a surfactant and hydrolytically unstable, the result should be considered as estimated value.

Test Facility NOTOX (2012a)

Adsorption/Desorption – screening test log K_{oc} = 3.2

Method OECD 121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (2001)
EC no. 440/2008, C.19 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (2008)

Remarks HPLC method at neutral pH was used. The dead time was determined by using

Formamide.
In the chromatogram of the test solution (in 30/70 (v/v) methanol/water mobile phase), one major peak (93% of the total area) at 8.87 min (corresponds to a Koc value of 1.7×10^3) and several smaller peaks (each ≤ 6% of the total area) were observed. It was considered that the notified chemical corresponds to the major component and the K_{OC} value is therefore, determined to be 1.7×10^3.
Because the notified chemical is a surfactant, the result should be considered as estimated value.

Test Facility NOTOX (2012a)

Dissociation Constant No pK_a values in the pH range of 1 – 14

Method Perrin Calculation method.
Remarks Calculation of pKa from the structural formula showed no pKa values. The notified chemical does not contain any dissociable functionality.
Test Facility NOTOX (2012a)

Flash Point > 100ºC

Method In house method details not provided
Remarks Measured using a Pensky Martens closed cup
Test Facility DSM Desotech (2012)

Flammability Not highly flammable

Method EC Council Regulation No 440/2008 A.12 Flammability (Contact with Water).
Remarks Test not conducted. The notified chemical does not contain groups that might lead to spontaneously ignition in contact with water and/or to the evolution of a flammable gas. The notified chemical was also dissolved in water during the determination of the water solubility demonstrating that it does not ignite spontaneously in contact with water.
Test Facility NOTOX (2012a)

Pyrophoric Properties Does not ignite in contact with air

Method EC Council Regulation No 440/2008 A.13 Pyrophoric Properties of Solids and Liquids.
Remarks Test not conducted. The notified chemical does not contain groups that might lead to ignition in contact with air. The notified chemical was also handled under normal laboratory conditions for the determination of the physical-chemical properties demonstrating that the notified chemical does not ignite in contact with air.
Test Facility NOTOX (2012a)

Autoignition Temperature 440ºC

Method EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks The experiment was run in triplicate and the lowest auto-ignition temperature obtained was 440°C. To decrease viscosity, the notified chemical was placed in an oil bath at a temperature of approximately 70°C.
Test Facility NOTOX (2012a)

Explosive Properties Not expected to be explosive

Method EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks Test not conducted. The notified chemical does not contain chemical groups which are associated with explosive properties. The calculated oxygen balance of -187% is due to the presence of groups in the molecular structure of the test substance, which are not chemically instable or highly energetic.
Test Facility NOTOX (2012a)

Oxidizing Properties Not expected to be oxidising

Method	EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids).
Remarks	Test not conducted. The notified chemical does not contain functional groups that are expected to act as an oxidizing agent. The oxygen atoms that are present in the molecular structure of the notified chemical are chemically bonded to carbon or hydrogen.
Test Facility	NOTOX (2012a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method. EC Council Regulation No 440/2008 B.1 tris Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Wistar strain Crl:WI (Han)
Vehicle	Propylene glycol
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	3 Female	2,000	0/3
II	3 Female	2,000	0/3

LD50	> 2,000 mg/kg bw
Signs of Toxicity	Hunched posture was observed for all animals on Day 1 and for three animals on Days 2 and 3. In addition, three animals showed one or more of the following symptoms on Day 1: lethargy, uncoordinated movements, piloerection and ptosis.
Effects in Organs	No abnormalities were noted at necroscopy.
Remarks - Results	No significant variations in body weight gains were observed.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	NOTOX (2011a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity. EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/Wistar strain Crl:WI (Han)
Vehicle	Propylene glycol
Type of dressing	Occlusive
Remarks - Method	The formulation containing the notified chemical was applied to approximately 10% of the surface area of the test subjects and kept in place for 24 hours, after which the skin was cleaned with tap water. No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5 per sex	2,000	0/10

LD50	> 2,000 mg/kg bw
Signs of Toxicity - Local	General or maculate erythema, scales and/or scabs were seen in the treated skin-area of all animals during the observation period.
Signs of Toxicity - Systemic	Lethargy, hunched posture, shallow respiration, piloerection and/or chromodacryorrhoea were noted for all animals. The animals had fully recovered from these symptoms by Day 3 or 4.

Effects in Organs	Incidental findings at macroscopic post mortem examination consisted of many, dark red foci on the thymus of one male and several scab formations in the thoraco-dorsal region of the skin of another male. These effects were not considered toxicologically significant at the incidence seen.
Remarks - Results	No significant variations in body weight gains were observed.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	NOTOX (2011b)

B.3. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	Analogous to OECD TG 439: In Vitro Skin Irritation: Reconstituted Human Epidermis Test Method Evaluation of Skin Irritation Potential using the EPISKIN Reconstituted Human Epidermis Model. The principle of the assay is based on the measurement of cytotoxicity in reconstituted human epidermal cultures following topical exposure to the test material. The irritation potential of the test substance was assessed by applying the notified chemical undiluted onto the surface of 3 EpiSkin reconstructed human epidermis (RHE) units for 15 mins. The notified chemical was then washed from the surface of the EpiSkin units which were incubated for a recovery period of approximately 44 hrs at 37 °C. Triplicate Episkin samples with 25 µL of negative (Phosphate Buffered Saline (PBS)) and positive (Sodium Dodecyl Sulfate (SDS) 5%) controls were treated similarly. After incubation, the tissues were transferred to the MTT filled wells and incubated for 3 hours before being transferred to micro tubes containing 500 µL isopropanol for extraction of formazan crystals out of the MTT loaded tissues. The amount of extracted formazan was determined spectrophotomically at 540 nm in duplicate. Data are presented in the form of % viability (MTT reduction in the test material treated tissue relative to negative control tissues).
Remarks - Method	Phosphate Buffered Saline (PBS) was used as the negative control and Sodium Dodecyl Sulphate (SDS) (5% aqueous solution) as the positive control. A preliminary test was conducted to assess the ability of the notified chemical to reduce MTT (2H-tetrazolium, 2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-, bromide (1:1)). The notified chemical did not directly reduce MTT. The test is considered to be acceptable if the following criteria are met: 1. The absolute mean optical density at 570 nm (OD_{570}) for the samples of the negative control is within the historical range. 2. The mean % viability of the positive control is ≤ 40% that of the negative control. 3. The standard deviations (SD) should be within the 1-sided 95% tolerance interval calculated from historical data, the SD for the historical data must be < 18%.

RESULTS

Substance	OD_{570} of tissues*	Mean OD_{570} of triplicate tissues	± SD of OD_{570}	Relative mean % viability
Negative Control	1.352 1.226 1.277	1.285	0.063	100**
Test Substance	0.077 0.084 0.074	0.078	0.006	6.1
Positive Control	0.064 0.106 0.077	0.082	0.021	6.4

* The mean of the duplicate measurements and corrected for background absorption (0.041).
** The mean viability of the negative control tissues is set as 100%.

Remarks - Results

The relative mean tissue viability obtained after treatment with the notified chemical compared to the negative control tissues was 6.1%. Since this is below 50%, the chemical is considered to be an irritant.
The positive control had a mean cell viability 6.4%, the absolute mean OD_{570} of the negative control tissues was within the historical range and the standard deviation value of the percentage viability of three tissues treated identically was less than 5% confirming the validity of the test system.

CONCLUSION

The notified chemical is moderately irritating to the skin.

TEST FACILITY

NOTOX (2012b)

B.4. Irritation – eye

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 405 Acute Eye Irritation/Corrosion.
EC Council Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation).

Species/Strain: Rabbit/New Zealand White
Number of Animals: 3 Males
Observation Period: 14 Days
Remarks - Method: No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	2	1.3	1.3	2	< 14 days	0
Conjunctiva: chemosis	0.7	0.3	0.3	1	< 72 hours	0
Conjunctiva: discharge	1	0.3	0.3	1	< 14 days	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0.3	0	0	1	< 7 days	0

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

Remarks - Results

A single application of the test material to the non-irrigated eye of three rabbits produced iridial inflammation in one animal and moderate conjunctival irritation in all animals. One treated eye appeared normal at the 72 hour observation with the remaining two eyes appearing normal at the 14 day observation.

CONCLUSION

The notified chemical is slightly irritating to the eye.

TEST FACILITY NOTOX (2011c)

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

TEST SUBSTANCE Notified chemical

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)

Species/Strain Mouse/CBA/J strain, female

Vehicle Propylene glycol

Remarks - Method No concurrent positive control group was added to the study. A six-monthly reliability check is conducted by the testing facility using the same methods, animal strain and supplier with α-hexylcinnamaldehyde in Acetone:Olive oil (4:1) as the positive control.

No significant protocol deviations.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	142 (± 27)	1
10	1,317 (± 458)	9.3
25	2,436 (± 330)	17.2
50	2,957 (± 190)	20.8
Positive Control		
0	133 (± 38)	1
5	181 (± 55)	1.4
10	269 (± 48)	2.0
25	988 (± 142)	7.4

Remarks - Results There was no mortality and no clinical signs of toxicity were observed in the test subjects. Mild erythema was noted in animals treated with a concentration of 50% of the notified chemical. All auricular lymph nodes of animals treated with the test substance appeared larger in size as compared to nodes of the control animals, except for one node of one animal treated at 10% which was considered normal in size. No macroscopic abnormalities of the surrounding area were noted in any of the animals.

The stimulation index (SI) for all three test groups dosed with the notified chemical was above 3 and hence the notified chemical is considered to be a potential skin sensitiser. The SI value of 3 (also referred to as EC3) of the notified chemical, which is used as the classification cut-off for skin sensitisation, is < 10%.

The positive control confirmed the sensitivity of the test system.

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

TEST FACILITY NOTOX (2011d)

B.6. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Council Regulation No 440/2008 B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/Crl:WI(Han)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: none
Vehicle	Propylene glycol
Remarks - Method	Doses were chosen on the basis of a 5-day range-finding study. Several protocol deviations were detailed, including changes to the clinical observations carried out in an arena, and small differences in the heating protocol used to homogenise the dosing formulation. The study authors considered that the study integrity was not adversely affected by the deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 per sex	0	0/10
low dose	5 per sex	100	0/10
mid dose	5 per sex	300	0/10
high dose	5 per sex	1,000	0/10

Mortality and Time to Death

There were no unscheduled deaths during the study.

Clinical Observations

Hunched posture was noted in all male animals in the high dose group during the second week of treatment. Increased salivation was noted in the majority of animals in the low dose group and all animals in the higher dose groups. Incidental findings included fur loss, scabs and a wound. These isolated, incidental external changes were considered of no toxicological importance.

There were no toxicologically significant changes in the functional performance parameters measured.

There were no significant differences in the bodyweight gain and food consumption between the control and treated groups.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There was a statistically significant higher mean corpuscal haemoglobin concentration (MCHC) in male animals in the low dose group, the value was within the historical control range and in the absence of any dose response relationship is considered to be of no toxicological significance.

Female animals in the low and mid dose groups had higher protein levels and also in mid dose females higher albumin levels. Male animals in the mid dose group had lower protein levels and higher chloride and calcium levels. The values were within the historical control range and in the absence of any dose response relationship are considered to be of no toxicological significance.

Effects in Organs

There were higher liver weights (19%) and liver to body weight ratios (14%) in female animals in the high dose group. These findings were not accompanied with findings in clinical laboratory investigations or microscopic examination that suggested organ dysfunction and therefore no toxicological significance was ascribed to these findings.

Lower seminal vesicles weights and lower seminal vesicles to body weight ratios for males in the low and high dose groups were considered not to represent a change of toxicological significance, because they were within the range considered normal for male rats of this age and strain and occurred in the absence of a dose response relationship.

A range of macroscopic findings were noted in individual animals including in the control group, however there was no dose response relationship seen and the findings were considered to be part of the normal background pathology of rats of this age and strain.

Three females in the high dose group had singular minimal foci of myelin fragmentation in the sciatic nerve. The low and mid dose animals were not examined. The study authors considered that this effect was not toxicologically significant because of the singularity of the effect and the fact that it is commonly seen in control animals. There were no other microscopic findings recorded which could be attributed to treatment with the test substance.

Remarks – Results
No adverse treatment related effects were clearly identified at any dose level and hence the No Observed Adverse Effect Level (NOAEL) can be regarded as the highest dose level tested.

CONCLUSION
The NOAEL was established as > 1,000 mg/kg bw/day in this study, based on the absence of any adverse effects at any of the dose rates tested.

TEST FACILITY NOTOX (2012c)

B.7. 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.
Plate incorporation procedure

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	Rat S9 fraction from aroclor induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 3 – 5,000 µg/plate b) Without metabolic activation: 3 – 5,000 µg/plate
Vehicle	Dimethyl sulfoxide
Remarks - Method	The negative control values for WP2uvrA were just above historical control values. This deviation was not expected to affect the results of the study.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5,000	> 5,000	≥ 5,000	negative
Test 2		> 5,000	≥ 3,330	negative
Present				
Test 1	> 5,000	> 5,000	≥ 5,000	negative
Test 2		> 5,000	≥ 3,330	negative

Remarks - Results Precipitation was observed at 5,000 µg/plate in the first experiment and 3,330 µg/plate in the second experiment. Slight toxicity was observed in the absence of metabolic activation at a dose of 5000 µg/plate.

The test material was tested up to the maximum recommended dose level

of 5000 µg/plate. No toxicologically significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation.

All the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

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

TEST FACILITY NOTOX (2012d)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
EC Council Regulation No 440/2008 B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.

Species/Strain	Human
Cell Type/Cell Line	Peripheral lymphocytes
Metabolic Activation System	Rat S9 fraction from phenobarbitone/β-napthoflavone induced rat liver
Vehicle	Ethanol
Remarks - Method	No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	1*, 10*, 15*, 20, 25, 30	3	24
Test 2	3, 10, 30, 50, 70, 90	24	24
Test 2	3, 10, 30, 50, 70, 90	48	48
Test 2A	1*, 3, 7, 10*, 15*, 25	24	24
Test 2A	1*, 3, 7, 10*, 15, 25*	48	48
Present			
Test 1	1*, 30*, 40*, 50, 60, 80	3	24
Test 2	1*, 30*, 40*, 50, 60, 80	3	48

*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	≥ 33	≥ 15	> 30	negative
Test 2		≥ 30	> 90	negative
Test 2		≥ 30	> 90	negative
Test 2A		≥ 25	> 25	negative
Test 2A		≥ 25	> 25	negative
Present				
Test 1	≥ 100	≥ 40	> 80	negative
Test 2		≥ 50	> 80	negative

Remarks - Results The positive and vehicle controls gave satisfactory responses, confirming the validity of the test system.

A precipitate was seen to form at 333 µg/mL in the dose finding test.

	The test material did not induce any statistically significant increases in the frequency of cells with aberrations, or in the numbers of polyploid cells. Slight increases in aberrations were seen in some of the assays, but were not statistically significant and were not clearly dose related.
CONCLUSION	The notified chemical was not clastogenic to human peripheral lymphocytes treated *in vitro* under the conditions of the test.
TEST FACILITY	NOTOX (2012e)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test (1992). EC No.440/2008 Part C, C.4 Biodegradation: determination of the 'ready' biodegradability, C.4-C: Carbon dioxide (CO_2) evolution test (Modified Sturm Test) (2008).
Inoculum	Activated sludge from a domestic sewage treatment plant
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Total Organic Carbon (TOC) analysis. The CO_2 produced reacted with the barium hydroxide in the gas scrubbing bottle and precipitated out as barium carbonate. The amount of CO_2 produced was determined by titrating the remaining $Ba(OH)_2$ with 0.05 M standardized HCl.
Remarks - Method	The notified chemical was tested in duplicate at 15 mg/L, corresponding to 9 mg TOC/L, 20.1 – 21.5˚C and pH 7.5 – 7.8 in darkness. The test solutions were continuously stirred during the test, to ensure optimal contact between the test substance and the test organisms. A blank control in duplicates with inoculum only, a single reference control with sodium acetate, and a single toxicity control with the notified chemical and the reference item (40 mg sodium acetate/L) were conducted for validation purposes.

RESULTS

Test substance		*Sodium acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
9	0	9	59
29	0	14	66

Remarks - Results	All the test validity criteria were met. The test concentration of the notified chemical (9 mg TOC/L) is below the recommended range of 10 – 20 mg TOC/L. As no biodegradation of the notified chemical was measured, the deviation was considered to have no effect on the final outcome of this study (criterion for ready biodegradability). In the toxicity control, the notified chemical was found not to inhibit microbial activity. The notified chemical is considered not to be readily biodegradable based on the test results.
CONCLUSION	The notified chemical is not readily biodegradable based on the test results.
TEST FACILITY	NOTOX B.V. (2011e)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test - Static (1992). EC No.440/2008 Part C, C.1 Acute Toxicity for Fish- Static (2008).
Species	*Cyprinus carpio*, Teleostei, Cyprinidae (Carp)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	180 mg $CaCO_3$/L
Analytical Monitoring	HPLC-MS/MS for analysis of the sample concentrations
Remarks – Method	Following a range-finding test, the definitive test was conducted at loading rates of 10, 18, 32, 56 and 100% of a water soluble fraction (WSF) at 20.9 – 22.1˚C, pH 7.4 – 7.8, and O_2 > 6.4 mg/L. Preparation of test solutions started with a loading rate of 100 mg/L applying two days of magnetic stirring to ensure maximum dissolution in test medium. This resulted in a clear solution with a floating layer and the notified chemical precipitate appearing as lumps. The mixture was kept still for just over an hour and then the WSF was collected by means of siphoning. The clear and colourless WSF was used as highest test concentration. Lower concentrations were prepared by serial dilution of the above prepared WSF. A blank control test was also conducted. The LC50 was calculated as $(AB)^{1/2}$, with A and B respectively being the highest 0% mortality concentration and lowest 100% mortality concentration, and also being the limits of the 95% confidence interval.

RESULTS

Concentration		*Number of Fish*	*Mortality*				
Nominal[*]	*Actual*[**] *(mg/L)*		*3.5 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	0	7	0	0	0	0	0
10% of WSF	2.9	7	0	0	0	0	0
18% of WSF	4.8	7	0	0	0	0	0
32% of WSF	8.5	7	0	0	0	0	0
56% of WSF	15	7	0	0	0	0	0
100% of WSF	26	7	0	7	7	7	7

[*] Percentage of a WAF prepared at a loading rate of 100 mg/L
[**] Initial concentrations

LC50	20 (95% CI 15 – 26) mg/L at 96 hours
NOEC	8.5 mg/L at 96 hours
Remarks – Results	All the test validity criteria were met. Analysis of samples at the start showed measured concentrations of 2.9, 4.8, 8.5, 15 and 26 mg/L in the 10, 18, 32, 56 and 100% WSFs, respectively. After 24 hours (100% WSF) or 96 hours (WSF dilutions) of exposure, the measured notified chemical concentrations remained constant (91 – 100% of initial). Therefore, all toxicity parameters were based on initial measured concentrations. Fish exposed to the 15 mg/L test group showed clinical effects from 48 hours onwards. All fish generally appeared more orange coloured with observations of very small hemorrhages on or just behind their heads. Three fish in the same group were continuously swimming at the surface showing extremely hyperactive swimming behaviour from time to time. This behaviour was observed at 72 hours, but was no longer present after 96 hours of exposure.

Under the conditions of the test, the notified chemical induced no visible effects in carp at or below an initial measured concentration of 8.5 mg/L. Therefore, the NOEC was determined to be 8.5 mg/L (initial concentration).

The 96h LC50 based on initial measured exposure concentrations was estimated to be 20 mg/L with 0% mortality at 15 mg/L and 100% mortality at 26 mg/L.

The notified chemical is considered to be harmful to fish based on the test results.

CONCLUSION The notified chemical is harmful to fish

TEST FACILITY NOTOX B.V. (2012f)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia Sp. Acute Immobilisation Test –Static (2004).
EC No.440/2008 Part C, C.2 Daphnia Sp. Acute Immobilisation Test - Static (2008).
ISO Standard 6341: "Water quality - Determination of the inhibition of the mobility of Daphnia magna Straus - Acute toxicity test (1996).
OECD series on testing and assessment number 23, Guidance document on aquatic toxicity testing of difficult substances and mixtures 2000).

Species *Daphnia magna*
Exposure Period 48 hours
Auxiliary Solvent None
Water Hardness 180 mg $CaCO_3$/L
Analytical Monitoring HPLC-MS/MS for analysis of sample concentrations
Remarks - Method Following a range-finding test, the definitive study was conducted at 10, 18, 32, 56 and 100% of a WSF prepared at a loading rate of 100 mg/L at pH 7.8 – 7.9, and O_2 = 8.7 – 9.2 mg/L. The test temperature was not provided. Preparation of test solutions followed the same method for the previous fish study.

A blank control was also conducted.
The LC50 was calculated as $(AB)^{1/2}$, with A and B respectively being the highest 0% mortality concentration and lowest 100% mortality concentration, and also being the limits of the 95% confidence interval.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal[*]	*Actual*		*24 h*	*48 h*
Control	Not measured	20	0	0
10% of WSF	Not measured	20	0	0
18% of WSF	Not measured	20	0	0
32% of WSF	Not measured	20	0	0
56% of WSF	33 mg/L[**]	20	0	0
100% of WSF	60 mg/L[**]	20	6	20

[*] Percentage of a WSF prepared at a loading rate of 100 mg/L
[**] Initial concentrations

EC50 44 (95% CI 33 – 60) mg/L at 48 hours
NOEC 33 mg/L at 48 hours
Remarks - Results All the test validity criteria were met.

Samples of 0%, 56% and 100% of WSF were analysed at the start and the end of the test. The measured concentrations at the start were 33 and 60

mg/L respectively for the solutions 56 and 100% of the WSF. During the test the measured concentrations remained constant (95 – 96% of the initial). Hence, toxicity parameters were based on initial measured concentrations.

During the test, the notified chemical did not induce acute immobilization of *Daphnia magna* at an initial measured concentration of 33 mg/L after 48 hours of exposure. Therefore, the NOEC was determined to be 33 mg/L.

The 48h EC50 was determined as 44 mg/L with 0% immobility at 33 mg/L and 100% immobility at 60 mg/L.

The notified chemical is considered to be harmful to *Daphnia magna* based on the test results.

CONCLUSION The notified chemical is harmful to aquatic invertebrates.

TEST FACILITY NOTOX B.V. (2012g)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test-Static (2006)
EC No.440/2008 Part C, C.3 Algal Inhibition Test-Static, amended by EC No. 761/2009 (2009)
ISO International Standard 8692: "Water quality - Freshwater algal growth inhibition test with unicellular green algae"(2004)
OECD series on testing and assessment number 23, Guidance document on aquatic toxicity testing of difficult substances and mixtures 2000)

Species	*Pseudokirchneriella subcapitata*, strain: NIVA CHL 1 (Algae)
Exposure Period	72 hours
Concentration Range	Nominal: 4.6%, 10%, 22%, 46% and 100% of 100 mg/L WSF (see below in Remarks – Method) Actual: 2.0, 2.9, 9.0, 19 and 45 mg/L
Auxiliary Solvent	None
Water Hardness	24 mg $CaCO_3$/L
Analytical Monitoring	HPLC-MS/MS for analysis of sample concentrations
Remarks - Method	Following a range-finding test, the definitive study was conducted with an initial cell density of 1×10^4, a light intensity of 77 – 89 µE m^{-2} s^{-1}, and at pH 7.2 – 7.9. The test temperature was not provided. Preparation of test solutions followed the same method for the previous fish study, except the mixture after agitation was kept still for 1¼ hour before the WSF was collected by means of siphoning. A blank control and a reference control using potassium dichromate were conducted. For analysis of the test data to determine the NOEC and the EC50 values, TOXATAT Release 3.5 was used.

RESULTS

Biomass		*Growth*	
E_bC50 (mg/L at 72 h)	*NOEC (mg/L)*	*E_rC50 (mg/L at 72 h)*	*NOEC (mg/L)*
8.1 (95% CI 3.7 – 18)	2.0	37 (95% CI 21 – 66)	2.0

Remarks - Results

Analysis of the samples at the start of the definitive test showed measured concentrations of 2.0, 2.9, 9.0, 19 and 45 mg/L for the solutions 4.6, 10, 22, 46 and 100% of WSF, respectively. After 72 hours of exposure the test substance concentrations measured had generally remained constant (86-

	99% of initial) except for the test group representing 10% of the WSF that had decreased to 71% of initial. Despite the slightly higher decrease in one test group, it was justified to base toxicity parameters on initial measured concentrations. All the test validity criteria were met. The test substance reduced growth rate and inhibited the yield of the fresh water algae species *Pseudokirchneriella subcapitata* significantly at measured concentrations of 2.9 mg/L and higher. Therefore, the NOEC for both growth rate reduction and yield inhibition was 2.0 mg/L. The 72h E_rC50 was determined as 37 mg/L (95% confidence interval ranging from 21 to 66 mg/L. The 72 h E_bC50 was determined as 8.1 mg/L with a 95% confidence interval ranging from 3.7 to 18 mg/L. The notified chemical is considered to be harmful to alga based on the obtained 72 h E_rC50 value.
CONCLUSION	The notified chemical is harmful to alga
TEST FACILITY	NOTOX B.V. (2012h)

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