File No: LTD/1856

November 2015

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Exolit OP 222

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

TABLE OF CONTENTS

SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/1856	Clariant (Australia) Pty Ltd and Connell Brothers Australasia Pty Ltd	Exolit OP 222	No	< 1 tonne per annum	Flame retardant

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 of 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
Based on the low hazard and reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical during blending into plastic articles:
  - Enclosed, automated processes, where possible
  - Ventilation system, including local exhaust ventilation

- 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 during blending into plastic articles:
  - Avoid contact with eyes
  - Avoid inhalation of dusts
  - Use low-dust handling techniques

- 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 during blending into plastic articles:
  - Eye protection
  - Respiratory protection, if ventilation is inadequate, when handling the notified chemical in powder form

  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 of 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

- Where reuse or recycling are unavailable or impracticable, dispose of the chemical in an environmentally sound manner in accordance with relevant Commonwealth, State, Territory and local government legislation.

Storage

- The following precautions should be taken regarding storage of the notified chemical:
  - Avoid alkali
  - Keep container tightly closed
  - Keep in cool place

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if
  - the importation volume exceeds one tonne per annum notified chemical;

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from flame retardant, 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)SDSs of the notified chemical and a product containing the notified chemical provided by the notifier were reviewed by NICNAS. The accuracy of the information on the (M)SDSs remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Clariant (Australia) Pty Ltd (ABN: 30 069 435 552)
Level 3, Olympus Building
3 Acacia Place
296-324 Ferntree Gully Road
NOTTING HILL VIC 3168

Connell Brothers Australasia Pty Ltd (ABN: 53 079 159 327)
3/32 Windorah Street
STAFFORD QLD 4053

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

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

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: boiling point, vapour pressure, dissociation constant, adsorption/desorption, flash point, and ready biodegradability.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Canada, China, EU, Japan, Korea, Switzerland, Taiwan, USA

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Exolit OP 222
Exolit OP product (contains < 35% notified chemical)

MOLECULAR WEIGHT
< 500 Da

ANALYTICAL DATA
Reference NMR and IR spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: white powder

Property	Value	Data Source/Justification
Melting Point	Decomposes without melting from ~429 °C	Measured
Boiling Point	Not determined	Expected to decompose before boiling based on melting point
Density	2,140 kg/m^3 at 2.6 ± 0.2 °C	Measured
Vapour Pressure	Not determined	Expected to have a low vapour

		pressure based on melting point
Water Solubility	> 0.3 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	The hydrolysis study was not feasible due to low water solubility. However, no significant hydrolysis is expected under environmental conditions.
Partition Coefficient (n-octanol/water)	Not determined	The notified chemical is inorganic therefore determination of partition coefficient is not suitable.
Surface Tension	63.7 m/N at 20 °C	Measured
Adsorption/Desorption	Not determined	The adsorption/desorption study was not feasible due to low water solubility. Based on its expected low solubility in water, the notified chemical is expected to settle to sediment and sludge
Dissociation Constant	Not determined	The dissociation constant study was not feasible due to low water solubility.
Particle Size	D_{10} = 1.97 µm D_{50} = 12.79 µm D_{90} = 28.07 µm Inhalable fraction (< 100 µm): 100% Respirable fraction (< 10 µm): ~ 40.3%	Measured
Particle Size	D_{50} = 3.502 µm D_{10} = 0.824 µm D_{90} = 12.65 µm	Measured
Flammability	Not highly flammable	Measured
Autoignition Temperature	400 °C	Measured
Explosive Properties	Estimated to be negative	Based on structural analysis
Oxidising Properties	Estimated to be negative	Based on structural analysis

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

Reactivity
The notified chemical is expected to be stable under normal conditions of use. It reacts with alkali and hazardous phosphorus oxides such as phosphorus pentoxide may be produced in case of fires.

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 of 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 powder as a component of a flame retardant formulation (Exolit OP product) at < 35% concentration.

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

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

PORT OF ENTRY
Melbourne and Sydney

TRANSPORTATION AND PACKAGING
The solid product containing the notified chemical at < 35% concentration will be packed in 20 kg paper bags (three paper layers with plastic inner liner) and will be transported by road and/or rail.

USE
The imported flame retardant product containing the notified at < 35% concentration will be used for manufacture of plastic articles, mainly for the electronic industry.

OPERATION DESCRIPTION
The notified chemical will be imported as a component of a formulation for end-use. No manufacture or reformulation will occur in Australia.

End use
The imported formulation containing the notified chemical will be blended with other materials to form plastic articles through processes involving weighing and transferring into a mixer, mixing, extruding, QA testing, moulding, and routine cleaning and maintenance. The mixing, extruding and moulding process will be performed in a closed system and in a controlled area with local exhaust ventilation.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Stevedores	2-3	10-15
Transport	6	260
Warehousing	6	260
Industrial users	8	260
Cleaners	8	260

EXPOSURE DETAILS
Transport and storage workers may come into contact with the notified chemical at < 35% concentration only in the unlikely event of an accident.

End-use
Processes for plastics production are expected to be largely enclosed and automated; however, dermal, ocular and inhalation exposure to the notified chemical at < 35% concentration may occur during weighing and transferring the product containing notified chemical to the mixer or the moulding machine, during quality control testing and during maintenance and cleaning tasks. Exposure is expected to be minimised by the use of local exhaust ventilation and the use of personal protective equipment (PPE) such as coveralls, impermeable gloves, eye protection and a respirator (if required). Once blended into plastic articles, the notified chemical will be bound within an inert matrix and will not be bioavailable.

6.1.2. Public Exposure
The formulation containing the notified chemical at < 35% concentration will be used in industrial settings only and will not be sold to the public. The public may come into contact with plastic articles containing the notified chemical. However, once moulded into plastic articles, the notified chemical will be bound into an inert matrix and will not be available for exposure.

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 > 5 mg/L/4 hour; low toxicity
Skin corrosion (in vitro)	non-corrosive
Skin irritation (in vitro)	non-irritating
Eye irritation (in vitro)	slightly irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Rat, repeat dose oral toxicity – 14 days	NOAEL = 1000 mg/kg bw/day
Rat, repeat dose oral toxicity – 28 days	NOAEL = 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian cell gene mutation test	non genotoxic
Genotoxicity – in vitro mammalian chromosome aberration test	non genotoxic
Genotoxicity – in vivo mammalian erythrocyte micronucleus test	non genotoxic
Rat, reproductive and developmental toxicity	NOAEL = 1000 mg/kg bw/day

Toxicokinetics.
No toxicokinetic data on the notified chemical were submitted.

The notified chemical is a salt therefore dermal absorption is expected to be limited. However given the low molecular weight (< 500 Da) there is potential for absorption via the oral and inhalation routes.

Acute toxicity.
The notified chemical is of low acute oral, dermal and inhalation toxicity based on studies conducted in rats.

Irritation.
The notified chemical was found to be not corrosive or irritating to the skin based on two *in vitro* studies conducted using the reconstructed human epidermis model.

In an *in vitro* bovine corneal opacity and permeability (BCOP) assay the notified chemical was found to be a mild eye irritant. The notified chemical was also found to be slightly irritating in an eye irritation study in rabbits. Mild to moderate conjunctival irritation was observed that was completely resolved at the 7-day observation period.

Sensitisation.
The notified chemical was not found to be a skin sensitiser when tested at up to 25% concentration in a local lymph node assay (LLNA).

In the LLNA study a 25% test concentration of the notified chemical resulted in a stimulation index (SI) of 0.8. A dose response was not observed in this study as the other two test concentrations of 6.25% and 12.5% resulted in a SI of 1.2 and 1.1, respectively.

Repeated dose toxicity.
A No Observed Adverse Effect Level (NOAEL) of 1000 mg/kg bw/day (the highest dose level tested) was established for the notified chemical in a 28-day repeated dose oral gavage toxicity study in rats based on no treatment-related, toxicologically relevant findings were noted.

Mutagenicity/Genotoxicity.
The notified chemical was negative in a bacterial reverse mutation assay, in an *in vitro* mammalian cell gene mutation test in Chinese hamster V79 cells and in an *in vitro* chromosomal aberration study in Chinese hamster V79 cells. The notified chemical was also negative in an *in vivo* mouse micronucleus assay.

Toxicity for reproduction.
A reproductive/developmental NOAEL of 1000 mg/kg bw/day (the highest dose level tested) was established for the notified chemical in a one generation reproduction toxicity (oral gavage) study in rats based on an absence of toxicologically relevant effects.

Health hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System of 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
Workers may be exposed to the notified chemical at < 35% concentration during manufacture of plastic articles containing the notified chemical.

Based on the available information, the notified chemical is of low hazard, presenting only as a slight eye irritant. At the proposed handling concentrations the notified chemical may have the potential to cause eye irritation effects. In addition, given that the notified chemical is a powder (with particle sizes in the inhalable and respirable size range) there is also potential for temporary lung overloading effects from exposure to dusts of the notified chemical.

Operations to blend the notified chemical into plastic articles are expected to be in well ventilated areas and to use enclosed and automated processes where possible. Workers are expected to wear personal protective equipment (PPE) such as coveralls, impermeable gloves, eye protection and a respirator (if required) when handling the notified chemical. These control measures should reduce the risk of irritation and the potential risk of temporary lung overloading effects from exposure to dusts of the notified chemical.

Therefore, under the occupational settings described, the risk of the notified chemical to occupational health is not considered to be unreasonable.

6.3.2. Public Health
Members of the public may experience repeated exposure to plastic articles (mainly electronic devices) containing the notified chemical. However, once blended into plastics, the notified chemical will be bound into a solid matrix and will not be bioavailable. In addition, the notified chemical is not expected to release harmful or toxic substances at elevated temperatures (such as in automobile interiors which can reach around 80 ºC in hot summer conditions) and during accidental fires, based on a study on similar Exolit phosphorus flame retardants (Beard A and Thomas M, 2003).

Therefore, when used in the proposed manner, the risk to public health 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 or reformulated in Australia. Therefore, no releases from these activities are expected. Spills or accidental release of the notified chemical are expected to be collected and disposed of in accordance with local authorities.

RELEASE OF CHEMICAL FROM USE
The product containing the notified chemical will be used to manufacture plastic articles, mainly for the electronic industry e.g. computer devices. Sampling, mixing and processing will occur in a closed system and in a controlled area with local exhaust and ventilation. Release of the product during use may occur due to accidental spills. The notified chemical could therefore potentially contaminate waterways through sewers or stormwater system. However, accidental release of product from use, is most likely to be in very low volumes, which when reaches large water bodies is diluted down, thereby resulting in very low environmental implications.

RELEASE OF CHEMICAL FROM DISPOSAL
The notified chemical is expected to share the fate of the manufactured products it is incorporated into i.e. manufacture plastic articles, mainly for the electronic industry e.g. computer devices. These products are

expected to be disposed to landfill or in accordance with state and/or federal regulations. Environmental release is therefore anticipated to be low.

7.1.2. Environmental Fate

There are no environmental fate data for the notified chemical. The majority of the notified chemical is expected to be chemically incorporated into plastic articles and not expected to exist in the original form. Therefore, notified chemical incorporated into plastic articles is not expected to leach or be bioavailable, and no significant release is expected based on the reported use pattern. A small proportion of the notified chemical may be released to landfill via the disposal of empty containers. In the unlikely case if the notified chemical is released in the environment, it is assumed that under environmental conditions in aqueous media, only minor amounts of it will be present in bioavailable form due to low water solubility. Therefore, the notified chemical is not expected to be significantly bioaccumulative in the environment.

7.1.3. Predicted Environmental Concentration (PEC)

The notified chemical is not expected to be present at significant concentrations in the aquatic environment because of the very low potential for direct release to surface waters when used in closed reactors. A Predicted Environmental Concentration (PEC) has therefore not been calculated.

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	EC50 (96 h) > 100 mg/L	Not harmful to fish
	NOEC (28 d) = 100 mg/L	Not harmful to fish
Daphnia Toxicity	EC50 (48 h) > 100 mg/L	Not harmful to aquatic invertebrates
	NOEC (21 d) = 100 mg/L	Not harmful to aquatic invertebrates
Algal Toxicity	ErC50 (72 h) > 100 mg/L	Not harmful to algae
Inhibition of Bacterial Respiration	NOEC (3 h) > 1000 mg/L	Not expected to inhibit bacterial respiration

Based on the endpoints for toxicity of the notified chemical to aquatic organisms, the notified chemical is not considered to be harmful to aquatic organisms under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009). Therefore, the notified chemical is not formally classified under the GHS. Based on its measured chronic toxicity, lack of biodegradability and expected low bioaccumulation potential, the notified chemical is not formally classified under the GHS for long-term hazard.

7.2.1. Predicted No-Effect Concentration

A Predicted No-Effect Concentration (PNEC) for the aquatic compartment has not been calculated since the notified chemical is not considered to be harmful up to the limit of its solubility in water.

7.3. Environmental Risk Assessment

The Risk Quotient (PEC/PNEC) was not calculated as limited release of the notified chemical to the aquatic compartment is expected based on the reported use pattern. The majority of the imported notified chemical will be chemically incorporated into plastic articles and is not expected to leach or be bioavailable. Therefore, on the basis of limited aquatic exposure and the assessed use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point Decomposes without melting from ~429 °C

Method EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks Differential Scanning Calorimetry
Test Facility Siemens (2012a)

Density 2,140 kg/m^3 at 2.6 ± 0.2 °C

Method OECD TG 109 Density of Liquids and Solids.
Remarks Pycnometer method
Test Facility Siemens (2012b)

Water Solubility > 0.3 g/L at 20 °C

Method OECD TG 105 Water Solubility.
Remarks Flask Method
Test Facility Siemens (2012c)

Surface Tension 63.7 mN/m at 20 °C

Method OECD TG 115 Surface Tension of Aqueous Solutions.
Remarks Concentration: 1,004 mg/L
Test Facility Siemens (2012d)

Particle Size

Method ISO13320:2009: Particle Size Analysis – Laser Diffraction Methods

Range (µm)	*Mass (%)*
1.97	10
12.79	50
28.07	90

Remarks Determined by laser diffraction (light scattering)
Test Facility Siemens (2012e)

Particle Size

Method Not reported

Range (µm)	*Mass (%)*
0.824	10
3.502	50
12.65	90

Test Facility Mastersizer (2014)

Flammability Not highly flammable

Method EC Council Regulation No 440/2008 A.10 Flammability (Solids).
Remarks The test substance could not be ignited with a flame in a preliminary test. Therefore, no main test was performed.
Test Facility Siemens (2012f)

Autoignition Temperature 400 °C

Method EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids.
Test Facility Siemens (2012g)

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 Crl: WI(Han)
Vehicle Physiological saline 0.9% NaCl
Remarks - Method No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3F	300	0/3
2	3F	2000	0/3
3	3F	2000	0/3

LD50 > 2000 mg/kg bw
Signs of Toxicity No signs of systemic toxicity.
Effects in Organs No treatment-related abnormalities were noted at necropsy.
Remarks - Results All animals showed expected body weight gains over the study period.

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

TEST FACILITY Bioservice (2012a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE Notified chemical

METHOD OECD TG 402 Acute Dermal Toxicity – Limit Test.
Species/Strain Rat/ Wistar Crl: WI(Han)
Vehicle Water
Type of dressing Semi-occlusive
Remarks - Method No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5M, 5F	2000	0/10

LD50 > 2000 mg/kg bw
Signs of Toxicity - Local No signs of dermal irritation were noted.
Signs of Toxicity - Systemic No signs of systemic toxicity were noted.
Effects in Organs No abnormalities were noted at necropsy.
Remarks - Results All animals showed expected body weight gains over the study period, except 1/5 female animals showed a slight weight loss during the first week but all of the females showed weight gain during the second week.

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

TEST FACILITY Bioservice (2012b)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 436 Acute Inhalation Toxicity – Acute Toxic Class Method
Species/Strain	Rat/Crl:WI(Han)
Vehicle	None
Method of Exposure	Oro-nasal exposure
Exposure Period	4 hours
Physical Form	Solid particulate
Particle Size	MMAD 3.4/3.5 µm (GSD 2.1)
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Concentration <mg/L>*		*Mortality*
		Nominal	*Actual*	
1	3 per sex	15.6	5.1 ± 0.3	0/6

LC50	> 5 mg/L/4 hours
Signs of Toxicity	Slow and shallow breathing was seen in all treated animals during exposure. No clinical signs of systemic toxicity were noted after exposure.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks - Results	All male animals showed normal body weight gain. Two female animals showed body weight loss up to 7 days post-exposure, with 1 showing recovery during the second week.

CONCLUSION	The notified chemical is of low toxicity via inhalation.
TEST FACILITY	WIL (2014)

B.4. Corrosion – 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	Water
Remarks - Method	The test substance (25 mg moistened with 25 µL water) was applied to the tissues in duplicate. Following exposure periods of 3 minutes (room temperature; test 1) and 1 hour (37 ºC; test 2), the tissues were rinsed, treated with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolim bromide] and then incubated at 37 °C for 3 hours. In a preliminary test the test substance was shown not to directly reduce MTT. Positive and negative controls were run in parallel with the test substance: - Negative control (NC): distilled water - Positive control (PC): potassium hydroxide (8N)

RESULTS

Test material	*Test 1 (3 minute exposure period)*		*Test 2 (1 hour exposure period)*	
	Mean OD_{550} of duplicate tissues	*Relative mean viability (%)*	*Mean OD_{550} of duplicate tissues*	*Relative mean viability (%)*
Negative control	1.834	100	1.773	100
Test substance	1.634	89	1.726	97
Positive control	0.395	22	0.304	17

OD = optical density

Remarks - Results	The test substance showed no corrosive effects. The positive 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.
TEST FACILITY	Bioservice (2012c)

B.5. Irritation – skin (in vitro)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 439 In vitro Skin Irritation: Reconstructed Human Epidermis Test Method EPISKIN-SM™ Reconstructed 3D Human Epidermis Model
Vehicle	Distilled water
Remarks - Method	The test substance (10 mg) was applied to the tissues (pre-moistened with 5 µL distilled water) in triplicate. Following exposure periods of 15 minutes (room temperature) and 1 hour (room temperature; test 2), the tissues were rinsed, treated with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolim bromide] and then incubated at 37 °C for 3 hours. In a preliminary test the test substance was shown not to directly reduce MTT. Positive and negative controls were run in parallel with the test substance: - Negative control (NC): phosphate buffered saline - Positive control (PC): 5% sodium dodecyl sulphate in distilled water

RESULTS

Test material	*Mean OD_{550} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	1.233	100	4.8
Test substance	1.259	102.1	5
Positive control	0.096	7.8	0.4

OD = optical density; SD = standard deviation

Remarks - Results	The test substance showed no irritation effects. The positive and negative controls gave satisfactory results, confirming the validities of the test systems.
CONCLUSION	The notified chemical was non-irritating to the skin under the conditions of the test.
TEST FACILITY	Bioservice (2012d)

B.6. Irritation – eye (in vitro)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants.
Vehicle	Physiological saline 0.9% NaCl
Remarks - Method	No significant protocol deviations.

Negative control was physiological saline and positive control was 20% imidazole in physiological saline.

RESULTS

Test material	*Mean opacities of triplicate tissues**	*Mean permeabilities of triplicate tissues**	*IVIS**
Vehicle control	0	0.025	0.38
*Test substance**	16.67	-0.001	16.65
*Positive control**	212	1.837	239.56

SD = Standard deviation; IVIS = in vitro irritancy score
*Corrected for background values

Remarks - Results: The controls gave satisfactory results confirming the validity of the test system.

CONCLUSION: The notified chemical was a mild irritant to eyes under the conditions of the test.

TEST FACILITY: Bioservice (2012e)

B.7. Irritation – eye

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain: Rabbit/New Zealand White
Number of Animals: 3
Observation Period: 7 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	1.7	0.7	1.7	2	< 7 d	0
Conjunctiva: chemosis	0.7	0.3	0.7	1	< 72 h	0
Conjunctiva: discharge	0.3	0.3	0.7	1	< 72 h	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

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

Remarks - Results: No corneal effects were noted during the study. Iridial inflammation was noted in 2 treated eyes 1 hour after treatment. Mild to moderate conjunctival irritation was noted in all treated eyes at the 24-hour observation. At the 72-hour observation 2 treated eyes appeared normal and 1 treated eye had mild redness only which appeared normal at the 7-day observation.

CONCLUSION: The notified chemical is slightly irritating to the eye.

TEST FACILITY: Harlan (2014)

B.8. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 429 Skin Sensitisation: Local Lymph Node Assay

Species/Strain	Mouse//CBA/CaOlaHsD
Vehicle	Acetone/olive oil (4:1)
Preliminary study	Yes
Positive control	Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using p-phenylenediamine.
Remarks - Method	No significant protocol deviations.

RESULTS

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	5F	941.3 ± 151.1	-
6.25	5F	1086.5 ± 339.8	1.2
12.5	5F	1037 ± 241.1	1.1
25	5F	724.5 ± 243.7	0.8
Positive Control			
1%	5	6726.5 ± 2062	10.9 ± 3.3

EC3	> 25%
Remarks - Results	There were no mortalities or clinical abnormalities. All treated animals showed the expected weight development which includes a weight loss of up to 2 g throughout the study.

CONCLUSION There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY Bioservice (2012f)

B.9. Repeat dose toxicity

TEST SUBSTANCE Notified chemical

METHOD Similar to OECD TG 407 Repeated Dose 28-Day Oral Toxicity Study in Rodents.

Species/Strain	Rat/Wistar
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 14 days Dose regimen: 7 days per week Post-exposure observation period: none
Vehicle	Water
Remarks - Method	No significant protocol deviations except for the exposure period of 14 days.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	3 per sex	0	0/6
low dose	3 per sex	100	0/6
mid dose	3 per sex	300	0/6
high dose	3 per sex	1000	0/6

Mortality and Time to Death
There were no unscheduled deaths.

Clinical Observations
No clinical signs were noted in all animals.

All animals showed body weight development within the normal range of variation for this strain.

No significant effects on food consumption were noted.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology: Values of female animals of RBC, HB, HCT, PLT, WBC and the percentages of Neu, Lym, Mono, Eos and Baso were comparable with those of the control group. One male animal had changes values in the amount of Eos, RBC, HB, HCT and PLT were considered by the study authors to be possibly of toxicological relevance but a conclusion could not be given due to the low animal number.

Clinical biochemistry: Values of male animals of ASAT, ALAT, Crea, TP, Alb, Urea, Chol, Na and K were comparable with those of the control group. Values of female animals of ALAT, AP, TP, Alb, Urea, Na and K were comparable with those of the control group. A dose dependent decrease for ASAT values in female animals of all treated groups were assumed to be test substance-related by the study authors but could not be assumed to be of toxicological relevance due to all changed values were still in the range of the historical control data in the testing facility.

Effects in Organs

A female animal of the high-dose group showed a fluid distension in uterus with oviduct and cervix. The effect was considered by the study authors to be possibly treatment-related but its toxicological relevance was considered to be unclear due to the low animal number.

Adrenals of male animals in the high-dose group were slightly decreased in weight when compared to the controls. The change was mainly attributed to 1 of the 3 animals and therefore not considered by the study authors to be test substance-related. No other biologically relevant changes were noted in treated male animals.

Increases in absolute adrenal weights of female animals in low-dose and high-dose groups were considered by the study authors as incidental due to the mildness of the increases and no increase in the mid-dose group.

Absolute and relative weights of ovaries were slightly but dose dependently decreased in mid-dose and high-dose groups. The changes were considered by the study authors to be possibly test-substance-related but their toxicological relevance was unclear.

Absolute and relative weights of uteri of female animals of low-dose and mid-dose groups decreased when compared to the controls. The changes were considered by the study authors to be possibly test-substance-related but their toxicological relevance was unclear.

Remarks – Results

Overall, there were no signs of severe toxicity or mortality.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 1000 mg/kg bw/day in this study, based on no treatment-related, toxicologically relevant findings were noted.

TEST FACILITY	Bioservice (2012g)

B.10. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-Day Oral Toxicity Study in Rodents.
Species/Strain	Rat/Wistar
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	Water for injection
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10 per sex	0	0/10
low dose	5 per sex	100	0/5
mid dose	5 per sex	300	0/5
high dose	10 per sex	1000	0/10
control recovery	5 per sex	0	0/5
high dose recovery	5 per sex	1000	0/5

Mortality and Time to Death

There were no unscheduled deaths.

Clinical Observations

No test substance-related clinical signs were noted in all animals. No ophthalmoscopic findings or effects in parameters of the functional observation battery were noted.

All animals showed body weight development within the normal range of variation for this strain.

No biologically relevant effects on food consumption were noted.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology and blood coagulation: Changes included increase in percentage of neutrophils in male animals of all treated groups and in female animals of the high-dose group, decreases in mean percentage of lymphocytes of animals of high-dose group, a statistically significant increase in the HB value in the low-dose group, a slight decrease in PLT values in treated females and statistically differences in prothrombin time of high-dose recovery male animals. However, all haematological parameters were within the normal range of variation and the differences were not considered by the study authors to be biologically relevant.

Clinical biochemistry: Increases in mean values of total bile acids in low-dose and high-dose males and females of all treated groups were considered by the study authors to be test substance-related but not toxicologically relevant, based on no other supportive findings in liver organ weights, histopathology and other biochemical parameters. A decrease in AP for treated females was not considered by the study authors to be toxicologically relevant due to the decrease was inhomogeneous and the values were still in the range of the test facility's historical data.

Urinalysis: Erythrocytes in the urine were found in several treated animals but no treatment related effects were noted. Increases in amount of proteins in treated individuals including 4/5 male animals and 2/5 female animals of the high-dose recovery group were assumed by the study authors to be accidental based on a lack of supportive changes/effects. A slight change in specific gravity of the urine in high-dose males was not considered by the study authors to be biologically relevant due to the mildness of increase and a lack of supportive symptoms.

Effects in Organs

A statistical significant increase in absolute weight of kidneys in the mid-diose group was not considered by the study authors to be toxicologically relevant due to lack of dose dependency.

Decreases in the absolute mean weight of prostate in high-dose male were not considered by the study authors to be toxicologically relevant due to the mildness of the changes and lack of support in histopathology.

Decreases in weights of hearts in female animals of the high-dose group were assumed by the study authors to be not test-substance-related and not toxicologically relevant.

Decreases in weights of adrenals in female animals of the mid-dose and high-dose groups were assumed by the study authors to be accidental due to the mildness of the changes.

A slight decrease in the mean weight of spleens in the high-dose female animals was not considered by the study authors to be toxicologically relevant as the change was not found in recovery animals.

Absolute weight increases of thymus noted in animals (mainly females) of all treated groups were not dose dependent and not considered by the study authors to be clearly related to the test substance.

Absolute weight losses of uteri noted in animals (mainly females) of all treated groups were not dose dependent and assumed by the study authors to be accidental.

Pathology
No treatment-related findings in gross pathological evaluations.

Histopathology
No histopathological findings were clearly related to the test substance. A minimal increase, when compared to the control group, in the incidence and degree of minimal submuscosal mixed cell infiltrates in the glandular part of the stomach in the high-dose group was considered by the study authors to be possibly related to a local irritant effect of the rest substance formulation when administered repeatedly by oral gavage and was not relevant to humans. The difference was not seen in the recovery period.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 1000 mg/kg bw/day in this study, based on no treatment-related, toxicologically relevant findings were noted.

TEST FACILITY Bioservice (2013a)

B.11. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
Plate incorporation procedure (Test 1)/Pre incubation procedure (Test 2)

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 mix from phenobarbital/β-naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 31.6-5000 µg/plate b) Without metabolic activation: 31.6-5000 µg/plate
Vehicle	Dimethyl sulfoxide
Remarks - Method	Tests with negative controls (A. dest.), vehicle controls and positive controls were run concurrently. Positive controls were: - With metabolic activation: 2-aminoanthracene - Without metabolic activation: sodium azide (TA100, TA1535); 4-nitroquinoline-1-oxide (TA98, TA1537); methylmethanesulfonate (WP2uvrA)

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5000	> 2500	> 5000	negative
Test 2		> 5000	> 5000	negative
Present				
Test 1	> 5000	> 5000	> 5000	negative
Test 2		> 5000	> 5000	negative

Remarks - Results
Toxic effects of the test substance were noted in TA1535 and TA1537 at 5000 µg/plate in Test 1, in the absence of metabolic activation.

In both tests, no increases in the frequency of revertant colonies were observed in the presence or absence of metabolic activation.

The positive and negative controls gave a satisfactory response confirming the validity of the test system.

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

TEST FACILITY Bioservice (2012h)

B.12. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
Species/Strain Chinese hamster
Cell Type/Cell Line V79 cell lines
Metabolic Activation System S9 mix from phenobarbital/β-naphthoflavone induced rat liver
Vehicle Cell culture medium (MEM + 0% FBS 4 h treatment)
Cell culture medium (MEM + 10% FBS 20 h treatment)
Remarks - Method

Metabolic Activation	*Test Substance Concentration (mM)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	0.025*, 0.05*, 0.1*, 0.25*, 0.5*, 1.0*, 2.5*, 5.0*, 7.5*, 10*	4 h	48-72 h	7-10 days
Test 2	0.025*, 0.05*, 0.1*, 0.25*, 0.5*, 1.0*, 1.4*, 1.6*, 1.8*, 2.0*	20 h	48-72 h	7-10 days
Present				
Test 1	0.1*, 0.25*, 0.5*, 1.0*, 2.5*, 5.0*, 6.25*, 7.5*, 8.75*, 10*	4 h	48-72 h	7-10 days
Test 2	0.5*, 1.0*, 2.0*, 4.0*, 5.0*, 6.0*, 7.0*, 8.0*, 9.0*, 10*	4 h	48-72 h	7-10 days

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (mM) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 10	≥ 10	≥ 0.5	negative
Test 2	≥ 0.5	≥ 2.0	≥ 0.5	negative
Present				
Test 1	-	> 10	≥ 0.5	negative
Test 2	-	> 10	≥ 0.5	negative

Remarks - Results In both tests, no dose-response relationship was noted and no biologically relevant increases in the frequency of revertant colonies were observed in the presence or absence of metabolic activation.

CONCLUSION The notified chemical was not clastogenic to Chinese hamster V79 cells treated in vitro under the conditions of the test.

TEST FACILITY Bioservice (2012i)

B.13. 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 cell lines
Metabolic Activation System S9 mix from phenobarbital/β-naphthoflavone induced rat liver

Vehicle	MEM cell culture medium
Remarks - Method	A dose range-finding study was carried out at 0.02 – 10 mM. The dose selection for the main experiments was based on the solubility and results from the preliminary study. Vehicle and positive controls (ethylmethanesulfonate and cyclophosphamide) were run concurrently with the notified chemical.

Metabolic Activation	*Test Substance Concentration (mM)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0.0063, 0.02, 0.063, 0.2*, 0.63*, 1.25*, 2.5, 5.0, 7.5, 10	4 h	20 h
Test 2	0.0063, 0.02*, 0.063*, 0.2*, 0.63, 1.25, 2.5, 5.0, 7.5, 10	20 h	20 h
Present			
Test 1	0.0063, 0.02*, 0.063*, 0.2, 0.63, 1.25*, 2.5*, 5.0*, 7.5, 10	4 h	20 h
Test 2	0.0055, 0.018, 0.055*, 0.18, 0.554, 1.75, 3.5*, 4.5*, 5.5, 6.5	4 h	20 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (mM) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 1.25	≥ 0.63	≥ 0.2	negative
Test 2		> 10	≥ 0.2	negative
Present				
Test 1	≥ 5	> 10	≥ 0.063	negative
Test 2		≥ 4.5	≥ 0.055	negative

Remarks - Results	In both tests, no biologically relevant increases in the frequency of cells with structural or numerical chromosome aberrations were observed in the presence or absence of metabolic activation. The positive and negative controls gave a satisfactory response confirming the validity of the test system.
CONCLUSION	The notified chemical was not clastogenic to Chinese hamster V79 cells treated in vitro under the conditions of the test.
TEST FACILITY	Bioservice (2012j)

B.14. Genotoxicity – in vivo

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
Species/Strain	Mouse/NMRI
Route of Administration	Intraperitoneal
Vehicle	0.9% aqueous sodium chloride
Remarks - Method	The selection of the highest dose for the main test was based on a preliminary study. Cytotoxicity was assessed by relative PCE (polychromatic erythrocytes) (rel. PCE = proportion of PCE among total erythrocytes). Mutagenic response was indicated by the relevant increase of micronucleated PCEs.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
vehicle control 1	5 per sex	0	24 h
vehicle control 2	5 per sex	0	48 h

low dose	5 per sex	400	24 h
mid dose	5 per sex	1000	24 h
high dose 1	5 per sex	2000	24 h
high dose 1	5 per sex	2000	48 h
positive control, CP	5 per sex	40	24 h

CP=cyclophosphamide

RESULTS

Doses Producing Toxicity	No premature death occurred. Animals in all treatment groups showed dose-dependent mild to moderate signs of systemic toxicity. The signs of moderate systemic toxicity shown in the 2000 mg/kg groups included reduction of spontaneous activity, constricted abdomen, bradykinesia, opisthotonos, piloerection, ataxia, recumbency and half eyelid closure. There was no evidence of cytotoxicity in any treatment groups based on the comparison of relative PCE between treatment groups and negative control groups.
Genotoxic Effects	There were no statistically significant increases in the frequency of micronucleated PCEs.
Remarks - Results	The positive and negative controls gave a satisfactory response confirming the validity of the test system.

CONCLUSION The notified chemical was not clastogenic under the conditions of this in vivo mammalian erythrocyte micronucleus test.

TEST FACILITY Bioservice (2013a)

B.15. Toxicity to reproduction – one generation study

TEST SUBSTANCE Notified chemical

METHOD OECD TG 412 Reproduction/Developmental Toxicity Screening Test

Species/Strain	Rat/Wistar
Route of Administration	Oral – gavage
Exposure Information	Exposure period - female: 14 days pre-mating, 14 days of mating, during gestation and until day 3 post-natal (total 54 days) Exposure period - male: 14 days pre-mating and 14 days of mating
Vehicle	Water
Remarks – Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	10M/10F	0	0/20
Low dose	10M/10F	100	0/20
Mid dose	10M/10F	300	0/20
High dose	10M/10F	1000	0/20

Effects on Parental (P) animals and 1st Filial Generation (F1):

Clinical symptoms including slight piloerection and salivation recorded in male and female animals of the mid-dose and high-dose groups at clinical observations were considered by the study authors to be treatment-related but not toxicologically relevant.

The overall body weight gain was within the normal range of variation of the rat strain tested. Decreases in body weights of male animals of the high-dose group during pre-mating days 7-14 were considered by the study authors to be possibly treatment-related but not toxicologically relevant.

Significant increases in food consumption for high-dose females were not considered by the study authors to be toxicologically relevant. No significant effects on food consumption were noted in other groups.

No treatment-related effects on the litter data (such as the total number of pups born, number of males and females, sex ratio, live pubs on Day 0 and Day 4 post-natal). One still birth in the mid-dose group was considered by the study authors to be incidental.

No remarkable change was noted in litter weight data between the treatment groups and the control group.

No treatment-related effects were seen during the precoital internal or during the gestation when compared with the control group.

The group mean number of corpora lutea, number of implantation sites, number of live pups born on Day 0 post-natal and percentage of post-implantation loss remained unaffected when compared with the control group. Increases in the pre-implantation loss observed in low-dose and high-dose groups were considered by the study authors to be incidental due to lack of dose-response or the increase was rather slight.

Reproductive indices (fertility index, copulation index and delivery index) remained comparable with among all groups.

No significant effects on survival of the pubs from Day 0 to Day 4 post-natal.

No treatment-related findings were seen in any of the treated groups at gross external and gross pathological evaluations.

No remarkable differences in the absolute and relative organ weights of the treatment groups when compared with the control group.

No effects were seen at histopathological evaluation of reproductive organs for parental animals.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 1000 mg/kg bw/day for reproductive/developmental toxicity in this screening study, based on an absence of toxicologically relevant effects.

TEST FACILITY Bioservice (2013b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Ecotoxicological Investigations

C.1.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test -Static.
Species	Zebra fish (*Danio rerio*)
Exposure Period	96 hours
Auxiliary Solvent	Not reported
Water Hardness	10-250 mg $CaCO_3$/L
Analytical Monitoring	HPLC analysis with MS detection.
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

Concentration mg/L	*Number of Fish*	*Mortality*				
Nominal		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	7	0	0	0	0	0
100	7	0	0	0	0	0

LC50	> 100 mg/L at 96 hours
NOEC (or LOEC)	1 mg/L at 96 hours
Remarks – Results	All validity criteria for the test were satisfied. The result is based on the nominal concentration.
CONCLUSION	The notified chemical is not harmful to fish.
Test Facility	DUNAL (2012a)

C.1.2. Chronic toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	The guidelines for the testing of chemicals, SEPA (HJ/T 153-2004) and OECD TG 215 Fish, Juvenile Growth Test- Semi-static.
Species	Rare minnow, (*Gobiocypris rams*),
Exposure Period	28 days (72 h-renewal)
Auxiliary Solvent	Not reported
Water Hardness	122.3 mg $CaCO_3$/L
Analytical Monitoring	ICP-MS detection.
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

Concentration mg/L	*Number of Fish*	*Mortality*				
Nominal		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	10	0	0	0	0	0
10	10	0	0	0	0	0
20	10	0	0	0	0	0
40	10	0	0	0	0	0
80	10	0	0	0	0	0
100	10	0	0	0	0	0

NOEC	100 mg/L at 28 days
LOEC	> 100 mg/L at 28 days
Remarks – Results	All validity criteria for the test were satisfied. The result is based on the nominal concentration.

CONCLUSION The notified chemical is not harmful to fish.

Test Facility NIES (2013)

C.1.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

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

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None reported
Water Hardness	160-180 mg $CaCO_3$/L
Analytical Monitoring	HPLC analysis with MS detection.
Remarks - Method	The test was conducted in accordance with the guideline above and in compliance with GLP standards and principles.

RESULTS

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

LC50	> 100 mg/L at 48 hours
Remarks - Results	The validity criteria were met. There was no effect observed, neither in the limit concentration nor in the control group.

CONCLUSION The notified chemical is not harmful to aquatic invertebrates.

TEST FACILITY DUNAL (2012b)

C.1.4. Chronic toxicity to aquatic invertebrates

Test Substance Notified chemical

Method OECD TG 211 *Daphnia magna* Reproduction Test- Semi-static.

Species	*Daphnia magna*
Exposure Period	21 days
Auxiliary Solvent	None reported
Water Hardness	160-180 mg $CaCO_3$/L
Analytical Monitoring	HPLC analysis with MS detection.
Remarks - Method	The test was conducted in accordance with the guideline above and in compliance with GLP standards and principles.

RESULTS

Concentration mg/L	*Number of D. magna*	*Number Immobilised*		
Nominal		*7 days*	*14 days*	*21 days*
Control	10	0	0	0
100	10	0	0	0

LOEC	> 100 mg/L at 21 days
NOEC	100 mg/L
Remarks - Results	The validity criteria were met. The measured concentrations of the component aluminium in the fresh media (0 h) were the range of 68 to 85 % of the nominal value. The measured concentrations of the notified chemical in the old media (48 and 72 h) were the range of 85 to 99 % of the nominal value, which demonstrates that the test item was fully present throughout the exposure.
CONCLUSION	The notified chemical is not harmful to aquatic invertebrates.
TEST FACILITY	DUNAL (2013a)

C.1.5. 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: 2.56, 6.40, 16.0, 40.0, 100 mg/L (factor 2.50) Geometric Mean Measured: 0.538, 1.10, 2.20, 4.40, 15.5 mg/L
Auxiliary Solvent	None reported
Water Hardness	240 mg $CaCO_3$/L
Analytical Monitoring	HPLC analysis with MS detection
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. Six replicates (without test item) were tested under the same test conditions as the test replicates.

RESULTS

	Biomass		*Growth*	
	E_yC50 mg/L at 72h (95% confidence limit)	*NOEC mg/L at 72 h*	*E_rC50 mg/L at 72h*	*NOEC mg/L at 72 h*
Nominal	59.5 (46.5 - 85.2)	16	> 100	40
Measured	6.11 (4.90 -10.4)	2.20	> 15.5	4.40

Remarks - Results	The test was considered reliable as all validity criteria of the OECD test guideline were satisfied. The results above were based on nominal and geometric mean measured concentrations of the test item. The measured concentration of the homogenized sample was in the range of the nominal value. After a separation phase of 3 hours the concentration of the key element (used to measure the test substance concentration) of the notified chemical in the supernatant corresponded to 17 % of the nominal value. The supernatant was used as the highest test concentration and for the preparation of all other test concentrations. The observed growth inhibition (33.6 %) and yield inhibition in the highest test concentration are not considered substance related but can be interpreted from the lack of essential nutrient salts as a result of the key element in the notified chemical forming insoluble salts with other ions present in the test medium. This has also resulted in the reduced measured test substance concentration in the test medium.
CONCLUSION	The notified chemical is not expected to be harmful to algae.
TEST FACILITY	DUNL (2013b)

C.1.6. Inhibition of microbial activity

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 100, 320, 1000 mg/L
Remarks – Method	The test was conducted according to the guidelines above. No significant deviations from the test guidelines were reported.
RESULTS	
IC50	> 1000 mg/L
NOEC	1000 mg/L
Remarks – Results	All validity criteria for the test were satisfied. The mean inhibition of respiration for the test item replicates ranged from 2% to 24%.
CONCLUSION	The notified chemical is not expected to inhibit microbial respiration.
TEST FACILITY	DUNAL (2012c)

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