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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Nitric acid, calcium potassium salt, hydrate

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 and Energy.

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

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

Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1606	Yara Australia Pty Ltd	Nitric acid, calcium potassium salt, hydrate	Yes	≤ 3,500 tonnes per annum	Component of cement/concrete premix

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 of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

Hazard classification	*Hazard statement*
Acute toxicity, oral (Category 4)	H302 – Harmful if swallowed
Serious eye damage/eye irritation (Category 1)	H318 – Causes serious eye damage

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

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

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

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Acute toxicity, oral (Category 4): H302 – Harmful if swallowed
  - Serious eye damage/eye irritation (Category 1): H318 – Causes serious eye damage

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

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 reformulation:
  - Enclosed, automated processes where possible

- 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 reformulation processes:
  - Avoid contact with skin and eyes
  - Avoid dust formation

- 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 reformulation processes and application:
  - Safety goggles or full face shield
  - Protective clothing
  - Impervious gloves
  - Boots
  - Respiratory protection when dust is likely to be generated

  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 not appropriate, dispose of the notified chemical in an environmentally sound manner in accordance with relevant Commonwealth, state, territory and local government legislation.

Storage

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

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by 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(2) of the Act; if
  - the function or use of the chemical has changed from a component of cement/concrete premix 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.

No additional secondary notification conditions are stipulated.

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Yara Australia Pty Ltd (ABN: 77 076 301 221)
Level 1, 6 Holt Street
MCMAHONS POINT NSW 2060

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: molecular and structural formulae, analytical data, degree of purity, impurities, additives/adjuvants and import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: autoignition temperature and bioaccumulation.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
European Union (REACH) 2010
South Korea (AREC) 2016
New Zealand (NZIoC) 2013

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
NITCAL®-K (product containing the notified chemical at ~85% by weight)

CAS NUMBER
905593-70-6

CHEMICAL NAME
Nitric acid, calcium potassium salt, hydrate

OTHER NAME
Potassium-pentacalcium-nitrate decahydrate

MOLECULAR WEIGHT
Unspecified

ANALYTICAL DATA
Reference IR, UV, XRD and ICP-OES spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 96%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: white solid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	91 – 94 °C	Measured
Boiling Point	> 400 °C at 101.3 kPa	Measured
Density	2090 kg/m^3 at 20 °C	Measured
Vapour Pressure	1.47 × 10^{-11} kPa at 25 °C (or 20 °C)	Measured
Water Solubility	1400 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	The chemical is a salt and is not expected to significantly hydrolyse under environmental conditions (pH 4-9)
Partition Coefficient (n-octanol/water)	log Pow = -1.34 at 20 °C	Measured. Expected to stay in water phase as it is highly soluble in water
Surface Tension	74.1 mN/m at 20 °C	Measured
Adsorption/Desorption	Not determined	Expected to be mobile in water and not expected to absorb to soil or sediment significantly
Dissociation Constant	Not determined	The notified chemical is a salt and will be ionised under environmental conditions
Particle Size	Inhalable fraction (< 100 μm): < 5.5% Respirable fraction (< 10 μm): 1.78% MMAD* = 1420 μm	Measured
Flash Point	Not determined	Inorganic substance.
Flammability (solid)	Not highly flammable	Measured
Flammability (contact with water)	Not highly flammable	Not expected based on chemical structure and solubility in water
Autoignition Temperature	Not self-ignitable	Measured.
Explosive Properties	Not determined	Not expected to be explosive based on chemical structure.
Oxidising Properties	Oxidising	Measured

* MMAD = Mass Median Aerodynamic Diameter

DISCUSSION OF PROPERTIES

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

Reactivity

The notified chemical is expected to be stable under normal conditions of use. The notified chemical has oxidizing potential that may cause or contribute to combustions of other materials.

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 as a component of a concrete additive (at a concentration of 85%) for reformulation into end-use products.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤ 3,500	≤ 3,500	≤ 3,500	≤ 3,500	≤ 3,500

PORT OF ENTRY
Brisbane, Perth, Melbourne, Sydney.

IDENTITY OF RECIPIENTS
Yara Australia Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemical will be imported into Australia in powder form (at 85% concentration) in 1200 kg bulk bags. The notified chemical will be stored at Notifier's warehouse and transported by road to customers for reformulation.

USE
The notified chemical is intended for use as a setting accelerator and corrosion inhibitor in concrete. It will be reformulated into a cement premix at 20 – 50% by weight which will be further blended into cement to contain the notified chemical at 1 – 4% by weight. As the cement containing the notified chemical will be used at ≤ 20% by weight in the final concrete, the concentration of the notified chemical in final concrete products is expected to be ≤ 0.8%.

OPERATION DESCRIPTION
The notified chemical will not be manufactured within Australia. The imported product containing the notified chemical at (85% by weight) will be reformulated with additional components to form the cement premix (containing the notified chemical at ≤ 50% by weight). Reformulation facilities are expected be a mix of automated and manual systems.

The notified chemical may also be directly supplied to construction sites where the chemical will be directly blended into concrete mix for building work.

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)*
Warehouse workers	< 8	12
Blending operators	< 8	< 200
Transport operators	< 8	50
End-users	< 8	< 200

EXPOSURE DETAILS
It is anticipated that transport and warehouse/store personnel would only be exposed to the notified chemical (at up to 85% by weight) in the event of an accident.

Reformulation workers
Dermal, ocular and inhalation exposure to the notified chemical (at up to 85% by weight) may occur during transfer, mixing and blending operations which are expected to use a mix of manual and automated systems. Exposure to the notified chemical is expected to be minimised by the use of safe work practices and personal protective equipment (PPE) including protective clothing, chemical resistant gloves, and chemical goggles as indicated by the notifier. Inhalation exposure to the notified chemical in powder form may occur where dusts containing the chemical are formed. Inhalation exposure may be mitigated using dust suppression measures or through the use of protective face masks.

End-use Workers
When preparing the concrete, dermal and ocular exposure to the notified chemical (at ≤ 50% by weight) are expected to be the main routes of exposure with some potential for inhalation exposure if dusts or mists containing the chemical are formed. Final concrete products (containing the notified chemical at ≤ 0.8%) may be

applied by rolling/brushing, dipping, pouring or spraying. Exposure to the notified chemical is expected to be mitigated by the use of dust suppression measures, enhanced general ventilation and the use of PPE by workers.

Workers may also experience extensive exposure to solidified concrete or cement. However, the notified chemical will be reacted and bound into the concrete matrix during the curing process and is not expected to be available for exposure.

6.1.2. Public Exposure

The notified chemical is primarily intended for industrial use only. However, some do-it-yourself (DIY) construction products (containing the notified chemical at ≤ 50% by weight) will be available to the general public. When preparing the concrete, dermal and ocular exposure to the notified chemical (at ≤ 50% by weight) are expected to be the main routes of exposure with some potential for inhalation exposure if dusts or mists containing the chemical are formed. Final concrete products (containing the notified chemical at ≤ 0.8%) may be applied by rolling/brushing, dipping, pouring or spraying. Exposure to the notified chemical is expected to be mitigated by relevant product labelling and safe use instructions. The notifier stated in the submission that DIY users will be advised to use certain PPE during applications including safety glasses.

Public exposure to solidified concrete containing the notified chemical (added at < 0.8% concentration) is likely to occur. However, the notified chemical will be reacted and bound into the concrete matrix during the curing process and is not expected to 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 300 - 2000 mg/kg bw; harmful
Rat, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	corrosive
Mouse, skin sensitisation – local lymph node assay	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL – 150 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro chromosome aberration test in human lymphocytes	non clastogenic
Genotoxicity – in vitro chromosome aberration test in mouse lymphoma cells	non clastogenic (Analogue 1)
Genotoxicity – in vitro chromosome aberration test in mouse lymphoma cells	non clastogenic (Analogue 2)

Toxicokinetics, metabolism and distribution

No toxicokinetic data on the notified chemical were submitted. For dermal and gastrointestinal absorption, dermal uptake is likely to be high if the water solubility is > 0.1 g/L. Moderate dermal uptake through the epidermis is favoured if the partition coefficient (log P) values are between -1 and 4 (ECHA, 2014). Absorption of the notified chemical through the skin and gastrointestinal tract is not expected based on the partition coefficient (-1.34) and water solubility (1,400 g/L).

Acute toxicity

The notified chemical is of low acute dermal toxicity, and harmful by the oral route based on studies conducted in rats.

Irritation and sensitisation

The notified chemical was non-irritating to the skin, but was corrosive to the eye based on an acute study conducted on rabbits. Moderate to severe conjunctival irritation and slight iridial irritation were observed throughout the study period.

The notified chemical did not show sensitising effects at up to 50% concentration in a local lymph node assay on mice.

Repeated dose toxicity
In a repeated dose (oral) toxicity study in rats, the No Observed Adverse Effect Level (NOAEL) for systemic toxicity was established as 150 mg/kg bw/day based on adverse effects observed in the stomach, spleen and haematology of males and females exposed to the highest dose tested.

Mutagenicity/Genotoxicity
The notified chemical was non-genotoxic in a bacterial reverse mutation assay and in an *in vitro* mammalian chromosome aberration test in human lymphocytes. Two analogues of the notified chemical (which share a similar structure and chemical composition to the notified chemical, and may be present in the end-use product), were also non-mutagenic in *in vitro* mammalian chromosome aberration tests in mouse lymphoma cells.

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

Hazard classification	*Hazard statement*
Acute toxicity, oral (Category 4)	H302 – Harmful if swallowed
Serious eye damage/eye irritation (Category 1)	H318 – Causes serious eye damage

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The notified chemical is considered to cause serious eye damage and be harmful if ingested. Therefore, caution should be exercised when handling the notified chemical.

Workers may experience dermal and accidental ocular exposure to the notified chemical at up to 85% by weight during transfer, mixing and blending operations with the imported product. During the preparation of concrete products exposure to the notified chemical will be at ≤ 50% by weight and when applying the final concrete products at ≤ 0.8% by weight. The use of PPE (including protective clothing, chemical resistant gloves, chemical goggles or full face shield and respiratory protection if necessary) should minimise the potential for exposure. Provided adequate control measures including the use of automated processes, PPE and safe work practices are in place to minimise worker exposure, the risk to workers from the notified chemical is not considered to be unreasonable.

6.3.2. Public Health
Members of the public may be exposed to the notified chemical in solidified concrete (added at < 0.8% concentration). However, as the notified chemical will be reacted and bound into the concrete matrix, exposure to the notified chemical is not expected.

Members of the public may also be exposed to the notified chemical where using do-it-yourself (DIY) construction products (containing the notified chemical at ≤ 50% by weight), similar to end-use workers. The main routes of exposure are expected to be dermal and ocular exposure, with some potential for inhalation exposure if dusts or mists containing the chemical are formed. The notified chemical is considered to cause serious eye damage and be harmful if ingested. However, use by the public is expected to on an infrequent basis and hence, the risk to the public associated with the use of the notified chemical at ≤ 50% by weight 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 into Australia for further formulation into cement admixtures. Environmental release of the notified chemical is expected to be limited to accidental spills arising during the production of cement admixtures, cleaning of equipment and transfer of the additive, and during transport to customers. It is expected that storage containers and mixing vessels will be rinsed with water and the rinsate will

be either used in the formulation of the next batch of additive or processed by licensed waste disposal contractors. Any spills will be collected using adsorbent material and sent to landfill by licensed waste contractors.

RELEASE OF CHEMICAL FROM USE
Release is expected to be minimal at concrete mixing sites and during use by the public, where workers will shovel and rake, consolidate and trowel finish the wet concrete containing low level of the notified chemical. Once the treated cement is incorporated into the concrete, the potential for release of notified chemical into the environment is expected to be negligible. Release of the notified chemical to the environment during use of the concrete admixture by the general public would be limited to the discharge of nominal quantities of water used to clean residual amounts of concrete from equipment. The release into waterways is not expected to be significant.

RELEASE OF CHEMICAL FROM DISPOSAL
The notified chemical in cement admixtures is expected to share the fate of the concrete within which it is bound, and is predominantly expected to be disposed of to landfill at the end of its useful life.

Wastes containing the notified chemical include equipment wash water, empty packaging, container residues, old concrete, and spilt materials. Residues on concrete application equipment are expected to be rinsed, and the wash water collected and allowed to cure before disposal as solid wastes to landfill. Empty packaging, residues, old concrete, and spilt materials will be disposed of as builder's rubble in accordance with local government regulations, most likely to landfill. As a worst case scenario, it is assumed that up to 5% of cement admixture containing the notified chemical used by DIY users may be incorrectly disposed of to the sewer, drains or ground from waste and washing of application equipment.

7.1.2. Environmental Fate

No environmental fate data were submitted for the notified chemical as the substance is inorganic. The notified chemical is a water-soluble salt and will readily dissociate to form ions of nitrate, potassium and calcium upon release into environment. The notified chemical is not expected to be bioaccumulative, based on its high water solubility and low partition coefficient (log P_{OW} = -1.34).

The majority of the notified chemical is expected to be cured within an inert concrete matrix and is expected to share the fate of the concrete, which will involve eventual disposal to landfill. The notified chemical is also expected to enter landfill as collected wastes and residues. Once cured, the notified chemical is not expected to be bioavailable or biodegradable, and thus there will be no significant release to the environment.

7.1.3. Predicted Environmental Concentration (PEC)

The calculation for the predicted environmental concentration (PEC) is summarised in the table below. Based on the reported use in concrete for industrial and DIY-users, a conservative release of 5% of the notified chemical to sewers on a nationwide basis over 365 days per year is used. It also assumes a worst case scenario where none of the notified polymer is removed during STP processes.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	3,500,000	kg/year
Proportion expected to be released to sewer	5%	
Annual quantity of chemical released to sewer	175,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	479.45	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	0%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	106.01	µg/L
PEC - Ocean:	10.6	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1,000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1,500 kg/m^3). Using these assumptions, irrigation with a concentration of 106.01 µg/L may potentially result in a soil concentration of approximately 0.71 mg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 3.53 mg/kg and 7.07 mg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 > 100 mg/L	Not harmful to fish
Daphnia Toxicity	48 h EC50 > 100 mg/L	Not harmful to aquatic invertebrates
Algal Toxicity	48 h E_rC50 > 100 mg/L	Not harmful to algae
Inhibition of Bacterial Respiration	3 h IC50 = 100 mg/L	Not inhibitory to microbial respiration

Based on the above ecotoxicological endpoints, the notified chemical is not expected to be harmful to aquatic life. Therefore, the notified chemical is not formally classified under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009) for acute and chronic toxicities.

7.2.1. Predicted No-Effect Concentration

The predicted no-effects concentration (PNEC) has been calculated using the endpoint of LC50 > 100 mg/L (for fish, Daphnia and algae). A safety factor of 100 was used given acute endpoints for three trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50	> 100	mg/L
Assessment Factor	100	
Mitigation Factor	1.00	
PNEC:	> 1,000	µg/L

7.3. Environmental Risk Assessment

The Risk Quotient (Q = PEC/PNEC) has been calculated based on the predicted PEC and PNEC.

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	106.01	1,000	< 0.106
Q - Ocean	10.60	1,000	< 0.011

The risk quotient for discharge of treated effluents containing the notified chemical to the aquatic environment indicates that the notified chemical is unlikely to reach ecotoxicologically significant concentrations in surface waters, based on its maximum annual import quantity. When used as a component of concrete admixtures, the notified chemical will be irreversibly bound within the inert concrete matrix, and is not likely to be released into the aquatic environment in a bioavailable form. The notified chemical is expected to have a low potential for bioaccumulation based on its high water solubility and low measured log P_{OW}. Therefore, on the basis of its limited aquatic exposure, low predicted toxicity to aquatic organisms, and assessed use pattern, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point 91 – 94 °C

Method	OECD TG 102 Melting Point/Melting Range. EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks	Differential scanning calorimetry
Test Facility	NOTOX (2007a)

Boiling Point > 400 °C at 101.3 kPa

Method	OECD TG 103 Boiling Point. EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks	Differential scanning calorimetry. Boiling was not observed below 400 °C.
Test Facility	NOTOX (2007a)

Density D^{20}_4 = 2.09 (2090 kg/m^3 at 20 °C)

Method	OECD TG 109 Density of Liquids and Solids. EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks	Gas comparison stereopycnometer
Test Facility	NOTOX (2007a)

Vapour Pressure < 1.47 × 10^{-11} kPa at 20 °C

Method	OECD TG 104 Vapour Pressure EC Council Regulation No 440/2008 A.4 Vapour Pressure
Remarks	Isothermal thermogravimetric effusion method
Test Facility	NOTOX (2007a)

Water Solubility 1400 g/L at 20 °C

Method	OECD TG 105 Water Solubility.
Remarks	Flask Method
Test Facility	NOTOX (2007a)

Partition Coefficient (n-octanol/water) log Pow = -1.34 at 20 °C

Method	OECD TG 117 Partition Coefficient (n-octanol/water).
Remarks	Flask Method
Test Facility	NOTOX (2007a)

Surface Tension 74.1 mN/m at 20 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions. EC Council Regulation No 440/2008 A.5 Surface Tension.
Remarks	Ring method Concentration: 1.045 g/L
Test Facility	NOTOX (2007a)

Particle Size MMAD = 1420 µm

Method Laser Diffraction Analysis following manual sieve analysis.

Manual Sieve Analysis

Range (µm)	*Mass (%)*
> 2000	86.42
1000 – 2000	10.14
< 1000	3.44

Laser Diffraction Particle Size Analysis (< 2000 μm fraction)	
Particle size (μm)	*Mass (%)*
< 1586.954	90%
< 982.292	50%
< 204.169	10%

Remarks While the sample was hygroscopic, the 'wet' small volume dispersion system used for the analysis was not suitable for the sample. Only one run was performed. Manual sieving indicated 13.6% of the sample was ≤ 2000 μm. The volume weighted mean was calculated as 946.194 μm with a median (d50) of 982.292 μm and mode of 1189.948 μm.

1.78% by volume of the < 2000 μm fraction was < 10 μm.

Test Facility Chilworth (2007)

Flammability Not highly flammable

Method EC Council Regulation No 440/2008 A.10 Flammability (Solids).

Remarks Test substance did not ignite, but melted when in contact with flame. No smouldering observed when ignition source removed.

Test Facility NOTOX (2007a)

Autoignition Temperature Not self-ignitable

Method EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids.

Remarks Test substance was not self-ignitable between 15 °C and its melting point (91 – 94 °C). Testing for self-ignition as a liquid was not performed as the test substance was too viscous to be injected into the test apparatus when melted.

Test Facility NOTOX (2007a)

Oxidizing Properties Oxidising

Method EC Council Regulation No 440/2008 A.17 Oxidizing Properties (Solids).

Barium Nitrate/Cellulose Mixture (w/w)	*Mean Burning Time (mm/s)*
3:7	0.75
2:3	0.68
1:1	0.67
3:2	0.78

Test Substance/Cellulose Mixture (w/w)	*Mean Burning Time (mm/s)*
1:9	0.28
1:4	0.40
3:7	0.45
2:3	4.00
1:1	5.41
3:2	4.44
7:3	2.82
4:1	0.87
9:1	Not ignited

Remarks The maximum burning rate (1:1 mixture of an analogue to the test substance ($KNO_3.5Ca(NO_3.10H_2O)$ of 5.41 mm/s was significantly higher than the positive control (0.78 mm/s).

Test Facility NOTOX (2007a)

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 Directive 67/548/EEC, Annex V, B.1 tris Acute Oral Toxicity
Species/Strain	Rat/Wistar Crl:WI
Vehicle	Water
Remarks - Method	GLP compliant. No deviations from the protocol.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 F	2,000	3/3
2	3 F	300	0/3
3	3 F	300	0/3

LD50	300 – 2,000 mg/kg bw
Signs of Toxicity	One animal in group 1 was euthanised 2 hours after exposure, while the remaining two animals were found dead 4 hours after exposure. No unscheduled deaths were observed in groups 2 and 3. Prior to death, animals in group 1 exhibited lethargy (3/3), flat (2/3) or hunched (1/3) posture, uncoordinated movements (1/3), shallow respiration (1/3), slow breathing and laboured respiration (2/3), hypothermia (2/3), salivation and red nasal secretions (1/3), piloerection (3/3) and/or pale (2/3) or dark (1/3) snout. Hunched posture was observed in all animals in groups 2 (2 and 4 hours after exposure) and 3 (immediately following exposure, and at the 2 and 4 hour observations). Piloerection was observed in 1/3 animals in group 2, at the 4 hour observation. Recovery from all effects was observed at the Day 2 observations.
Effects in Organs	All animals in group 1 exhibited dark red discolouration of the glandular mucosa of the stomach. No macroscopic abnormalities were observed in animals in groups 2 and 3.
Remarks - Results	All animals in groups 2 and 3 made the expected body weight gains.
CONCLUSION	The notified chemical is harmful via the oral route.
TEST FACILITY	NOTOX (2007b)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity. EC Council Directive 67/548/EEC, Annex V, B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/Wistar Crl:WI
Vehicle	Water
Type of dressing	Occlusive.
Remarks - Method	GLP compliant. No deviations from the protocol.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 M	2,000	0/5
2	5 F	2,000	0/5

LD50	> 2,000 mg/kg bw
Signs of Toxicity - Local	The treated skin area of the animals exhibited slight scales and scabs (5/5 animals, group 1) or scales (4/5) or scabs (1/5) in animals in group 2. Slight maculate erythema was seen with scales in one animal in group 2. These effects were observed from day 3 (5/5 animals in group 1 and 4/5 animals in group 2) of the observation period with the effects persisting in 5/5 animals in group 1 (one animal in this group exhibited recovery from scales at day 6, and onset of scabs at day 15) and 1/5 animals in group 2 (this animal did not start exhibiting scabs until day 10 of the observation period). Recovery was noted in 4/5 animals in group 2.
Signs of Toxicity - Systemic	Animals in group 1 exhibited lethargy (4/5), flat (4/5) and/or hunched (3/5 posture, uncoordinated movements (2/5), shallow respiration (3/5), piloerection (5/5), red nasal secretions (3/5), ptosis (5/5) and/or hypothermia (2/5). Recovery from these effects were noted in all animals by Day 3. Hunched posture (1/5) or red nasal secretions (2/5) were noted among animals in group 2. Recovery from these effects were noted in all animals by Day 3.
Effects in Organs	No abnormalities were found at macroscopic post-mortem examination.
Remarks - Results	No unscheduled deaths occurred. All animals made the expected body weight gains.

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

TEST FACILITY NOTOX (2007c)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Council Directive 67/548/EEC Annex V, B.4 Acute Toxicity (Dermal Irritation/Corrosion).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	Water
Observation Period	72 hours
Type of Dressing	Semi-occlusive.
Remarks - Method	GLP compliant. No significant deviations from the protocol.

RESULTS

Remarks - Results	No skin irritation effects were observed at the 1, 24, 48 or 72 hour observations.

CONCLUSION The notified chemical is non-irritating to the skin.

TEST FACILITY NOTOX (2007d)

B.4. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Council Directive 67/548/EEC Annex V, B.5 Acute Toxicity (Eye Irritation/Corrosion).
Species/Strain	Rabbit/New Zealand White
Number of Animals	1 male
Observation Period	21 days
Remarks - Method	GLP compliant. No deviations from the protocol

RESULTS

Lesion	*Mean Score* Animal No.*	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1			
Conjunctiva: redness	2.6	3	> 21 days	2
Conjunctiva: chemosis	2.6	3	> 21 days	2
Conjunctiva: discharge	2	3	> 21 days	3
Corneal opacity	1	4	> 21 days	4
Iridial inflammation	1	1	> 21 days	1

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

Remarks - Results	Based on the severity of the effects, only one animal was tested. Moderate to severe conjunctival irritation was observed throughout the observation period. The nictitating membrane exhibited signs of necrosis (grey/white discolouration) at the 48 hour observation with recovery indicated at the 14 day observation. Reduced elasticity of the eyelids (72 hour observation) and neovascularisation of the cornea (Day 7 observation) persisted to the end of the observation period. Slight corneal opacity was observed 24 hours after exposure with recovery indicated at the day 7 observation. However, at the Day 21 observation, the cornea was opaque. Slight iridial irritation was observed 1 hour following exposure, and persisted through the observation period (except on Day 7).
CONCLUSION	The notified chemical is corrosive to the eye.
TEST FACILITY	NOTOX (2007e)

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

TEST SUBSTANCE	Notified chemical
METHOD	OECD Section 4, Health Effects, No 429 (2002) Skin Sensitisation: Local Lymph Node Assay EC Council Directive 67/548/EEC Annex V, B.42
Species/Strain	Mouse/CBA
Vehicle	1% watery pluronic L92
Preliminary study	Yes
Positive control	Positive control (α-Hexylcinnamaldehyde) run concurrently with experimental animals.

Remarks - Method
GLP compliant.
No significant protocol deviations.

A preliminary irritation study tested the test substance at 25% and 50% concentration. Slight erythema was observed in both animals. No signs of systemic toxicity were observed in either animal.

RESULTS

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response (mean DPM)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	5 F	161 ± 54	-
5	5 F	166 ± 66	1.0
25	5 F	175 ± 19	1.1
50	5 F	433 ± 149	2.7
Positive Control			
25	5 F	679 ± 224	4.2

Remarks - Results
Slight erythema was observed in all animals exposed to the test substance. No signs of oedema were observed.

Body weight gains in animals exposed to the test substance were not significantly different to animals in the control groups.

Positive and negative controls performed as expected. Slight to well-defined erythema was noted on all ears of animals exposed to the positive control.

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

TEST FACILITY
NOTOX (2007f)

B.6. Repeat dose toxicity

TEST SUBSTANCE
Notified chemical

METHOD
OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).

Species/Strain
Rat/Wistar Crl:(WI) BR

Route of Administration
Oral – gavage

Exposure Information
Total exposure days: 28 days
Dose regimen: 7 days per week

Vehicle
Water

Remarks - Method
GLP compliant.
No significant deviations from the protocol.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 M, 5 F	0	0/5, 0/5
low dose	5 M, 5 F	50	0/5, 0/5
mid dose	5 M, 5 F	150	0/5, 0/5
high dose	5 M, 5 F	1000	0/5, 0/5

Mortality and Time to Death
There were no unscheduled deaths.

Clinical Observations

All animals made the expected body weight gains. No clinical signs of toxicity were attributed to exposure to the test substance. Slight salivation was observed in animals in the high-dose group (3/5 males and 5/5 females) between days 6 and 15. However, this effect is often observed in rats of this age and strain following oral gavage and was considered to be a physiological response by the study authors.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

A statistically significant increase in the percentage of reticulocytes and in the red blood cell distribution width was observed in high-dose males. Females in the high-dose group exhibited a significant decrease in red blood cell count and haematocrit levels.

Significantly lower levels of mean corpuscular haemoglobin concentration (males, mid-dose group) and prothrombin times (females, low- and mid-dose groups). Females in the high-dose group exhibited a statistically significant increase in inorganic phosphate levels. However, a dose-response relationship was not observed with these effects or the study authors indicated that the lower levels were within the physiological range.

Effects in Organs

Thickened limiting ridge (2/5 males and 3/5 females in the high-dose group) and foci in glandular mucosa (1/5 males in the high-dose group exhibited this effect in addition to a thickened limiting ridge) were noted in the stomach. These observations correlated to microscopic observations of hyperplasia/hyperkeratosis of the limiting ridge of the stomach (minimal or slight degree in 4/5 males and 3/5 females in the high-dose group). These effects were not observed in the control, low- or mid-dose groups.

Male animals exhibited pelvic dilation of the kidney (1/5 in the control and high-dose groups), enlarged testis with agenesis of testes and epididymides (1/5 mid-dose group), reddish foci in thymus (1/5 males), while females exhibited reddish foci (1/5 control group) discolouration of the thymus (1/5 mid-dose group), uterus containing fluid (1/5 in each of the control, low- and mid-dose groups) and constricted spleen (1/5 low-dose group).

A slight increase in the severity (minimal or slight to minimal to moderate) of haemopoietic foci (primarily erythroid) was observed in the spleen of males and females in the high-dose group. The increase was not statistically significant, although a positive trend was recorded in females. These observations were not considered to be of toxicological relevance by the study authors as no microscopic correlate was recorded, a dose-response relationship was not observed, the effects were within the range of background pathology observed in rats of this age and strain, and the effect occurred at similar incidences and severity in both control and exposed rats.

No toxicologically significant changes were observed in the organ weights and organ to body weight ratios of animals exposed to the test substance.

Remarks – Results

No toxicologically significant changes were noted in clinical appearance, functional observations, body weight, food consumption and organ weights.

A thickened limiting ridge and/or foci in glandular mucosa in the stomach of animals in the high-dose group was correlated by the presence of minimal to slight hyperplasia/hyperkeratosis.

A slight increase in the severity of (primarily erythroid) haemopoietic foci (minimal or slight to minimal to moderate) in the spleen was observed in males and females in the high-dose group. While the observation was not statistically significant, a positive trend was observed in females. Slight changes in the haematology of males and females in the high-dose group (increased reticulocyte percentage and red blood cell distribution width in males and decreased red blood cell turnover in females) were correlated with the microscopic changes observed in the spleens of this group. The study authors observed that this combination of observations may be an indication of increased red blood cell turnover with the potential for slight extramedullary haemopoiesis in the spleen.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 150 mg/kg bw/day in this study, based on adverse effects observed in the stomach, spleen and haematology of males and females exposed to the highest dose.

TEST FACILITY NOTOX (2007g)

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	S9 fraction from phenobarbital/β-naphthoflavone induced rat liver
Concentration Range in Preliminary Test (Test 1)	a) With metabolic activation: 3, 10, 33, (TA100 only), 100, 333, 1,000, 3,300, 5,000 µg/plate b) Without metabolic activation: 3, 10, 33, (TA100 only), 100, 333, 1,000, 3,330, 5,000 µg/plate
Concentration Range in Main Test (Test 2)	a) With metabolic activation: 100, 333, 1,000, 3,300, 5,000 µg/plate b) Without metabolic activation: 100, 333, 1,000, 3,330, 5,000 µg/plate
Vehicle	Water
Remarks - Method	GLP compliant. No significant deviations from the protocol.

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	-	≥ 3,330	negative
Test 2		> 5,000	> 5,000	negative
Present				
Test 1	> 5,000	-	≥ 3,330	negative
Test 2		> 5,000	> 5,000	negative

Remarks - Results
In test 1, precipitation of the test substance was observed at the start of the incubation period at concentrations of 3,330 µg/plate and 5,000 µg/plate. No precipitate was observed at the end of the incubation period.

Under the conditions of test 1 and 2, no reduction in the bacterial background lawn or biologically relevant decrease in the number of revertants was observed in the presence or absence of metabolic activation. No significant dose-related increase in the number of revertant colonies, in the presence or absence of metabolic activation was observed.

Positive and negative controls performed as expected

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

TEST FACILITY NOTOX (2007h)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test. EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.
Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 fraction from phenobarbital/β-naphthoflavone induced rat liver.
Vehicle	Water
Remarks - Method	GLP compliant. No significant deviations from the protocol. Dose range finding study performed. Positive controls: mitomycin C (absence of metabolic activation) and cyclophosphamide (presence of metabolic activation).

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	333*, 1,000*, 3,330*	3	24
Test 2a	333*, 1,000*, 3,000*, 4,000, 5,000	24	24
Test 2b	100*, 333, 1,000*, 2,000*, 3,000, 4,000, 5,000	48	48
Present			
Test 1	333*, 1,000*, 3,330*	3	24
Test 2	333*, 1,000*, 3,330*	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	≥ 3,330	> 3,330	> 3,330	negative
Test 2a		≥ 3,000	≥ 3,000	negative
Test 2b		≥ 2,000	≥ 2,000	negative
Present				
Test 1	> 3,330	> 3,330	> 3,330	negative
Test 2		> 3,330	> 3,330	negative

Remarks - Results	No statistically significant or biologically relevant increase in the number of cells with chromosome aberrations, polyploid cells and cells with endoreduplicated chromosomes were observed in the presence or absence of metabolic activation in test 1 or test 2. Positive and negative controls performed as expected.
CONCLUSION	The notified chemical was not clastogenic to human lymphocytes treated in vitro under the conditions of the test.
TEST FACILITY	NOTOX (2007i)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE	Analogue 1
METHOD	OECD TG 476 In vitro Mammalian Cell Gene Mutation Test. EC No. 440/2008 B.17 Mutagenicity - In vitro Mammalian Cell Gene Mutation Test.

Species/Strain	Mouse
Cell Type/Cell Line	Lymphocytes/ L5178Y/TK$^{+/-}$-3.7.2C
Metabolic Activation System	S9 fraction from phenobarbital/β-naphthoflavone induced rat liver.
Vehicle	Dimethyl sulfoxide
Remarks - Method	GLP compliant. No significant deviations from the protocol. Dose range finding study performed. Positive controls: methyl methane sulfonate (absence of metabolic activation) and cyclophosphamide (presence of metabolic activation).

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1a	10, 100, 333, 666, 1,000, 1,250, 1,500, 1,750, 2,000, 2,250, 2,500	3	48	48
Test 1b	10, 50*, 100*, 200*, 300*, 350*, 375*, 400*, 450*, 475, 500, 525, 550, 575	3	48	48
Test 2a	100, 125, 150, 175, 200, 225, 250, 275, 300, 315, 333	24	48	48
Test 2b	10, 100, 150, 200, 250, 275, 300, 310, 320, 325, 330, 335, 340	24	48	48
Test 2c	10, 100, 200, 250, 300*, 310, 320*, 330, 340, 350*, 360*, 370*, 380*, 390*, 400*	24	48	48
Present				
Test 1a	10, 100, 333, 1,000, 1,750, 2,500, 3,000, 3,250, 3,500, 3,750, 4,000, 4,250	3	48	48
Test 1b	10, 100, 300, 1,000, 2,000, 2,500, 3,000, 3,250, 3,500, 3,750, 4,000	3	48	48
Test 1c	10*, 100*, 600*, 1,000*, 2,000*, 2,250, 2,500*, 2,750*, 3,000*, 3,250, 3,500, 3,750, 4,000	3	48	48
Test 2	10*, 100*, 600*, 1,000*, 2,000*, 2,250, 2,500, 2,750*, 3,000*, 3,250*, 3,500, 3,750, 4,000	3	48	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 1b	≥ 2,510	≥ 400	> 450	negative
Test 2c		≥ 350	≥ 320	negative
Present				
Test 1c	≥ 2,510	≥ 2,750	≥ 2,000	negative
Test 2		≥ 3,250	≥ 2,000	negative

Remarks - Results

Test 1 was repeated twice in the absence of metabolic activation and three times in the presence of metabolic activation in order to obtain suitable levels of cell survival and cytotoxicity. Test 2 was repeated three times in the absence of metabolic activation in order to obtain suitable levels of cell survival and cytotoxicity.

In the absence or presence of metabolic activation, the relative total growth of the highest test substance was reduced by 80 - 88% (test 2 and 1 respectively) or 67 – 80% (test 2 and 1 respectively) respectively.

No significant increase in the mutation frequency at the TK locus, or in the numbers of small and large colonies was observed in the presence or

absence of metabolic activation in either of test 1 or 2.

Positive and negative controls performed as expected in the absence and presence of metabolic activation.

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

TEST FACILITY NOTOX (2010a)

B.10. Genotoxicity – in vitro

TEST SUBSTANCE Analogue 2

METHOD OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
EC No. 440/2008 B.17 Mutagenicity - In vitro Mammalian Cell Gene Mutation Test.

Species/Strain	Mouse
Cell Type/Cell Line	Lymphocytes/ L5178Y/TK$^{+/-}$-3.7.2C
Metabolic Activation System	S9 fraction from phenobarbital/β-naphthoflavone induced rat liver.
Vehicle	RPMI 1640 medium
Remarks - Method	GLP compliant. No significant deviations from the protocol. Dose range finding study performed. Positive controls: methyl methane sulfonate (absence of metabolic activation) and cyclophosphamide (presence of metabolic activation).

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	1*, 3*, 10*, 33*, 100*, 333*, 666*, 1011*	3	48	48
Test 2	1*, 3*, 10*, 33*, 100*, 333*, 666*, 1011*	24	48	48
Present				
Test 1	1*, 3*, 10*, 33*, 100*, 333*, 666*, 1011*	3	48	48
Test 2	1*, 3*, 10*, 33*, 100*, 333*, 666*, 1011*	3	48	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	> 1011	> 1011	> 1011	negative
Test 2		> 1011	> 1011	negative
Present				
Test 1	> 1011	> 1011	> 1011	negative
Test 2		> 1011	> 1011	negative

Remarks - Results No toxicity was observed in the presence or absence of metabolic activation in tests 1 or 2.

No significant increase in the mutation frequency at the TK locus, or in the numbers of small and large colonies was observed in the presence or absence of metabolic activation in tests 1 or 2.

Positive and negative controls performed as expected in the absence and presence of metabolic activation.

CONCLUSION	The test substance was not clastogenic to mouse lymphoma treated in vitro under the conditions of the test.
TEST FACILITY	NOTOX (2010b)

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	*Cyprinus carpio* (Carp)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	180 mg $CaCO_3$/L
Analytical Monitoring	Not reported
Remarks – Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. A test solution was prepared at a nominal concentration of 100 mg/L. No special treatment other than thorough mixing was necessary to completely dissolve the test substance in test medium.

RESULTS

Concentration mg/L Nominal	*Number of Fish*	*Mortality (%)*			
		24 h	*48 h*	*72 h*	*100 h*
Control	7	0	0	0	0
100	7	0	0	0	0

LC50	> 100 mg/L at 96 hours
NOEC	100 mg/L at 96 hours.
Remarks – Results	All validity criteria of the test guideline were satisfied. Analytical confirmation of actual exposure concentrations were not performed as the test substance consisted of inorganic salts which are partly present in the test medium or could only be analysed by non-specific spectrophotometric analytical method. Results were based on nominal concentrations as the test substance was soluble and the inorganic salts present are all known to be stable in aquatic media.
CONCLUSION	The notified chemical is not considered to be harmful to fish.
TEST FACILITY	NOTOX (2007j)

C.1.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	180 mg $CaCO_3$/L
Analytical Monitoring	Not reported
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. A test solution was prepared at a nominal concentration of 100 mg/L. No special treatment other than thorough mixing was necessary to completely dissolve the test substance in test medium.

RESULTS

Concentration mg/L *Nominal*	*Number of D. magna*	*Cumulative Immobilised (%)* *24 h*	*48 h*
Control	20	0	0
100.0	20	0	0

LC50	> 100 mg/L at 48 hours
NOEC (or LOEC)	100 mg/L at 48 hours
Remarks - Results	All validity criteria of the test guideline were satisfied. Analytical confirmation of actual exposure concentrations were not performed as the test substance consisted of inorganic salts which are partly present in the test medium or could only be analysed by non-specific spectrophotometric analytical method. Results were based on nominal concentrations as the test substance was soluble and the inorganic salts present are all known to be stable in aquatic media.
CONCLUSION	The notified chemical is not considered to be harmful to aquatic invertebrates.
TEST FACILITY	NOTOX (2007k)

C.1.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Fresh Water Algal, Growth Inhibition Test - Static
Species	*Pseudokirchneriella subcapitata (green alga)*
Exposure Period	… hours
Concentration Range	Nominal: 100 mg/L Actual: Not determined
Auxiliary Solvent	None
Water Hardness	24 mg $CaCO_3$/L
Analytical Monitoring	Not reported
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. A test solution was prepared at a nominal concentration of 100 mg/L. No special treatment other than thorough mixing was necessary to completely dissolve the test substance in test medium.

RESULTS

Biomass		*Growth*	
E_yC50 *mg/L at 48 h*	*NOE_yC* *mg/L*	*E_rC50* *mg/L at 48 h*	*NOE_rC* *mg/L*
> 100	100	> 100	100

Remarks - Results	All validity criteria of the test guideline were satisfied. Analytical confirmation of actual exposure concentrations were not performed as the test substance consisted of inorganic salts which are partly present in the test medium or could only be analysed by non-specific spectrophotometric analytical method. Results were based on nominal concentrations as the test substance was soluble and the inorganic salts present are all known to be stable in aquatic media.
CONCLUSION	The notified chemical is not harmful to algae.
TEST FACILITY	NOTOX (2007l)

C.1.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sewage sludge
Exposure Period	3 hours
Concentration Range	Nominal: 100 mg/L Actual: Not determined
Remarks – Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. 0.5 g/L stock solution was prepared by addition of 124.5 mg test substance to 250 mL of Milli-RO water followed by thorough mixing. A concentration of 100 mg/L, corresponding to 100 mL of the prepared stock solution in 500 mL final volume was tested in duplicate. 3,5-Dichlorophenol was used as the reference substance.
RESULTS	
IC50	> 100 mg/L at 3 hours
NOEC	Not determined
Remarks – Results	All validity criteria of the test guideline were satisfied. The 3 h IC50 was determined to be > 100 mg/L based on nominal concentrations. The duplicate measurement confirmed the result of the first measurement.
CONCLUSION	The notified chemical is not inhibitory to microbial respiration.
TEST FACILITY	NOTOX (2007m)

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