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April 2009

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

L-Glutamic acid, N,N-bis(carboxymethyl)-, tetrasodium salt

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

For the purposes of subsection 78(1) of the Act, this Full Public Report may be inspected at our NICNAS office by appointment only at 334-336 Illawarra Road, Marrickville NSW 2204.

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

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Director
NICNAS

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Akzo Nobel Pty Ltd (ABN 59 000 119 424 51)
8 Kellaway Place Wetherill Park NSW 2164

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: Details of use, Purity, Import volume, Spectral data

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: Water solubility, Hydrolysis as a function of pH, Adsorption/desorption, Flammability, Acute dermal toxicity, Acute inhalation toxicity.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
No

NOTIFICATION IN OTHER COUNTRIES
USA

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Dissolvine GL-38 (contains 38% notified chemical in an aqueous solution)
Dissolvine GL-74 (contains 74% notified chemical as a powder)

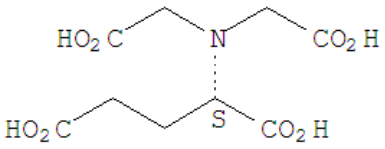
CAS NUMBER
51981-21-6

CHEMICAL NAME
L-Glutamic acid, N, N-bis(carboxymethyl)-, tetrasodium salt

OTHER NAME(S)
Tetrasodium glutamate diacetate (INCI)
Tetrasodium, N,N-bis(carboxylatomethyl)-L-glutamate
Glutamic acid diacetate tetrasodium salt
Glutamic acid N, N-diacetic acid sodium salt
L-Glutamic acid-N, N-di(acetic acid) tetrasodium salt
GLDA
GBS-5
Dissolvine GL-PD
GLDA-Na_4

MOLECULAR FORMULA
$C_9H_{13}NO_8.4Na$

STRUCTURAL FORMULA



• 4 Na

MOLECULAR WEIGHT
351 Da

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

3. COMPOSITION

DEGREE OF PURITY > 90%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS

Chemical Name	Sodium hydroxide		
CAS No.	1310-73-2	*Weight %*	0.5-1.9 (in the products Dissolvine GL-38 and Dissolvine GL-74)
Hazardous Properties	Conc ≥ 5% C; R35 2% ≥ Conc > 5% C; R34 0.5% ≥ Conc > 2% Xi; R36/38		

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (>1% by weight) None

ADDITIVES/ADJUVANTS None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Light yellow liquid (Dissolvine GL-38) or white powder (Dissolvine GL-74)

Property	Value	Data Source/Justification
Melting Point/Freezing Point	Not determined	Decomposes without melting at 380-400°C
Boiling Point	Not determined	Decomposes prior to boiling
Density	1150-1380 kg/m^3 at	MSDS for Dissolvine GL-38
Vapour Pressure	0.08 kPa at 20°C	Measured
Water Solubility	~ 500 g/L at 20°C	The solubility limit has not been determined because concentrated aqueous solutions act like a gel.
Hydrolysis as a Function of pH	Stable at pH 4, 7 and 9	The notified chemical contains no readily hydrolysable functionality
Partition Coefficient (n-octanol/water)	log Pow = - 12 at 20°C	Estimated. The negative value reflects the high water solubility and low solubility in lipids (< 0.1%).
Adsorption/Desorption	Not determined	The notified chemical is expected to show very weak soil adsorption because of its hydrophilicity.
Dissociation Constant	pKa = 9.36, 5.03, 3.49 and 2.56	The acid dissociation constants were determined from the neutralisation

		curve.
Particle Size	≤ 400 μm. *Particle size distribution:* ≤ 240 μm: 90% ≤ 142 μm: 50% ≤ 68 μm: 10%	Technical data (Dissolvine GL-PD: Particle size distribution & bulk density, Akzo Nobel)
Flash Point	Not determined	Not expected to be flammable as it is an organic salt
Flammability	Not determined	Not expected to be flammable as it is an organic salt.
Autoignition Temperature	> 600°C	Measured
Explosive Properties	Not determined	Not expected to be explosive based on lack of structural groups for explosivity
Dust Explosivity	Minimal sensitivity on ignition to dust explosions	Measured

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

Reactivity
Stable under the following recommended storage conditions: Avoid contact with strong oxidisers, aluminium, nickel, zinc, copper alloys and store in PVC, PE, stainless steel or bituminised tanks only.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
Imported into Australia at 38% in aqueous solution or at 74% in solid powder.

MAXIMUM INTRODUCTION VOLUME OF NOTIFIED CHEMICAL OVER NEXT 5 YEARS

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 100	< 100	< 100	< 125	< 150

PORT OF ENTRY
Sydney

TRANSPORTATION AND PACKAGING
The notified chemical will be imported in 250 kg net plastic drums or 1100 kg net bulk containers. The finished product will be packed in containers ranging from 1 to 5 L.

USE
The notified chemical will be used as a chelating agent in commercial cleaning products at concentrations < 10%.

OPERATION DESCRIPTION

The notifier provided the following descriptions outlining the typical scenario involving reformulation of the notified chemical:

The aqueous solution containing 38% notified chemical will be pumped into stainless steel water-jacketed vessels using air-operated diaphragm pumps and mixed with water and other components to give the finished cleaning product containing the notified chemical at < 10%. The powder form of the notified chemical will be manually transferred out of the import container and weighed before being added to the blending tank. After mixing, the product will be automatically filled into plastic bottles and capped. Technical personnel may check the product for quality control purposes throughout the operation. Samples of the raw material are taken from the drum using a dipper and transferred to a labelled plastic container and the final product is sampled via a sample port in the batch tank to a plastic container.

Sales personnel will use the finished cleaning products during demonstration to end-users (cleaning staff, cafes/restaurants, car washes etc). End-users will use the products on surfaces using cleaning equipment such as sponges or mops depending on the application.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	6-8	4	100
Production	15	4	50
Technical	3	1	50
Sales	100	1	250
End users	5000^{+}	1	250

EXPOSURE DETAILS
Waterside workers, truck drivers and warehouse workers are not likely to be exposed to the notified chemical except in the event of an accident.

Dermal, ocular and inhalation exposure may occur during transfer of the notified chemical to the water-jacketed vessel and during the mixing and formulation stages. The powder form of the notified chemical is expected to be weighed within a fume hood thereby reducing inhalation exposure. Aerosols and vapours could potentially be generated during mixing but exposure is expected to be minimised by the use of ventilation extractors in the area above the mixing vessels. Workers are expected to wear overalls, protective gloves, safety glasses and protective footwear to avoid contact from drips, spills and splashes when connecting and disconnecting lines and during system cleaning and maintenance.

Technical staff may experience inhalation and dermal exposure when checking raw materials or finished goods for specification compliance. These tasks are expected to be performed in fume cupboards and gloves and safety glasses are worn when handling chemicals to minimise exposure.

Sales personnel and end users are likely to come into skin contact with finished products containing < 10% notified chemical. Exposure to end-users may be limited by wearing overalls, enclosed shoes and gloves.

6.1.2. Public exposure
The notified chemical and finished products are not sold directly to the general public and exposure is not expected.

6.2. Human health effects assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	oral LD50 > 2000 mg/kg bw low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation – adjuvant test	no evidence of sensitisation
Rat, repeat dose oral toxicity – 90 days	NOAEL = 300 mg/kg/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro*, mammalian cell gene mutation test	non genotoxic
Genotoxicity – *in vitro*, mammalian chromosome aberration test	weakly genotoxic at high doses
Genotoxicity – *in vivo*, micronucleus test	non genotoxic

Acute toxicity
There were no signs of toxicity and no deaths in rats given an oral dose of up to 2000 mg/kg bw/day. The notified chemical has low acute oral toxicity. No acute toxicity data are provided on dermal and inhalation toxicity of the notified chemical.

Irritation and Sensitisation
In a dermal irritation test on rabbits, very slight erythema was observed in 2 animals 1 hour after exposure, but this had resolved by 24 hours. In an eye irritation test, conjunctival redness was observed in 3/3 animals tested 1 hour after exposure. One animal showed conjunctival redness at the 24-hour observation point, but this resolved within 48 hours. The notified chemical is considered only slightly irritating to the skin or eyes.

A Magnusson and Kligman Maximisation study in guinea pigs was conducted using challenge doses of up to 50% notified chemical. There was no dermal response in any animal, thus the notified chemical is considered to be non-sensitising to the skin of guinea pigs.

Repeated Dose Toxicity (sub acute, sub chronic, chronic)
There were no signs of toxicity in clinical observations and no changes were observed during macroscopic and microscopic organ examinations. At 1000 mg/kg bw/day, changes to blood cell parameters, blood chemistry and urine were observed in animals of both sexes. At 300 mg/kg bw/day, a limited number of statistically significant changes were observed on haematology (reduced MCH) and clinical and urine biochemistry (increased sodium concentration, decreased alkaline phosphatase and bilirubin, decreased calcium levels). These were considered to be non-adverse and so the NOAEL is considered to be 300 mg/kg bw/day in this study.

Mutagenicity
An Ames test on *Salmonella* strains showed no significant increase in the numbers of revertant colonies for any strain at any dose, either with or without metabolic activation. In an *in vitro* mammalian gene forward mutation test in Chinese hamster ovary (CHO) cells, no significant increase in the mutant frequency was observed at any dose level, either with or without metabolic activation. The notified chemical is considered non mutagenic under the conditions of these tests.

An *in vitro* mammalian chromosome aberration test on Chinese hamster lung (CHL) cells showed a small but statistically significant increase in aberrant cells at the highest doses (1825 and 3650 μg/ml) in three exposure groups in the presence and absence of metabolic activation. The notified chemical is considered weakly clastogenic to mammalian CHL cells at high doses in this test. The test material was shown to be toxic to CHL cells *in vitro* with a very steep dose response curve and the weak response is considered to be a result of toxicity-induced cell cycle delay.

An *in vivo* micronucleus test using mice bone marrow on doses up to 400 mg/kg bw did not show a significant increase in the frequency of polychromatic erythrocytes in the 24 and 48-hour sampling groups, but a small increase was observed in the 400 mg/kg test group at 72 hours after dosing. This increase was still within the normal range for the vehicle control and the result was not considered to be toxicologically significant. The notified chemical is not clastogenic under the conditions of this micronucleus test.

Health hazard classification
Based on the available data the notified chemical is not classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
There is potential for workers to experience dermal, ocular and inhalation exposure during formulation of the notified chemical, including exposure to sodium hydroxide which is present as an impurity in the imported product and presents a hazard for skin and eyes. The concentration of sodium hydroxide is not significant in formulated products and is not expected to pose a health risk for end-users. Exposure will be minimised by the use of proposed engineering controls such as ventilation and fume hood and PPE (overalls, protective gloves, safety glasses and protective footwear). The risk is not considered to be unacceptable when engineering controls and PPE are used.

6.3.2. Public health

As the public is unlikely to come into contact with the notified chemical, the risk of adverse effects on public health is not expected.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE
Mixing tanks are cleaned with water after each batch. On-site treatment (neutralisation and sedimentation) of the resulting washings is not expected to remove much of the notified chemical before discharge to sewer.

RELEASE OF CHEMICAL FROM USE
The notified chemical is a component in cleaning products, and hence, ultimately all of the imported volume of the chemical could enter the aquatic environment when the cleaning products or scouring pads are rinsed down the sink and into the sewer during cleaning application.

RELEASE OF CHEMICAL FROM DISPOSAL
Wastes from spills and container residues are expected to be washed to sewer.

7.1.2 Environmental fate

The notified chemical is not volatile, is highly water soluble, and therefore is expected to partition mainly into the aquatic compartment. However, owing to its chelating ability, the chemical is expected to have a high affinity to the metal cations in the sewer and in soils and sediments, and hence some of the chemical may form insoluble precipitates that will settle out into sludge. As the chemical is readily biodegradable, with 77% being degraded in a ready biodegradation test, some biodegradation may also occur in the sewer.

A substantial proportion of the imported quantity of the notified chemical is likely to be discharged from sewage treatment works into receiving waters. In the natural aquatic environment, the notified chemical will disperse and biodegrade. A fraction may partition into sediments, most likely through complexing with mineral cations such as calcium and magnesium on the surfaces of suspended sediments. In soil/sediment environments, the notified chemical is expected to undergo fairly rapid biodegradation.

7.1.3 Predicted Environmental Concentration (PEC)

The PECs can be determined based on the assumption of complete release to receiving waters, as outlined below.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	150,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	150,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	411	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	21.374	million
Removal within STP	0%	
Daily effluent production:	4,275	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.096	µg/L
PEC - Ocean:	0.0096	µg/L

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 > 70.7 mg/L	Not harmful
Daphnia Toxicity	EC50 > 70.7 mg/L	Not harmful
Algal Toxicity	EC50 > 100 mg/L	Not harmful
Inhibition of Bacterial Respiration	EC50 > 412 mg/L	Not harmful

The EC50s in fish and daphnids have been corrected to reflect the purity of the test substance. As the test substance has good water solubility, and no harmful effects were seen in any of the test organisms, the EC50s in these species would be expected to exceed 100 mg/L. The notified chemical is not harmful to aquatic life, consistent with its high water solubility.

7.2.1 Predicted No-Effect Concentration

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
Acute toxicity to fish and daphnids	> 70.7	mg/L
Assessment Factor	100	
PNEC:	> 707	µg/L

7.3. Environmental risk assessment

The risk quotients (PEC/PNEC) are tabulated below.

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.096	> 707	**< 1.36 x 10^{-4}**
Q - Ocean	0.0096	> 707	**< 1.36 x 10^{-5}**

The PEC/PNEC ratios in the natural aquatic environment are much less than 1, indicating low risk for aquatic organisms.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

Based on the data provided, the notified chemical is not classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* [NOHSC: 1008(2004)].

Human health risk assessment

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

When used in the proposed manner, the notified chemical is not considered to pose an unacceptable 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 a risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- Avoid skin and eye contact with the notified chemical.
- Avoid inhalation of dust of the notified chemical.

- A copy of the MSDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Disposal

- The notified chemical should be disposed of by landfill.

Storage

- The following precautions should be taken regarding storage of the notified chemical:
  - Avoid contact with strong oxidisers, aluminium, nickel, zinc, copper alloys
  - Store in PVC, PE, stainless steel or bituminised tanks only.

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from an ingredient in commercial cleaning products or is likely to change significantly;
  - the amount of chemical being introduced has increased from 150 tonnes or is likely to increase, significantly;
  - if the chemical has begun to be manufactured in Australia;
  - additional information has become available of 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 MSDS of the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Vapour Pressure 0.08 kPa at 20ºC

Method Isothermal measurement of the vapour pressures of a solid (in-house method, Akzo Nobel. The dust is inserted in a stainless steel vessel that is equipped with a thermo stated heating/cooling jacket and is connected to a vacuum system. After evacuating the air from the vessel, the pressure is measured over a period of time using a micromembrane pressure transducer.
Remarks A constant increase in pressure was observed over time with increasing temperature.
Test Facility Akzo Nobel Technology & Engineering (2008a)

Water Solubility > 500 g/L at 20ºC

Method Experience and observation.
Remarks A formal test has not been conducted. The notified chemical evidently has high water solubility, but the solubility limit cannot be determined as concentrated solutions act like a gel. The imported material containing 38% of the notified chemical is an aqueous solution.

Hydrolysis as a Function of pH

Method A formal test has not been conducted. The notified chemical evidently has high hydrolytic stability, as it is imported in aqueous solution and has no readily hydrolysable functionality.

Partition Coefficient (n-octanol/water) log P_{ow} = - 11.95 at 20ºC

Method Computer based estimation (fragment method).
Remarks The estimation method may be somewhat inaccurate for ionic compounds, but the conclusion that the log P_{ow} will be negative is considered sound.
Test Facility Akzo Nobel Technology & Engineering (2007a)

Adsorption/Desorption
– screening test

Remarks The test was not conducted. Sorption is expected to be weak based on the low partition coefficient.

Dissociation Constant pKa = 9.36, 5.03, 3.49, 2.56

Method The four acid dissociation constants of the notified chemical can be determined from the neutralisation curve of the tetra-acetic acid with hydroxide.
Remarks The neutralisation curve is presented in a brochure dated March 2004 on Dissolvine GL. The acid dissociation constants are similar to those determined for EDTA (10.29, 6.16, 2.67, 1.99).

Autoignition Temperature > 600ºC

Method Determination of the Autoignition temperature (in-house method, Akzo Nobel).
Dust is sprayed in a vertically positioned electrical furnace. The air pulse pressure and concentration is optimised to find the lowest temperature at which a dust cloud of the test material can be ignited.
Remarks No ignition occurred at any temperature up to 600ºC when subjected to various air pressures.
Test Facility Akzo Nobel Technology & Engineering bv (2008b)

Dust Explosivity Minimal sensitivity on ignition to dust explosions

Method	Determination of the dust explosion data (in-house method, Akzo Nobel). *Dust explosion*: Notified chemical was blown through a dispersion system using an air push into a 20 L sphere, which was then evacuated to compensate for the pressure rise due to the air push. After a short time delay, chemical ignition sources (10kJ) were activated and two pressure transducers recorded the pressure-time history. Several tests were performed over a wide dust concentration range. From the pressure-time curve, the average maximum pressure (P_{max}) and maximum rate of pressure rise (K_{max}) in a standardised 1m^3 vessel was calculated. *Minimum ignition energy*: Dust samples of known concentrations were suspended in a 1.2 L tube. After a short time delay, a spark was generated between the two electrodes inside the dust/air mixture, and the ignition energy varied until no ignition occured. Visual observation of ignition of the mixture was conducted.
Remarks	Minimum ignition energy > 1000 mJ (at ambient temperature) Maximum explosion pressure, P_{max} = 1.62 bar at a dust concentration of 250-500 g/m^3 Maximum dust explosion class value, K_{max} = 24 bar.m.s^{-1} at dust concentration range of 62.5-500 g/m^3
Test Facility	Akzo Nobel Technology & Engineering bv (2008b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical (> 70% purity)
METHOD	EC Directive 92/69/EEC B.1bis Acute Toxicity (Oral) Fixed Dose Method
Species/Strain	Rat/Sprague-Dawley
Vehicle	Distilled water
Remarks - Method	Two dose levels (500 and 2000 mg/kg bw) were tested in the range-finding study on two female rats. Based on the lack of signs of toxicity, 5 female and 5 male rats were dosed at 2000 mg/kg bw in the main study.
RESULTS	
LD50	> 2000 mg/kg bw
Signs of Toxicity	No sign of toxicity.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks - Results	There were no deaths and all animals gained expected bodyweight over the study period.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Safepharm (1994a)

B.2. Irritation – skin

TEST SUBSTANCE	Notified chemical (> 70% purity)
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of animals	3
Vehicle	Moistened with distilled water
Observation Period	72 hours
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations.
RESULTS	
Remarks - Results	Very slight erythema was observed in 2 animals at the 1 hour observation, but these reactions had cleared by 24 hours.
CONCLUSION	The notified chemical is slightly irritating to the skin.
TEST FACILITY	Safepharm (1994b)

B.3. Irritation – eye

TEST SUBSTANCE	Notified chemical (> 70% purity)
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).
Species/Strain	Rabbit/New Zealand White
Observation Period	72 hours
Remarks - Method	The study report did not specify the use of a vehicle for the test material.

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	0.3	0.0	0.0	1.0	< 48 hours	0
Conjunctiva: chemosis	0.0	0.0	0.0	0.0	0	0
Conjunctiva: discharge	0.0	0.0	0.0	0.0	0	0
Corneal opacity	0.0	0.0	0.0	0.0	0	0
Iridial inflammation	0.0	0.0	0.0	0.0	0	0

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

Remarks - Results: All three treated eyes showed conjunctival redness 1 hour after treatment, and one displayed chemosis. These symptoms resolved in two animals by the 24-hour observation.

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

TEST FACILITY: Safepharm (1994c)

B.4. Skin sensitisation

TEST SUBSTANCE: Notified chemical (> 70% purity)

METHOD: Japanese Ministry of Agriculture, Forestry and Fisheries Testing Guidelines for Toxicology Studies, 59 NohSan No. 4200 (1985)
US EPA Pesticides Assessment Guidelines Subdivision F; Hazard Evaluation and Domestic Animals Section 81-6 – Dermal Sensitisation Study (1984)
US EPA Health Effects Testing Guidelines; Subpart E, Section 798.4100 – Dermal Sensitisation

Species/Strain: Guinea pig/albino Dunkin Hartley

PRELIMINARY STUDY: Maximum Non-Irritating Concentration:
topical: 50%
Maximum Concentration Causing Mild Irritation:
intradermal: 1%

MAIN STUDY

Number of Animals: Test Group: 20 Control Group: 10

INDUCTION PHASE: Induction Concentration:
intradermal: 1%
topical: 50 %

Signs of Irritation: Slight redness of the treated skin site was observed in all animals 1 hour after application when treated with 50% topical dose of the test material. All treated skin sites appeared normal at the 24-hour observation.

CHALLENGE PHASE

1st challenge: topical: 25 % , 50 %

Remarks - Method: 2,4-dinitrochlorobenzene (DNCB) was used as the positive control substance in a concurrent study. Distilled water was the vehicle for the test material and arachis oil B.P. and absolute ethanol was the vehicle for DNCB in the positive control study. 50% w/w of the test material was the maximum attainable concentration that was suitable for topical application.

RESULTS

Animal	*Challenge Concentration (%)*	*Number of Animals Showing Skin Reactions after:*	
		24 h	*48 h*
Test Group	25	0/19	0/19
	50	0/19	0/19
Positive Control Group	0.25	10/10	8/10
	0.5	10/10	9/10

Remarks - Results: One animal in the test group was found dead on day 13 of the study. The cause of death was not determined however the investigators state that this did not affect the integrity of the study. The positive and vehicle control group produced a predicted response in test animals and confirms the validity of this study.

CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Safepharm (1995a)

B.5. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical (> 90% purity)
METHOD	OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents. EC Directive 67/548/EEC B.26 Sub-Chronic Oral Toxicity Test: 90-Day Repeated Oral Dose Study using Rodent Species.
Species/Strain	Rat/Wistar Crl:(WI) BR
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 90 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Water
Remarks - Method	From day 78 onwards, a different batch of test substance was used. This was noted as being identical in composition to the original test substance except for the batch number and expiry date.

RESULTS

Group	*Number and Sex of Animals*	Dose (mg/kg/day)	Mortality
vehicle control	10M, 10F	0	0
low dose	10M, 10F	100	0
mid dose	10M, 10F	300	0
high dose	10M, 10F	1000	0
control recovery	10M, 10F	0	0
high dose recovery	10M, 10F	1000	0

Clinical Observations

There were no clinical signs of toxicity. Salivation was noted after dosing among most animals at 1000 mg/kg/day and in some animals at 100 and 300 mg/kg/day, however this type of response is often associated with oral gavaging. Alopecia of the abdomen, chest, shoulders and legs were observed in up to 25% of the animals from week 5 but mostly resolved during the recovery period. Other findings included scabs and wounds on the neck, rales, chromodacryorrhoea, exopthalmos and swelling or discharge from the eye. The study authors noted that these observations were common in rats of this age and strain under the conditions of this test.

Laboratory Findings

Haematology

Statistically significant changes include increased red blood cell counts in males at 1000 mg/kg/day, reduced mean corpuscular volume (MCV) in males and females at 1000 mg/kg/day and reduced mean corpuscular haemoglobin (MCH) in males and females at 1000 mg/kg/day and in males dosed at 300 mg/kg/day. Increased red cell distribution width and increased platelet counts were noted in females at 1000 mg/kg/day.

Clinical Biochemistry

A number of changes were observed in males and females at the end of the treatment period. Males and females dosed at 1000 mg/kg/day had reduced creatinine levels and males at 1000 mg/kg had elevated albumin and reduced inorganic phosphate levels. Males in all dose groups (100, 300 and 1000 mg/kg) showed an increase in sodium concentration. In each case, the changes were absent at the end of the recovery period. Slightly reduced potassium levels were noted in males at 1000 mg/kg, but only at the end of the recovery period.

In females, changes that were observed at the end of the treatment were: a decrease in alkaline phosphatase enzyme levels at 100, 300 and 1000 mg/kg, a reduction in bilirubin in the 300 and 1000 mg/kg group, a small decrease in chloride levels at 1000 mg/kg and calcium levels at 300 mg/kg. Elevated cholesterol levels were noted at 1000 mg/kg. These changes reversed after the cessation of treatment and were absent at the end of the recovery period. Potassium levels were reduced in females at 300 and 1000 mg/kg at the end of treatment and did not revert to normal levels in the recovery group.

Urinalysis

Males at 300 and 1000 mg/kg and females at 1000 mg/kg had increased sodium concentration and excretion levels

but these changes were absent at the end of the recovery period. Females at 1000 mg/kg showed increased potassium concentration, specific gravity and protein levels. Reduced urine clarity and volume was also observed in females at the highest dose. These changes were absent at the end of the recovery period. Males at 100 mg/kg/day showed a decreased white blood cell count in urinary sediment however there was no dose-related effect as would be expected in the case of organ toxicity.

Effects in Organs
Microscopic examinations of organs did not reveal any pathologically related changes.

A macroscopic examination revealed a thickened limiting ridge of the stomach in 3/10 females at 1000 mg/kg/day but this was not observed in males. Other findings in test animals include discoloured lungs in 1/10 female, discoloured oesophagus in 1/10 female, kidney cysts in 1/10 female, ectopic splenic tissue in 1/10 female and gelatinous lacrimal glands in 1/10 females dosed at 1000 mg/kg. One male at 1000 mg/kg had a discoloured liver. In the vehicle control group of females, 3/10 had fluid in the uterus, 4/10 had skin alopecia and 1/10 had dessicated eyes at the end of the recovery. In the high dose recovery group of females, only 1/10 animal had fluid in the uterus and no other changes were observed. The study authors stated that these findings were occasionally observed among rats used in these types of studies and that in the absence of microscopic changes and dose-based distribution, the changes were not considered to be of toxicological significance.

An increase in kidney weight and kidney to body weight ratio was evident in males at 1000 mg/kg, which resolved by the end of the recovery phase. Females at 1000 mg/kg had normal kidney weights at the end of treatment but elevated kidney weight at the end of the recovery period. Males at 100 mg/kg had elevated adrenal weight and adrenal to body weight ratio but this was not observed at any other dose. This change occurred in the absence of a dose-related response and the mean was still within the normal range for rats of this age and strain. Females dosed at 1000 mg/kg had statistically significant decrease in brain weight and higher liver to body weight ratio at the end of the recovery period, but the values were regarded as being within the normal range.

Remarks – Results
There were no treatment-related changes in clinical observations, functional observations, body weight and food consumption. No toxicological changes were observed during macroscopic and microscopic organ examinations. At 1000 mg/kg/day, a range of haematological, blood chemistry, urine changes and increased kidney weights were observed in animals of both sexes. At 300 mg/kg/day, a limited number of changes were observed on haematology (reduced MCH) and clinical and urine biochemistry (increased sodium concentration, decreased alkaline phosphatase and bilirubin, decreased calcium levels).

CONCLUSION
Based on the range of haematological, blood, urine chemistry and kidney weight changes observed at 1000 mg/kg and limited non-adverse effects seen at 300 mg/kg bw/day, the No Observed Adverse Effect Level (NOAEL) was established as 300 mg/kg bw/day in this study.

TEST FACILITY NOTOX (2007a)

B.6. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical (> 70% purity)
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 92/69/EEC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1538, TA1535, TA1537, TA98, TA100
Metabolic Activation System	Aroclor 1254-induced rat liver preparation
Concentration Range in Main Test	a) With metabolic activation: 0-5000 µg/plate b) Without metabolic activation: 0-5000 µg/plate
Vehicle	Distilled water
Remarks - Method	No *E.coli* strains were used in this study. Positive controls used were N-ethyl-N'-nitrosoguanidine (ENNG), 9-Aminoacridine (9AA), 4-Nitro-o-phenyldenediamine (4NOPD), 4-Nitroquinoline-1-oxide (4NQO). 2-Aminoanthracene and Benzo(a)pyrene, which are non-mutagenic in the absence of metabolising enzymes were also used as control substances.

RESULTS

Remarks - Results	No toxicity was observed at any dose to any of the *Salmonella* strains tested. There were no significant increases in the numbers of revertant colonies for any of the strains used, at any dose level either with or without metabolic activation.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Safepharm (1994d)

B.7. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical (> 70% purity)
METHOD	OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test. EC Directive 87/302/EEC B.17 Mutagenicity - *In vitro* Mammalian Cell Gene Mutation Test.
Cell Type/Cell Line	Chinese hamster ovary (CHO-K1 BH4)
Metabolic Activation System	Aroclor-induced rat liver S9 preparation
Vehicle	Distilled water
Remarks - Method	Doses up to 3650 µg/ml were tested. Each culture was exposed to the test material for a period of 4 hours at 37°C, followed by a 7-day expression period.
RESULTS	
Remarks – Results	*Cytotoxicity* - In the preliminary test, there was no evidence of dose-related cytotoxicity when the cultures were exposed to up to 3650 µg/mL, either in the presence of absence of S9. In the main tests, there was some evidence of cytotoxicity at the highest dose in the absence of S9 when compared to the vehicle controls, but this finding was mostly confined to the first day of the main test and was less evident at day 7. *Mutagenicity* – There were no significant or dose-related increases in the frequency of mutation either in the absence or presence of metabolic activation when compared to the control. The positive controls all showed the expected increases in mutant frequency, which confirms that the test was functional.
CONCLUSION	The notified chemical was not clastogenic to Chinese hamster ovary cells treated *in vitro* under the conditions of the test.
TEST FACILITY	Safepharm (1995b)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical (> 70% purity)
METHOD	Safepharm Standard Method Number EEC 28B EU Annex V B.10 Mutagenicity - *In vitro* Mammalian Chromosome Aberration Test
Cell Type/Cell Line	Chinese hamster lung (CHL) cells
Metabolic Activation System	Aroclor-induced rat liver S9 preparation
Vehicle	Minimal Essential Media (MEM)
Remarks - Method	The positive control materials were: Mitomycin C (in the absence of S9, Cyclophosphamide (with and without S9).

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period (hrs)*	*Harvest Time (hrs)*
Absent			
Test 1	456.3*, 912.5*, 1825*	12	12

Test 2	456.3, 912.5*, 1825*, 3650*	6	24
Test 3	456.3*, 912.5*, 1825*, 3650*	24	24
Test 4	456.3*, 912.5*, 1825*, 3650*	48	48
Test 5	456.3*, 912.5*, 1825*, 3650	12	12
Present			
Test 1	456.3, 912.5*, 1825*, 3650*	4	12
Test 2	456.3*, 912.5*, 1825*, 3650*	6	24
Test 3	456.3, 912.5*, 1825*, 3650*	4	12

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*		
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Genotoxic Effect*
Absent	3650		
Test 1		> 1825	Negative
Test 2		> 3650	Equivocal
Test 3		3650	Negative
Test 4		3650	Equivocal
Test 5		3650	Negative
Present	3650		
Test 1		> 3650	Negative
Test 2		> 3650	Equivocal
Test 3		> 3650	Negative

Remarks – Results

Cytotoxicity – In the preliminary tests, there were no scorable metaphases above 14.25 μg/ml with 24-hour continuous exposure and above 228 μg/ml with 48-hour continuous exposure. However further experiments showed that the toxicity was over-estimated for the continuous exposure groups and the toxicity was similar to that of other dose groups. In the main tests, there was a general dose-related increase in cytotoxicity, most notably at the highest concentration (3650 μg/ml), either in the presence or absence of metabolic activation.

Mutagenicity – There was a small statistically significant increase in the number of aberrant cells at 1825 μg/ml in the 48-hour continuous exposure and 6-hour exposure group at 3650 μg/ml, both with and without S9. There was no significant increase in the numbers of polyploid cells at any treatment dose.

The positive controls all showed the expected increases in mutant frequency, which confirms that the test was functional.

CONCLUSION

The notified chemical was weakly clastogenic to Chinese hamster lung cells at the highest doses when treated *in vitro* under the conditions of the test. The weak response seen in the 12-hour treatment group was typical for this time-point and is considered to be a result of toxicity-induced cell cycle delay.

TEST FACILITY

Safepharm (1995c)

B.9. Genotoxicity – in vivo

TEST SUBSTANCE

Notified chemical (> 70% purity)

METHOD

OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
EC Directive 84/449/EEC B.12 Mutagenicity - Mammalian Erythrocyte Micronucleus Test.

Species/Strain	Mouse/CD1
Route of Administration	Intraperitoneal injection
Vehicle	Distilled water

Remarks - Method	A preliminary range-finding toxicity study was conducted using intraperitoneal doses ranging 50 -5000 mg/kg and oral doses of 2500 and 5000 mg/kg. The OECD TG 474 recommends that samples of bone marrow be collected only up to 48 hours after treatment; however this test has included samples up to 72 hours.

Dose Group (mg/kg bw)	*Number and Sex of Animals*	*Sacrifice/sampling time (hours)*
0 (vehicle control)	5M, 5F (Group 1); 5M, 5F (Group 2); 5M, 5F (Group 3)	72 (Group 1), 48 (Group 2), 24 (Group 3)
100 (low dose)	5M, 5F	24
200 (mid dose)	5M, 5F	24
400 (high dose)	5M, 5F (Group 1); 5M, 5F (Group 2); 5M, 5F (Group 3)	72 (Group 1), 48 (Group 2), 24 (Group 3)
50 (positive control, CP)	5M, 5F	24

CP=cyclophosphamide.

RESULTS

Doses Producing Toxicity	*Preliminary toxicity study* – Animals dosed via the intraperitoneal route at and above 400 mg/kg exhibited the following signs immediately after dosing: increased activity, loss of righting reflex, decreased respiratory rate, laboured respiration, gasping respiration, pallor of the extremities, splayed gait, lethargy and ataxia. All animals given an intraperitoneal dose of ≥ 800 mg/kg died within 5 minutes of being dosed. Mice given an intraperitoneal dose of 400 mg/kg showed hunched posture 1 hour after dosing but appeared normal thereafter. There were obvious signs of irritation at the injection site. There was no death among mice dosed via the oral route although clinical signs included hunched posture, lethargy, decreased respiratory rate, ptosis and splayed gait. *Main study* – 2/10 animals in the 400 mg/kg test group in the 72 hour sampling time died prematurely after dosing. Animals showed similar clinical signs as that seen in the preliminary toxicity study.
Genotoxic Effects	The 24 and 48-hour test groups at 400 mg/kg did not show any significant increase in the frequency of micronucleated polychromatic erythrocytes (PCE). A small but statistically significant increase ($p < 0.05$) in the frequency of micronucleated PCE in the 400 mg/kg test group (at 72 hours) was observed compared to the vehicle control. However as this increase was within the range of 0 - 4 micronuclei per 1000 PCEs for vehicle control animals, and because the value for the 72 hour control group was very low (0.4) compared to the other control groups (1.0 and 1.1), this increase is not considered to be toxicologically significant. The positive control group showed an expected increase in the incidence of micronucleated PCE and confirms the functionality of this test.
Remarks - Results	

CONCLUSION	The notified chemical was not clastogenic under the conditions of this *in vivo* mice micronucleus test.
TEST FACILITY	Safepharm (1995d)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical (> 80% purity)
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test.
Inoculum	Rhine water: aerated for 10 days, and filtered through filter paper before use
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Oxygen depletion (dissolved oxygen concentrations measured electrochemically using an oxygen electrode).
Remarks – Method	The inoculum was diluted with three volumes of mineral medium. The concentration of notified chemical was 4 mg/L.

RESULTS

Test substance		*Sodium acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	67	7	68
14	82	14	75
28	97	28	82

Remarks - Results	No inhibition of respiration was observed. The results tabulated above reflect oxidation of carbon only. If oxidation of nitrogen to nitrate is considered, the respective values are 53, 65 and 77%.
CONCLUSION	The notified chemical can be classed as readily biodegradable.
TEST FACILITY	Notox (2007b)

C.1.2. Ready biodegradability

TEST SUBSTANCE	Notified chemical (> 80% purity)
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test.
Inoculum	Secondary activated sludge from WWTP Nieuwgraaf, Duiven, Netherlands
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Oxygen depletion (dissolved oxygen concentrations measured electrochemically using an oxygen electrode).
Remarks – Method	The inoculum was diluted with nutrient medium, with the omission of ammonium chloride (this omission clearly did not result in nitrogen limitation). The concentration of notified chemical was 4 mg/L.

RESULTS

Test substance		*Sodium acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	2	7	72
14	0	14	72
21	2		
28	76		

Remarks - Results	No inhibition of respiration was observed. The results tabulated above reflect oxidation of carbon, hydrogen and nitrogen.
CONCLUSION	The notified chemical can be classed as readily biodegradable.

TEST FACILITY Akzo Nobel Technology & Engineering (2007b)

C.1.3. Bioaccumulation

METHOD No test was conducted. The notified chemical has a low bioaccumulation potential because of its high water solubility and ready biodegradability.

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE Notified chemical (> 70% purity)

METHOD OECD TG 203 Fish, Acute Toxicity Test – semi static
Species Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period 96 hours
Auxiliary Solvent None
Water Hardness 100 mg $CaCO_3$/L
Analytical Monitoring Not conducted
Remarks – Method A limit test only was conducted.

RESULTS

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

LC50 > 70.7 mg/L at 96 hours.
NOEC 70.7 mg/L at 96 hours.
Remarks – Results The result has been corrected for the purity of the test substance.

CONCLUSION The test substance is not harmful to rainbow trout

TEST FACILITY Safepharm (1994e).

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical (> 70% purity)

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 100 mg $CaCO_3$/L
Analytical Monitoring HPLC
Remarks – Method Measured concentrations were close to nominal (87% at initiation, 97% at termination)

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	0	20	0	0

100	92	40	0	0

LC50	> 70.7 mg/L at 48 hours
NOEC	70.7 mg/L at 48 hours
Remarks - Results	The results are expressed as nominal concentrations, after correcting for purity of the test substance.

CONCLUSION The test substance is not harmful to *Daphnia magna*.

TEST FACILITY Safepharm (1995e).

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical (> 70% purity)

METHOD OECD TG 201 Alga, Growth Inhibition Test.

Species	*Scenedesmus subspicatus*
Exposure Period	72 hours
Concentration Range	100 mg/L (nominal)
Auxiliary Solvent	None
Water Hardness	Macronutrient concentrations in the culture medium were doubled, so as to ensure adequate algal nutrition under chelation by the notified chemical. Significant growth inhibition was initially recorded in range finding studies at 10 and 100 mg/L, but there were no effects on growth with modified macronutrient concentrations.
Analytical Monitoring	HPLC
Remarks – Method	A limit test only was conducted. Measured concentrations were close to nominal (102% at initiation, 101% at termination). Cell concentrations increased by a factor of 21 during the test, meeting the validity criterion of a 16 fold increase. The pH increased from 8-8.4 to 9.9-10.1, consistent with good algal growth. The modification to macronutrient concentrations is not considered to have compromised the study, given their low concentrations in algal culture media. The OECD guideline specifies a concentration of 18 mg/L calcium chloride dihydrate (equivalent to 4.9 mg/L calcium) and the US EPA guideline 4.4 mg/L. Even when doubled, these concentrations remain below ambient levels in most natural environments. For example, Canberra's drinking water is soft, containing 40 mg/L calcium carbonate (equivalent to 16 mg/L calcium). A cautious approach is warranted when applying assessment factors to algal toxicity data for chelating agents such as the notified chemical, as such application is likely to change inhibitory to stimulatory concentrations. This is evident from the foregoing guideline requirements for algal culture media, which include the addition of the disodium salt of EDTA (0.1 and 0.3 mg/L, respectively). The chelating agent is added to prevent iron precipitation, while at the same time minimising heavy metal complexation.

RESULTS

Biomass *EC50 (mg/L at 72h)*	*Growth* *EC50 (mg/L at 24 h)*
> 100	> 100

Remarks - Results	The results are expressed as nominal concentrations. The NOEC can be reported as 100 mg/L, as the growth curve data showed no significant differences between control and test replicates, and there were no abnormalities seen in any culture.

CONCLUSION The test substance is not harmful to *Scenedesmus subspicatus*.

TEST FACILITY	Safepharm (1995f).

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical (> 80% purity)
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Secondary activated sludge from WWTP Nieuwgraaf, Duiven, Netherlands
Exposure Period	0.5 hours
Concentration Range	412 mg/L
Remarks – Method	
RESULTS	
IC50	> 412 mg/L
NOEC	412 mg/L
Remarks – Results	The EC50 of 3,5-dichlorophenol (8.7 mg/L) met the prescribed criterion (5-30 mg/L).
CONCLUSION	The test substance is considered harmless to activated sludge.
TEST FACILITY	Akzo Nobel Technology & Engineering (2007c)

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