File No: STD/1424

December 2012

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Amines, N-C10-16-alkyltrimethylenedi-, reaction products with chloroacetic acid

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

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

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS SUBSTANCE	INTRODUCTION VOLUME	USE
STD/1424	Brenntag Australia Pty Ltd	Amines, N-C10-16-alkyltrimethylenedi-, reaction products with chloroacetic acid	Yes	≤ 2 tonnes per annum	Component of hard surface industrial cleaners

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard classification	*Hazard statement*
Acute Toxicity (Category 4)	H302 – Harmful if swallowed
Skin Corrosion (Category 1)	H314 - Causes severe skin burns and eye damage

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:

R22: Harmful if swallowed
R34: Causes burns

The environmental hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals* (GHS) is presented below. Environmental classification under the GHS is not mandated in Australia and carries no legal status but is presented for information purposes.

Hazard classification	*Hazard statement*
Aquatic Acute 1	Very toxic to aquatic life
Aquatic Chronic 1	Very toxic to aquatic life with long lasting effects

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

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

Environmental risk assessment
On the basis of the PEC/PNEC ratio and the reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment provided the import volume does not exceed 2 tonnes per annum.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- The notified chemical should be classified as follows:

  - H302 - Harmful if swallowed
  - H314 - Causes severe skin burns and eye damage
- The following classifications should be used for products/mixtures containing the notified chemical:
  - Conc. ≥ 25%: H302; H314
  - ≥ 5% Conc. < 25%: H314
  - ≥ 3% Conc. < 5%: H315, H318
  - ≥ 1% Conc. < 3%: H319, H318

  H315 – Causes skin irritation
  H318 – Causes serious eye damage
  H319 – Causes serious eye irritation

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 as introduced:
  - Enclosed, automated processes, where possible
  - Local exhaust ventilation

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced:
  - Avoid skin and eye contact
  - Avoid inhalation of vapours and aerosols

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced:
  - Protective coveralls
  - Impervious gloves
  - Goggles

  Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

- 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 *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Environment

- The following control measures should be implemented by reformulators, users, transporters and handlers to minimise environmental exposure of the notified chemical:
  - Notified chemical or waste water containing the notified chemical is not to be released, directly or indirectly, to freshwater.

Disposal

- The notified chemical should be disposed of to landfill.

Storage

- The following precautions should be taken regarding storage of the notified chemical:
  - Store in a well-ventilated place.
  - Keep container tightly closed.

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if
- the importation volume exceeds two tonnes per annum of notified chemical;
- the concentration of the notified chemical exceeds or is intended to exceed 0.5% in hard surface industrial cleaners;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component of hard surface industrial cleaners, or is likely to change significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Brenntag Australia Pty Ltd (ABN: 84 117 996 595)
262 Highett Road
HIGHETT VIC 3190

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: analytical data, degree of purity, impurities and import volume.

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Germany (2009)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Rewocid WK 30 (contains notified chemical at < 50%).

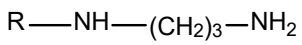
CAS NUMBER
139734-65-9

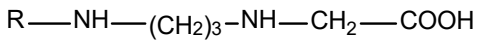
CHEMICAL NAME
Amines, N-C10-16-alkyltrimethylenedi-, reaction products with chloroacetic acid

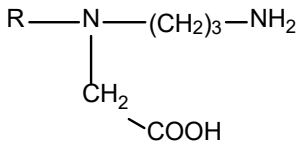
OTHER NAME(S)
Ampholyt 20/100 (99% notified chemical)
Ampholyt 20 (20% aqueous solution of notified chemical)
TEGO 2000 (20% aqueous solution of notified chemical)
Tegol 2000 (20% aqueous solution of notified chemical)

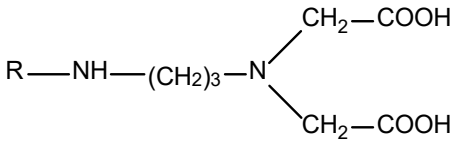
MOLECULAR FORMULA
Unspecified

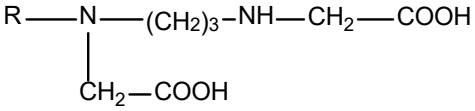
STRUCTURAL FORMULA
The notified chemical is a reaction product of N-C10-16-alkyltrimethylenediamines (CAS No. 179865-15-7) with chloroacetic acid and consists of 5 components. The major components are 1 (~55%), 2 (~19%) and 3 (~17%) based on a HPLC analysis of Ampholyt 20.

1. 

2. 

3. 

4. 

5. 

The alkyl moiety R is of natural origin (vegetable fats) and corresponds to carbon portions of C_{10}-C_{16}.

C_{12}	(70-83%)
C_{14}	(12-25%)
Other carbons	(0-2%)

MOLECULAR WEIGHT
241 - 414 Da
Average weighted molecular mass: 280.79 Da

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

3. COMPOSITION

DEGREE OF PURITY > 95%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: White solid

Property	Value	Data Source/Justification
Melting Point	Melts with decomposition at 140-145 °C	Measured
Density	1030 kg/m^3 at 4 °C	Measured
Viscosity	7.40 mm^2/s at 20 °C	Measured
Vapour Pressure	4 x10^{-7} kPa at 25 °C	Measured
Water Solubility	> 208 g/L	Measured
Hydrolysis as a Function of pH	t ½ > 1 year at 25 °C	Measured
Partition Coefficient	log Kow ≤ -0.76 at 25 °C	Measured

(n-octanol/water)		
Surface Tension	27.2 mN/m	Measured
Adsorption/Desorption	log Koc = 2.70 – 3.99	Measured
Dissociation Constant	Not determined	Expected to be ionised in the environmental pH range (4 – 9) based on the presence of acidic (pKa ~ 4) and basic (pKa ~ 10) functional groups
Particle Size	Not determined	Introduced as an aqueous solution
Flash Point	Not determined	Introduced as an aqueous solution
Flammability	Not determined	Introduced as an aqueous solution
Autoignition Temperature	> 402 °C	Measured
Explosive Properties	Not expected to be explosive	Contains no functional groups that would imply explosive properties.
Oxidising Properties	Not expected to be explosive	Contains no functional groups that would imply oxidative properties.

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

Reactivity
The notified chemical is considered to be stable at room temperature.

Dangerous Goods classification
Based on the submitted physico-chemical data in the above table, the notified chemical is not classified according to the Australian Dangerous Goods Code (NTC, 2007). However, the data above do not address all Dangerous Goods endpoints. Therefore, consideration of all endpoints should be undertaken before a final decision on the Dangerous Goods classification is made by the introducer of the chemical.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as an aqueous solution at < 50% concentration.

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

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

PORT OF ENTRY
Melbourne, Sydney and Brisbane by sea.

TRANSPORTATION AND PACKAGING
The notified chemical (< 50% concentration) will be imported in 200 L drums and transported by road to reformulation sites. The finished products containing the notified chemical at concentrations < 0.5% will be packaged in various sizes suitable for end use in industrial settings.

USE
The notified chemical will be used in hard surface industrial cleaners at concentrations of < 0.5%.

OPERATION DESCRIPTION
Reformulation
It is expected that the reformulation processes will involve blending operations that will be highly automated and occur in a fully enclosed environment, followed by automatic filling of the reformulated end-use product into containers of various sizes suitable for end use in industrial settings.

End-use
The finished cleaning products containing the notified chemical at < 0.5% will be used in industrial applications and will be applied to surfaces by a variety of methods including spray.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

Transport and storage workers may come into contact with the notified chemical at concentrations < 50% only in the event of accidental rupture of containers.

During reformulation dermal, ocular and perhaps inhalation exposure of workers to the notified chemical may occur at concentrations of < 50% during transfer of the notified chemical to the mixing vessels and at concentrations of < 0.5% during quality control analysis, cleaning and maintenance of equipment and packaging. Exposure is expected to be minimised through the use of coveralls, chemical resistant gloves, and safety glasses.

During end-use, there is potential for dermal, ocular and inhalation exposure to the notified chemical at < 0.5% concentration when using the cleaning products.

6.1.2. Public Exposure

The cleaning products containing the notified chemical will only be used in industrial settings and will not be sold to the public. Hence, public exposure to the notified chemical 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	LD50 300-2000 mg/kg bw; harmful
Rat, acute dermal toxicity*	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation	corrosive
Rabbit, eye irritation	corrosive
Guinea pig, skin sensitisation – adjuvant test	no evidence of sensitisation
Beagle dog, repeat dose oral toxicity – 90 days	NOEL = 5 mg/kg bw/day
Mutagenicity- bacterial reverse mutation	non mutagenic
Mutagenicity- bacterial reverse mutation	non mutagenic
Genotoxicity- *in vitro* mammalian chromosome aberration test	non genotoxic
Genotoxicity- *in vitro* mammalian cell gene mutation test in Chinese hamster ovary cells	non genotoxic

* For 20% aqueous solution of notified chemical

Toxicokinetics.

Based on the partition coefficient (log P_{ow} = -0.76 at 20 ^{0}C) and the low molecular weight (< 500 Da) of the notified chemical passive diffusion across the gastrointestinal tract (GI) is expected to occur. This is supported by the systemic toxicity observed in the 90 day repeated dose oral toxicity study in beagle dogs. Dermal absorption is also expected to occur particularly given the notified chemical is surface active and corrosive. The notified chemical may also be absorbed across the respiratory tract.

Acute toxicity.

The notified chemical was found to be harmful by the oral route in a study conducted in rats (LD50 = 300-2000 mg/kg bw). As the chemical is skin corrosive, animals were not treated with the standard dose of 2000 mg/kg bw. At a dose level of 300 mg/kg bw, one out of six animals died during the study. Clinical signs of toxicity consisted of sluggishness, vocalisation, nose encrustation and piloerection.

A 20% aqueous solution of the notified chemical was found to be of low acute dermal toxicity (LD50 > 2000 mg/kg bw).

No data was submitted on the acute inhalation toxicity of the notified chemical.

Irritation and Sensitisation.

The notified chemical was corrosive to the skin of rabbits. One of the three treated animals suffered full

thickness destruction of the skin from the 48-hour observation period and was sacrificed for ethical reasons after the 72-hour reading. Of the two surviving animals, only very slight erythema at the 1-hour observation period was noted for one animal and mild erythema and oedema as well as scaling was noted for the remaining animal, which was resolved at the end of the observation period.

A 20% aqueous solution of the notified chemical was corrosive to the eyes of rabbits. Conjunctival redness (Grade 3), chemomis (Grade 4) and iris lesions (Grade 2) were observed for all treated animals at the 1-hour observation period that persisted to the end of the 3 day study.

The notified chemical was not a skin sensitiser in guinea pigs.

Repeated Dose Toxicity.
In a 90-day repeat dose gavage study in beagle dogs the NOEL was established as 5 mg/kg bw/day based on changes in the adrenals, brain, kidneys, thyroids, genital system as well as haematological and biochemical parameters.

Mutagenicity.
The notified chemical was not mutagenic in two bacterial reverse mutation tests, and was not clastogenic to human lymphocytes in an *in vitro* mammalian chromosome aberration test or to Chinese hamster ovary cells in an *in vitro* mammalian cell gene mutation test.

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

Hazard classification	*Hazard statement*
Acute Toxicity (Category 1)	H302 - Harmful if swallowed
Skin Corrosion (Category 1)	H314 - Causes severe skin burns and eye damage

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:

R22: Harmful if swallowed
R34: Causes burns

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The notified chemical is corrosive to the skin and eyes and harmful by the oral route. It is not a skin sensitiser and is not expected to be genotoxic. A 20% aqueous solution of the notified chemical was determined to be of low acute dermal toxicity. Based on its physico-chemical properties, the notified chemical is likely to have potential for absorption by all routes. If absorbed, the notified chemical has potential for systemic toxicity (NOEL = 5 mg/kg bw/day).

Workers most at risk of irritation and toxic effects from repeated exposure will be those handling the notified chemical as introduced at up to 50% concentration during reformulation processes. This risk should be minimised by the expected use of PPE (gloves, safety glasses/face shield and protective coveralls), local exhaust ventilation and enclosed blending vessels.

The risk from exposure to the notified chemical is not expected once reformulated as workers will only be exposed to low concentrations (< 0.5%) of the notified chemical.

Overall, under the conditions of the occupational settings described, the risk presented by the notified chemical to the health and safety of workers is not expected to be unreasonable.

6.3.2. Public Health

The finished cleaning products containing the notified chemical will only be available to industrial end users, hence public exposure to the notified chemical is not expected. Therefore the notified chemical is not expected to pose an unreasonable risk to public health when used in the proposed manner.

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 formulation into industrial cleaning products. Empty packaging containing notified chemical residues is expected to be recycled, reused or disposed of according to local regulations.

RELEASE OF CHEMICAL FROM USE

The notified chemical will be used in hard surface industrial cleaners at a concentration of up to 0.5%. The majority of notified chemical is expected to be released to sewage treatment plants (STPs) during use as a hard surface industrial cleaner.

RELEASE OF CHEMICAL FROM DISPOSAL

Empty packaging containing notified chemical residues is expected to be recycled, reused or disposed of according to local regulations.

7.1.2. Environmental Fate

The majority of the notified chemical is expected to be released to sewer when used in industrial hard-surface cleaners. Some of the notified chemical is anticipated to partition to hard surfaces based on its measured adsorption/desorption coefficient and surface activity. The results of the ready biodegradability test indicate that in sewage treatment plants (STPs) the notified chemical is expected to be rapidly removed from STP influent. Notified chemical bound to sewage sludge is expected to be disposed to landfill or used for soil remediation where it will have limited mobility. Notified chemical in treated effluent that is released to surface water is expected to sorb to organic carbon and disperse and degrade. The notified chemical is expected to eventually degrade by abiotic and biotic processes to form water and oxides of carbon and nitrogen. For the details of the environmental fate study please refer to Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)

A worst-case predicted environmental concentration (PEC) was calculated assuming that all of the total import volume of notified chemical will be released to sewers with removal of the notified chemical by sewage treatment plants (STPs) estimated by SimpleTreat (European Commission, 2003). It is assumed the release of the notified chemical will occur over 260 days per annum into the total Australian effluent volume corresponding to a working week of 5 days per week.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import	2,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	2,000	kg/year
Days per year where release occurs	260	days/year
Daily chemical release	7.69	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	87%	
Daily effluent production:	4,523	ML
PEC – STP (before mitigation)	1.70	µg/L
Dilution Factor - River	1.0	
Dilution Factor - Ocean	□10.0	
PEC - River	0.22	µg/L
PEC- Ocean	0.022	µg/L

The PEC of the notified chemical in STPs, before mitigation, was calculated to be 1.70 µg/L. After mitigation (removal within the STP) of 87%, the PEC was calculated to be 0.22 and 0.022 µg/L in the riverine and marine compartments, respectively.

The notified chemical that is not removed from waste water during STP processes may be released to the environment in STP effluent. STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.22 µg/L may potentially result in a soil concentration of approximately 1.47 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 7.35 µg/kg and 14.7 µg/kg, respectively.

The notified chemical removal in STPs is expected to be due to biodegradation and adsorption to sludge. These mechanisms were not distinguished in the biodegradation study on the notified chemical, hence as a worst case it is assumed 87% of the notified chemical sorbs to biosolids in STPs. Partitioning to biosolids in STPs Australia-wide may result in an average biosolids concentration of 14.8 mg/kg (dry wt). Biosolids are applied to agricultural soils, with an assumed average rate of 10 t/ha/year. Assuming a soil bulk density of 1500 kg/m^3 and a soil-mixing zone of 10 cm, the concentration of the notified chemical may approximate 0.099 mg/kg in applied soil. This assumes that degradation of the notified chemical occurs in the soil within 1 year from application. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated biosolids application, the concentration of notified chemical in the applied soil in 5 and 10 years may approximate 0.495 mg/kg and 0.99 mg/kg, respectively.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on the notified chemical are summarised in the table below. It was noted that in the acute ecotoxicity studies the concentration of the notified chemical was not measured and hence the toxicity of the notified chemical may be confounded due to the physico-chemical properties of the notified chemical. For example, in the environment cationic substances are expected to sorb to organic carbon and be less bioavailable than under normal laboratory test conditions, where organic carbon levels are typically low. Hence the endpoints from the acute studies should be treated with caution and are reported as upper limits. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Acute		
Fish Toxicity (96 h)	LC50 ≤ 0.43 mg/L	Very toxic
Daphnia Toxicity (48 h)	EC50 ≤ 0.11 mg/L	Very toxic
Algal Toxicity (72 h)	E_rC50 ≤ 0.05 mg/L	Very toxic
Inhibition of Bacterial Respiration (3 h)	IC50 = 22 mg/L	Not expected to be inhibitory to bacterial respiration ≤ 22 mg/L
Chronic		
Fish Toxicity (28 d)	NOEC ≥ 0.0523 mg/L	At most, toxic with long lasting effects
Daphnia Toxicity (21 d)	NOEC = 0.0023 mg/L	Very toxic with long lasting effects
Algal Toxicity (72 h)	NOEC ≤ 0.009 mg/L	Very toxic with long lasting effects

Under the Globally Harmonised System of Classification and Labelling of Chemicals (United Nations, 2009) the notified chemical is considered to be very acutely toxic to fish, aquatic invertebrates and algae. Based on the acute toxicity to aquatic organisms the notified chemical is formally classified for the aquatic environment under the GHS as “Acute category 1; Very toxic to aquatic life”. Two adequate chronic toxicity endpoints were available (fish and daphnia). Therefore, the long-term classification for the notified chemical was determined based on the most stringent outcome by comparing the long-term hazard classification using either the acute or chronic data. The most stringent outcome resulted from classification based on the chronic endpoint for daphnia. The notified chemical is therefore formally classified under the GHS as “Chronic category 1; Very toxic to aquatic life with long lasting effects”. Although the endpoints from the acute studies were upper limits, in this case the GHS classifications were unambiguous, as all the endpoints were below the thresholds for the most toxic GHS hazard categories.

7.2.1. Predicted No-Effect Concentration

The endpoint of the most sensitive species, 21 day daphnia NOEC (No Observed Effect Concentration), determined from ecotoxicological studies submitted for the notified chemical was used to calculate the Predicted No-Effect Concentration ($PNEC_{daphnia}$). An assessment factor of 10 was used as chronic toxicity endpoints were available for the effects of the notified chemical.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
NOEC (invertebrate reproduction)	0.0023	mg/L
Assessment Factor	10	
PNEC:	0.23	µg/L

When the notified chemical is expected to be released through a sewage treatment plant (STP) and there is evidence for toxicity of the chemical towards STP micro-organisms a Predicted No-Effect Concentration for micro-organisms ($PNEC_{micro\text{-}organisms}$) should be considered (EPHC, 2009). In lieu of a NOEC or EC10, the EC50 was used with an assessment factor of 100.

Predicted No-Effect Concentration (PNEC) for STP micro-organisms		
EC50 (micro-organisms)	22	mg/L
Assessment Factor	100	
$PNEC_{micro\text{-}organisms}$:	220	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - STP	1.70	220	0.008
Q - River	0.22	0.23	0.956
Q - Ocean	0.022	0.23	0.096

The Risk Quotient (Q = PEC/PNEC) has been calculated to be < 1 for STP micro-organisms and the aquatic compartment. The risk quotients take into account an estimated 87% removal of the notified chemical from sewage treatment plants due to adsorption and biodegradation. The Q value of < 1 indicates the notified chemical is not expected to pose an unreasonable risk to the aquatic environment from its assessed use pattern. To ensure the notified chemical does not pose an unreasonable risk to the environment, i.e. to ensure RQs ≤ 1, the limit for import volume of the notified chemical should not exceed 2,000 kg/annum.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point Melts with decomposition at 140-145 °C

METHOD OECD TG 102 Melting Point/Melting Range.
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks Differential Scanning Calorimetry At a temperature of 140-145 °C, the test item turned from a white solid to black viscous liquid. The test item also frothed and there was a mass loss indicating the release of volatile decomposition products.
Test Facility Siemens (2005a)

Density 1030 kg/m^3 at 4 °C

Method OECD TG 109 Density of Liquids and Solids.
EC Council Directive 67/548/EEC Annex V (Council Directive 92/669/EEC).
Remarks Gas comparison pycnometer.
Test Facility Siemens (2005b)

Viscosity 7.40 mm^2/s at 20 °C

Method OECD TG 114 Viscosity of Liquids.
Remarks The test substance was a 20% aqueous solution of the notified chemical.
Test Facility Siemens (2007)

Vapour Pressure 4 $x10^{-7}$ kPa at 25 °C

Method OECD TG 104 Vapour Pressure.
EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks Effusion method.
Test Facility Siemens (2005c)

Water Solubility > 208 g/L at 20 °C

Method In-house method
Remarks An aqueous solution of test substance (approximately 20%; colourless to slightly yellow) was weighed, freeze dried and then reweighed. The non-aqueous component of the solution was found to be 20.83% by weight and the solubility was concluded to be > 20.83% (w/w). This is consistent with a solubility of > 208 g/L given the density of the test substance is approximately 1 g/mL. The pH dependence of the water solubility was checked by the addition by adjusting three solutions of test substance to pH 4.0, 7.0 and 9.0. At pH 4 and 7 the solution appeared clear. At pH 9 the solution appeared viscous and foamy and appeared clear after several hours. It was concluded the solubility of test substance was not pH dependent over the range tested. DSEWPaC notes the notified chemical is expected to be surface active and therefore the notified chemical is likely to be water dispersible rather than water soluble.
Test Facility Infracor (2002a)

Hydrolysis as a Function of pH – Test 1 > 1 year at 25 °C at pH 4, 7 and 9

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

pH	*T (°C)*	$t_{½}$
4	25	> 1year
7	25	> 1year
9	25	> 1 year

Remarks Sample solutions were prepared at nominal concentration of 29.4 µg/L in three buffer solutions (pH 4.09, 7.05 and 9.16). The test substance consisted of five ^{14}C-labelled substances representative of the notified chemical. The solutions were kept in darkness whilst maintained at the test temperature of 50.0 ± 0.5 °C for a period of 5 days. Aliquots of sample solutions were taken from flasks at various times and the pH and concentration of test substance was measured. The test substance components as well as potential hydrolysis products were quantified by liquid scintillation counting (LSC) and HPLC MS/MS. Minor amounts of unidentifiable residues were detected at < 2.5% of the applied radioactivity, hence it was concluded that no major hydrolysis products were formed. Less than 10% hydrolysis of the test substance was observed after 5 days at 50 °C at pH 4, 7 and 9 and therefore the estimated half-life at 25 °C is > 1 year. It was noted that from days 1 to 5 recoveries of ^{14}C decreased from approximately 100% to 59.3% (pH 7) and 58.6% (pH 9) compared with 105.6% at pH 4. Rinsing the walls of the glass vials with acetonitrile/water lead to recovery rates of 92.9% and 102% at pH 7 and 9, demonstrating the test substance adsorbed to the glass vials.

Test Facility Fraunhofer-Institute (2008a)

Hydrolysis as a Function of pH – Test 2 > 1 year at 25 °C at pH 9

Method EC Directive 92/69/EEC C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH

pH	*T (°C)*	*$t_{½}$*
4	–	n.d.*
7	–	n.d.*
9	25	> 1 year

*Not determined. See remarks.

Remarks An attempt to dissolve the test substance (50 mL; 20% aqueous solution of notified chemical) in standard pH 7 buffer (450 mL) resulted in a white precipitate forming. Due to the low sensitivity of the analytical method for the test substance (HPLC) a lower concentration of test substance would not have been suitable and hence a preliminary test was not carried out at pH 7. Solutions of test substance (50 mL; 30% aqueous solution of notified chemical) in pH 4 or 9 buffer were kept in darkness whilst maintained at the test temperature of 50.0 ± 0.5 °C for a period of 5 days. At pH 4 the hydrolysis results were inconclusive, most likely due to a milky white haze observed after dissolution of the test item and throughout the HPLC analysis. After 5 days at pH 9 the degree of hydrolysis was found to be < 10%.

Test Facility Infracor (2002a)

Partition Coefficient (n-octanol/water) log Kow ≤ -0.76 at 25 °C

Method EC Directive 92/69/EEC A.8 Partition Coefficient.

Remarks The partition coefficient was estimated based on the ratio of the individual solubilities of the test substance (20% aqueous solution of notified chemical) in water and n-octanol. Three tests were performed with twice distilled water (approximately 3.1, 5.3 and 10.4 g test substance added to 5 mL water). A clear solution was observed hence the test substance water solubility of the test substance was found to be ≥ 200 g active substance/L. Test substance (1.54 g) was mixed in 4 mL n-octanol and stirred for 75 min. After phase separation by centrifugation (10 min, 3000 rpm, 7000 g), the clear saturated n-octanol layer was analysed by UV spectrophotometry. The concentration of active substance in the octanol layer was found to be 35.0 mg/mL.

Test Facility TNO (1994)

Surface Tension 27.2 mN/m at 20 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions.
Remarks	Concentration of test substance: 1.004 g/L The test substance was a 20% aqueous solution of the notified chemical.
Test Facility	NOTOX (2000)

Adsorption/Desorption log K_{oc} = 2.70 – 3.99

Method	OECD TG 121: Estimation of the Adsorption Coefficient (K_{oc}) on Soil and Sewage Sludge using High Performance Liquid Chromatography
Remarks	HPLC Method. The adsorption coefficient was determined by interpolation from a calibration curve constructed from known standards (log K_{oc} range 1.32 – 3.92) in accordance with the guidelines above. The upper and lower limits of K_{oc} were determined as the test substance (20% aqueous solution of notified chemical) consisted of a mixture of substances with different K_{oc} values. DSEWPaC notes that the results may have been affected by the surfactant properties of the test substance.
Test Facility	Infracor (2002a)

Autoignition Temperature > 402 ºC

Method	EC Council Regulation No 67/548/EEC Annex V (Council Directive 92/69/EEC) A.16 Auto-flammability (solids- determination of relative self-ignition temperature).
Remarks	No self-ignition temperature was observed up to the maximum test temperature of 402 ºC.
Test Facility	Siemens (2005d)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical (purity 99%)

METHOD: OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.

Species/Strain: Rat/Wistar
Vehicle: Water
Remarks-Method: Animals were not treated with the standard dose of 2000 mg/kg/bw as it was considered that such a dose would result in extreme pain, distress and possible death in animals given the notified chemical is skin corrosive.

RESULTS

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

LD50: 300-2000 mg/kg bw
Signs of Toxicity: One animal died on Day 1. Clinical signs of toxicity observed were sluggishness, nose encrustation, piloerection and vocalization. No signs of toxicity were noted after Day 2.

All animals gained bodyweight except for the one dead animal that showed a body weight loss.
Effects in Organs: Nil in all animals.

CONCLUSION: The notified chemical is harmful via the oral route.

TEST FACILITY: TNO (2003a).

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Notified chemical (20% aqueous solution)

METHOD: OECD TG 402 Acute Dermal Toxicity-Limit Test

Species/Strain: Rat/Sprague-Dawley
Vehicle: Undiluted
Type of dressing: Semi-occlusive.
Remarks - Method: No significant protocol deviations. The dose was based on the test substance as supplied and was not corrected for the concentration of the notified chemical.

Results-Main Study

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

LD50: LD50 > 2000 mg/kg bw
Signs of Toxicity - Local: Females: Moderate to very slight erythema was noted from Days 2 to 8. Superficial eschar was noted in 3 animals at the end of study (Day 15).
Males: A very slight to well raised erythema was observed from Days 2 to 4 as well as desquamation of the skin to the application area on Days 4

	and Day 5. No cutaneous lesions were noted from Day 6 onwards.
Signs of Toxicity - Systemic	No systemic signs of toxicity were observed.
Effects in Organs	No macroscopic signs.

CONCLUSION The notified chemical at 20% concentration is of low toxicity via the dermal route.

TEST FACILITY Hazleton (1988)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical (purity 99%)

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.

Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	Undiluted
Observation Period	14 days
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	4**	0	2	4	< 14 days	0
Oedema	2	0	1.3	3	< 7 days	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.
** Based on 24 hour score only as not assessable due to necrosis at 48- and 72-hour observation.

Remarks - Results One animal was noted with deep necrosis (full thickness destruction of the skin) from the 48-hour observation period and was sacrificed for ethical reasons after the 72-hour reading. Of the two surviving animals, only very slight erythema at the 1-hour observation period was noted for one animal and mild erythema and oedema as well as scaling was noted for the remaining animal, which was resolved at the end of the observation period.

CONCLUSION The notified chemical is corrosive to the skin.

TEST FACILITY RCC (2005)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical (20% aqueous solution)

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.

Species/Strain	Rabbit/New Zealand White
Number of Animals	6
Observation Period	72 hours
Remarks - Method	Observations for conjunctiva discharge were not recorded.

RESULTS

Lesion	*Mean Score**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Conjunctiva: redness	2.96	3	> 72 h	3
Conjunctiva: chemosis	3.79	4	> 72 h	4

Corneal opacity	0	0	-	0
Iridial inflammation	2	2	> 72 h	2

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

Remarks - Results: Conjunctival redness (Grade 3), chemomis (Grade 4) and iris lesions (Grade 2) were observed for all treated animals at the 1-hour observation period that persisted to the end of the 3 day study.

CONCLUSION: The notified chemical at 20% concentration is corrosive to the eye.

TEST FACILITY: IBR (1988)

B.5. Skin sensitisation

TEST SUBSTANCE: Notified chemical (purity 99%)

METHOD: OECD TG 406 Skin Sensitisation – Maximisation Test.

Species/Strain: Guinea pig/Pirbright white

PRELIMINARY STUDY: Maximum Non-irritating Concentration:
intradermal: 0.5%
topical: 1%

MAIN STUDY

Number of Animals: Test Group: 10 Control Group: 5

INDUCTION PHASE: Induction Concentration:
intradermal: 0.5%
topical: 10%

Signs of Irritation: None

CHALLENGE PHASE

1st challenge: topical: 1%

Remarks - Method: No deviations from the study protocol. Prior to the topical induction the animals were treated with 10% sodium lauryl sulphate.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: 1st challenge*	
		24 h	*48 h*
Test Group	1%	0/10	0/10
Control Group	0%	0/5	0/5

Remarks - Results: No allergic skin reactions occurred in test animals 24 and 48 hours after the end of the challenge procedure with the test article at the concentration of 1%.

CONCLUSION: There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.

TEST FACILITY: Harlan (2004).

B.6. Repeat dose toxicity

TEST SUBSTANCE: Notified chemical (20% aqueous solution)

METHOD: OECD TG 409 Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents.

Species/Strain: Dog/Beagle

Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 90 days Dose regimen: 7 days per week Post-exposure observation period: Not conducted
Vehicle	Tap Water
Remarks - Method	No significant protocol deviations. The dose was adjusted for the concentration of the notified chemical in the test substance. Hence all doses reported are for the notified chemical.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	4/sex	0	0
low dose	4/sex	5	0
mid dose	4/sex	15	0
high dose	4/sex	45	0

Mortality and Time to Death

There were no mortalities.

Clinical Observations

No clinical observations were noted for the low dose group.

In the mid-dose group, two of four female animals revealed emesis on 1 and 5 test days, respectively, starting on Day 8. In the high-dose group, repeated emesis (up to 4 times daily) from Day 5 onwards was observed in all male and female animals on 51 to 79 test days.

Body-weight decreased dose-dependently in the male and female animals in both the mid- and high-dose groups; however this observation was only statistically significant for the females in the high dose group in test weeks 6 and 11 to 13.

In the mid- and high-dose groups, the average amount of the weekly food consumption was slightly decreased in the male and female animals, being statistically significant only in the females in the mid dose group in test weeks 2 and 9 and high dose group in test weeks 2 to 4.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No test-item related changes were noted in animals of the low-dose group.

In the mid- and high-dose groups, there were elevations in the following haematological parameters: WBC, neutrophils, monocytes and leucocytes.

In the mid- and high-dose groups there were decreases in the following biochemical parameters: albumin, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and total protein.

In the high dose group, there was a slight increase of the urinary pH value and the haemoglobin content in the urine.

Effects in Organs

No test-item related changes were noted in the low-dose group.

Gross Pathology:

In nearly all males of the mid- and high-dose group, downsized prostates and discolourations in the kidneys, liver and pancreas were observed. In three of the four high-dosed females, discolouration of the kidneys, liver, pancreas, spleen and/or lungs, and a reduction in the size of the uterus, thymus and/or spleen were noted.

Changes in absolute organ weights for the heart and thymus were noted for the male and female animals in the mid- and high-dose groups. Relative organ weights of adrenals, brain, kidneys and thyroids were increased in the mid- and high-dose groups caused by the reduced body weight.

Histopathology:
Histomorphological examination revealed a dose-related atrophy in the male and female genital system (cervix, epididymis, ovary, prostrate, uterus and vagina) and a slightly more pronounced involution of the thymus in both sexes of the mid- and high-dose group and an atrophy of the germinative epithelium in the testis of the high dose group.

Remarks – Results
The oral administration of the notified chemical to dogs resulted in treatment-related effects at 15 and 45 mg/kg bw/day. There were no treatment-related effects at 5 mg/kg bw/day.

CONCLUSION
The No Observed Effect Level (NOEL) for the notified chemical was established as 5 mg/kg bw/day in this study, based on dose-related atrophy in the male and female genital system, and changes in haematological and biochemical parameters in the mid- and high-dose groups.

TEST FACILITY LPT (2008)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical (purity 99%)

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
Plate incorporation method.
Species/Strain *S. typhimurium*: TA1535, TA1537, TA98, TA100
E coli: WP2 *uvr*A
Metabolic Activation System S9 fraction from Aroclor 1254 induced rat liver
Concentration Range in Main Test
Test 1
a) With metabolic activation: 62-5000 µg/plate
b) Without metabolic activation: 62-5000 µg/plate
Test 2
a) With metabolic activation: 62-5000 µg/plate
b) Without metabolic activation: 62-5000 µg/plate
Vehicle Water
Remarks - Method A preliminary cytotoxicity test was not conducted.

Vehicle and positive controls (2-nitrofluorene, N-ethyl-N-nitrosourea, sodium azide, and 9-aminoacridine without metabolic activation; 2-aminoanthracene and benzo(a)pyrene with metabolic activation) were used in parallel with the test material.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	*Cytotoxicity in Preliminary Test**	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	-	≥ 185	> 5000	Negative
Test 2		≥ 100		Negative
Present				
Test 1	-	≥ 556	> 5000	Negative
Test 2		≥ 200		Negative

* Not conducted

Remarks - Results No significant increases in the frequency of revertant colonies were noted for any of the bacterial strains, either with or without metabolic activation.

The positive controls gave satisfactory responses, confirming the validity of the test system.

CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	TNO (2003)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical (20% aqueous solution)
METHOD	OECD TG 471 Bacterial Reverse Mutation Test Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 0.004-0.25 µl/plate b) Without metabolic activation: 0.004-0.25 µl/plate
Vehicle	Water
Remarks - Method	The test substance concentration was based on the test substance as supplied and was not corrected for the concentration of the notified chemical. *E.coli* WP2 strains were not used in the study, hence oxidising mutagens and cross-linking agents may not be detected. In a preliminary toxicity determination test, the survival Salmonella strain TA100 was reduced by ~95%. Hence the mutagenicity experiments were performed in a concentration range from 0.0004 to 0.25 µl/plate. Vehicle and positive controls (2-nitrofluorene, sodium azide, and 9-aminoacridine without metabolic activation; 2-aminoanthracene with metabolic activation) were used in parallel with the test material.

RESULTS

Metabolic Activation	*Test Substance Concentration (µl/plate*) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 0.25	≥ 0.25	> 0.25	Negative
Present				
Test 1		≥ 0.25	> 0.25	Negative

* Concentration was reported as µl/plate.

Remarks - Results	No significant increases in the frequency of revertant colonies were noted for any of the bacterial strains, either with or without metabolic activation. The positive controls gave satisfactory responses, confirming the validity of the test system.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Labor (1988)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical (20% aqueous solution)
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test.

Species/Strain	Human volunteer
Cell Type/Cell Line	Human lymphocytes
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat liver
Vehicle	Culture medium
Remarks - Method	No significant protocol deviations.

The test substance concentration was based on the test substance as supplied and was not corrected for the concentration of the notified chemical.

Vehicle and positive controls (mitomycin C without metabolic activation and cyclophosphamide with metabolic activation) were used in parallel with the test material.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	1.11*, 3.33*, 10.0*, 30.0*	24 h	24 h
Test 2			
Present			
Test 1	1.11*, 3.33*, 10.0*, 30.0*	2 h	24 h
Test 2			

*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	≥ 27.4	≥ 30.0	> 30.0	Negative
Test 2				
Present				
Test 1	≥ 27.4	≥ 30.0	> 30.0	Negative
Test 2				

Remarks - Results

The test substance did not induce any statistically significant increases in the frequency of cells with aberrations in the presence or absence of metabolic activation in any exposure group.

In the absence of the S-9 mix, the mitotic index was reduced to about 60% of that of the vehicle control value at the highest concentration used (30 μg/mL), whereas in the presence of the S-9 mix, the mitotic index was reduced to about 54% of that of the vehicle control value at the highest concentration used (30 μg/mL), which indicates that the test substance was toxic to cultured human lymphocytes.

The positive controls gave satisfactory responses, confirming the validity of the test system.

CONCLUSION

The notified chemical was not clastogenic to human lymphocytes treated *in vitro* under the conditions of the test.

TEST FACILITY

TNO-CIVO (1989 b)

B.10. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical (20% aqueous solution)

METHOD OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.

Species/Strain	Chinese Hamster
Cell Type/Cell Line	Chinese hamster ovary (CHO) cells
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat liver
Vehicle	Culture medium
Remarks - Method	No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	2.0, 4.0, 6.0, 8.0, 10.0	4 h	22-24 h
Test 2	4.0, 6.0, 8.0, 10.0, 12.0	4 h	22-24 h
Present			
Test 1	20, 40, 60, 80, 100	4 h	22-24 h
Test 2	40, 60, 80, 100, 120	4 h	22-24 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 13.7	≥ 10.0	> 10.0	Negative
Test 2		≥ 12.0	> 12.0	Negative
Present				
Test 1	≥ 124	≥ 100	> 100	Negative
Test 2		≥ 100	> 120	Negative

Remarks - Results In both absence and presence of a metabolic activation system, the test substance neither induced a reproducible positive response nor a concentration-related increase in mutant frequency at any one of the test susbstance concentrations.

CONCLUSION The notified chemical was not clastogenic to CHO cells treated *in vitro* under the conditions of the test.

TEST FACILITY TNO-CIVO (1989a)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical (purity 99%)
METHOD	OECD TG 301 A Ready Biodegradability: DOC Die-Away Test
Inoculum	Activated sludge from a domestic sewage treatment plant
Exposure Period	28 days
Auxiliary Solvent	None reported
Analytical Monitoring	DOC analysis
Remarks - Method	Based on the results of a bacterial toxicity test (EC_{20} = 11.43 mg test substance / L) an initial test item concentration of 5.17 mg DOC/L was used which equates to approximately 7 mg/L of notified chemical. Duplicates of the inoculum and inoculum blank, and a single reference sample and toxicity control were run in parallel. The flasks (all at pH 7.4) were incubated at 22.1°C in the dark on a mechanical shaker for 28 days.

RESULTS

Test substance		*Sodium Benzoate*	
Day	*% Degradation**	*Day*	*% Degradation*
7	72	7	89
14	91	14	94
21	95	21	91
28	94	28	97

*Removal was likely, in part, due to adsorption – see Remarks-Results

Remarks - Results	All validity criteria for the test were satisfied and no significant deviations from test guidelines were reported. The reference substance, sodium benzoate, reached the pass level (70%) by day 14 and thus confirmed the suitability of the inoculum and test conditions. The toxicity control attained 94% degradation after 14 days indicating the notified chemical is non-inhibitory to micro-organisms used in the test. The test substance surpassed the 70% degradation pass level within a 10 day window and is therefore ready biodegradable. A DOC determination was carried out after 3 hours to determine if there was adsorption of the test substance. No significant decrease in DOC occurred after 3 hours. However hydrolysis test 1 conducted on the notified chemical (see Appendix A) indicated significant adsorption of the notified chemical after 5 days at pH 7. DSEWPaC therefore considers that the reported degradation may be, in part, due to removal of the test substance by adsorption to glassware, sludge etc. and the results be treated with caution. It is concluded the test substance is rapidly removed from the test system.
CONCLUSION	The test substance is rapidly removed from the test system
TEST FACILITY	Infracor (2002b)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Notified chemical (purity 99%)

METHOD
- Species: *Cyprinus carpio* (common carp)
- Exposure Period: 96 hours
- Auxiliary Solvent: None reported
- Water Hardness: 250 mg/L $CaCO_3$
- Analytical Monitoring: TOC
- Remarks – Method: A definitive test was conducted at concentrations 0.11 – 0.99 mg/L under semi-static conditions (changed daily) according to the guidelines above. Test conditions were: 19.7°C – 20.2°C, pH 7.7 – 8.3, dissolved O_2 92 – 101% of saturation, 8 h/16 h dark/light period. The 96 h LC50 was determined graphically.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*			
Nominal	*Actual*		*24 h*	*48 h*	*72 h*	*96 h*
Control	< LOQ*	10	0	0	0	0
0.11	< LOQ	10	0	0	0	0
0.19	< LOQ	10	0	0	0	0
0.33	< LOQ	10	0	0	0	0
0.57	< LOQ	10	8	10	10	10
0.99	< LOQ	10	10	10	10	10

*Limit of quantification (value not reported)

- LC50: ≤ 0.43 mg/L at 96 hours
- NOEC: ≤ 0.33 mg/L at 96 hours
- Remarks – Results: No significant deviations from test guidelines were reported. The test substance was below the limit of quantification (LOQ) of the analytical method in all test vessels. Hence an additional series of test substance concentrations above the LOQ (nominally 2, 5 and 10 mg/L) were analysed in a separate stability test. After 24 h, the maximum deviation from nominal observed was 16%. Therefore the study authors concluded the test substance was present over the entire test duration.

  However, DSEWPaC considers the results of the stability study are not indicative of the concentration of test substance expected to be present in the fish study. This is because it was not conducted under the same conditions and at higher concentrations of test substance than the fish study. Moreover, a hydrolysis test (hydrolysis test 1, Appendix A) demonstrated significant adsorption of the test substance (approximately 40%) to glass after 5 days. Therefore this fish study may not meet the validity criteria, as there is no direct evidence that the concentration of test substance was maintained over the duration of the study. The results should therefore be treated with caution and the reported LC50 and NOEC are considered to be upper limits.

CONCLUSION: The test substance is very toxic to fish

TEST FACILITY: Infracor (2002c)

C.2.2. Chronic toxicity to fish

TEST SUBSTANCE	Notified chemical (20% aqueous solution)
METHOD	OECD TG 215 Fish, Juvenile Growth Test – flow-through
Species	*Oncorhynchus mykiss* (rainbow trout)
Exposure Period	28 days
Auxiliary Solvent	None reported
Water Hardness	0.7 – 1.0 mmol/L Ca^{2+} and Mg^{2+}
Analytical Monitoring	HPLC-MS/MS
Remarks – Method	A definitive test was conducted under flow-through conditions in accordance with the guidelines above. No significant deviations to protocol were reported. The concentration of 4 representative compounds (which accounted for 65.92% (w/w) of the active substance) and this was extrapolated to the concentration of the active substance in the test substance. Test conditions were: 14 ± 1.5°C, pH 7.51 – 8.09, 65% - 98% O_2 saturation of test media. Ten fish were placed in each test vessel. The NOEC was determined by comparing the pseudo-specific growth rate for each test concentration with the rate for the controls. Statistical tests were not performed as the tank specific growth rates were equal across all test concentrations with considerable overlap of standard deviations.

RESULTS

Day 28				
Nominal concentration of active substance (µg/L)	*Mean measured active substance concentration (µg/L)**	*Mortality (%)*	*Mean Fish Weight (standard deviation) / g*	*Mean Fish Length (standard deviation) / cm*
Control	–	0	8.33 (1.64)	8.9 (0.5)
4.69	Nd	0	8.69 (1.13)	8.8 (0.5)
9.38	Nd	0	8.50 (2.11)	8.8 (0.6)
18.75	Nd	0	8.44 (1.32)	8.7 (0.5)
37.5	13.6	10**	8.70 (1.89)	8.9 (0.6)
75.00	52.3	0	8.49 (2.09)	8.7 (0.7)

Nd – not determined. *Only the two highest test substance concentrations were measured as no adverse effects on juvenile growth were observed. **Lost due to handling.

NOEC	≥ 0.0523 mg active substance/L (based on mean measured concentrations)
Remarks – Results	All validity criteria were satisfied. The measured concentrations of the test substance were 5 – 96% of the nominal concentrations. No fish showed any clinical signs of intoxication, abnormal condition or behaviour. No test substance related mortality occurred and hence no statistical evaluation was applied. One fish was lost due to handling. The test substance concentrations were not high enough to observe a LOEC and hence only a lower limit for the NOEC was reported. Therefore a definitive long-term hazard classification could not be applied and the test substance is considered to be at most toxic to fish with fish with long lasting effects.
CONCLUSION	The test substance is, at most, toxic to fish with long lasting effects.
TEST FACILITY	Fraunhofer-Institute (2008b)

C.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical (purity 99%)
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None reported
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	TOC
Remarks - Method	A definitive test was conducted according to the guidelines above under static conditions. Test conditions: 20.04 – 20.32°C, pH 7.9 – 8.1, conducted in the dark, dissolved oxygen concentration 7.6 – 8.6 mg/L. In a separate test, test organisms were exposed to a reference toxicant (potassium dichromate). The 48 h EC50 was determined graphically.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
control	< LOQ*	4 × 5	0	0
0.08	< LOQ	4 × 5	0	0
0.14	< LOQ	4 × 5	2	20
0.24	< LOQ	4 × 5	20	20
0.42	< LOQ	4 × 5	20	20
0.72	< LOQ	4 × 5	20	20
1.2	< LOQ	4 × 5	20	20

*Limit of quantification (value not reported)

LC50	≤ 0.11 mg/L at 48 hours
NOEC	≤ 0.08 mg/L at 48 hours
Remarks - Results	No significant deviations from test guidelines were reported. All daphnids were immobilised with a reference toxicant concentration of 2.0 mg/L, which was considered to be within the normal range. The test substance was below the limit of quantification (LOQ) of the analytical method in all test vessels. Hence an additional series of test substance concentrations above the LOQ (nominally 2, 5 and 10 mg/L) were analysed in a separate stability test. After 48 h, the maximum deviation from nominal observed was 15%. Therefore the study authors concluded the test substance was present over the entire test duration. DSEWPaC considers the results of the stability study are not indicative of the concentration of test substance expected to be present in the daphnia study. This is because it was not conducted under the same conditions and at higher concentrations of test substance than the daphnia study. Moreover, a hydrolysis study (hydrolysis test 1, Appendix A) demonstrated significant adsorption of the test substance (approximately 40%) to glass after 5 days. Therefore this daphnia study may not meet the validity criteria, as there is no direct evidence the concentration of test substance was maintained over the duration of the study. The results should therefore be treated with caution and the reported LC50 and NOEC are considered to be upper limits.
CONCLUSION	The test substance is very toxic to aquatic invertebrates
TEST FACILITY	Infracor (2002d)

C.2.4. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical (20% aqueous solution)
METHOD	OECD TG 211 *Daphnia magna* Reproduction Test – semi static
Species	*Daphnia magna*
Exposure Period	21 days
Auxiliary Solvent	None reported
Water Hardness	0.6 – 0.9 mmol/L Ca^{2+} and Mg^{2+}
Analytical Monitoring	HPLC-MS/MS
Remarks - Method	A definitive test with daily renewal of solutions was conducted in accordance with the guidelines above. No significant deviations to protocol were reported. The concentration of 4 representative compounds (which accounted for 65.92% (w/w) of the active substance) and this was extrapolated to the concentration of the active substance in the test substance. Test conditions were: 20°C, pH 7.6 – 8.4, 7.6 – 9.0 mg O_2/L. For each endpoint the NOEC, LOEC and, if possible, the EC50 and EC10 were determined. Calculations were performed with the software ToxRat Professional (v2.09). A NOEC was calculated using ANOVA followed by Williams' test or an appropriate non-parametric test suggested by the ToxRat program. When the data showed a concentration-response relationship, the data were analysed by regression to determine the EC50 using Probit-analysis assuming log-normal distribution of the values.

RESULTS

Day 21				
Nominal concentration of active substance (µg/L)	*Time weighted mean of measured active substance concentration (µg/L)**	*Mean Percent Adult Survival*	*Mean Number of Living Offspring Produced per female – cumulative (standard deviation)*	*Mean Total Body Length in mm (standard deviation)*
Control	–	100	67.6 (9.5)	4.73 (0.28)
0.92	0.8	100	65.7 (7.2)	4.70 (0.37)
2.30	2.3	100	62.9 (9.1)	4.67 (0.35)
5.75	2.4	90	57.4 (13.2)	4.62 (0.31)
14.40	11.4	40	53.5 (13.0)	4.35 (0.36)
36.00	27.5	20	28.0 (8.5)	4.63 (0.28)

*LOQ of each component of test substance was < 0.1 µg/L

EC50 (immobilisation)	10.6 µg active substance/L at 21 days based on time weighted mean (95% CI 6.5 – 17.1 µg notified chemical/L)
EC50 (reproduction, cumulative offspring)	24.6 µg active substance /L at 21 days based on time weighted mean (95% CI 11.3 – >27.5 µg notified chemical/L)
NOEC (immobilisation)	2.4 µg active substance/L at 21 days based on time weighted mean
NOEC (reproduction, cumulative offspring)	2.3 µg active substance/L at 21 days based on time weighted mean
Remarks - Results	Due to decrease of the exposure concentrations during the test period, the time weighted means of the measured concentrations were used for the evaluation of the effect of concentration. All validity criteria for the test were satisfied and no significant deviations to protocol were reported. No adult mortality nor any sub-lethal effects

were observed up to a measured concentration of 2.4 µg active substance/L based on the time weighted mean (TWM) concentrations. This concentration was therefore identified as the immobilisation NOEC. Adult body length exhibited no significant differences up to the highest concentration tested. All surviving specimens appeared to be healthy.

The cumulative number of offspring per parent animal was 28.0 – 67.6 across treatment levels, showing an apparent concentration-dose relationship. Significant effects were not observed up to a measured concentration of 2.3 µg active substance/L (TWM) which was therefore identified as the reproduction NOEC. The reproduction EC50 was 24.6 µg active substance/L (TWM).

CONCLUSION The notified chemical is very toxic to aquatic invertebrates with long lasting effects

TEST FACILITY Fraunhofer-Institute (2008c)

C.2.5. Algal growth inhibition test

TEST SUBSTANCE Notified chemical (purity 99%)

METHOD OECD TG 201 Alga, Growth Inhibition Test.

Species	*Desmodesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: control, 0.003, 0.005, 0.009, 0.017, 0.030, 0.055, 0.1, 1 ,2 mg/L Measured: Not determined
Auxiliary Solvent	Not reported
Water Hardness	0.15 mmol/L Ca^{2+} and Mg^{2+}
Analytical Monitoring	TOC
Remarks - Method	A definitive test was conducted at nominal concentrations 0.003 – 2 mg/L according to the guidelines above. Test conditions were: 23.12 – 23.72°C, pH 7.4 – 9.8, photoperiod 24 hours, light intensity 6000 – 10,000 lux. The E_yC50 and E_rC50 were determined by probit analysis. The NOEC was determined on the area under the growth curve during the whole exposure time using the Student t-test.

RESULTS

Biomass		*Growth*	
E_yC50 mg/L at 72 h	*NOEC mg/L*	*E_rC50 mg/L at 72 h*	*NOEC mg/L*
≤ 0.03 95% CI 0.02 – 0.03	Not reported	≤ 0.05 95% CI 0.05 – 0.06	≤ 0.009

Remarks - Results The test substance concentration range was below the limit of quantification (LOQ) of the analytical method. Hence an additional series of test substance concentrations above the LOQ (nominally 2 mg/L) were analysed in a separate stability test. After 72 h in the 2 mg/L sample, the deviation from nominal observed was 14%. Therefore the study authors concluded the test substance was present over the entire test duration.

DSEWPaC considers the results of the stability study are not indicative of the concentration of test substance expected to be present in the algae study. This is because it was not conducted under the same conditions and at higher concentrations of test substance than the algae study. Moreover, a hydrolysis study (hydrolysis test 1, Appendix A) demonstrated significant adsorption of the test substance (approximately 40%) to glass after 5 days. Therefore this algae study may not meet the

	validity criteria, as there is no direct evidence the concentration of test substance was maintained over the duration of the test. The results should therefore be treated with caution and the reported EC50 and NOEC are considered to be upper limits.
CONCLUSION	The test substance is very toxic to algae. The test substance is very toxic to algae with long lasting effects.
TEST FACILITY	Infracor (2002e)

C.2.6. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical (purity 99%)
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test
Inoculum	Activated sewage sludge from a domestic sewage treatment plant
Exposure Period	3 hours
Concentration Range	Nominal: 5, 12.5, 32, 80, 200, 500 mg/L Measured: Not reported
Remarks – Method	A definitive test was conducted according to the guidelines above at test substance concentrations of 5 – 500 mg/L. A blank control and reference (3,5-dichlorophenol) control were run in parallel. No significant deviations to the test protocol were reported. Test conditions were: 18.0 – 20.0°C, pH 8.3 – 8.6 (after 3 h).
RESULTS	
IC50	22 mg/L (95% CI 19 –25 mg/L)
IC20	11 mg/L
NOEC	Not reported
Remarks – Results	All validity criteria for the guidelines were satisfied.
CONCLUSION	The notified chemical is not expected to be inhibitory to bacterial respiration ≤ 22 mg/L
TEST FACILITY	Infracor (2002f)

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