File No: STD/1425

September 2012

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Oxazolidine, 3- butyl-2-(1-ethylpentyl)-

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 CHEMICAL	INTRODUCTION VOLUME	USE
STD/1425	Chemicalia Pty Ltd Akzo Nobel Pty Ltd	Oxazolidine, 3- butyl-2-(1-ethylpentyl)-	No	< 50 tonnes per annum	Component of coatings for industrial use

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

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*
Acute Category 2	Toxic to aquatic life
Chronic Category 2	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 assessed use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- Due to the combustible properties of the notified chemical, the notifier should consider their obligations under the Australian Dangerous Goods Code.

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 and its hydrolysis products:
  - Enclosed systems (where possible) and local exhaust ventilation during paint manufacture
  - Spray applications to be conducted within a spray booth

- 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 or its hydrolysis products:
  - Avoid skin and eye contact
  - Avoid breathing mists or vapours

- 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 or its hydrolysis products:
  - Coveralls, impervious gloves, goggles
  - Respiratory protection during spray application

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

- Spray applications should be carried out in accordance with the Safe Work Australia *National Guidance Material for Spray Painting* (NOHSC, 1999) or relevant State and Territory Codes of Practice.

- A copy of the (M)SDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from a component of coatings for industrial use, or is likely to change significantly;
  - the amount of chemical being introduced has increased from 50 tonnes per annum, or is likely to increase, significantly;
  - the chemical has begun to be manufactured in Australia;
  - additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

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

No additional secondary notification conditions are stipulated.

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Chemicalia Pty Ltd (ABN: 17 100 190 270)
7 Cremin Court
MT WAVERLEY VIC 3149

Akzo Nobel Pty Ltd (ABN: 59 000 119 424)
115 Hyde Road
YERONGA QLD 4104

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: Degree of purity, residual monomers/ impurities, and import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: Water solubility, adsorption/desorption, dissociation constant, particle size, flammability and autoignition.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Incozol 2 (containing > 95% notified chemical)

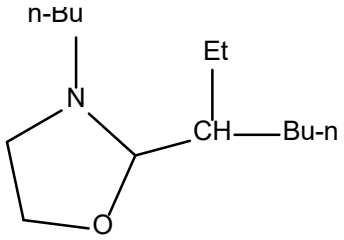
CAS NUMBER
165101-57-5

CHEMICAL NAME
Oxazolidine, 3-butyl-2-(1-ethylpentyl)-

OTHER NAME(S)
N-Butyl- 2-(1-ethylpentyl)-1 ,3-oxazolidine
2-(3-Heptyl-n-butyl-1, 3 oxazolane
2-(1-Ethyl pentanal)-N-butyl- 1, 3-oxazolane

MOLECULAR FORMULA
$C_{14}H_{29}NO$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
227.39 Da

ANALYTICAL DATA
Reference, IR, GC, UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY: > 95%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Light yellow liquid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	< -50 °C	MSDS
Boiling Point	259.9 °C at 101.3 kPa	MSDS
Density	872 kg/m^3 at 20 °C	Measured
Vapour Pressure	0.0025 kPa at 25 °C	MSDS
Water Solubility	Not determined	Test was not conducted due to the rapid degradation of the notified chemical in water.
Hydrolysis as a Function of pH	$t_{1/2}$ < 4 hours at pH 4, 7 and 9	Measured
Partition Coefficient (n-octanol/water)	log Pow = 4.47	Calculated (KOWWIN v1.68, US EPA 2011).
Adsorption/Desorption	Not determined	Test was not conducted due to the rapid hydrolysis of the notified chemical in water.
Dissociation Constant	Not determined	The notified chemical hydrolyses rapidly in water to yield a chemical, (2-(butylamino)-ethanol), that is potentially cationic in the environmental pH range of 4-9.
Particle Size	Not applicable	Liquid
Flash Point	82 °C (closed cup)	Measured
Flammability	Not expected to be flammable	Based on measured flash point.
Autoignition Temperature	> 196 °C	Analogue data
Explosive Properties	Not expected to be explosive	The structure formula contains no explosophers.
Oxidising Properties	Not expected to oxidise	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 rapidly hydrolyses in water to form 2-ethylhexanal (CAS No. 123-05-7) and 2-(butylamino)-ethanol (CAS No. 111-75-1). 2-Ethylhexanal is a flammable liquid.

Physical hazard classification
Based on the submitted physico-chemical data depicted in the above table, 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***
Flammable Liquids (Category 4)	H227-Combustible Liquid

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported neat (> 95% purity) as a liquid.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	1-3	3-10	10-30	10-30	< 50

PORT OF ENTRY
Brisbane and Melbourne

TRANSPORTATION AND PACKAGING
Incozol 2 (containing the notified chemical at > 95% concentration) will be imported in 200 L steel drums by sea. These drums will be transported by road to the notifier's warehouse facilities at various locations across Australia.

USE
The notified chemical will be used in two-part high solids polyurethane coatings and/or polyaspartic coatings as a water scavenger.

OPERATION DESCRIPTION
Manufacture of Part A paint
At the customers' paint manufacturing plants, Incozol 2 (containing the notified chemical at > 95% concentration) will be transferred using a spear pump to a closed mixing kettle containing polyols/and or polyaspartic esters, pigments, fillers and other additives. The amount of Incozol 2 to be added to the paint will vary depending on the moisture content present in the paint but is typically ~ 2%. After one hour of mixing, small samples will be taken for quality assessment purposes. The Part A paint will then be transferred using a pump from the mixing kettle to 200 L steel drums.

Application of Part A paint by end users
The Part A paint (containing the notified chemical at ~ 2%), packaged in 200 L steel drums, will be dispatched by road from the paint companies to industrial steel fabrication companies throughout Australia. At these sites, the Part A paint will be connected to a two-part paint mixer and sprayer (containing the Part B isocyanate curing agent). The two-part polyurethane or polyaspartic coating paint system will then be applied onto primed steel structures by spray paint operators in spray booths. After the two-part paint has been applied onto the steel structures they are left to cure at ambient temperature (which typically takes less than one hour). Once the coatings are fully cured the steel structures will be packed and transported to various locations throughout Australia for use.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport workers	4	4	6
Distribution workers	6	4	10
Warehouse staff	4	2	24
Paint production workers	2	3	24
Paint quality control technicians	2	1	24
End use spray painters	10	1	60

EXPOSURE DETAILS

Transport and storage workers may come into contact with the notified chemical (at concentrations > 95%) 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 > 95% during transfer of the notified chemical to the mixing vessels and at concentrations of up to 2% during blending, quality control analysis, and cleaning and maintenance of equipment. Exposure is expected to be minimised through the use of local exhaust ventilation and/or enclosed systems and through the use of personal protective equipment (PPE) such as coveralls, safety glasses and impervious gloves.

Exposure to the notified chemical at concentrations < 2% in end-use products may occur when used by professional spray paint operators. Exposure is expected to be minimised through the use of PPE including appropriate respiratory protection and conducting spray applications within spray booths.

During curing the notified chemical will react with moisture in the air and in the paint to form 2-ethylhexanal and 2-(butylamino)-ethanol. 2-(Butylamino)-ethanol will react with the Part B curing agent which contains isocyanates and therefore will be incorporated into the paint matrix. 2-Ethylhexanal is expected to be released from the coatings as a volatile by-product. Hence once the paint is cured and dried worker exposure to the notified chemical or 2-(butylamino)-ethanol is not expected to occur. There is potential for inhalation exposure to 2-ethylhexanal during curing and drying; however this should be minimised through the use of local exhaust ventilation.

6.1.2. Public Exposure

Paints containing the notified chemical will not be used by the public. The general public may come in contact with steel structures coated with paints containing the notified chemical; however once the paints are cured and dried public exposure to the notified chemical or its hydrolysis products is not expected to occur (see occupational exposure).

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 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	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 – 28 days	NOAEL = 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro chromosome aberration	non genotoxic

Toxicokinetics.
The notified chemical has a relatively low molecular weight (227.39 Da) and rapidly hydrolyses to form 2-ethylhexanal and 2-(butylamino)-ethanol (half-life < 4 hours at 25°C and pH 4, 7 and 9). Due to this reaction, the notified chemical is expected to undergo hydrolysis if in contact with mucous membranes lining the respiratory system, eyes and to a lesser extent the skin. The effects seen in the toxicological studies therefore likely represent a mixture of the notified chemical and its hydrolysis products.

Acute toxicity.
The notified chemical is of low acute oral (LD50 > 2000 mg/kg bw) and dermal toxicity (LD50 > 2000 mg/kg bw) in rats. There is no data available on the acute inhalation toxicity of the notified chemical. However, the vapour pressure of the notified chemical is very low (≤ 4 x 10^{-3} kPa) and therefore inhalation of the vapour of the notified chemical is not expected to occur under normal environmental conditions unless aerosols are formed.

Irritation and Sensitisation.
The notified chemical is only slightly irritating to the eye and skin of rabbits. In the skin irritation study slight irritation was observed in only one animal that persisted to the 72 hour observation period but was resolved at Day 8. In the eye irritation study, only slight conjunctival irritation (redness and discharge) was observed in all treated animals immediately after exposure. All signs of irritation were resolved at the 24 hour observation period.

In the Guinea Pig Maximisation Test, hardening of the dose site and very slight erythema was observed in test animals challenged with 10% and 20% of the notified chemical. However, a similar degree of hardening and erythema was observed in a proportionally similar number of control animals. Hence, under the conditions of the test there was no evidence of reactions indicative of skin sensitisation to the notified chemical.

Repeated Dose Toxicity.
In a 28-day repeat dose gavage study, rats were administered the notified chemical at 0, 15, 150 or 1000 mg/kg bw/day. Hepatocyte enlargement was observed in the high dose group and reduced alkaline phosphate levels were detected for both sexes treated with 1000 and 150 mg/kg bw/day, extending into the female 15 mg/kg bw/day dose group. These effects were considered an adaptive response to a xenobiotic and hence the NOAEL was established as 1000 mg/kg bw/day.

Mutagenicity.
The notified chemical was not mutagenic in a bacterial reverse mutation study, and was not genotoxic in an *in vitro* chromosome aberration study in human lymphocytes.

Health hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the toxicological studies provided, the notified chemical is of low toxicity but is slightly irritating to the skin and eyes. The notified chemical is non-volatile but reacts readily with moisture to produce the volatile products 2-ethylhexanal (CAS No. 123-05-7) and 2-(butylamino)-ethanol (CAS No. 111-75-1) having a predicted vapour pressure of 10.4 and 291 Pa, respectively (MPBPVP v1.43, US EPA, 2011).

2-Ethylhexanal has been classified by the notifier as a skin, eye and a respiratory irritant. 2-(Butylamino)-ethanol is harmful by the oral route (rat acute LD50 = 1150 mg/kg) and is a skin and severe eye irritant (Wiley, 2012).

Workers at risk of irritation effects from the notified chemical or its hydrolysis products are those handling the notified chemical as introduced at > 95% for formulation of the Part A paint. However, the expected use of PPE should minimise this risk.

There is the potential for inhalation exposure to the notified chemical and its hydrolysis products during formulation and more likely during spray application of the paints containing the notified chemical at < 2%. The inhalation toxicity of the notified chemical or 2-(butylamino)-ethanol is not known. 2-Ethylhexanal is

moderately toxic by inhalation (LCLo = 4000 ppm/4 hr). However, exposure to the notified chemical or its hydrolysis products during spray application is expected to be low due to the reduced concentration of the notified chemical (< 2%) and control measures in place (i.e. PPE including respiratory protection during spray application and spray booths) to minimise exposure to the isocyanate curing agent in Part B of the paint. Inhalation exposure during formulation is expected to be minimised by enclosed systems and the use of local exhaust ventilation.

Given the control measures in place to minimise exposure to the notified chemical during paint formulation and spray application, the risk to workers is not considered to be unreasonable.

6.3.2. Public Health

The public will not be exposed to the notified chemical except in the unlikely event of an accident or spill, hence the risk to the public is not considered unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported in steel drums. The environmental release of the chemical during transportation is expected to occur only in the unlikely event of an accident. Any spilled material is expected to be adsorbed on an appropriate substrate and placed in recovery drums for disposal to landfill.

The reformulation of the notified chemical into the Part A paint of a two-part coating is expected to be carried out in a closed mixing kettle, by trained workers. Spills and residues from the reformulation equipment are not expected to be significant and are expected to be collected for disposal to landfill.

RELEASE OF CHEMICAL FROM USE

During the use of the notified chemical in the Part A paint formulation, it will be mixed with Part B curing agent. The mixed coating will be applied onto primed steel structures by spray paint operators in spray booths. After the spray application, the chemical will react with water present in the pigments and fillers in the paint or in the atmosphere to form 2-ethylhexanal and 2-(butylamino)-ethanol. No notified chemical is expected to be present in the final dry crosslinked polyurethane or polyaspartic coating paint system. The only release that can be expected is from washing of the spray equipment, which is commonly expected to be up to 1% of the used volume, and may be released into the sewer for the worst case scenario consideration. Any spills from the spray application are expected to be collected for proper disposal to landfill. No significant quantity of the notified chemical or its hydrolysis products are expected to be released to the aquatic environment.

RELEASE OF CHEMICAL FROM DISPOSAL

The residues remaining in the used drums are estimated to be less than 0.01% of the use volume and are expected to be collected for disposal to landfill, or to be pre-mixed with a waste fuel to use the caloric value (e.g. in a cement kiln), where they will be thermally decomposed into water and oxides of carbon and nitrogen.

7.1.2. Environmental Fate

The notified chemical cannot be classified as readily biodegradable; however, it can be considered inherently biodegradable. It is also expected to hydrolyse rapidly in water to form 2-ethylhexanal and 2-(butylamino)-ethanol. For the details of the environmental fate studies please refer to Appendix C.

The instability of the notified chemical in the environment is not considered to be a concern given no significant release to the aquatic environment is expected from the use pattern. Bioaccumulation is not expected for the notified chemical since it hydrolyses rapidly in water. The two hydrolysis products, 2-ethylhexanal and 2-(butylamino)-ethanol, are not expected to have potential for bioaccumulation based on the predicted low n-octanol/water partition coefficient (log P_{OW}) values of 2.71 and 0.33, respectively (KOWWIN v1.68, US EPA 2011).

Most of the notified chemical is expected to be incorporated into an inert solid coating of steel structures. The hydrolysis product 2-(butylamino)-ethanol is expected to react with the Part B curing agent and be incorporated into the coating paint system by cross-linking. 2-Ethylhexanal is expected to be released from the coating as a volatile by-product, and is expected to be partially released into the atmosphere and partially thermally

decomposed during the recycling process of the metal substrates at the end of their useful life, forming water and oxides of carbon. A small amount of the notified chemical may be disposed of to landfill as collected spills from reformulation and use, to undergo biotic or abiotic degradation. The two hydrolysis products, 2-ethylhexanal and 2-(butylamino)-ethanol, have a predicted vapour pressure of 10.4 and 291 Pa respectively (MPBPVP v1.43, US EPA, 2011), and are expected to be volatile to highly volatile. They may be released into the air after application. However, they are not considered to be persistent in the atmospheric compartment given the predicted half-life of 3.8 hours for 2-ethylhexanal and 1.4 hours for 2-(butylamino)-ethanol (AOPWIN v1.92, US EPA, 2011). In either landfill or atmosphere, the notified chemical or the hydrolysis products are expected to be finally decomposed into water and oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has not been calculated since no significant release of the notified chemical, or its hydrolysis products, to the aquatic environment is expected from the proposed use pattern.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 = 20 mg/L	Harmful to fish
Daphnia Toxicity	48 h EC50 = 9.5mg/L	Toxic to daphnids
Algal Toxicity	72 h EC50 = 5.6 mg/L	Toxic to algae
	72 h NOEC = 1 mg/L	Harmful to algae with long lasting effects
Inhibition of Bacterial Respiration	3 h EC50 = 1400 mg/L	Not inhibitory to the respiration of sludge micro-organisms

Considering the notified chemical hydrolyses rapidly in water, the above endpoints are considered to represent a mixture of the notified chemical and its hydrolysis products. Where degradation is rapid, it is considered acceptable to use this data to classify the parent substance in the normal way (Annex 9, United Nations, 2009). Thus, the notified chemical is considered acutely toxic to algae and daphnids, and harmful to fish.

Under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009) the notified chemical is considered acutely toxic to aquatic organisms and is formally classified as Acute Category 2; Toxic to aquatic life. The algal 72 hour no observed effect concentration (NOEC) is available as a chronic endpoint for aquatic organisms. Therefore, the long-term classification for the notified chemical was determined based on the most stringent outcome by comparing the long-term hazard classification using either the acute or chronic data. Based on the acute endpoints for daphnids and algae, and the predicted log P_{OW} of > 4 for the notified chemical, the notified chemical is formally classified under the GHS as Chronic Category 2; Toxic to aquatic life with long lasting effects.

7.2.1. Predicted No-Effect Concentration

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

7.3. Environmental Risk Assessment

The Risk Quotient (PEC/PNEC) was not calculated since no significant release of the notified chemical or its hydrolysis products to the aquatic environment is expected. The notified chemical or its hydrolysis products are not expected to pose any unreasonable risks to aquatic environment based on the assessed use pattern.

For consideration of persistent, bioaccumulative and toxic (PBT) substances, the notified chemical or its hydrolysis products are not expected to be persistent. It is also not considered to meet the criteria for bioaccumulation given it hydrolyses rapidly in water and the hydrolysis products are expected to have low potential for bioaccumulation based on calculated n-octanol/water partition coefficients. The notified chemical and its hydrolysis products are not considered to meet the criterion for toxicity based on the provided chronic endpoint for algae.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Density 872 kg/m^3 at 20oC

Method OECD TG 109 Density of Liquids and Solids.
EC Council Regulation No 440/2008 A.3 Relative Density.
Test Facility SafePharm (2005a)

Hydrolysis as a Function of pH

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

pH	*T (°C)*	*$t_{½}$ <hours or days>*
4	10.0 ± 0.5	< 4 hours
7	10.0 ± 0.5	< 4 hours
9	10.0 ± 0.5	< 4 hours

Remarks Sample solutions of 20 mg/L were maintained at 10.0 ± 0.5°C for a period of 4 hours at pH 4, 7 and 9. A co-solvent, acetonitrile, was used at 1% to aid solubility. The concentration of the sample solution was determined by gas chromatography (GC).
Test Facility SafePharm (2005a)

Flash Point 82 °C

Method EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks Closed cup method
Test Facility Incorez (2011)

Explosive Properties

Method EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks Based on the chemical structure and oxygen balance, the notified chemical was predicted not to have explosive properties.
Test Facility Safepharm (2005b)

Oxidizing Properties

Method EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids).
Remarks Based on the chemical structure, the notified chemical was predicted not to have oxidising properties.
Test Facility Safepharm (2005b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure. EC Council Regulation No 440/2008 B.1 bis Acute toxicity (oral) fixed dose method.
Species/Strain	Rat/Crl:CD.BR
Vehicle	Corn oil
Remarks - Method	No significant protocol deviations. A preliminary sighting study was conducted using groups of two female rats dosed at 500 or 2000 mg/kg bw. Given no mortalities were observed in the sighting study, the dose chosen for the main study was 2000 mg/kg bw.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity	No clinical signs of toxicity were observed.
Effects in Organs	No macroscopic signs
Remarks - Results	
CONCLUSION	The notified chemical is of low acute toxicity via the oral route.
TEST FACILITY	Corning Hazelton (1995a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test. EC Council Regulation No 92/69/EEC B.3 Acute Toxicity (Dermal) – Limit Test.
Vehicle	None
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	No local signs of toxicity were observed.
Signs of Toxicity - Systemic	No systemic signs of toxicity were observed.
Effects in Organs	No abnormalities noted during necropsy.
Remarks - Results	All animals gained weight over the 14 day observation period.
CONCLUSION	The notified chemical is of low acute toxicity via the dermal route.
TEST FACILITY	SafePharm (2005c)

B.3. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Council Regulation No 92/69/EC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	New Zealand White Rabbit/Crl:NZW/Kbl.BR

Number of Animals	3
Vehicle	None
Observation Period	8 days
Type of Dressing	Semi-occlusive.
Remarks - Method	On two occasions the maximum recorded temperature of the holding room exceeded the expected range (16-22^{0}C) by 2^{0}C, but had no adverse effect on animal.

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	1	0	0	2 (1 hr)	< 8 days	0
Oedema	0	0	0	0	-	0

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

Remarks - Results	Well defined erythema and very slight oedema was observed for one animal immediately after the four hour exposure period. Very slight erythema persisted in this animal up to the 72 hour observation period but all signs of irritation were resolved at Day 8. No signs of irritation were observed in the other two test animals.

CONCLUSION	The notified chemical is slightly irritating to the skin.
TEST FACILITY	Corning Hazleton (1995b)

B.4. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation. EC Directive No 92/69/EEC B.5
Species/Strain	New Zealand White Rabbit/Crl:NZW/Kbl.BR
Number of Animals	3
Observation Period	72 hours
Remarks - Method	No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0	0	0	1	< 24 hrs	0
Conjunctiva: chemosis	0	0	0	0	-	0
Conjunctiva: discharge	0	0	0	2	< 24 hrs	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

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

Remarks - Results	Instillation of notified chemical elicited a slight initial sting response in one animal but no reaction from the other two animals. Only slight conjunctival irritation (redness and discharge) was observed in all treated animals immediately after exposure. All signs of irritation were resolved at the 24 hour observation period.

CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	Covance (1997a)

B.5. Skin sensitisation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 406 Skin Sensitisation – Maximisation Test EC Directive 92/69/EEC B.6 Skin Sensitisation
Species/Strain	Guinea pig/Dunkin-Hartley
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: 1% topical: 20%
MAIN STUDY	
Number of Animals	Test Group: 10 Control Group: 5
INDUCTION PHASE	Induction Concentration: intradermal: 2.5% topical: 60%
Signs of Irritation	Intradermal induction: Moderate to well-defined erythema was observed at injection sites for both the test and control animals that included treatment with Freund's Complete Adjuvant. Only slight erythema was observed at injection sites for both the test and control animals receiving the test substance in vehicle or vehicle only. Topical induction: No signs of irritation were noted in test or control animals after topical induction with 60% of the test substance or vehicle, respectively.
CHALLENGE PHASE	
1st challenge	topical: 10% and 20%
Remarks - Method	The vehicle used for the test substance was Alembicol D.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after1st challenge*	
		24 h	*48 h*
Test Group	0%	2/10	1/10
	10%	3/10	1/10
	20%	4/10	4/10
Control Group	0%	0/5	0/5
	10%	0/5	0/5
	20%	2/5	2/5

Remarks - Method	Hardening of the dose site and some cases of slight erythema were observed in two cases of the control group challenged with 20% of the test substance. A similar degree of hardening and erythema was observed in the test group in a proportionally similar number of animals. None of the dermal reactions in the test animals were considered to be more marked or persistent than in the controls.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Covance (1997b)

B.6. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.

	EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/Sprague-Dawley Crl:CD(SD) IGS BR
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: none
Vehicle	Dried arachis oil BP
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5/sex	0	0/10
low dose	5/sex	15	0/10
mid dose	5/sex	150	0/10
high dose	5/sex	1000	0/10

Mortality and Time to Death
There was no mortality in the test group during the course of the study.

Clinical Observations
No clinically observable signs of toxicity were detected.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Reduced alkaline phosphate levels were detected for both sexes treated with 1000 and 150 mg/kg/day, extending into the female 15 mg/kg/day dose group. The reductions were considered by the study authors to be a result of altered metabolism of a xenobiotic and were considered to be adaptive and of no toxicological importance.

Effects in Organs
The absolute and relative liver weights of male and female rats treated with 1000 mg/kg/day were elevated; however, statistical significance was only achieved for males. Centrilobular hepatocyte enlargement was observed in relation to treatment for males treated with 1000 mg/kg/day. One female treated with 1000 mg/kg/day was similarly affected. Hepatocyte enlargement is commonly observed in the rodent liver following treatment with xenobiotics and, in the absence of degenerative or inflammatory changes, is generally considered an adaptive response.

Additionally, absolute and relative kidney weights treated with 1000 mg/kg/day were elevated. However, given the absence of pathological changes, the toxicological significance of this finding is minimal.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1000 mg/kg bw/day in this study, based on microscopic hepatic changes in the liver in the high dose group and reduced alkaline phosphate levels detected at all dose levels being considered adaptive in nature.

TEST FACILITY Safepharm (2005d)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure/Pre incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, TA102.

Metabolic Activation System	Aroclor induced rat liver S9 fraction
Concentration Range in Main Test	a) With metabolic activation: 8-5000 µg/plate b) Without metabolic activation: 8-5000 µg/plate
Vehicle	DMF
Remarks - Method	Only Test 2 with metabolic activation employed a pre-incubation step. *E.coli* WP2 strains were not employed.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5000	> 5000	> 5000	negative
Test 2		> 5000	> 5000	negative
Present				
Test 1	> 5000	> 5000	> 5000	negative
Test 2*		> 200	> 5000	negative

* Employed a pre-incubation step

Remarks - Results	There was no sign of toxicity up to the limit of 5000 µg/plate through the normal plate incorporation method, but toxic effects were observed in all strains following the use of a pre-incubation step in the presence of S-9 where the maximum concentrations were reduced to 50-200 µg/plate. No substantial increase in revertant colony numbers of any of the tester strains were observed following treatment with the notified chemical at any dose level, with and without metabolic activation, in either mutation test. The concurrent positive control compounds demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Corning Hazleton (1995c)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test. EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.
Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 fraction from rat liver induced with phenobarbitone/β-napthoflavone
Vehicle	Acetone
Remarks - Method	No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 35.47, 70.94*, 141.88*, 283.75*, 567.5, 851.25	4 h	24 h
Test 2	0*, 17.74, 35.47, 70.94*, 106.41*, 141.88, 212.82*	24 h	24 h
Present			
Test 1	0*, 17.74, 35.47, 70.94, 141.88*, 212.82*, 283.75*	4 h	24 h
Test 2	0*, 35.47, 70.94*, 141.88*, 283.75*, 567.5*, 1135	4 h	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	≥ 1135	≥ 567.5	> 851.25	negative
Test 2	≥ 141.8	≥ 212.82	> 212.82	negative
Present				
Test 1	≥ 283.75	> 283.75	> 283.75	negative
Test 2		≥ 567.5	≥ 1135	negative

Remarks - Results

The notified chemical was toxic and did not induce chromosomal aberrations or polyploidal cells with and without metabolic activation using a dose range that included dose levels that induced mitotic inhibition.

The concurrent positive control compounds demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

CONCLUSION

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

TEST FACILITY

Safepharm (2006)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	CO_2 Evolution
Remarks - Method	The test was conducted in duplicates at a nominal concentration equivalent to 15 mg C/L, 21.1°C to 23.7°C and pH 7.41 – 7.43 at the end of the incubation. The final suspended solids nominal concentration in each test vessel was 30 mg/L. A reference control in duplicates was conducted using sodium benzoate at a concentration corresponding to 15 mg C/L. A toxicity control was also performed in a single vessel.

RESULTS

Test substance		*<Reference Substance>*	
Day	*% Degradation*	*Day*	*% Degradation*
28	63	28	91.5

Remarks - Results

Measurements of pH were made at the start of incubation in the blank and reference vessels only. The reason for omitting the vessels which contained the test substance was the risk that the pH electrode would become coated with the undissolved test substance to the extent that sufficient might be removed when the electrode was withdrawn to prejudice the eventual yield of CO_2. Final pH readings were made in all vessels on Day 28, immediately before their contents were acidified to release any residual CO_2 remaining in solution. The pH in the blank vessels was in the range of 7.49 – 7.61, which did not differ significantly from the test vessels at Day 28. Therefore, the pH is not considered to have any effects of concern on the test outcome.

The toxicity control results (139% by day 14) showed that the test substance is not inhibitory (> 25% by day 14).
Carbon dioxide evolution from the test substance reached a mean of 63% (66% maximum) at the applied concentration over the course of the 28 day incubation. This exceeds the 60% level that conventionally represents complete mineralisation. However, as CO_2 production failed to meet the ten day window requirement the test substance cannot therefore be classified as readily biodegradable. Although the test substance has failed to qualify for classification as readily biodegradable under the conditions employed in this study, the extent of mineralisation achieved in 28 days suggests that it is unlikely to persist in the aerobic environment.

CONCLUSION	The notified chemical cannot be classified as readily biodegradable.
TEST FACILITY	Covance (1997c)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test 96 hours - Semi-static. EC Directive 92/69/EEC C.1 Acute Toxicity for Fish 96 hours - Semi-static.
Species	*Oncorhyncus mykiss* (rainbow trout)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	The test substance was expected to rapidly hydrolyse in water to form 2-ethylhexanal and 2-(butylamino)-ethanol. The concentrations of the notified chemical and two hydrolysis products were determined using gas chromatography (GC).
Remarks – Method	Following a range-finding test, the definitive test was performed at nominal concentrations of 10, 18, 32, 56 and 100 mg/L with daily renewal of the test preparations. The test was conducted at 13.6 – 15.3°C, with a photoperiod of 16 hours light and 8 hours darkness with 20 minute dawn and dusk transition periods. The dissolved oxygen content was greater than or equal to 9.8 mg/L. The vessels were covered to reduce evaporation. The test material preparations were observed to be clear, colourless solutions throughout the duration of the test. It is expected that the test substance reacts with water to yield 2-ethylhexanal and 2-(butylamino)-ethanol as products of hydrolysis and full decomposition typically takes 6 hours. Therefore, the test media were analysed for the parent test material and the two hydrolysis products 2-ethylhexanal and 2-(butylamino)-ethanol. The median lethal concentration (LC50) values and associated confidence limits at 3, 6, 24, 72 and 96 hours were calculated by the trimmed Spearman-Karber method (Hamilton *et al* 1977) using the ToxCalc computer software package (ToxCalc 1999) and at 48 hours the LC50 value was calculated using the geometric mean method when there are no mortalities between 0% and 100% mortality.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality(hours)*					
Nominal	*Actual*		*3*	*6*	*24*	*48*	*72*	*96*
0	See below for Remarks – Results	7	0	0	0	0	0	0
10		7	0	0	0	0	0	0
18		7	0	0	0	0	1	2***
32		7	1*	1	1	7**	7	7
56		7	7*	7	7	7	7	7
100		7	7*	7	7	7	7	7

* Increased pigmentation.
** Swimming at the bottom with increased pigmentation.
*** Loss of equilibrium with increased pigmentation.

LC50	20 mg/L (95% CL 17 - 25 mg/L) at 96 hours
NOEC	10 mg/L at 96 hours
Remarks – Results	Test validity criteria were met, however evidence of maintained test substance concentration was not provided for reasons discussed below.

No significant protocol deviations except for temperature control for the control and test vessels and the pH of the control group. The slight deviation was considered not to have adversely affected the outcome of the test as no mortalities or sub-lethal effects of exposure were observed in the control animals. The pH of the control group was observed to vary between 7.4 and 8.2. The test vessels showed an increase in pH with increasing test concentration at 0 hours (9.1 for the 100 mg/L load). This may have been a contributing factor to the toxic nature of the test substance but it was considered not to affect the integrity of the study given that the purpose of the study was to determine the effect that the test substance would have on the test species.

Recoveries lower than the accepted range of 80-120% (70 – 75%) was achieved with 2-ethylhexanal. However, the analytical method has been considered to be adequate for the purposes of this test.

The parent test substance was expected to yield the hydrolysis products 2-ethylhexanal and 2-(butylamino)-ethanol in the proportions 56% and 52% respectively. Based on this information, analysis of the freshly prepared test media at 0, 24, 48 and 72 hours showed the concentrations of 2-ethylhexanal to range from 65% to 148 % of the theoretical nominal value. The values outside the range of 80 - 120% of the theoretical nominal value may have been due to the test material degrading differently in the test system than to the expected degradation path. Mean measured test concentrations of the old or expired test media at 24, 48, 72 and 96 hours showed the test concentrations of 2-ethylhexanal to range from 26% to 71% of the theoretical nominal value.

The decline in the measured test concentration of 2-ethylhexanal over each 24-hour exposure period may be due to its highly volatile property, unstable nature in culture medium and/or adsorption to surfaces.

The 2-(butylamino)-ethanol was detected by gas chromatography but could not be quantified in the test samples. Various procedures were attempted in method development but found to be of little value and suggested the chemical did not remain in the test medium.

As the test media contained the test substance and/or its hydrolysis products the toxicity cannot be calculated in terms of the hydrolysis products alone but of the test mixture as a whole. Therefore, it was considered justifiable to calculate the LC50 values in terms of the nominal test concentrations of the parent test substance only.

The 96-hour LC50 based on nominal test concentrations was 20 mg/L with 95% confidence limits of 17 - 25 mg/L. The no observed effect concentration (NOEC) was 10 mg/L. The notified chemical and/or the hydrolysis product are expected to be harmful to fish based on the test results.

CONCLUSION	The notified chemical is harmful to fish
TEST FACILITY	Safepharm (2005e)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test - Static
Species	*Daphnia magna*
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	The test substance was expected to rapidly hydrolyse in water to form 2-

ethylhexanal and 2-(butylamino)-ethanol. The concentrations of the notified chemical and two hydrolysis products were determined using gas chromatography (GC).

Remarks - Method

Following a range-finding test, twenty daphnids (2 replicates of 10 animals) were exposed to an aqueous solution of the test material at nominal concentrations of 1.0, 1.8, 3.2, 5.6, 10, 18, 32, 56 and 100 mg/L for 48 hours at a temperature of 21.1°C to 21.7°C, with a photoperiod of 16 hours light and 8 hours darkness with 20 minute dawn and dusk transition periods.

A positive control was conducted using potassium dichromate as the reference material at concentrations of 0.32, 0.56, 1.0, L8 and 3.2 mg/L. The EC50 value and associated confidence limits at 48 hours were calculated using the trimmed Spearman-Karber method (Hamilton *et al.*, 1977) using the ToxCalc computer software package (ToxCalc 1999).

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	See below for	20	0	0
1.0	Remarks –	20	0	0
1.8	Results	20	0	0
3.2		20	0	0
5.6		20	0	5
10		20	0	10
18		20	7	17
32		20	17	20
56		20	20	20
100		20	20	20

LC50 9.5 mg/L at 48 hours (95% confidence limits of 7.8-11 mg/L).

NOEC 3.2 mg/L at 48 hours

Remarks - Results

All test validity criteria were met. No significant protocol deviations except for temperature control. The water temperature throughout the test was shown to be slightly above the temperature range specified in the protocol of 20 ± 1°C. This slight deviation was considered not to have adversely affected the outcome of the test as no immobilisation or sub-lethal effects of exposure were observed in the control animals. The pH for the duplicate control vessels was 7.9 throughout the test. The test vessels showed an increase in pH with increasing test concentration at the start of the test (9.2 for the 100 mg/L load). This may have been a contributing factor to the toxic nature of the test substance but it was considered not to affect the integrity of the study given that the purpose of the study was to determine the effect that the test substance would have on the test species.

2-(Butylamino)-ethanol could not be quantified by the analytical method. Analysis of the freshly prepared test media at 0 hours showed measured test concentrations of 2-ethylhexanal to range from 98% to 123% of the theoretical nominal value. Analysis of the old or expired test media at 48 hours showed measured test concentrations of 2-ethylhexanal to range from 35% to 64% of the theoretical nominal value, with the exception of the 1.0 mg/L test concentration which showed a measured test concentration less than the limit of quantification. The result for the 1.0 mg/L test concentration was considered not to affect the results of the test as this concentration was below the NOEC.

The decline in the measured test concentration of 2-ethylhexanal over the 48-hour exposure period was considered may be due to its highly volatile property, unstable nature in culture medium and/ or adsorption to surfaces.

As the test media contained the test substance and/or its hydrolysis

products the toxicity cannot be calculated in terms of the hydrolysis products alone but of the test mixture as a whole. Therefore, the results are based on nominal test concentrations of the parent test substance only.
The 48 hour EC50 for the test material to *Daphnia magna* based on nominal test concentrations of parent test material was 9.5 mg/L with 95% confidence limits of 7.8 - 11 mg/L. The 48 hour no observed effect concentration (NOEC) was 3.2 mg/L. The notified chemical and/or hydrolysis products are considered to be toxic to daphnids based on the test results.

CONCLUSION The notified chemical is toxic to daphnids

TEST FACILITY Safepharm (2005f)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test.
Species *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*)
Exposure Period 72 hours
Concentration Range Nominal: 1.0, 3.2, 10, 32, 100 mg/L
Actual: Not applicable
Water Hardness Not reported
Analytical Monitoring Cell densities were determined using a Coulter® Multisizer Particle Counter;
The test preparations were analysed by gas chromatography (GC) for the parent test material and the two hydrolysis products 2-ethylhexanal and 2-(butylamino)-ethanol
Remarks - Method Following a preliminary range-finding test, alga were exposed to aqueous solutions of the test substance at concentrations of 1.0, 3.2, 10, 32 and 100 mg/L in three replicates, under constant illumination at 7000 lux and shaking at a temperature of 24 ± 1°C. The initial cell density used was about 1×10^4 cells/mL.
All statistical analyses were performed using the SAS computer software package (SAS 1999 - 2001) utilising Dunnett's and Bartlet's tests. The 72 hour median effective concentration for inhibition of growth (E_rC50) value was determined from the equation for the fitted line. The 95% confidence limits were calculated using the method of Litchfield and Wilcoxon (Litchfield and Wilcoxon 1949).

RESULTS

Biomass		*Growth*	
E_bC50	*NOEC*	*E_rC50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
5.6	1.0	12	1.0
95% CL 4.8 - 6.5		95% CL 9.7 – 15	

Remarks - Results

All test validity criteria were met. No significant protocol deviations.
The control vessels had a pH range of 7.3 – 7.4 through the test. The test vessels showed an increase in pH with increasing test concentration at 0 hours (9.6 for the 100 mg/L load). This may have been a contributing factor to the toxic nature of the test material but it was considered not to affect the integrity of the study given that the purpose of the study was to determine the effect that the test material would have on the test species.

Analysis of the test preparations at 0 hours showed measured test concentrations of 2-ethylhexanal to range from 0.712 to 73.3 mg/L (127% to 131% of the theoretical nominal value). Analysis of the test preparations at 72 hours showed a decline in measured test concentrations of 2-

ethylhexanal to less than the limit of quantification (LOQ) of the analytical method employed. 2-(Butylamino)-ethanol could not be detected by the analytical method. The decline in the measured test concentration of 2-ethylhexanal may be due to its highly volatile property, unstable nature in culture medium and/or possible adsorption to algal cells.

Exposure of *Scenedesmus subspicatus* to the test material gave a 72 hour E_bC50 of 5.6 mg/L with 95% confidence limits of 4.8 - 6.5 mg/L and a 72 h E_rC50 of 12 mg/L with 95% confidence limits of 9.7- 15 mg/L values in terms of the nominal test concentrations of the test substance. The no observed effect concentration (NOEC) was 1.0 mg/L. The notified chemical and/or its hydrolysis products are considered toxic to alga based on the test results.

CONCLUSION The notified chemical is toxic to algae

TEST FACILITY Safepharm (2005g)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum Sewage sludge from domestic sewage treatment plant.
Exposure Period 3 hours
Concentration Range Nominal: 180, 320, 560, 1000, 1800, and 3200 mg/L
Remarks – Method Following range-finding tests for the notified chemical and its hydrolysis products (after 24 hours stirring of the notified chemical's solution), a definitive test was performed. Activated sewage sludge was exposed to an aqueous dispersion of the test substance at nominal concentrations ranging 180 – 1000 mg/L for a period of 3 hours at a temperature of 21°C.

At all of the test concentrations, an oily slick of test substance was visible on the surface of the test media. The test substance was dispersed with the aid of ultrasonication in the test diluent for approximately 30 minutes prior to the addition of the synthetic sewage, activated sewage sludge and water. Each vessel was aerated with compressed air to ensure that there was maximum contact between the test substance and activated sewage sludge in the test medium.

A blank control and a reference control using 3,5-dichlorophenol were also conducted.

The percentage inhibition values were plotted against concentration, a line fitted using the Xlfit3 software package (IDBS 2002) and the median effective concentration (EC50) values determined from the equation for the fitted line.

RESULTS
EC50 1400 mg/L
NOEC 180 mg/L
Remarks – Results In some instances, the initial and final dissolved oxygen concentrations were below those recommended in the test guidelines (6.5 mg O_2/L and 2.5 mg O_2/L respectively). This was considered to have had no adverse effect on the results of the study given that in all cases the oxygen consumption rate was determined over the linear portion of the oxygen consumption trace.
The control vessels had pH values 8.1 – 8.2 at the test termination. The test vessels showed a concentration dependent increase in pH values with the higher test concentrations having higher pH values (9.2 for the 3200 mg/L load). This may have been a contributing factor to the toxic nature of

	the test substance but it was considered not to affect the integrity of the study given that the purpose of the study was to determine the effect that the test substance would have on a waste water treatment facility where pH adjustment of the incoming effluent would not occur. The effect of the test substance on the respiration of activated sewage sludge micro-organisms gave a 3 h EC50 of 1400 mg/L in term of nominal concentration of the test substance. The NOEC after 3 hours exposure was 180 mg/L. The notified chemical is considered not to inhibit the sludge micro-organisms respiration based on the test results.
CONCLUSION	The notified chemical is not expected to be inhibitory to sludge micro-organism respiration.
TEST FACILITY	SafePharm (2005h)

BIBLIOGRAPHY

Corning Hazleton (1995a) Incozol 2 - Acute Oral Toxicity Study in the Rat (Report No. 988/1-1032, 7 December 1995). Harrogate, England, Corning Hazleton (Europe) (Unpublished report submitted by Chemicalia Pty Ltd).

Corning Hazleton (1995b) Incozol 2 - Skin Irritation Study in the Rabbit (Report No. 988/2-1032, 7 December 1995). Harrogate, England, Corning Hazleton (Europe) (Unpublished report submitted by Chemicalia Pty Ltd).

Corning Hazleton (1995c) Incozol 2 – Reverse mutation in 5 histidine-requiring strains of *Salmonella typhimurium* (Report No. 988/3-1052, 7 December 1995). Harrogate, England, Corning Hazleton (Europe) (Unpublished report submitted by Chemicalia Pty Ltd).

Covance (1997a) Incozol 2 - Eye Irritation Study in the Rabbit (Report No. 988/006-1032, 6 August 1997). Harrogate, England, Covance Laboratories Ltd (Unpublished report submitted by Chemicalia Pty Ltd).

Covance (1997b) Incozol 2 - Skin Sensitisation Study in the Guinea Pig (Report No. 988/005-1032, 8 August 1997). Harrogate, England, Covance Laboratories Ltd (Unpublished report submitted by Chemicalia Pty Ltd).

Covance (1997c) Incozol 2 - Assessment of ready biodegradability by measurement of carbon dioxide evolution, (Report No. 988/7-1018, 8 September 1997). Harrogate, England, Covance Laboratories Ltd (Unpublished report submitted by Chemicalia Pty Ltd).

Hamilton, M A, Russo, R C and Thurston, R V (1977) Trimmed Spearman-Karber Method for Estimating Median Lethal Concentration m Toxicity Bioassays. *Environ Sci Technolll,* 714-719.

Incorez (2011) Determination of the flash point for Incozol 2 (April, 2011). Preston, Lancashire, UK, Incorez custom chemistry (Unpublished report provided by Chemicalia Pty Ltd).

Litchfield, J T and Wilcoxon, F (1949) A Simplified Method of Evaluating Dose-Effect Experiments. *J Pharmacal Exp Ther,* 96, 99-113.

NOHSC (2004) Approved Criteria for Classifying Hazardous Substances, 3rd edition [NOHSC:1008(2004)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

NTC (National Transport Commission) 2007 Australian Code for the Transport of Dangerous Goods by Road and Rail (ADG code), 7th Edition, Commonwealth of Australia

Safepharm (2005a) Incozol 2 - Determination of General Physico-Chemical Properties, (Report No. 2090/001, 2 June 2005). Derbyshire, England, Safepharm Laboratories Ltd (Unpublished report submitted by Chemicalia Pty Ltd).

Safepharm Laboratories (2005b) Incozol 2 - Explosive Properties and Oxidising Properties (Liquids), (Report No. 2090/009, 23 June 2005). Derbyshire, England, Safepharm Laboratories Ltd (Unpublished report submitted by Chemicalia Pty Ltd).

Safepharm (2005c) Incozol 2 - Acute Dermal Toxicity (Limit Test) in the Rat, (Report No. 2090/003, 2 June 2005). Derbyshire, England, Safepharm Laboratories Ltd (Unpublished report submitted by Chemicalia Pty Ltd).

Safepharm (2005d) Incozol 2 - Twenty-Eight Day Repeated Dose Oral (Gavage) Toxicity Study in the Rat, (Report No. 2090/0004, 5 December 2005). Derbyshire, England, Safepharm Laboratories Ltd (Unpublished report submitted by Chemicalia Pty Ltd).

Safepharm (2005e) Incozol 2 - Acute Toxicity to Rainbow Trout *(Oncorhynchus mykiss)*, (Report No. 2090/006, 3 October 2005). Derbyshire, England, Safepharm Laboratories Ltd (Unpublished report submitted by Chemicalia Pty Ltd).

Safepharm (2005f) Incozol 2: Acute Toxicity to *Daphnia Magna* (Project No. 2090/010, September, 2005). Shardlow, UK, Safepharm Laboratories (Unpublished report submitted by Chemicalia Pty Ltd).

Safepharm (2005g) Incozol 2: Algal Inhibition Test (Project No. 2090/007, September, 2005). Shardlow, UK, Safepharm Laboratories (Unpublished report submitted by Chemicalia Pty Ltd).

Safepharm (2005h) Incozol 2: Assessment of the Inhibitory Effect on the Respiration of Activated Sewage Sludge (Project No. 2090/008, October, 2005). Shardlow, UK, Safepharm Laboratories (Unpublished report submitted by Chemicalia Pty Ltd).

Safepharm (2006) Incozol 2 - Chromosome Aberration Test in Human Lymphocytes *In Vitro*, (Report No. 2090/005, 26 January 2006). Derbyshire, England, Safepharm Laboratories Ltd (Unpublished report submitted by Chemicalia Pty Ltd).

SAS/STAT Proprietary Software Release 8.02 (1999- 2001), SAS Institute Inc, Cary, NC, USA.

ToxCalc Version 5.0.23C (1999), Tideponl Scientific Software, McKinleyville, CA 95519, USA.

US EPA (2011) Estimation Programs Interface (EPI) Suite™ for Microsoft® Windows, v 4.10. United States Environmental Protection Agency. Washington DC, USA, <http://www.epa.gov/oppt/exposure/pubs/episuite.htm >.

Xlfit3 Version 3.0.5 Build 12 (2002), ID Business Solutions Ltd.

United Nations (2009) Globally Harmonised System of Classification and Labelling of Chemicals (GHS), 3rd revised edition. United Nations Economic Commission for Europe (UN/ECE), <http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html >.

Wiley (2012) 2-Butylaminoethanol 111-75-1 Sax's Dangerous Properties of Industrial Materials. Wiley Online Library http://onlinelibrary.wiley.com/doi/10.1002/0471701343.sdp04320/full. Last accessed 21 August 2012.