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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Chemical in AERO® XD 5002 Promoter

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

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

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

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Director
NICNAS

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Chemical in AERO® XD 5002 Promoter

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Cytec Australia Holdings Pty Limited (ABN: 45 081 148 629)
Suite 1, Level 1 Norwest Quay
21 Solent Circuit
Norwest Business Park
Baulkham Hills NSW 2153

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: Chemical names, Other names, CAS Number, Molecular formula, Structural Formula, Molecular weight, Spectral data, Purity, Non-hazardous impurities, Additives/adjuvants, Manufacture/import volume, and identity of sites.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: Chronic aquatic toxicity

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
US PMN (2005)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
AERO® XD5002 Promoter (imported product containing 75-85% notified chemical)

MOLECULAR WEIGHT
< 500 g/mol

ANALYTICAL DATA
A reference IR spectrum was provided.

3. COMPOSITION

DEGREE OF PURITY > 90%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

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

Property	Value	Data Source/Justification
Melting Point/Freezing Point	-7°C	Measured
Boiling Point	238°C at 101.3 kPa	Measured
Density	1060 kg/m^3 at 20°C	Measured
Vapour Pressure	2.8 x 10^{-5} kPa at 25°C	Measured
Water Solubility	6.48 ×10^{-2} g/L at 20°C	Measured
Hydrolysis as a Function of pH	pH 4 = 77.8 d at 25°C pH 7 = 100 d at 25°C pH 9 = 102 d at 25°C	Measured
Partition Coefficient (n-octanol/water)	log P_{OW} = 3.20 at 20°C	Measured
Surface tension	56.1 mN/m at 22°C (5.74 x 10^{-2} g/L solution)	Measured
Adsorption/Desorption	log K_{OC} = 3.13 at 30°C	Measured
Dissociation Constant	Not applicable	The notified chemical does not contain dissociable functionality.
Particle Size	Not applicable	The notified chemical is a liquid
Flash Point	125°C at 99.1 kPa	Measured
Flammability	Not determined	Not expected to be flammable based on vapour pressure and flash point values
Autoignition Temperature	336°C	Measured
Explosive Properties	Not explosive	Estimated based on chemical structure
Oxidising Properties	Not oxidising	Estimated based on chemical structure

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

Reactivity
Stable under normal conditions of use. Polymerisation will not occur.
Materials to avoid: Strong acids, bases, oxidizing agents.
Hazardous decomposition products include: oxides of sulphur (includes sulphur di- and trioxides), carbon monoxide, carbon dioxide, oxides of nitrogen, hydrogen sulphide and carbonyl sulfide

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as a component of AERO® XD5002 Promoter (75-85% concentration).

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 800	< 800	< 800	< 800	< 800

PORT OF ENTRY
Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
It is envisaged that the notified chemical will be used at mines around Australia.

TRANSPORTATION AND PACKAGING
The product AERO® XD5002 Promoter (75-85% notified chemical) will be imported by ship in 200 L drums or 1 000 L intermediate bulk containers (IBC). The product will be transported from the dock to the Cytec warehouse, and then to the customer site by road.

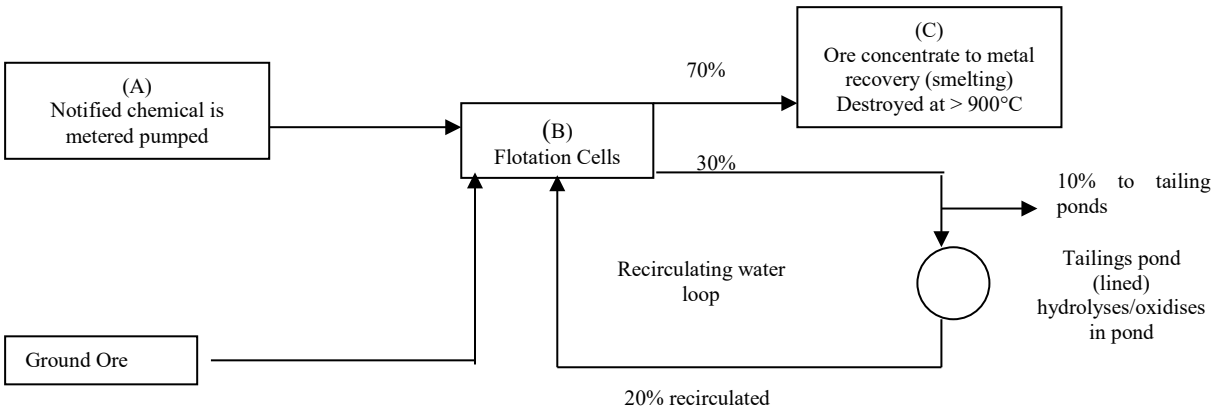
USE
The notified chemical will be used as a mineral processing reagent.

OPERATION DESCRIPTION
The imported product will be transported from the dockside to Cytec's contract warehouse where it will be stored in a chemical warehouse prior to being transported to the customer site. Initially the imported product will only be used in the mining industry without reformulation. However in the future the imported product (75-85% notified chemical) may be reformulated into more dilute solutions (10-50% notified chemical) by a toll manufacturer. A typical procedure for reformulation is therefore described below.

Reformulation of AERO® XD5002 Promoter
The AERO® XD5002 Promoter is received at the local blending site in 200 L drums or 1000 L IBC. All material is expected to be stored in a bunded, dedicated Dangerous Goods Area. When required the AERO® XD5002 Promoter is transferred on a pallet by forklift from the warehouse area to the blending area. AERO® XD5002 Promoter is pumped using an automated pumping system into a 20,000 L isotainer. The blending vessels are sealed at all times during the blending of a batch, except during the charging of the vessels. During the blending process, quality control samples may be taken from the 20,000 L isotainer via a valve. Once the blending process is completed the 20,000 L isotainer containing the finished product (10-50% notified chemical) is closed off and transported by road to the customer site for use. Blending will take approximately two hours, and will not require the use of heat.

End-User
At the end-user site metered quantities of the notified chemical will be pumped or gravity fed from the 200 L drums, 1000 L IBC or 20,000 L isotainer to a flotation cell which will be in a closed-loop water recirculation circuit. An ore slurry feeds into the same flotation cell. The notified chemical will be used at a rate of 12 g/tonne of ore (dry basis). The notified chemical selectively adsorbs to and enhances the floatability of the metal sulphide particles. Adsorption of the notified chemical is highly selective for metal bearing particles. The metal sulphides will then generally be mechanically collected and further concentrated by succeeding 'cleaner' flotation cells. The concentrated floated sulphides will finally be drawn off and transported to a smelter for metal recovery where the notified chemical will be destroyed within the smelting process. Settling ponds are part of the water recirculation loop, where depleted ore will be deposited.

The reagent storage/mixing and flotation processes are automated, continuous and recycling, thus minimising worker exposure. The reagent storage and flotation areas are open and well ventilated. Typically, within the reagent storage area, where other hazardous chemicals are also handled, the plant operators are required to wear respirators, impervious gloves, coveralls and eye protection.



6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration*	*Exposure Frequency*
Waterside and Transport	3-6	2-3 hours/day	10-15 days/year
Warehouse	2-3	2-3 hours/day	10-15 days/year
At blending site			
Blending	2	8 hours/day	25 days/year
Quality control	1	0.75 hours/day	25 days/year
End-users: Mining Industry			
Plant operators	6-12	1-8 hours/day	300 days/year

Storage and transportation
It is anticipated that waterside workers, transport drivers and warehouse workers would only be exposed to the material in the event of an accident

Reformulation of AERO® XD5002 Promoter
Dermal exposure to the notified chemical (up to 85% concentration) may occur as result of drips and spills during the sampling process or during the connection/disconnection of pumps. Inhalation exposure is not considered to be significant due to the low vapour pressure of the notified chemical. Manufacturing areas are equipped with general and local exhaust ventilation. Blending workers are expected to wear chemical resistant overalls, chemically resistant gloves, safety glasses/face shield, and safety shoes. The laboratory worker undertaking the QC activities wears a lab coat, chemically resistant gloves and safety glasses.

End-use: Mining Industry
The transfer, mixing and flotation processes are automated, continuous and recycling, with little need for worker intervention. However there is potential for dermal and possibly ocular exposure to the notified chemical (up to 85% concentration) while connecting and disconnecting lines and cleaning pumping and ancillary apparatus. Inhalation exposure is not considered to be significant due to the low vapour pressure of the notified chemical. The reagent storage and flotation areas are open and well ventilated. The plant operators in the reagent storage area are expected to wear respirators, impervious gloves, coveralls and eye protection due to the presence of other hazardous chemicals. The personnel in other areas will be expected to wear impervious gloves, coveralls and chemical splash goggles.

The quantitative exposure estimate is expected to be similar for both reformulation and mining industry personnel (if it is assumed that in the worst case an 85% solution of the notified chemical will be handled at both sites) since the worker processes are similar. EASE modeling of these processes (connecting/disconnecting hoses and cleaning operations) was performed to estimate the dermal exposure of the workers to the notified chemical. The following assumptions were made: direct handling, non-dispersive use (only used by workers with knowledge of the processes and use of controls), incidental contact level (assumed to be one event per day), and 85% concentration. The predicted dermal exposure is 0-0.085 mg/cm^2/day. Assuming exposure to a surface area equivalent to one hand (420 cm^2), a body weight of 70 kg, and 100% dermal absorption, this is equivalent to a systemic exposure of 0-0.51 mg/kg bw/day. This estimate does not take into account the use of PPE.

6.1.2. Public exposure
As the notified chemical will only be used for the process of flotation extraction in the mining industry, public exposure is unlikely.

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 and Result	*Assessment Conclusion*
Rat, acute oral	Harmful, LD50 ~ 500 mg/kg bw
Rat, acute dermal	Low toxicity, LD50 > 2000 mg/kg bw
Rat, acute inhalation	Not determined
Rabbit, skin irritation	Slightly irritating
Rabbit, eye irritation	Slightly irritating
Guinea pig, skin sensitisation – Maximization test (Magnusson-Kligman)	Limited evidence of sensitisation.
Rat, Oral (Gavage) repeat dose toxicity - 28 days.	No NOEL/NOAEL was determined, as toxicologically significant effects were observed at all dose levels (100, 40 and 15 mg/kg/day)
Genotoxicity - bacterial reverse mutation	Non mutagenic
Genotoxicity – in vivo Mammalian bone marrow chromosome aberration test in the rat	Non-Clastogenic
Genotoxicity – in vivo Micronucleus test in the mouse	Weakly genotoxic at maximum tolerated dose (320 mg/kg bw/day)

Toxicokinetics
The notified chemical is expected to be absorbed across biological membranes, based on the relatively low molecular weight (<500 g/mol) and the favourable physical-chemical properties (log Pow = 3.20, water solubility of 64.8 mg/L). Absorption across the gastrointestinal tract was confirmed by the observation of toxic effects after both acute and sub-acute oral exposure. While no evidence for acute toxic effects was observed in the acute dermal toxicity study, given the properties of the notified chemical the possibility of dermal absorption cannot be ruled out.

Acute toxicity
The notified chemical was found to be harmful to rats via the oral route. Adverse effects after oral exposure included hunched posture, lethargy, ataxia, decreased respiratory rate, laboured respiration, increased salivation, ptosis, loss of righting reflex, coma, increased lachrymation, dehydration and hypothermia. All animals treated at a dose level of 2000 mg/kg were found dead or were killed *in extremis*.

The notified chemical was found to be of low toxicity to rats after dermal exposure. No signs of local or systemic toxicity were observed during the study.

Irritation and Sensitisation
The notified chemical was found to be only slightly irritating to the eyes and skin of rabbits. While skin reactions were observed during the challenge phase of the sensitisation study in guinea pigs, the number of animals affected (15%) was below the cut-off for classification as a sensitiser (30%).

Repeated Dose Toxicity (sub-acute)
In a 28-day oral repeat dose study in rats adverse effects were observed at all dose levels (15, 40, and 100 mg/kg/day). The main effects observed involved the liver (including centrilobular hepatocyte degeneration and necrosis at all dose levels), the haemopoietic system and the kidney. Although the effects observed at the lowest dose (15 mg/kg/day) were relatively minimal when compared to animals treated at the higher doses, they were still considered to be dose-related and therefore precluded a determination of a NO(A)EL. The LOAEL determined in this study was therefore the lowest dose, 15 mg/kg/day. The notified chemical is therefore considered to have the potential to cause serious damage to health by prolonged exposure, which warrants a classification of R48 according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

Genotoxicity
The notified chemical was found to be non-mutagenic in a bacterial reverse mutation test, and non-clastogenic in an *in vivo* bone marrow chromosome aberration test in the rat. However in an *in vivo* micronucleus test in the mouse the notified chemical was found to be genotoxic at the maximum tolerated dose (320 mg/kg), although not at the lower doses in the study (80, 160 and 250 mg/kg). The testing laboratory has hypothesised that the

weak positive response in this assay was due to an aneugenic effect, rather than a clastogenic effect (i.e. a numerical aberration rather than a structural aberration), and therefore indicate that a threshold below which the notified chemical would not induce genotoxic effects may be able to be established. However the genotoxic mechanism cannot be proven conclusively from the studies presented, and the studies are not designed to reliably establish a lowest effective dose. Although no genotoxic effects were observed in the *in vivo* chromosome aberration test in the rat, if the notified chemical was aneugenic and only induced chromosome loss then this would not be picked up in this metaphase analysis assay. Therefore, based on the available information the notified chemical would be considered to be a concern for mutagenicity.

Carcinogenicity
The notified chemical has not been investigated for its carcinogenicity potential. Given the concern for genotoxic effects described above carcinogenic effects cannot be ruled out.

Classification
Based on the observed acute oral toxicity, sub-acute oral toxicity and genotoxicity the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following classification:

Xn: R22 Harmful if swallowed
T: R48/25 Danger of serious damage to health by prolonged exposure if swallowed
Xn: R68 Possible risk of irreversible effects

6.3. Human health risk characterisation

6.3.1. Occupational health and safety

The main route of exposure to the notified chemical (up to 85% concentration) for both reformulation and mining industry workers is expected to be dermal exposure, during processes such as connecting/disconnecting hoses, cleaning and sampling.

As the effects seen in the irritation and sensitisation tests were not sufficient to warrant classification, the risk of the notified chemical causing these effects in workers is considered to be low. In addition the workers are expected to be wearing PPE, which would further minimise the risk.

Although the notified chemical was found to be harmful via the oral route, this route of exposure is not expected to be significant for these workers as long as good hygiene practices are maintained. As the notified chemical was found to be of low acute toxicity via the dermal route the risk of health effects after a single dermal exposure to the notified chemical is expected to be low.

While the notified chemical was found to be of low acute toxicity via the oral route, health effects after repeated dermal exposure cannot be ruled out, particularly given the systemic toxicity observed following repeated oral exposure and the favourable physical-chemical properties for dermal absorption. EASE modelling of the reformulation/mining processes estimated the exposure as 0-0.51 mg/kg/day. No dermal NOAEL was determined. In the oral sub-acute toxicity study only a LOAEL (15 mg/kg/day) could be established as adverse effects were observed at all dose levels. Use of this LOAEL results in an MOE (margin of exposure) of 29. MOE greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. This MOE therefore indicates that the risk is not acceptable if workers are exposed to the notified chemical repeatedly on the skin. The MOE is based on conservative assumptions (e.g. 100% dermal absorption, no PPE) and may overestimate the risk.

In addition, the notified chemical was found to be a concern for mutagenicity, based on the positive result obtained in an *in vivo* micronucleus test in the mouse. While the testing laboratory hypothesised that the result was due to an aneugenic mechanism and attempted to establish a threshold dose, this could not be proven conclusively and so a NOEL cannot be used in the assessment of genotoxicity risk. Based on the potential for genotoxicity the risk of carcinogenicity cannot be ruled out.

Given the risk of causing serious damage to health by prolonged exposure, and the concern for mutagenicity the risk to workers is likely to only be acceptable when used under highly controlled conditions, and with the appropriate PPE. As the notifier has described the operations to be highly controlled, and good worker practices (including PPE) are in place during limited activities where worker handling is required, the risk of adverse effects is significantly reduced and is considered acceptable under the occupational settings described.

6.3.2. Public health

As the public are not expected to be exposed to the notified chemical the risk to public health is considered to be negligible.

7. ENVIRONMENTAL IMPLICATIONS

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported as a finished product and will not be reformulated in Australia. However, it is envisaged that in the future AERO® XD5002 Promoter containing the notified chemical will be blended in Australia by a toll manufacturer.

During blending process, water used to flush the pump is reused as part of the finished batch. The 20 000 L Isotainers containing the finished product are returned to blender for reuse. It is estimated that a maximum of 2% of the notified chemical (<16 tonne per annum) would be lost during spillage as a result of connecting and disconnecting pump. Spill kits are in place in the storage and production areas. Spills are contained in a bunded area and collected with inert absorbent material and disposed of through a licensed waste disposal contractor. No notified chemical enters the sewer system.

RELEASE OF CHEMICAL FROM USE

Release to the environment of the notified chemical is expected to be minimal. The areas where the chemical will be handled, pumped and stored will be bunded and any spilt material will be collected and disposed of appropriately in accordance with local, state and federal regulations.

The flotation reagent is added from the 200 L drums or IBC through a metered dosing pump to a flotation cell which will be in a closed-loop water recirculation circuit, and thoroughly mixed with the slurry. The typical usage of the notified chemical in the closed-loop water recirculation circuit is in the range of 2.5-25 ppm. The residence time in this tank is sufficient to allow the reagent(s) to react with (adsorb to) the surface of the desirable sulphide minerals. Approximately 70% of the notified chemical is expected to adhere to the beneficiated mineral surface with the remainder adhering to the gangue material or remaining in the solution. After conditioning, the slurry is usually diluted to around 30% solids with more water, and pumped to the flotation machines where the sulphide minerals attach themselves to air bubbles (generated by an impellor or gas sparging at the bottom of the flotation chamber), and float to the surface of the pulp. Here they are skimmed off, collected and filtered. The solids are then further dried to produce the final mineral concentrate which is then transported to a smelter to be refined into metal.

The gangue material (which has not been made sufficiently hydrophobic to attach to the bubbles) remains in the slurry, and is pumped out of the flotation cells to the tailings thickener. Here this waste is allowed to settle (usually with the aid of flocculants) into a high solids pulp, and then pumped to the tailings storage dam for final disposal. The excess water overflows from the thickener, and is returned to the flotation process. The tailings slurry is then pumped to tailings storage dams where the solids settle to the bottom and the excess water forms a shallow layer overlying these solids. This water usually becomes highly polluted with acid and dissolved heavy metals and is allowed to evaporate in shallow, large surface area ponds called evaporation dams. These may be eventually smelted for recovery of metal and the high temperature of the furnaces would destroy the compound. Some of the remaining reagent becomes attached to the surface of the gangue (waste) minerals, which are deposited into the tailings dams. However, the compound has a low affinity for the surface of these particles, and only a fraction of the reagent is released in this manner. It is expected that the 30% of the reagent that is discarded would typically comprise of 10% of the reagent adsorbed to the tailings (gangue) and the remaining 20% being dissolved in the water and is reused in the flotation process. At steady state it is expected that 20% of the dosed amount will remain in the tailings dam, this equates to 0.5-5 ppm (20% × 2.5-25 ppm).

The tailings dams are sealed with clay, special geo-textile lining fabric or a combination of both designed to greatly reduce the influx or efflux of water. A well designed and maintained clay liner is expected to have a permeability of 10^{-6} cm/sec or less and be between 2-4 feet (61-132 cm thick). Similarly synthetic liners are expected to have permeability of 10^{-9} to 10^{-14} cm/sec and have thickness of 40 to 60 mils (0.10-0.15 cm, A mil is defined as $1/1000^{th}$ of an inch, although it occasionally referred to incorrectly as a millimetre) (US EPA 1994). Liners may fail due to poor design with differential settling of the foundation; drying of the clay; or

possible weathering of synthetic liners (*ibid*). Catastrophic failures of tailings dams occur, but should be avoided by adequate design.

RELEASE OF CHEMICAL FROM DISPOSAL

The majority of the notified chemical will be destroyed by oxidation in the smelting process. Residual product left in the drums (~0.5%; ~4 tonnes per annum) will be rinsed out with water and the rinsate will be fed into the recirculating water loop. The rinsed drums are expected to contain ~1% residue (40 kg per annum) in the drums, which will be disposed of by licensed drum contractors.

7.1.2 Environmental fate

A single ready biodegradability study was supplied for the notified chemical, which demonstrated 48% degradation after 28 days. Therefore, the notified chemical cannot be classified as ready biodegradable. For the details of the environmental fate study please refer to Appendix C.

7.1.3 Predicted Environmental Concentration (PEC)

There is expected to be minimal release of the notified chemical from reformulation or spills at mine sites during use to the environment. Most of the compound will become associated with the surface of mineral particles in metal concentrates, and will be destroyed during smelting. The compound will decompose to water vapour and oxides of carbon, nitrogen and sulphur.

The new chemical will be used at approximately 6 mine sites within Australia the major release is expected to be from seepage from tailings dams. The PEC may be calculated assuming the maximum concentration of the notified chemical (5 ppm) in the dam and the degradation of the notified chemical as it permeates through the tailings liner. Sulphidic tailings dams are expected to be acidic with minimal microbiological life; and the main route of degradation is therefore expected to be hydrolysis. The minimum time taken to permeate through the liners is the depth (61 cm) ÷ by the highest permeability rate 1×10^{-6}cm, which results in 61×10^6 seconds or 706 days. For synthetic liners the time is 100×10^6 or ~1157 days. At acidic pH values (4) the notified chemical has a half-life of 77.4 days. Accordingly it will undergo approximately 9.1 half lives (706 ÷ 77.4) during its permeation through the liner. A worst case PEC at release from the tailings dam may calculated by 5 ppm $\times 0.5^{9.1} = 9.0$ µg/L.

7.2. Environmental effects assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	EC50 > 0.78 mg/L	Not toxic close to the limit of its solubility
Daphnia Toxicity	EC50 > 1.00 mg/L	Not toxic close to the limit of its solubility
Chronic daphnia toxicity	EC50 estimated as >0.1 mg/L (acute ÷ 10)	Slightly chronically toxic
Algal Toxicity	E_rC50 > 0.64 mg/L	Very toxic to algae
	E_bC50 = 0.32 mg/L	Very toxic to algae
Inhibition of Bacterial Respiration	EC50 240 mg/L	Not harmful to microorganisms

7.2.1 Predicted No-Effect Concentration

As acute ecotoxicity tests for three trophic levels of aquatic species was made, a safety (assessment) factor of 100 is used on the lowest measured EC50, which is 0.32 mg/L (E_bC50 algae).

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
E_bC50 algae	0.32	mg/L
Assessment Factor	100	
PNEC:	3.2	µg/L

7.3. Environmental risk assessment

The worst case Risk quotient (RQ) may be calculated as the PEC (9.0 µg/L) ÷ the PNEC (3.2 µg/L), resulting in an RQ of 2.8. This shows a potential hazard at the point of release from the liner of the tailings dam. However, such water will not immediately contact the aquatic environment containing aquatic life. It is expected that the notified chemical will travel underground after release from the dam's liner and will adsorb strongly to any organic carbon (log Koc = 3.13; Koc 1349) present in soil. Even at low organic carbon levels (0.5%) the Kd may be estimated as 0.005 × 1349 = 6.7. The percentage adsorbed to soil may therefore be calculated as [1 ÷ (6.7+1)] × 100 = 13%. The concentration in water after one cycle of adsorption to organic carbon in soil is expected to be 1.2 µg/L with an RQ of 0.38, showing that soon after leaving the tailings dam liner the risk is likely to fall below 1; and well before the notified chemical reaches the aquatic environment containing aquatic life. Further the half-life is expected to be an overestimate as the notified chemical is expected to be more rapidly hydrolysed in the highly acidic environment of a sulphidic tailings dam. In most scenarios the concentration of the notified chemical is expected to be even lower in the tailings dam and the liner thicker or less permeable.

Catastrophic failure of tailings dams or poor design and maintenance of liners may see the notified chemical contaminate the aquatic environment. However, this should normally be prevented.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available data the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)]. The classification and labelling details are: …

Xn: R22 Harmful if swallowed
T: R48/25 Danger of serious damage to health by prolonged exposure if swallowed
Xn: R68 Possible risk of irreversible effects

S36 Wear protective clothing
S37 Wear suitable gloves
S45 In case of accident or if you feel unwell seek medical advice immediately (and show the label where possible)
S57 Use appropriate containment to avoid environmental contamination

and

As a comparison only, the classification of the notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations 2003) is presented below. This system is not mandated in Australia and carries no legal status but is presented for information purposes.

	Hazard category	***Hazard statement***
Aquatic toxicity	1	Very toxic to aquatic life with long lasting effects
Acute toxicity	4	Harmful if swallowed
Germ cell mutagenicity	2	Suspected of causing genetic defects
Target organ systemic toxicity following repeat exposure	1	Causes damage to organs through prolonged or repeated exposure

Human health risk assessment
There is a risk of serious health effects after repeated exposure to the notified chemical, as well as a concern for mutagenicity. This risk to occupational health and safety is considered acceptable provided that the notified chemical is only used under controlled conditions by trained workers.

When used in the proposed manner the risk to the public is considered to be acceptable.

Environmental risk assessment
On the basis of the PEC/PNEC ratio the notified chemical is not considered to pose a risk to the environment based on its reported use pattern.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- The Office of the ASCC, Department of Employment and Workplace Relations (DEWR), should consider the following health hazard classification for the notified chemical:
  - Xn: R22 Harmful if swallowed
  - T: R48/25 Danger of serious damage to health by prolonged exposure if swallowed
  - Xn: R68 Possible risk of irreversible effects

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - Conc ≥ 25%: R48/25, R22, R68
  - 10% ≤ Conc < 25%: R48/25, R68
  - 1% ≤ Conc < 10%: R48/22, R68

- The following safety phrases should appear on the MSDS and label for the notified chemical:
  - S36 Wear protective clothing
  - S37 Wear suitable gloves
  - S45 In case of accident or if you feel unwell seek medical advice immediately (and show the label where possible)
  - S57 Use appropriate containment to avoid environmental contamination

Health Surveillance

- As the notified chemical is a health hazard (poses danger of serious damage to health by prolonged exposure if swallowed), employers should carry out health surveillance for any worker involved in its handling.

CONTROL MEASURES
Occupational Health and Safety

- Employers should ensure that the facility is equipped such that operations involving the notified chemical are performed in a highly controlled manner. The following isolation and engineering controls should be in place to minimise occupational exposure to the notified chemical:
  - Automated processes
  - Local exhaust ventilation
  - Sealed equipment

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical:
  - If swallowed, seek medical advice immediately
  - Avoid skin contact

- Workers must have adequate education and training before handling the notified chemical.

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical when worker handling is required for limited activities such as pipe disconnection and cleaning:
  - Safety glasses
  - Gloves
  - Coveralls

  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 *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Disposal

- The notified chemical should be disposed of by authorised landfill.

Storage

- Containers should be securely closed and stored according to container label instructions.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by adsorbing with inert adsorbent material. Sweep up and place in suitable container for disposal. Flush spill area with water and collect flush water with adsorbent material, do not allow entry into drains or waterways.

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
  - adverse incidents involving the notified chemical occur;
  - regulatory action on the notified chemical is undertaken by other jurisdictions;
  - details of the operation description are altered such that exposure to workers or the environment may be increased;
  - additional data becomes available on the genotoxicity or carcinogenicity of the notified chemical

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from a mineral processing reagent, or is likely to change significantly;

- the amount of chemical being introduced has increased from 800 tonnes, or is likely to increase, significantly;
- if 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.

AICS Annotation

- When the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS) the entry should be annotated with the following statement(s):
  - The notified chemical should only be used for industrial purposes under highly controlled conditions

Material Safety Data Sheet and Label

The MSDS and labels 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 MSDS and labels remains the responsibility of the applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point -7ºC

Method EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks The melting point was determined using differential scanning calorimetry. No significant protocol deviations.
Test Facility Safepharm Laboratories Ltd (2007a)

Boiling Point 238ºC at 101.85 kPa

Method EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks The boiling point was determined using differential scanning calorimetry. No significant protocol deviations.
Test Facility Safepharm Laboratories Ltd (2007a)

Density 1060 kg/m^3 at 20ºC

Method EC Directive 92/69/EEC A.3 Relative Density.
Remarks The relative density was determined using the pycnometer method, with distilled water as the comparison substance. No significant protocol deviations.
Test Facility Safepharm Laboratories Ltd (2007a)

Vapour Pressure 2.8 x 10^{-5} kPa at 25ºC

Method EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks The vapour pressure was determined using a vapour pressure balance at several temperatures, with linear regression analysis used to determine the vapour pressure at 25°C. No significant protocol deviations.
Test Facility Safepharm Laboratories Ltd (2007b)

Water Solubility 6.48 ×10^{-2} g/L at 20 ± 0.5°C (range 6.42-6.57 ×10^{-2} g/L)

Method EC Directive 92/69/EEC A.6 Water Solubility.
Remarks Flask Method, with analysis using HPLC. The pH of the solution was between 6.2-6.6.
Test Facility Safepharm Laboratories Ltd (2007a)

Hydrolysis as a Function of pH

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

pH	*T (°C)*	*$t_{½}$ (days)*
4	25	77.8
7	25	100
9	25	102

Remarks A preliminary test at 50°C was conducted and the half-lives at pH 4, 7 and 9 were 8.28, 7.48 and 5.53 days respectively. Methanol 0.8% was used as a co-solvent. Testing was continued at 60 and 70°C. The kinetics of the study were determined to be consistent with that of a pseudo-first order reaction as the graphs of $\log_{10}$ concentration versus time are straight lines.
Test Facility Safepharm Laboratories Ltd (2007a)

Partition Coefficient (n-octanol/water) log Pow = 3.20 at 20ºC

Method EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks HPLC Method. The notified chemical eluted between the reference substances toluene and naphthalene.

Test Facility Safepharm Laboratories Ltd (2007a)

Surface Tension 56.1 mN/m at 22°C

Method EC Directive 92/69/EEC A.5 Surface Tension.
Remarks The surface tension determination was conducted using a White Electrical Institute interfacial torsion balance and a procedure based on the ISO 304 ring method, which complies with Method A5 of the Commission Directive, except for the lack of correction (which is not applicable to the apparatus used). This deviation is not considered to have affected the integrity of the study.

The surface tension was determined using a 5.74 x 10^{-2} g/L solution. Although the substance is designated surface active according to Method A5 (since the surface tension is below 61 mN/m) it was considered that this value was not low enough to solely designate the material as surface active. The test material does not have a typical surfactant structure but does have a polar portion and non-polar portions suited to interaction with mineral surfaces and render them hydrophobic. To provide further information the emulsification properties were investigated with the test material prepared in water saturated n-octanol and partitioned with an equal amount of n-octanol saturated water. Two immiscible phases separated. Therefore the test material was considered to be surface active by method A5 definition but not significantly surface-active.
Test Facility Safepharm Laboratories Ltd (2007a)

Adsorption/Desorption log K_{oc} = 3.13 at 30°C

Method EC Directive 2001/59/EC C.19 Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography
Remarks The notified chemical eluted between the reference substances Endosulfan-diol and α-Endosulfan.
Test Facility Safepharm Laboratories Ltd (2007a)

Flash Point 125 ± 2°C at 101.3 kPa

Method EC Directive 92/69/EEC A.9 Flash Point.
Remarks The flash point was determined using a closed cup equilibrium method. No significant protocol deviations.
Test Facility Safepharm Laboratories Ltd (2007b)

Autoignition Temperature 336°C

Method EC Directive 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks No significant protocol deviations.
Test Facility Safepharm Laboratories Ltd (2007b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method. EC Directive 92/69/EEC B.1tris Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/ Sprague-Dawley CD (Crl: CD® (SD) IGS BR)
Vehicle	Administered as supplied for the 2000 mg/kg dose level. Administered as part of an arachis oil BP solution for the 300 mg/kg dose level.
Remarks - Method	No significant protocol deviations

RESULTS

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

* All animals treated at a dose level of 2000 mg/kg were found dead or killed *in extremis* one day after dosing.

LD50	~ 500 mg/kg bw
Signs of Toxicity	Signs of systemic toxicity noted dusting the study were hunched posture, lethargy, ataxia, decreased respiratory rate, laboured respiration, increased salivation, ptosis, loss of righting reflex, coma, increased lachrymation, dehydration and hypothermia. An isolated incident of red/brown staining around the mouth and diuresis were noted in one animal treated at a concentration of 300 mg/kg. Surviving animals appeared normal two or three days after dosing. The surviving animals showed expected gains in bodyweight over the study period.
Effects in Organs	Abnormalities noted at necropsy of animals that died or were killed *in extremis* during the study were haemorrhage of the lungs, dark liver, dark kidneys, haemorrhage and/or epithelial sloughing of the gastric mucosa. No abnormalities were noted at necropsy of animals that were killed at the end of the study.
Remarks - Results	

CONCLUSION	The notified chemical is harmful via the oral route.
TEST FACILITY	Safepharm Laboratories Limited (2005a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/Sprague-Dawley CD (Crl:CD® (SD) IGS BR)
Vehicle	Administered as supplied.
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 males	2000	0
2	5 females	2000	0

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

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

TEST FACILITY Safepharm Laboratories Limited (2005b)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 males
Vehicle	Administered as supplied
Observation Period	7 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	1	1	1	2	< 7 days	0
Oedema	0.3	0.3	0.3	1	< 48 hours	0

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

Remarks - Results A single 4-hour, semi-occluded application of the test material to the intact skin of the three rabbits produced very slight to well-defined erythema and very slight oedema at the 24 hour observation. Slight erythema was noted at all treated skin sites at 48 hours. Two treated skin sites appeared normal at the 72-hour observation and the remaining treated skin site appeared normal at the 7-day observation.
In 3-minute and 1-hour semi-occluded applications of the test material to the intact skin of one rabbit no evidence of skin irritation was produced.
No corrosive effects were noted.

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY Safepharm Laboratories Limited (2005c)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 males
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	0.33	0.33	0.33	1	< 48 hours	
Conjunctiva: chemosis	0	0	0	1	< 24 hours	
Conjunctiva: discharge	0	0.33	0.33	1	< 48 Hours	
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	A single application of the test material to the non-irrigated eye of three rabbits produced minimal conjunctival irritation. All animals showed mild conjunctival irritation at the 1-hour observation, with all reactions clearing by 48 hours.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY Safepharm Laboratories Limited (2005d)

B.5. Skin sensitisation

TEST SUBSTANCE Notified chemical

METHOD OECD TG 406 Skin Sensitisation – Guinea pig maximization test (Magnusson-Kligman).

Species/Strain	Guinea pig/Hartley Albino
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: 25% topical: 50% Maximum concentration causing mild-moderate irritation: topical: 100%
MAIN STUDY	
Number of Animals	Test Group: 20 Control Group: 10 (100% corn oil)
INDUCTION PHASE	Induction Concentration: intradermal injection: 25% topical application: 100% (with SLS (10%) pre-treatment)
Signs of Irritation	For test substance - erythema was discrete to intense. For vehicle control – erythema was absent to intense.
CHALLENGE PHASE	
1st challenge	topical application: 50%
2nd challenge	topical application: not conducted
Remarks - Method	The scoring time was not recorded for the topical screen animals at 24 hours post patch removal. Upon review of the data, it appeared that the topical screen animals were scored following observations of the intradermal screen animals. The oversight did not impact on the study results, since all screen animals were observed and the scores were recorded on the data.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*

		1st challenge		2nd challenge	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	50%	3/20	2/20	-	-
Control Group	50%	0/10	0/10	-	-

Remarks - Results: There were no deaths or substance-related signs of toxicity during the study. On first challenge 3/20 (15%) animals showed a score of 1 at 24 hours. This was below the 30% cut-off for evidence of positive responses to meet the classification criteria. The positive control confirmed the sensitivity of the test system.

CONCLUSION: There was limited evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.

TEST FACILITY: MB Research Laboratories (2005a)

B.6. Repeat dose toxicity

TEST SUBSTANCE

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	Sprague-Dawley Crl:CD® (SD) IGS BR strain
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days;
Vehicle	Arachis oil BP
Remarks - Method	No recovery period. Overnight fasting before collection of blood samples was not conducted.

RESULTS

Dose mg/kg bw/day	*Number and Sex of Animals*	*Mortality*
0	5 male, 5 female	0
15	5 male, 5 female	0
40	5 male, 5 female	0
100	5 male, 5 female	0

Mortality and Time to Death

There were no unscheduled deaths during the study.

Clinical Observations

Incidences of increased salivation immediately after dosing and, on occasions prior to dosing and up to one and five hours after dosing were detected for all treated animals throughout the study period. On Day 1 of the study, incidents of hunched posture were detected for animals of either sex treated with 100 or 40 mg/kg/day, while lachrymation was detected for animals of either sex treated with 100 mg/kg/day or noisy respiration detected for females only of this treatment group.

Behavioural Assessment - There were no treatment-related changes in the behavioural parameters measured.

Functional Performance Tests - There were no toxicologically significant changes in the functional performance parameters measured.

Sensory Reactivity Assessments - There were no treatment-related changes in sensory reactivity.

Bodyweight - A reduction in bodyweight development was detected for males treated with 100 and 40 mg.kg/day during Week 1 of the study. This reduction persisted throughout the remainder of the study for 100

mg/kg/day males, while subsequent recovery was observed after the first week of treatment for males treated with 40mg/kg/day.

Food Consumption - A reduction in food consumption was detected for animals of either sex treated with 100 mg/kg/day during the first week of treatment. This reduction subsequently recovered for 100 mg/kg/day females, while the reduction continued for 100 mg/kg/day males for the remainder of the study period.

Water Consumption - An increase in water consumption was detected for animals treated with 100 mg/kg/day during the first week of treatment and for all treated males for the remainder of the study when compared to controls. Females treated with 100 or 40 mg/kg/day had increased water consumption throughout the study period.

No such observations were detected for animals of either sex treated with 15 mg/kg/day.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology - Animals of either sex treated with 100 mg/kg/day showed a reduction in haemoglobin, haematocrit and haematocrit and erythrocyte count, and elevations in reticulocyte count. In addition males of this treatment group showed reductions in neutrophil count, while females showed an increase in man cell volume.

At 40 mg/kg/day reductions in haemoglobin and haematocrit were detected for animals of either sex, whilst females of this treatment group also showed a reduction in erythrocyte count and an elevation in reticulocyte count.

No such observations were detected for animals of either sex treated with 15 mg/kg/day.

Blood Chemistry - At 100 mg/kg/day, animals of either sex showed a decrease in total protein and an increase in alkaline phosphatase levels. In addition males had elevated cholinesterase and plasma urea levels; whilst females of this treatment group showed reductions in both glucose and cholesterol levels. The reduction in plasma cholesterol levels extended to females treated with 40 and 15 mg/kg/day.

Males treated with 40 mg/kg/day had reduced total protein levels and showed elevations in plasma glucose levels.

No treatment related changes were detected in the blood parameters measured for males treated with 15 mg/kg/day.

Effects in Organs

All treated males showed increases in both relative liver and kidney weights, while females treated with 100 and 40 mg/kg/day also showed elevations in liver weight. In addition all treated animals had reduced thymus weight, and animals of either sex treated with 100 mg/kg/day had elevated spleen weights.

Three males treated with 100 mg/kg/day had pallor of the liver and kidneys. In addition 3 females treated with 100 mg/kg/day and one treated with 40 mg/kg/day had pallor of the kidneys.

Liver - Histopathological changes characterised by centrilobular hepatocyte enlargement, vacuolation of centrilobular hepatocytes, centrilobular hepatocyte inflammatory cell infiltrates, pigment deposits, and a higher incidence and generally higher grades of severity of generalized hepatocyte vacuolation (glycogen type in appearance), were seen in relation to treatment for animals of either sex treated with 100 mg/kg/day, and to a lesser extent for animals treated with 40 and 15 mg/kg/day. Vacuolation of centrilobular hepatocytes was demonstrated to be a consequence of lipid accumulation by frozen sections stained with Oil Red O. Pigment deposits stained positively with Perl's stain and were thus likely to be haemosiderin.

Centrilobular hepatocyte degeneration and necrosis were observed for animals of either sex treated with 100 mg/kg/day; for one female and two males treated with 40 mg/kg/day and for three males treated with 15 mg/kg/day. This is considered to be an adverse morphological change.

Spleen – Higher grades of severity of extramedullary haemopoiesis and haemosiderin pigment deposition, positively stained with Perl's stain, were seen in relation to treatment for animals of either sex treated with 100 mg/kg/day. Higher grades of severity of extramedullary haemopoiesis were also seen for males treated with 40 and 15 mg/kg/day, and higher grades of severity of extramedullary haemopoiesis and pigment deposition were seen for females treated with 40 mg/kg/day.

Kidneys – Tubular basophilia and karyomegaly of tubular cells affecting tubules of the inner cortex were seen for animals of either sex treated with 100 mg/kg/day, but not convincingly at any other treatment level.

Thyroid - Follicular cell hypertrophy was observed in relation to treatment for animals of either sex treated with 100 and 40 mg/kg/day.

Thymus - Atrophy of the thymus was seen as a variable response among treated animals of either sex but there was no evidence of a dose relationship and although the condition may be associated with treatment as such it is regarded as a secondary effect.

Remarks – Results
Haemopoietic system – The haematological findings (i.e. reductions in haemoglobin, haematocrit and erythrocyte count) are associated with haemolytic anaemia, and are supported by the histopathological evidence of disruptions to the haemopoietic system (effects on spleen).

Liver – Adverse morphological changes were detected in the liver for animals from all treatment groups. These findings were supported by haematological and organ weight evidence.

Kidney – Macroscopic and microscopic evidence of kidney damage was correlated with the elevation in plasma urea and elevate kidney weights.

The primary dose related effects in the liver, kidney and spleen at the lowest dose of 15 mg/kg/day were relatively minimal when compared to animals treated with 100 or 40 mg/kg/day. However the occurrence of these effects still precludes the determination of a NO(A)EL.

CONCLUSION
Oral administration of the test material, N-Butylcarbonyl-O-butyl thionocarbamate, to rats for a period of 28 consecutive days at dose levels of 100, 40 and 15 mg/kg/day resulted in toxicologically significant effects for animals of either sex from all dose levels.

The "No Observed Effect Level (NOEL) was therefore, not achieved. The Lowest Observed Adverse Effect Level (LOAEL) was determined to be 15 mg/kg/day in this study.

TEST FACILITY	Safepharm Laboratories Limited (2007c)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100. *E. coli*: WP2uvrA$^-$.
Metabolic Activation System	Rat S9 fraction from phenobarbitone/β-napthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 5 - 5000 µg/plate (*S. typhimurium*) 50-5000 µg/plate (*E. coli*) b) Without metabolic activation: 5 - 5000 µg/plate (*S. typhimurium*) 50-5000 µg/plate (*E. coli*)
Vehicle	Dimethyl sulphoxide, test substance added as solution
Remarks - Method	No significant protocol deviations. In the preliminary toxicity test, conducted on TA100 and WP2uvrA$^-$, the test material was initially toxic at 500 µg/plate to TA100 and was non-toxic to WP2uvrA$^-$.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	500 µg/plate	500 µg/plate	>5000 µg/plate	negative
Test 2		1500 µg/plate	>5000 µg/plate	negative
Present				
Test 1	1500 µg/plate	500 µg/plate	>5000 µg/plate	negative
Test 2		1500 µg/plate	>5000 µg/plate	negative

Remarks - Results

The test material was tested up to the maximum recommended dose level of 5000 µg/plate. No toxicologically significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation

Small increases in revertant colony frequency were noted in bacterial strains TA100 (in the presence of S9) and TA1535 (in the absence of S9) at 150 µg/plate in the first experiment. These increases were not however, reproduced in experiment two and were considered to be of no biological relevance because there was no evidence of a dose-response relationship or reproducibility. Furthermore, the revertant counts at 150 µg/plate were well within the in-house range for the tester strains and the fold increases were less than 1.4 times the concurrent vehicle controls.

All the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

CONCLUSION

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

TEST FACILITY

SafePharm Laboratories (2005e)

B.8. Genotoxicity – in vivo

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 475 Mammalian Bone Marrow Chromosome Aberration Test.
EC Directive 2000/32/EC B.11 Mutagenicity - In vivo Mammalian Bone Marrow Chromosome Aberration Test.

Species/Strain: Rat/Sprague-Dawley Crl:CD®(SD) IGS BR
Route of Administration: Oral – gavage
Vehicle: Arachis oil
Remarks - Method: No significant protocol deviations. As it was suspected that the response, if any would be quite modest the animal numbers for the vehicle and test material groups were increased to ten/group. The preliminary toxicity test was conducted using only male animals at doses of 320 and 400 mg/kg/day, based on data from the Mouse Micronucleus test.

Dose mg/kg bw	*Number and Sex of Animals*	*Sacrifice Time Hours*
0 (vehicle control)	10 male	48
0 (vehicle control)	10 male	24
80	10 male	24
160	10 male	24
320	10 male	48
320	10 male	24

25 (Positive control, CP)	5 male	24

CP = cyclophosphamide

RESULTS

Doses Producing Toxicity

No premature deaths occurred in the preliminary toxicity test. However, the clinical signs observed after 24-hours at 400 mg/kg exceeded acceptable limits permitted by the Home Office for this type of study and, therefore, the animals were killed in *extremis*. The clinical signs observed at 400 mg/kg were as follows: hunched posture, ataxia, lethargy, ptosis, pilo-erection, decreased respiratory rate and laboured respiration. In animals dosed orally with test material at 320 mg/kg the clinical signs observed were acceptable, and as follows: pilo-erection and diuresis. 320 mg/kg was therefore chosen as the maximum tolerated dose.

There were no premature deaths seen in any of the test material dose groups. Clinical signs were observed in all animals dosed with the test material. These were as follows: hunched posture, ataxia, lethargy and diuresis.

Genotoxic Effects

The test material did not induce any significant or dose-related increases in the frequency of aberrations in any of the treatment groups. The test material did not induce a significant increase in the numbers of polyploidy cells in any of the treatment groups.

There were no statistically significant reductions in the mean mitotic index in any of the test material treatment groups when compared to their concurrent vehicle control groups. The mean mitotic index of the positive control group was statistically significantly lower than that of the 24-hour vehicle control group indicating a cytotoxic response in the bone marrow.

All the vehicle control animals gave values of chromosome aberrations within the expected range. The mean frequency of aberrations was consistent between the two vehicle control groups, the highest frequency (0.3% cells with aberrations excluding gaps) being seen in the 48-hour group.

The positive control group animals showed highly significant increases in the frequency of aberrations indicating that the test method itself was operating as expected.

Remarks - Results

Although there was no indication of cytotoxicity to the bone marrow, the observation of clinical signs of toxicity indicated that systemic absorption had occurred. It is therefore assumed that the target organ was reached.

CONCLUSION

The notified chemical was not clastogenic to rat bone marrow cells *in vivo* under the conditions of the test.

TEST FACILITY

SafePharm Laboratories (2005f)

B.9. Genotoxicity – in vivo Micronucleus

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
EC Directive 2000/32/EC B.12 Mutagenicity Mammalian Erythrocyte Micronucleus Test.

Species/Strain

Albino mice/Crl:CD-1™ (ICR)BR

Route of Administration	Oral – gavage
Vehicle	Arachis oil
Remarks – Method	No significant protocol deviations. In the preliminary toxicity test male and female rats were treated with 250, 330, 500 or 1000 mg/kg doses of the test substance. The test material showed no marked difference in its toxicity to male or female rats; therefore only male rats were used in the main test. In the main test animals were treated with the test substance once.

Dose mg/kg bw	*Number and Sex of Animals*	*Sacrifice Time Hours*
First experiment		
0 (vehicle control)	7	48
0 (vehicle control)	7	24
80	7	24
160	7	24
320	7	24
320	7	48
50 (Positive control, CP)	5	24
Second experiment		
0 (vehicle control)	7	24
250	7	24
50 (Positive control, CP)	5	24

CP = cyclophosphamide

RESULTS

Doses Producing Toxicity	In the preliminary toxicity test in animals dosed with the test material premature deaths occurred at and above 500 mg/kg, and severe clinical signs were observed at and above 500 mg/kg as follows: hunched posture, ptosis, ataxia, lethargy, splayed gait, decreased respiratory rate, laboured respiration, pallor of the extremities, prostrations and hypothermia. In animals dosed with the test material at 250 and 320 mg/kg there were no premature deaths, and the severity of the clinical signs was considered to be modest. In the 250 mg/kg group only hunched posture was observed, and in the 320 mg/kg dose group the clinical signs were as follows: hunched posture, ataxia, lethargy and ptosis after 1 hour, and included hunched posture, lethargy and pilo-erection at 24 and 48 hours. The maximum tolerated dose (MTD) of the test material was therefore selected as 320 mg/kg for use in the main test. In the main test there were no premature deaths seen in any of the dose groups. Clinical signs were observed in animals dosed with the test material at 320 mg/kg in both the 24 and 48 hour groups, these were as follows: hunched posture, lethargy, ataxia and ptosis. In the additional investigation clinical signs were seen with the test material at 250 mg/kg and were as follows: hunched posture, ptosis and ataxia.
Genotoxic Effects	There were no statistically significant decreases in the PCE/NCE ratio in the 24 or 48 hour test material groups when compared to their concurrent vehicle control groups. However, the observation of clinical signs of toxicity was taken to indicate that systemic absorption had occurred. A summary of the results for the micronuclei count is shown in the table below. There was a weak but statistically significant increase in the frequency of micronucleated PCEs observed in the 24 hour 320 mg/kg test material dose group when compared to the concurrent vehicle control group (4.7 micronuclei/2000 PCEs for test material group versus 1.3 micronuclei/2000 PCEs for the control group). The response was very modest and was not observed in the 48 hour test material dose group.

Therefore, it was decided that the duplicate slide for this group and the vehicle group would be scored for the incidence of micronucleated PCEs. Once again a small but statistically significant increase in the frequency of micronucleated PCEs was observed. It was therefore concluded that the test material was weakly genotoxic at moderately toxic dose levels in mice.

The testing laboratory has indicated that a chemical analogue of the test material has been investigated in an *in vitro* chromosome aberration study and was found not to be clastogenic. However this study (or the identity of the analogue) was not provided to NICNAS. Due to the result from this analogue test, the test laboratory hypothesised that the test material response in this assay was not a clastogenic effect but was possibly due to an aneugenic mechanism and therefore may be subject to threshold limits. A second experiment was performed to investigate a further dose level of the test material (250 mg/kg) to obtain a more accurate no effect dose level. The test material did not induce a statistically significant increase in the frequency of micronucleated PCEs at 250 mg/kg. A small increase in the frequency of micronucleated PCEs was observed (2.7 micronuclei/2000 PCEs for test material group versus 1.1 micronuclei/2000 PCEs for the control group), but the increase was within the historical range for vehicle control groups. The test laboratory therefore concluded that the maximum no effect dose level for the test material was 250 mg/kg, within the confines of this study.

The positive control showed a marked increase in the incidence of micronucleated polychromatic erythrocytes hence confirming the sensitivity of the system to the known mutagenic activity of cyclophosphamide under the conditions of the test.

Dose	*Number of PCE with Micronuclei per 2000 PCE*	
mg/kg bw	*Group Mean*	*Standard Deviation*
First experiment		
0 (vehicle control)	1.1	1.5
0 (vehicle control)	1.3	0.8
80	2.0	1.4
160	2.1	2.0
320	4.7**, 4.0*	3.3, 2.2
320	2.1	2.3
50 (Positive control, CP)	34.2***	4.8
Second experiment		
0 (vehicle control)	1.1	0.9
250	2.7	1.6
50 (Positive control, CP)	36.6**	20.9

* = p <0.05
** = p< 0.01
*** = p<0.001

Remarks - Results

The notified chemical was found to be weakly clastogenic at the maximum tolerated dose (320 mg/kg). At lower doses, including when only slight toxicity was observed (250 mg/kg) the test material was not genotoxic.

Although the test laboratory has hypothesised that the positive response in this assay was due to an aneugenic effect rather than a clastogenic effect, this cannot be proved conclusively in this study. In addition it should be noted that this micronucleus test is not designed to reliably identify lowest effective doses.

CONCLUSION The notified chemical was clastogenic to mouse bone marrow under the conditions of this in vivo mouse micronucleus test.

TEST FACILITY SafePharm Laboratories (2005f)

B.10. Genotoxicity – in vivo Chromosome Aberration

TEST SUBSTANCE Notified chemical

METHOD OECD TG 475 Mammalian Bone Marrow Chromosome Aberration Test.
EC Directive 2000/32/EC B.11 Mutagenicity - In vivo Mammalian Bone Marrow Chromosome Aberration Test.

Species/Strain Rat/Sprague-Dawley Crl:CD®(SD) IGS BR
Route of Administration Oral – gavage
Vehicle Arachis oil
Remarks - Method No significant protocol deviations. As it was suspected that the response, if any would be quite modest the animal numbers for the vehicle and test material groups were increased to ten/group. The preliminary toxicity test was conducted using only male animals at doses of 320 and 400 mg/kg/day, based on data from the Mouse Micronucleus test.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time Hours*
I – vehicle control	10 male	0	48
II – vehicle control	10 male	0	24
III – low dose	10 male	80	24
IV – mid dose	10 male	160	24
V – high dose	10 male	320	48
VI – high dose	10 male	320	24
VII - Positive control, CP	5 male	25	24

CP = cyclophosphamide

RESULTS

Doses Producing Toxicity No premature deaths occurred in the preliminary toxicity test. However, the clinical signs observed after 24-hours at 400 mg/kg exceeded acceptable limits permitted by the Home Office for this type of study and, therefore, the animals were killed in *extremis*. The clinical signs observed at 400 mg/kg were as follows: hunched posture, ataxia, lethargy, ptosis, pilo-erection, decreased respiratory rate and laboured respiration. In animals dosed orally with test material at 320 mg/kg the clinical signs observed were acceptable, and as follows: pilo-erection and diuresis. 320 mg/kg was therefore chosen as the maximum tolerated dose.

There were no premature deaths seen in any of the test material dose groups. Clinical signs were observed in all animals dosed with the test material. These were as follows: hunched posture, ataxia, lethargy and diuresis.

Genotoxic Effects The test material did not induce any significant or dose-related increases in the frequency of aberrations in any of the treatment groups. The test material did not induce a significant increase in the numbers of polyploidy cells in any of the treatment groups.

There were no statistically significant reductions in the mean mitotic index in any of the test material treatment groups when compared to their concurrent vehicle control groups. The mean mitotic index of the positive control group was statistically significantly lower than that of the 24-hour vehicle control group indicating a cytotoxic response in the bone

	marrow. All the vehicle control animals gave values of chromosome aberrations within the expected range. The mean frequency of aberrations was consistent between the two vehicle control groups, the highest frequency (0.3% cells with aberrations excluding gaps) being seen in the 48-hour group. The positive control group animals showed highly significant increases in the frequency of aberrations indicating that the test method itself was operating as expected.
Remarks - Results	Although there was no indication of cytotoxicity to the bone marrow, the observation of clinical signs of toxicity indicated that systemic absorption had occurred. It is therefore assumed that the target organ was reached.
CONCLUSION	The notified chemical was not clastogenic to rat bone marrow cells *in vivo* under the conditions of the test.
TEST FACILITY	SafePharm Laboratories (2005g)

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.
EC Directive 92/69/EEC C.4-C Biodegradation: Determination of the " Ready" Biodegradability: Carbon Dioxide Evolution Test

Inoculum	Mixed population of activated sewage sludge micro-organisms.
Exposure Period	28 Days
Auxiliary Solvent	None; adsorbed onto silica gel.
Analytical Monitoring	TOC analyser.
Remarks – Method	The test material, at a concentration of 10 mg C/L, was exposed to activated sewage sludge micro-organisms with culture medium in sealed culture vessels in the dark at 21ºC for 28 days. CO_2 was captured in two adsorbers arranged in series. The second adsorber was analysed on days 0 and 29 only. Due to apparent low water solubility (globules observed after ultrosonication and high shear mixing), the test material was adsorbed onto granular silica gel prior to dispersion in the test medium in order to aid dispersion of the test material in the test medium and to increase the surface area of the test material exposed to the test organisms. The degradation of the test material was assessed by the determination of carbon dioxide produced. Control solutions with inoculum and the standard material, sodium benzoate, together with a toxicity control were used for validation purposes.

RESULTS

Day	*Test substance % Degradation*	*Sodium Benzoate % Degradation*	*Test Material plus Sodium Benzoate % Degradation*
0	0	0	0
1	0	30	11
2	0	45	17
3	9	59	29
6	32	76	51
8	27	72	51
10	37	81	59
14	46	80	60
16	42	85	58
20	42	82	67
22	50	81	71
24	48	81	72
27	48	79	72
28	48	78	73
29*	57	84	73

* Based on the additional CO_2 trapped in the second adsorber.

Remarks – Results: The test material attained 48% degradation after 28 days and therefore cannot be considered to be readily biodegradable under OECD Guideline No. 301B. The toxicity control attained 60% degradation after 14 days and 73% after 28 days thereby confirming that the test material is non-toxic to sewage sludge organisms.

CONCLUSION The notified chemical cannot be classed as ready biodegradable.

TEST FACILITY Safepharm Laboratories (2005h)

C.1.2. Bioaccumulation

Remarks While the notified chemical is not ready biodegradable, it is subject to biodegradation, although at a slow rate. The physicochemical properties (log P_{OW} = 3.2 and solubility in water of 64.8 mg/L) indicates that the notified chemical has a potential for bioaccumulation. The risk of bioaccumulation is mitigated by the expected low exposure to natural waters.

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE Notified chemical.

METHOD OECD TG 203 Fish, Acute Toxicity Test – 96 hour, Semi-Static Limit Test.

Species Rainbow trout (*Oncorhynchus mykiss)*

Exposure Period 96 hours

Auxiliary Solvent Dimethylformamide (DMF)

Water Hardness 100 mg $CaCO_3$/L

Analytical Monitoring HPLC

Remarks – Method No significant deviations from standard protocol. The test concentration of 1.0 mg/L was the highest attainable test concentration due to the limited solubility of the test material and auxiliary solvent and having due regard for the amount of auxiliary solvent permitted in the test under the OECD Guidelines. During preliminary solubility work, fine particles of test material were observed dispersed throughout the test media at concentrations in excess of 1.0 mg/L indicating this to be the maximum limit of water solubility under these conditions.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*						*% Mortality*
Nominal	*Actual*		*3 h*	*6 h*	*24 h*	*48 h*	*72 h*	*96 h*	*96 h*
Control		10	0	0	0	0	0	0	0
Solvent control		10	0	0	0	0	0	0	0
1.0 R_1	0.78	10	0	0	0	0	0	0	0
1.0 R_2	0.78	10	0	0	0	0	0	0	0

LC50 > 0.78 mg/L at 96 hours.

NOEC 0.78 mg/L at 96 hours.

Remarks – Results In the range-finding test - there was no sub-lethal effects of exposure during the range-finding test. The results showed no mortalities at the test concentration of 1.0 mg/L.

A concentration of 1.0 mg/L was selected for the definitive test. This experimental design conforms to a "Limit Test" to confirm that at the highest attainable test concentration of 1.0 mg/L, no mortalities or sub-lethal effects of exposure were observed.

There were no mortalities in 20 fish exposed to a test concentration of 1.0 mg/L for a period of 96 hours. The results of the definitive test showed the highest concentration resulting in 0% mortality to be ≥1.0 mg/L and No Observed Effect Concentration (NOEC) to be 1.0 mg/L. This was based on zero mortalities and the absence of any sub-lethal effects of exposure at this concentration.

A reduction of concentration was observed after analysis of fresh and old media, and therefore, it was considered justifiable to base the results on the time-weighted mean measured test concentrations of the centrifuged test media to give a "worst case" analysis of the data.

CONCLUSION The notified chemical is at worst very toxic to *Oncorhynchus mykiss;* or better stated as not toxic to close to the limit of its solubility.

TEST FACILITY Safepharm Laboratories (2005i)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical.

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – 48 hour static

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	DMF
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	Following a preliminary range-finding test, 20 daphnids (4 replicates of 5 animals) were exposed to an aqueous solution of the test material at a concentration of 1.0 mg/L for 48 hours at a temperature of 21.3º to 21.9ºC under static conditions. Immobilisation and any adverse reactions to exposure were recorded after 24 and 48 hours. A positive control conducted approximately every 6 months used potassium dichromate as the reference material.

RESULTS

Concentration mg/L Nominal	*Number of D. magna*	*Number Immobilised*	
		24 h	*48 h*
Control	10	0	0
Solvent control	10	0	0
1.0	20	0	0

LC50	> 1.0 mg/L at 48 hours
NOEC	1.0 mg/L at 48 hours
Remarks – Results	Analysis of the centrifuged test preparations at 0 and 48 hours showed measured test concentrations of 81% to 85% of nominal value. Therefore, it was considered justifiable to estimate the EC50 values in terms of nominal test concentration only. The test material preparations were observed to be clear, colourless solutions throughout the duration of the test.

CONCLUSION The notified chemical is at worst toxic to *Daphnia magna;* or better stated as not toxic to close to the limit of its solubility.

TEST FACILITY Safepharm Laboratories (2005j)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical.

METHOD OECD TG 201 Alga, Growth Inhibition Test.

Species	Green alga (*Scenedesmus subspicatus)*
Exposure Period	72 hours
Concentration Range	0.0625, 0.125, 0.25, 0.50 and 1.0 mg/L.

Nominal Concentration Range Actual	0.018, 0.024, 0.082, 0.27 and 0.64 mg/L.
Auxiliary Solvent	DMF
Analytical Monitoring	HPLC
Remarks – Method	No significant deviations from standard protocol.

RESULTS

Biomass		*Growth*	
E_bC_{50} mg/L at 72 h	*NOEC mg/L*	*E_rC_{50} mg/L 0 – 72 h*	*NOEC mg/L*
0.32	0.024	> 0.64	0.024

Remarks – Results

Analysis of the test preparations at 72 hours showed a marked decline in measured test concentrations in the range of less than the limit of quantification (LOQ) of the analytical method employed to 40% of nominal for the untreated test samples and from less than the LOQ to 39% of nominal for the centrifuged test samples. These results were in-line with the preliminary stability analyses conducted that indicated the test material as unstable in culture medium over the test period.

Given this decline in measured concentrations it was considered justifiable to base the results on the geometric mean measured test concentrations of the centrifuged test media in order to give a "worst case" analysis of the data.

It was not possible to calculate 95% confidence limits for the EC_{50} values as the data generated did not fit the models available for the calculation of confidence limits. It was not possible to calculate an E_rC_{50} value as no concentration tested resulted in greater than 50% inhibition of growth rate, with a maximum of 19% at the highest concentration.

At the start of the test all control, solvent control and test cultures were observed to be clear colourless solutions. After the 72-hour test period all control, solvent control and test cultures were observed to be green dispersions.

CONCLUSION The notified chemical is very toxic to algae.

TEST FACILITY Safepharm Laboratories (2005k)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE Notified chemical.

METHOD OECD TG 209 Activated Sludge, Respiration Inhibition Test.
EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test.

Inoculum	Activated sewage sludge microorganisms
Exposure Period	3 hours
Concentration Range Nominal	56, 100, 180, 320, 560 and 1000 mg/L
Remarks – Method	Following preliminary range-finding tests, activated sewage sludge was exposed to an aqueous dispersion of the test material at concentrations of listed above for a period of 3 hours at a temperature of approximately 21°C with the addition of a synthetic sewage as a respiratory substrate. The rate of respiration was determined after 30 minutes and 3 hours contact time and compared to data for the control and a reference material

	3,5-dichlorophenol.
RESULTS	
EC50	240 mg/L (95% confidence limits 200 – 290 mg/L)
NOEC	56 mg/L
Remarks – Results	It was not possible to obtain an EC50 and an EC80 value (at 30 minutes) for the test material as no concentration tested resulted in greater than 50% inhibition after 30 minutes contact time. It was not possible to obtain a 95% confidence limit for the test material after 30 minutes contact time as the data generated did not fit the models available for the calculation of this limit. The reference had an EC50 of 8.7 mg/L (95% CL 7.1-11), which is within the accepted range of 5-30 mg/L.
CONCLUSION	The notified chemical is not harmful to sewage sludge microorganisms.
TEST FACILITY	SafePharm Laboratories (2007d)

BIBLIOGRAPHY

FORS (Federal Office of Road Safety) (1998) Australian Code for the Transport of Dangerous Goods by Road and Rail (ADG code), 6th Edition, Canberra, Australian Government Publishing Service

NOHSC (1994) National Code of Practice for the Labelling of Workplace Substances [NOHSC:2012(1994)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

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

NOHSC (2003) National Code of Practice for the Preparation of Material Safety Data Sheets, 2nd edition [NOHSC:2011(2003)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

SafePharm Laboratories (2005a) Acute oral toxicity in the rat – acute toxic class method (SPL Project Number: 971/293, 10 October 2005) Safepharm Laboratories Ltd, Derbyshire, UK (Unpublished report provided by notifier)

SafePharm Laboratories (2005b) Acute dermal toxicity (limit test) in the rat (SPL Project Number: 971/294). Safepharm Laboratories Limited, Derbyshire, UK (Unpublished report provided by notifier)

SafePharm Laboratories (2005c) Acute dermal toxicity irritation in the rabbit (SPL Project Number: 971/295). Safepharm Laboratories Limited, Derbyshire, UK (Unpublished report provided by notifier)

SafePharm Laboratories (2005d) Acute eye irritation in the rabbit (SPL Project Number: 971/296). Safepharm Laboratories Limited, Derbyshire, UK (Unpublished report provided by notifier)

MB Research Labs (2005) Guinea Pig Maximization Test (Magnusson-Kligman) (MB Research Project #: MB 05-13980.06). MB Research Laboratories, Pennsylvania, USA (Unpublished report provided by notifier)

SafePharm Laboratories (2005e) Reverse mutation assay "Ames Test" using Salmonella typhimurium and Escherichia coli: (SPL Project Number: 971/284). Safepharm Laboratories Limited, Derbyshire, UK (Unpublished report provided by notifier)

SafePharm Laboratories (2005f) Micronucleus test in the mouse: (SPL Project Number: 971/271). Safepharm Laboratories Limited, Derbyshire, UK (Unpublished report provided by notifier)

SafePharm Laboratories (2005g) Mammalian bone marrow chromosome aberration test: (SPL Project Number: 971/285). Safepharm Laboratories Limited, Derbyshire, UK (Unpublished report provided by notifier)

Safepharm Laboratories (2005h) Assessment of Ready Biodegradability; CO_2 Evolution Test (SPL Project Number: 971/301, 23 September 2005) Safepharm Laboratories Limited, Derbyshire, UK (Unpublished report provided by notifier)

Safepharm Laboratories (2005i) Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) (SPL Project Number: 971/298, 17 October 2005) Safepharm Laboratories Limited, Derbyshire, UK (Unpublished report provided by notifier)

Safepharm Laboratories (2005j) Acute Toxicity to *Daphnia magna* (SPL Project Number: 971/299, 17 October 2005) Safepharm Laboratories Limited, Derbyshire, UK (Unpublished report provided by notifier)

Safepharm Laboratories (2005k) Algal Inhibition Test (SPL Project Number: 971/300, 17 October 2005) Safepharm Laboratories Limited, Derbyshire, UK (Unpublished report provided by notifier)

SafePharm Laboratories (2007a) Determination of general physico-chemical properties (SPL Project Number: 0971/0331, 26 March 2007) Safepharm Laboratories Ltd, Derbyshire, UK (Unpublished report provided by notifier)

SafePharm Laboratories (2007b) Determination of hazardous physico-chemical properties (SPL Project Number: 0971/0332, 6 March 2007). Safepharm Laboratories Ltd, Derbyshire, UK (Unpublished report provided by notifier)

SafePharm Laboratories (2007c) Twenty-eight day repeated dose oral (gavage) toxicity study in the rat (SPL Project Number: 0971/0333, 14 June 2007). Safepharm Laboratories Ltd, Derbyshire, UK (Unpublished report provided by notifier)

Safepharm Laboratories (2007d) Assessment of the Inhibitory Effect on the Respiration of Activated Sewage Sludge (SPL Project Number: 971/0334, 12 February 2007) Safepharm Laboratories Ltd, Derbyshire, UK (Unpublished report provided by notifier)

United Nations (2003) Globally Harmonised System of Classification and Labelling of Chemicals (GHS). United Nations Economic Commission for Europe (UN/ECE), New York and Geneva.

US EPA (1994), Design and Evaluations of Tailings Dams, Technical Report, EPA530-R-94-038, NTISPB94-201845, August 1994.