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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Chemical in Reagent S-10104 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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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 name, Other names, CAS Number, Molecular formula, Structural Formula, Molecular weight, Spectral data, Purity, Hazardous impurities and Import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: acute dermal toxicity, acute inhalation toxicity, skin irritation, eye irritation, skin sensitisation, repeat dose toxicity, induction of point mutations, induction of chromosome damage, fish acute toxicity, chronic aquatic toxicity, bioaccumulation.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Reagent S-10104 Promoter (imported product containing < 70% notified chemical)

MOLECULAR WEIGHT
< 500 g/mol

ANALYTICAL DATA
A reference IR spectrum was provided.

3. COMPOSITION

DEGREE OF PURITY > 95%

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20ºC AND 101.3 kPa: yellow solid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	30.85°C	Measured
Boiling Point	211.85°C at 103.15 to 103.87 kPa	Measured
Density	1180 kg/m^3 at 20.3 ± 0.5°C	Measured
Vapour Pressure	9.8 x 10^{-6} kPa at 25°C	Measured
Water Solubility	2.58 ×10^{-3} g/L at 20.0 ± 0.5°C	Measured
Hydrolysis as a Function of pH	Half-lives at 25°C to be 46.3, 37.2 and 14.1 days at pH 4, 7 and 9, respectively.	Measured
Partition Coefficient (n-octanol/water)	log P_{OW} = 4.09	Measured
Surface tension	61.7 mN/m at 20.2 ± 0.5°C (2.16 x 10^{-3} g/L solution)	Measured
Adsorption/Desorption	log K_{OC} = 3.72	Measured
Dissociation Constant	Not applicable	The notified chemical does not contain any dissociable functionality.
Particle Size	Inhalable fraction (< 100 μm) = 11.3% Thoracic fraction (< 10 μm) = 0.65% Respirable fraction (< 5.5 μm) = 0.17%	Measured
Flash Point	134 ± 2°C at 101.3 kPa	Measured
Flammability	Not determined	Not expected to be flammable based on vapour pressure and flash point values
Autoignition Temperature	278 ± 5°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 Reagent S-10104 Promoter (< 70% concentration).

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	100-300	100-300	100-300	100-300	100-300

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 Reagent S-10104 Promoter (< 70% notified chemical) will be imported by ship in 20,000 L isotainers, 1 tonne totes or 20 L pails. The product will be transported from the dock to the formulator's warehouse for storage. After blending the notified chemical will be transported to the end-use sites by road in 20,000 L isotainers.

USE
The notified chemical will be used as a froth-generating reagent in the mineral processing industry.

OPERATION DESCRIPTION

Reformulation of Reagent S-10104 Promoter
The Reagent S-10104 Promoter is received at the local blending site in 20,000 L isotainers, 1 tonne totes or 20 L pails. All material is expected to be stored in a bunded, dedicated Dangerous Goods Area. When required the Reagent S-10104 Promoter is transferred on a pallet by forklift from the warehouse area to the blending area. Reagent S-10104 Promoter is pumped using an automated pumping system into a 20,000 L isotainer, where it is diluted. 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 (1-30% 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 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 dose range of the notified chemical into the flotation cell will be 5-75 ppm. 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.

Routine cleaning and minor maintenance of the plant is carried out regularly, and shut-downs occur for major maintenance.

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 Reagent S-10104 Promoter
Dermal exposure to the notified chemical (up to 70% 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 70% 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 a 70% 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 70% 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 0-0.42 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 and an analogue chemical are summarised in the table below. Details of these studies can be found in Appendix B.

Endpoint	*Test Substance*	*Assessment Conclusion*
Rat, acute oral	Notified chemical	Low toxicity, LD50 > 2000 mg/kg bw
Rat, acute dermal	Analogue	Low toxicity, LD50 > 2000 mg/kg bw
Rat, acute inhalation	-	Not determined
Rabbit, skin irritation	Analogue	Slightly irritating
Rabbit, eye irritation	Analogue	Slightly irritating
Guinea pig, skin sensitisation – Maximization test (Magnusson-Kligman)	Analogue	Limited evidence of sensitisation.

Rat, Oral (Gavage) repeat dose toxicity - 28 days.	Analogue	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	Notified chemical	Non mutagenic
Genotoxicity – in vivo Mammalian bone marrow chromosome aberration test in the rat	Analogue	Non-clastogenic
Genotoxicity – in vivo Micronucleus test in the mouse	Notified chemical	Non-genotoxic

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 = 4.09, water solubility of 2.58 mg/L). Absorption of the notified chemical across the gastrointestinal tract was confirmed by the observation of toxic effects after acute oral exposure. While no evidence for acute toxic effects was observed in the acute dermal toxicity study, given the properties of the analogue the possibility of dermal absorption for the analogue, and therefore the notified chemical, cannot be ruled out.

Acute toxicity
The notified chemical was found to be of low toxicity to rats after acute dosing via the oral route. One animal dosed at 2000 mg/kg was found dead 3 days after dosing. Adverse effects after oral exposure included hunched posture, lethargy, decreased respiratory rate, and laboured respiration.
The notified chemical is expected to be of low toxicity after dermal exposure based on the study conducted in rats using the analogue chemical. No signs of local or systemic toxicity were observed during the study.
The acute inhalation hazard of the notified chemical or of the analogue has not been determined. However, given the low volatility of the notified chemical (vapour pressure of 9.8×10^{-6} kPa at 25°C) and the fact that it is not introduced in its solid form it is not expected to pose a significant inhalation hazard.

Irritation and Sensitisation
The analogue 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 using the analogue chemical, the number of animals affected (15%) was below the cut-off for classification as a sensitiser (30%). Therefore the notified chemical is expected to be only slightly irritating to eyes and skin and not to be a skin sensitiser.

Repeated Dose Toxicity (sub-acute)
In a 28-day oral repeat dose study in rats adverse effects were observed at all dose levels of the analogue chemical (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 analogue chemical, and by analogy 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* erythrocyte micronucleus test in the mouse.
The analogue chemical was found to be non-clastogenic in an *in vivo* bone marrow chromosome aberration test in the rat.

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

T: R48/25 Danger of serious damage to health by prolonged exposure if swallowed

6.3. Human health risk characterisation

6.3.1. Occupational health and safety

The main route of exposure to the notified chemical (up to 70% 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 on the analogue chemical 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.

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 of the analogue chemical and the favourable physical-chemical properties for dermal absorption. EASE modelling of the reformulation/mining processes estimated the exposure as 0-0.42 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 36. 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.

Given the risk of causing serious damage to health by prolonged exposure 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 component of a finished product in 20,000 L isotainer, one tonne tote or 20 L pail. The imported product containing the notified chemical will be further blended.

The blending vessels are sealed at all times during the blending of a batch, except during the charging of the vessels. The transfer of the concentrated product containing the notified chemical between vessels occurs via pumps or by gravity. During the blending process, water used to flush the pumps is reused as part of the finished batch. The 20,000 L isotainers containing the finished product are returned to the blender for reuse. It is estimated that a maximum of 0.5% of the notified chemical would be lost during spillage as a result of connecting and disconnecting pumps. Spill kits are in place in the storage and production areas. Spills are contained in bunded areas or 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.

During ore treatment in a plant, the flotation reagent or the diluted product containing the notified chemical is transferred from the 20,000 L Isotainers through fixed lines and dosed by a metered dosing pump to a flotation cell. The floatation reagent will be in a closed-loop water recirculation circuit, and thoroughly mixed with the slurry to chelate the ore. The typical usage of the notified chemical in the closed-loop water recirculation circuit is in the range of 5-75 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 that has not been made sufficiently hydrophobic to attach to the bubbles will remain in the slurry, and be 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 solid pulp, and 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. The deposit in the evaporation dams may be eventually smelted for recovery of metal and the high temperature of the furnaces would decompose the notified chemical into water, oxides of carbon, sulphur and nitrogen. 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 1-15 ppm (20% × 5-75 ppm).

Settling dam walls are typically constructed using tailings and are designed to permit water to leach. It is, therefore, anticipated that some water will inevitably enter the groundwater. Based on the apparent relatively low water solubility of the notified chemical, it is expected that only a small proportion of the total annual import volume will be mobile and could enter groundwater. However, given the very large quantities used, this release could be significant. Settling dam walls occasionally breach during periods of intense precipitation, releasing the contents of the dam. It is possible, that in such an event, significant quantities of notified chemical may be released, and enter terrestrial waterways. Notified chemical that leaches into groundwater is expected to eventually degrade via biotic and abiotic means to form simple organic compounds.

RELEASE OF CHEMICAL FROM DISPOSAL
Imported Isotainers containing the notified chemical will be returned to supply for reuse.

The residual notified chemical in blending pumps will stay in the flush and cleaning water that will be reused as part of the diluted product. The release is estimated to be 0.5% and will be collected and disposed of properly.

The majority of the notified chemical will be destroyed by oxidation in the smelting process. Residual product left in the drums (~0.5%) will be rinsed out with water and the rinsate will be fed into the recirculating water loop and be reused.

During the floatation process, approximately 20% of the notified chemical will remain in the tailing dam and significant seepage is predicted to end up eventually with aquatic environment via groundwater.

7.1.2 Environmental fate

A single ready biodegradability study was supplied for the notified chemical, which demonstrated 46% degradation after 28 days. Therefore, the notified chemical cannot be classified as readily 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 most likely release is expected from seepage of

the notified chemical in the tailing dams.

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 1/1000th of an inch, although it occasionally referred to incorrectly as a millimetre) (US EPA 1994).

The PEC may be calculated assuming the maximum concentration of the notified chemical (15 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) ÷ 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 46.3 days. Accordingly it will undergo approximately 15.2 half lives (706 ÷ 46.3) during its permeation through the liner. A worst case PEC at release from the tailings dam may calculated by 15 ppm $\times 0.5^{15.2} = 0.4$ µg/L.

7.2. Environmental effects assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	LC50 > 0.78 mg/L (Acceptable analogue)	Not toxic up to the limit of its solubility
Daphnia Toxicity	EC50 > 1.0 mg/L (Acceptable analogue)	Not toxic up to the limit of its solubility
Algal Toxicity	E_rC50 = 0.15 mg/L	Very toxic to algae
Inhibition of Bacterial Respiration	EC50 = 240 mg/L (Acceptable analogue)	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 0.15 mg/L (E_rC50 algae).

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
E_rC50 algae	0.15	mg/L
Assessment Factor	100	
PNEC:	1.5	µg/L

7.3. Environmental risk assessment

Risk Assessment	***PEC µg/L***	***PNEC µg/L***	*PEC/PNEC*
River:	0.4	1.5	**0.27**
Ocean:	0.04	1.5	**0.03**

The calculated Risk Quotient is less than 1 even though it has been estimated based on the worst case. Therefore, the notified chemical is not predicted to pose an unacceptable risk to the aquatic environment.

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:

T: R48/25 Danger of serious damage to health by prolonged exposure if swallowed

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***
Systemic Toxicity Repeated Exposure	Category 1	Causes damage to organs through prolonged or repeated exposure
Environment	Acute Category 1 Chronic Category 1	Very toxic to aquatic life (with long lasting effects)

Human health risk assessment
There is a risk of serious health effects after repeated exposure to the notified chemical. This risk to occupational health and safety is not considered to be unacceptable provided that the notified chemical is only used under controlled conditions by trained workers.

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

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

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

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

- 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 to 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;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a froth-generating reagent in the mineral processing industry., or is likely to change significantly;
- the amount of chemical being introduced has increased from 300 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 30.85 ± 0.5°C

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 (2008a)

Boiling Point 211.85°C at 103.15 to 103.87 kPa

Method	EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks	The boiling point was determined using differential scanning calorimetry. The notified chemical underwent a colour change to a brown solid at 210°C which is indicative of decomposition. No significant protocol deviations.
Test Facility	Safepharm Laboratories (2008a)

Density 1180 kg/m^3 at 20.3 ± 0.5°C

Method	EC Directive 92/69/EEC A.3 Relative Density.
Remarks	The relative density was determined using the pycnometer method. No significant protocol deviations.
Test Facility	Safepharm Laboratories (2008a)

Vapour Pressure 9.8 x 10^{-6} kPa at 25°C

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 (2008b)

Water Solubility 2.58 ×10^{-3} g/L at 20.0 ± 0.5°C

Method	EC Directive 92/69/EEC A.6 Water Solubility.
Remarks	Flask Method, with analysis using HPLC. The pH of the solution was between 5.4 and 6.3.
Test Facility	Safepharm Laboratories (2008a)

Hydrolysis as a Function of pH Half-life estimated to be 46.3, 37.2 and 14.1 days at pH 4, 7 and 9, respectively, at 25 ˚C.

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	46.3
7	25	37.2
9	25	14.1

Remarks	HPLC was used for the analysis of solution concentrations. A preliminary test at 50°C was conducted at pH 4, 7 and 9. The preliminary test at pH 4 was found to be inconsistent with the 40, 60 and 70°C tests and was not included in the test report. The preliminary test results at pH 7 and 9 were 12.7 and 10.3 hours, respectively. The kinetics of the study were determined to be consistent with that of a pseudo-first order reaction as graphs of $\log_{10}$ concentration versus time are straight lines.
Test Facility	Safepharm Laboratories (2008a)

Partition Coefficient (n-octanol/water) $\log P_{OW} = 4.09$

Method EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks HPLC Method. The notified chemical eluted between the reference substances naphthalene ($\log P_{OW}$= 3.6) and phenanthrene ($\log P_{OW}$=4.5). The estimated $\log P_{OW}$ is considered reasonable based on the formula structure.
Test Facility Safepharm Laboratories (2008a)

Surface Tension 61.7 mN/m at 20.2 ± 0.5°C (2.16×10^{-3} g/L solution)

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 2.16 x 10^{-3} g/L solution at pH 5.4. The notified chemical is not considered to be significantly surface active based on the test result.
Test Facility Safepharm Laboratories (2008a)

Adsorption/Desorption $\log K_{OC} = 3.72$

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 Fenthion ($\log K_{OC}$=3.31) and α-Endosulfan ($\log K_{OC}$=4.09). The estimated $\log K_{OC}$ is considered in compliance with the formula structure.
Test Facility Safepharm Laboratories (2008a)

Particle Size

Method OECD TG 110 Particle Size Distribution/Fibre Length and Diameter Distributions.

Range (μm)	*Mass (%)*
< 100 μm	11.3%
< 10 μm	0.65%
< 5.5 μm	0.17%

Remarks Measured using a cascade impactor after a preliminary sieve test.
Test Facility Safepharm Laboratories (2008a)

Flash Point 134 ± 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 (2008b)

Autoignition Temperature 278 ± 5°C

Method EC Directive 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks No significant protocol deviations.
Test Facility Safepharm Laboratories (2008b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Method. EC Directive 2004/73/EC B.1bis Acute Oral Toxicity – Fixed Dose Method.
Species/Strain	Rat/ Sprague-Dawley CD (Crl: CD® (SD) IGS BR)
Vehicle	Arachis oil BP
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex Animals*	*Dose of mg/kg bw*	*Mortality*
1	1 female	300	0
2	1 female	2000	0
3	4 females	2000	1

LD50	> 2000 mg/kg bw
Signs of Toxicity	No signs of systemic toxicity were noted for the female treated at a dose level of 300 mg/kg bw. Signs of systemic toxicity in animals dosed at 2000 mg/kg bw were hunched posture, lethargy, piloerection, decreased respiratory rate, and laboured respiration. Surviving animals dosed at 2000 mg/kg bw 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 the animal that died were abnormally red lungs, dark liver, and dark kidneys. No abnormalities were noted at necropsy of animals that were killed at the end of the study.
Remarks - Results	One female animal dosed at 2000 mg/kg bw died.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Safepharm Laboratories Limited (2008c)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Analogue 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 analogue chemical is of low toxicity via the dermal route.

TEST FACILITY Safepharm Laboratories Limited (2005a)

B.3. Irritation – skin

TEST SUBSTANCE Analogue 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 analogue chemical is slightly irritating to the skin.

TEST FACILITY Safepharm Laboratories Limited (2005b)

B.4. Irritation – eye

TEST SUBSTANCE Analogue 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 analogue chemical is slightly irritating to the eye.

TEST FACILITY: Safepharm Laboratories Limited (2005c)

B.5. Skin sensitisation

TEST SUBSTANCE: Analogue 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 analogue chemical under the conditions of the test.

TEST FACILITY: MB Research Laboratories (2005)

B.6. Repeat dose toxicity

TEST SUBSTANCE: Analogue 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: 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 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 (2007a)

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 T100 in the absense of metabolic 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	1500 µg/plate	1500 µg/plate	negative
Test 2		1500 µg/plate	1500 µg/plate	negative
Present				
Test 1	1500 µg/plate	5000 µg/plate	1500 µg/plate	negative
Test 2		1500 µg/plate	1500 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

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 (2006a)

B.8. Genotoxicity – in vivo

TEST SUBSTANCE: Analogue 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 for the analogue.

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

TEST FACILITY

SafePharm Laboratories (2005d)

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 500 mg/kg 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
125	7	24
250	7	24
500	7	24
500	7	48
50 (Positive control, CP)	5	24

CP = cyclophosphamide

RESULTS

Doses Producing Toxicity: Clinical signs were observed in animals dosed at and above 250 mg/kg as follows: hunched posture, ptosis and ataxia. The maximum tolerated dose (MTD) of the test material was therefore selected as 500 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 at and above 250 mg/kg, both the 24 and 48 hour groups, 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.

Dose	*Number of PCE with Micronuclei per 2000 PCE*	
mg/kg bw	*Group Mean*	*Standard Deviation*
First experiment		
0 (vehicle control) - 48 h	1.1	1.5
0 (vehicle control) - 24 h	1.4	2.6
125	0.9	1.2
250	1.0	1.0
500 – 48 h	1.3	1.0
500 – 24 h	1.7	2.4
50 (Positive control, CP)	43.8*	17.3

* = $p < 0.001$

Remarks - Results: 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.
The notified chemical did not induce a significant increase in the frequency of the micronuclei at any dose level.

CONCLUSION: The notified chemical was not clastogenic to mouse bone marrow under

	the conditions of this in vivo mouse micronucleus test.
TEST FACILITY	SafePharm Laboratories (2006b)

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: CO2 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: Sodium benzoate
Analytical Monitoring:
Remarks - Method: The test material, at a concentration of 10 mg Carbon/L, was exposed to activated sewage sludge micro-organisms with culture medium in sealed culture vessels in the dark at 21ºC for 28 days.

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

Test substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
6	13	6	51
14	25	14	66
22	34	22	69
28	46	28	80

Remarks - Results: Study is considered valid since all validation criteria have been satisfied.

On Days 0, 6 and 13 the contents of both the test material and the toxicity control vessels were observed to be cloudy brown dispersions with fine particles of test material on the surface and on Days 20 and 27 no undissolved test material was visible.

The test material attained 46% degradation after 28 days and therefore cannot be considered to be readily biodegradable under OECD Guideline No. 301B.

CONCLUSION: The notified chemical cannot be classed as readily biodegradable.

TEST FACILITY: Safepharm Laboratories (2008d)

C.1.2. Bioaccumulation

Remarks: The physico-chemical properties (log P_{OW} = 4.09 and solubility in water of 2.58 x 10^{-3}g/L) and the nature of being not readily biodegradable indicate that the notified substance has 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	Acceptable Analogue
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. The test result is considered applicable to the notified chemical based on the formula similarity.
CONCLUSION	The analogue substance is not toxic up to the limit of its solubility.
TEST FACILITY	Safepharm Laboratories (2005e)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Acceptable Analogue
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 using 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. The test result is considered applicable to the notified chemical based on the formula similarity.
CONCLUSION	The analogue substance is not toxic to *Daphnia magna* up to the limit of its solubility.
TEST FACILITY	Safepharm Laboratories (2005f)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified substance
METHOD	OECD TG 201 Alga, Growth Inhibition Test. EC Directive 92/69/EEC C.3 Algal Inhibition Test.
Species	Green alga (*Desmodesmus subspicatus)*
Exposure Period	72 hours
Concentration Range	Nominal: 0.015, 0.048, 0.15, 0.48 and 1.5 mg/L Actual: 0.0018, 0.0045, 0.010, 0.43 and 1.4 mg/L (Geometric mean measured test concentration)
Auxiliary Solvent	None
Water Hardness	Not reported

Analytical Monitoring	HPLC for determination of test material concentrations.
Remarks - Method	No significant deviations from standard protocol.

RESULTS

*Biomass**		*Growth**	
E_bC50 *mg/L at 72 h*	*NOEC* *mg/L*	*E_rC50* *mg/L 0 -72 h*	*NOEC* *mg/L*
0.014	0.0018	0.15	0.0018

* Based on geometric mean measured test concentrations.

Remarks - Results

Analysis of the test preparations at 72 hours showed a marked decline in measured test concentrations in the range of less than 1% to 70% of the nominal. 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, particularly at the lower test concentrations employed.

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.

At the start of the test all control and test cultures were observed to be clear colourless solutions. After the 72-hour test period all control, 0.0018, 0.0045 and 0.010 mg/L test cultures were observed to be green dispersions whilst the 0.48 mg/L and 1.5 mg/L test cultures were observed to be clear colourless solutions.

Based on the geometric mean measured test concentrations the E_rC_{50} (0-72 h) value was 0.15 mg/L; 95% confidence limits 0.10 – 0.23 mg/L, the E_yC_{50} (0-72 h) value was 0.010 mg/L; 95% confidence limits 0.0070 – 0.014 mg/L and the E_bC_{50} (0-72) value was 0.014 mg/L; 95% confidence limits 0.010-0.021 mg/L. The LOEC based on growth rate, yield and biomass integral was 0.0045 mg/L and the NOEC was 0.0018 mg/L.

CONCLUSION The notified chemical is very toxic to algae.

TEST FACILITY Safepharm Laboratories (2008e)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE Acceptable Analogue

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. The test result is considered applicable to the notified chemical based on the formula similarity.

CONCLUSION	The analogue is not harmful to sewage sludge microorganisms.
TEST FACILITY	SafePharm Laboratories (2007b)

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