File No: STD/1469 and STD/1470

July 2013

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

STD/1469: Component 1 in S-10821
STD/1470: Component 2 in S-10821

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

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

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICALS	INTRODUCTION VOLUME	USE
STD/1469 and STD/1470	Cytec Australia Holdings Pty Ltd	Component 1 in S-10821 and Component 2 in S-10821	Yes	STD/1469: ≤300 tonnes per annum STD/1470: ≤150 tonnes per annum	Component of a mineral flotation agent

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard classification	*Hazard statement*
Acute Toxicity (Category 4)	H302 – Harmful if swallowed
Skin sensitisation (Category 1)	H317 – May cause an allergic skin reaction

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

R22 Harmful if swallowed
R38 Irritating to skin
R43 May cause sensitisation by skin contact
R31 Contact with acids liberates toxic gas

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

Hazard classification	*Hazard statement*
Acute (Category 1)	H400 – Very toxic to aquatic life
Chronic (Category 1)	H410 – Very toxic to aquatic life with long lasting effects

Human health risk assessment
Under the conditions of the occupational settings described, provided that exposure is minimised using automated processes and that the use of PPE (gloves, coveralls, eye and face protection) is enforced, the notified chemicals are not considered to pose an unreasonable risk to the health of workers.

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

Environmental risk assessment
On the basis of the PEC/PNEC ratio and the assessed use pattern, the notified chemicals are not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- The notified chemicals should be classified as follows:
  - Acute Toxicity (Category 4): H302 – Harmful if swallowed
  - Skin sensitisation (Category 1): H317 – May cause an allergic skin reaction

  Classification of products/mixtures containing the notified chemicals should be considered based on the concentration of the notified chemicals present.

Health Surveillance

- As the notified chemicals have skin sensitisation potential, employers should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of skin sensitisation.

CONTROL MEASURES
Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemicals:
  - Automated and enclosed processes, where possible
  - Enclosed processes and adequate ventilation if toxic gas is expected to form during use

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemicals:
  - Avoid contact with skin

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemicals:
  - Gloves
  - Coveralls
  - Eye and face protection
  - Adequate respiratory protection if toxic gas is expected to form during use

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

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

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

Disposal

- The notified chemicals should be disposed of to landfill.

Storage

- The handling and storage of the notified chemicals should be in accordance with the Safe Work Australia Code of Practice for *Managing Risks of Hazardous Chemicals in the Workplace* (SWA, 2012) or relevant State or Territory Code of Practice.

Emergency procedures

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

Regulatory Obligations

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

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

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

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

No additional secondary notification conditions are stipulated.

(Material) Safety Data Sheet
The (M)SDS of the notified chemicals provided by the notifier was reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Cytec Australia Holdings Pty Ltd (ABN 45 081 148 629)
21 Solent Circuit
Baulkham Hills NSW 2153

NOTIFICATION CATEGORY
STD/1469: Standard: Chemical other than polymer (more than 1 tonne per year)

STD/1470: Standard (Reduced fee notification): Chemical other than polymer (more than 1 tonne per year) – Chemical is being notified at the same time as a similar chemical

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: chemical name, other names, CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, impurities, additives/adjuvants, use details and import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: acute oral toxicity, skin irritation and bacterial reverse mutation assay.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
S-10821 (>90% mixture of the notified chemicals: contains up to 65% Component 1 [STD/1469] and up to 35% Component 2 [STD/1470])

MOLECULAR WEIGHT
STD/1469: <500 Da
STD/1470: <500 Da

ANALYTICAL DATA
Reference NMR, IR and GC/MS spectra were provided.

3. COMPOSITION

Inseparable mixture of the notified chemicals.

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Yellow-orange liquid*

Property	Value	Data Source/Justification
Melting Point/Freezing Point	<-100 °C	Measured*
Boiling Point	210 °C	Measured*
Density	1.015 kg/m^3 at 20 °C	Measured*
Vapour Pressure	1.1 × 10^{-3} kPa at 20 °C 1.8 × 10^{-3} kPa at 25 °C	Measured*
Water Solubility	0.7 × 10^{-3} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	$t_{½}$ = 32.6 hours (pH 4) 27.0 hours (pH 7) 30.7 hours (pH 9)	Measured
Partition Coefficient (n-octanol/water)	log Pow = 5.50 – 5.58	Measured
Adsorption/Desorption	log K_{oc} = 3.98 – 4.07	Measured
Dissociation Constant	Not determined	Does not contain dissociable functional groups
Flash Point	73.9 °C at 101 kPa (closed cup)	(M)SDS*
Flammability	Not determined	Not expected to be flammable based on flash point
Autoignition Temperature	> 73.9 °C	Based on flash point
Explosive Properties	Predicted negative	Not expected to be explosive based on structure
Oxidising Properties	Predicted negative	Not expected to be oxidising based on structure

*Inseparable mixture of the notified chemicals.

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

Reactivity
The notified chemicals are expected to be stable under normal conditions of use. The notifier has indicated that the reaction of the notified chemicals with acid is expected to produce toxic gas. The notifier has classified the notified chemicals as: R31 Contact with acids liberates toxic gas.

Physical hazard classification
Based on the submitted physico-chemical data depicted in the above table, the notified chemicals are not recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemicals will be imported neat (>90% combined concentration).

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

Year	*1*	*2*	*3*	*4*	*5*
STD/1469	≤300	≤300	≤300	≤300	≤300
STD/1470	≤150	≤150	≤150	≤150	≤150
Tonnes	≤450	≤450	≤450	≤450	≤450

PORT OF ENTRY
Import may occur through all main Australian ports.

IDENTITY OF MANUFACTURER/RECIPIENTS
Cytec Australia Holdings Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemicals (mixture containing >90% concentration) will be imported in 1300-1500 L totes and transported within Australia by rail or road.

USE
The notified chemicals (mixture containing >90% concentration) will be used for mineral floatation during ore processing at mining sites.

OPERATION DESCRIPTION
Manufacturing and/or reformulation will not occur in Australia.

The notified chemicals (mixture containing >90% concentration) will be pumped or gravity fed into a closed flotation cell containing ore slurry at a rate of 5 grams per tonne of ore. The notified chemicals adsorb to metal bearing particles to enable their collection for smelting. Workers will conduct routine maintenance operations.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Storage and transportation	2-4	24
Mineral processing	8	300

EXPOSURE DETAILS
Transport and storage workers
Transport and storage workers will only come into contact with the notified chemicals (>90% concentration) in the unlikely event of an accident.

Mineral flotation
Dermal and ocular exposure to the notified chemicals (>90% concentration) may occur to workers when connecting or disconnecting transfer hoses, or during cleaning and maintenance operations. Inhalation exposures are not expected based on the low vapour pressure of the inseparable mixture of the notified

chemicals (1.8×10^{-3} kPa) and because aerosols are not expected during mineral flotation processes. PPE is expected to be worn, including gloves, goggles and coveralls. The remainder of the flotation processes is expected to be automated and enclosed.

The majority of the notified chemicals will be destroyed during smelting and no exposure to workers is expected outside of the mineral flotation process.

6.1.2. Public Exposure

The notified chemicals will only be used by the mining industry and public exposure is not expected.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on an inseparable mixture of the notified chemicals or a suitable analogue chemical are summarised in the following table. For full details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity*	LD50 >300 and <2000 mg/kg bw; harmful
Rat, acute dermal toxicity	LD50 >2000 mg/kg bw; low toxicity
Rabbit, skin irritation*	irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
Rat, repeat dose oral toxicity – 7 days	NOAEL not established
Rat, repeat dose oral toxicity – 28 days	NOAEL = 30 mg/kg bw/day
Mutagenicity – bacterial reverse mutation*	non mutagenic
Genotoxicity – *in vivo* micronucleus test	non genotoxic

*Analogue chemical

Toxicokinetics, metabolism and distribution

No toxicokinetic data on the notified chemicals were submitted. Absorption across the gastrointestinal and respiratory tract is supported by evidence of systemic toxicity observed in the acute oral toxicity and the 28-day repeat dose oral toxicity studies in rats. Absorption across the skin may be limited based on the partition coefficient (Log Pow 5.50 – 5.58) of the inseparable mixture of notified chemicals.

Acute toxicity

The analogue chemical was harmful by the oral route to rats (LD50 >300 and <2000 mg/kg bw/day). Mortalities were observed in an *in vivo* micronucleus study with mice administered the inseparable mixture of the notified chemicals by oral gavage at 300 (2/18), 320 (1/2), 400 (1/2) and 600 (2/2) mg/kg bw/day. Overall, the inseparable mixture of the notified chemicals is considered to be harmful by the oral route.

The inseparable mixture of the notified chemicals was of low acute dermal toxicity to rats (LD50 >2000 mg/kg bw/day).

Irritation and sensitisation

The analogue chemical was a skin irritant to rabbits. The inseparable mixture of the notified chemicals was found to be a slight eye irritant to rabbits. The inseparable mixture of the notified chemicals was a skin sensitiser in an LLNA study.

Repeated Dose Toxicity

In a 7 day dose range-finding study (3/sex/dose), one female administered the inseparable mixture of the notified chemicals at 200 mg/kg bw/day died on the second day of dosing, after which the dosing was reduced to 150 mg/kg bw/day for the remainder of the study. All remaining animals survived.

In a 28-day oral gavage study, rats (5/sex/dose) were administered an inseparable mixture of the notified chemicals at 0, 30, 75 or 150 mg/kg bw/day. The NOAEL was established as 30 mg/kg bw/day, based on increased liver and kidney weights, centrilobular hepatocellular hypertrophy and tubular hypertrophy of the kidney in both sexes, and decreased body weight gain in males.

Mutagenicity/Genotoxicity

The analogue chemical was not mutagenic in a bacterial reverse mutation assay. The inseparable mixture of the

notified chemicals was not clastogenic in an *in vivo* micronucleus study in mice.

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

Hazard classification	*Hazard statement*
Acute Toxicity (Category 4)	H302 – Harmful if swallowed
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Based on the available information, the inseparable mixture of the notified chemicals is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase(s):

R22 Harmful if swallowed
R38 Irritating to skin
R43 May cause sensitisation by skin contact

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The inseparable mixture of the notified chemicals will be imported and used at >90% concentration. The toxicological effects of concern are acute oral toxicity, skin irritation and sensitisation, slight eye irritation and systemic toxicity. Based on the toxicity profile of the notified chemicals, particularly skin sensitisation and systemic toxicity, exposure should be minimised to the lowest level practicable.

In contact with acid, the notified chemicals may react to form a toxic gas. However, under normal conditions of use, the notified chemicals are not likely to be mixed with acidic chemicals and therefore production of toxic gas is not expected.

Dermal and ocular exposure may occur to workers connecting or disconnecting transfer hoses, or during cleaning/maintenance operations. Exposure will be minimised by the use of automated processes and PPE (gloves, coveralls and eye/face protection). Overall, provided exposure is minimised using automated processes and the PPE described above, the risk to workers is not considered to be unreasonable.

6.3.2. Public Health
As the public is not expected to be exposed to the notified chemicals, the risk to public health is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemicals, as an inseparable mixture, will be imported to Australia for direct use in mineral floatation during ore processing at mining sites. No reformulation is expected in Australia. No significant release is expected from transportation and storage of the notified chemicals.

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

The notified chemicals will be added from the imported totes 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 chemicals (mixture containing >90% concentration) in the closed-loop water recirculation circuit was reported to be 5 ppm. The residence time in this tank will be sufficient to allow the reagent(s) to react with (adsorb to) the surface of the desirable sulphide minerals. Approximately 70% of the notified chemicals are 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 will usually be 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 will be skimmed off, collected and filtered. The solids will then be further dried to produce the final mineral concentrate which will then be transported to a smelter to be refined into metal.

The gangue material (which 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 will be 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 will be returned to the flotation process. The tailings slurry will then be 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 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 compounds. Some of the remaining reagent becomes attached to the surface of the gangue (waste) minerals, which are deposited into the tailings dams. However, the notified chemicals were indicated to have a low affinity for the surface of these particles, and only a fraction of the reagent will be released in this manner. It is estimated 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 reused in the flotation process. At steady state it is expected that 20% of the dosed amount of notified chemicals will remain in the tailings dam, which equates to 1 ppm (20% × 5 ppm).

Settling dam walls are typically constructed using tailings and are designed to permit water to leach. In addition, settling dam walls occasionally breach during periods of intense precipitation, releasing the contents of the dam. Therefore, significant quantities of notified chemicals may be released into terrestrial waterways.

It is also anticipated that some water will inevitably enter the groundwater. Based on the readily hydrolysis property and the log K_{OC} of around 4, it is expected that only a small proportion of the total annual import volume will be mobile and could enter groundwater. Notified chemicals that leach into groundwater are expected to eventually degrade via biotic and abiotic means to form low molecular weight compounds.

RELEASE OF CHEMICAL FROM DISPOSAL

Imported totes that contained the notified chemicals are expected be returned to supply for reuse. The residual notified chemicals in blending pumps will stay in the flush and cleaning water that will be reused as part of the diluted product. The releases are expected to be collected and disposed of to landfill or to the tailing dam.

The majority of the notified chemicals will be destroyed by oxidation in the smelting process. Residual product left in the drums 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 chemicals will remain in the tailing dam and significant seepage, via groundwater, is predicted to result in some environmental exposure.

7.1.2. Environmental Fate

Most of the notified chemicals will become associated with the surface of mineral particles in metal concentrates and will be destroyed during smelting. The compounds will decompose to water vapour, oxides of carbon, and low molecular weight sulphur chemicals.

The remainder of the notified chemicals will be associated with the tailings solids and waters. The provided biodegradation study indicated that the inseparable mix of notified chemicals does not have a potential for biodegradation. However, the notified chemicals are not expected to be persistent in the environment due to their potential for rapid hydrolysis ($t_{1/2}$ ~30 h) under environmental conditions (pH 4 – 9). Whilst the provided hydrolysis study results should be treated with caution, this class of chemicals is known to hydrolyse under mild conditions. For details of the environmental fate studies please refer to Appendix C.

The physicochemical properties (log Pow = 5.50-5.58 and solubility in water of 0.7 ppm) indicate that the

notified chemicals could be bioaccumulative. However, the potential for bioaccumulation is expected to be significantly reduced due to the expected hydrolysis of the notified chemicals in the environment. Furthermore, no bioaccumulation is anticipated as there will be no aquatic exposure when the notified chemicals are used as intended.

The predicted hydrolysis products of the notified chemicals are not expected to be persistent in the environment, nor bioaccumulative.

7.1.3. Predicted Environmental Concentration (PEC)

There is expected to be minimal release of the notified chemicals to the environment from spills at mine sites during use. Most of the notified chemicals will become associated with the surface of mineral particles in metal concentrates, and will be destroyed during smelting. The notified chemicals will decompose to water vapour, oxides of carbon, and low molecular weight sulphur compounds. The most likely release is expected from seepage of the notified chemicals 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 61-132 cm thick. Similarly, synthetic liners are expected to have permeability of 10^{-9} to 10^{-14} cm/sec and have thicknesses of 0.10-0.15 cm (US EPA 1994).

The PEC may be calculated assuming the maximum concentration of the notified chemicals (1 ppm) in the dam and the degradation of the notified chemicals as they permeate through the tailings liner. Sulphidic tailings dams are expected to be acidic with minimal microbiological life; 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/sec), 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 chemicals have a half-life of 32.6 days. Accordingly they will undergo approximately 21.7 half-lives (706 ÷ 32.6) during their permeation through the liner. A worst case PEC from release from the tailings dam may be calculated by 1 ppm $\times 0.5^{21.7} = 0.3 \times 10^{-3}$ µg/L.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on an inseparable mixture of the notified chemicals are summarised in the table below. Details of these studies can be found in Appendix C. Since the notified chemicals are expected to hydrolyse over the duration of the ecotoxicity tests, the endpoints are considered to represent the notified chemicals and their hydrolysis products. Given that the toxicity cannot be attributed to a single component but to the test substance as a whole, classifications have been made based on the reported endpoints.

Endpoint	*Result for the test substance*	*Assessment Conclusion*
Fish Toxicity	96 h LL50 = 2.49 mg/L (WAF)	Toxic to fish
Daphnia Toxicity	48 h EL50 = 0.63 mg/L (WAF)	Very toxic to aquatic invertebrates
Algal Toxicity	72 h EL50 > 160 mg/L (WAF) 72 h NOEL = 10 mg/L (WAF)	Not harmful to algae
Inhibition of Bacterial Respiration	3 h IL50 = 100 mg/L	Significantly inhibits microbial respiration at ≤ 100 mg/L

Under the Globally Harmonised System of Classification and Labelling of Chemicals (United Nations, 2009) the notified chemicals and their hydrolysis products are classified as not harmful to algae, toxic to fish and very toxic to aquatic invertebrates.

Based on the acute endpoint for daphnids, the notified chemicals and their hydrolysis products are formally classified as 'Acute Category 1; very toxic to aquatic life' under the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS).

On the basis of its high oil/water partition coefficient (log Pow = 5.50-5.58) and acute toxicity to daphnids, the inseparable mixture of notified chemicals and their hydrolysis products are formally classified under the GHS as 'Chronic Category 1; very toxic to aquatic life with long-lasting effects'.

7.2.1. Predicted No-Effect Concentration

The Predicted No-Effect Concentration (PNEC) was calculated based on the most sensitive endpoint for daphnids and an assessment factor of 100. This assessment factor was used as effects endpoints are available

for three trophic levels.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EL50 (daphnids)	0.63	mg/L
Assessment Factor	100	
PNEC:	6.3	µg/L

7.3. Environmental Risk Assessment

The Risk Quotient (PEC/PNEC) has been calculated as shown below:

Risk Assessment	*PEC µg/L*	*PNEC µg/L*	*Q*
Q - River:	0.3×10^{-3}	6.3	<<0.001

Despite the relatively large quantities of the notified chemicals introduced and the toxicity to aquatic organisms, many factors preclude significant release to the aquatic environment. These factors include the proposed use pattern and the rapid hydrolysis of the notified chemicals. The notified chemicals and their hydrolysis products are not expected to be persistent in the environment, nor do they have significant potential for bioaccumulation. The calculated Risk Quotient is much less than 1 even though it has been estimated based on the worst case scenario. Therefore, when used in the proposed manner, the notified chemicals are not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point <-100 °C

Method	OECD TG 102 Melting Point/Melting Range
Remarks	Determined (for inseparable mixture of the notified chemicals) by differential scanning calorimetry and confirmed using cold stage microscopy.
Test Facility	Cytec (2012)

Boiling Point 210 °C

Method	OECD TG 103 Boiling Point
Remarks	Determined (for inseparable mixture of the notified chemicals) by differential scanning calorimetry.
Test Facility	Cytec (2012)

Density 1.015 kg/m^3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids
Remarks	Density (of inseparable mixture of the notified chemicals) referenced against the density of toluene using a pycnometer.
Test Facility	Cytec (2012)

Vapour Pressure 1.8×10^{-3} kPa at 25 °C

Method	OECD TG 104 Vapour Pressure
Remarks	Determined (of inseparable mixture of the notified chemicals) determined by thermogravimetric analysis.
Test Facility	Cytec (2012)

Water Solubility 0.7×10^{-3} g/L at 20 °C

Method	OECD TG 105 Water Solubility
Remarks	Flask Method. The test substance (an inseparable mixture of the notified chemicals) was dried in a vacuum oven at 65 °C before testing was conducted. The test substance was mixed with water at 20 ppm at 30 °C for 24 hours and was occasionally hand-shaken, followed by keeping for up to two more days at 20 – 30 °C. After centrifugation, supernatants were collected for concentration analysis using HPLC/UV (detection limit 0.1 ppm). A mean concentration of 0.7 ppm ± 0.2 ppm was determined. The pH of the supernatant was 7.0. The results indicated that the test substance was fully equilibrated within 24 hours. The test report did not detail any investigations into the instability of the test substance. Given the potential for the test substance to hydrolyse ($t_{½}$ of 27.0 hours at pH 7), as per the results of the study below, these measured results should be treated with caution. However, overlaid chromatograms of the standards and supernatants all show two peaks which suggest that the notified chemicals were present in solution. Furthermore, modelling results (WSKOW v1.42, US EPA 2011) indicate that the notified chemicals are predicted to have a water solubility of ~1 ppm, when estimations are based on the measured log Pow and melting point, thereby supporting the water solubility values determined in this test.
Test Facility	Cytec (2012)

Hydrolysis as a Function of pH $t_{½}$ = 32.6 hours at pH 4, 27.0 hours at pH 7, and 30.7 hours at pH 9

Method	A procedure designed to be compatible with OECD TG 111 Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$ (hours)*
4	25	32.6
7	25	27.0
9	25	30.7

Remarks Test solutions of nominal concentration 3.0 mg/L were prepared in the three buffer solutions. A co-solvent of methanol (1%) was used to aid solubility. As there was no detected test item after a tier 1 test at 50 °C for 144 hours, a tier 2 test was conducted at 50 °C (24 hours), 40 °C (up to 54 hours), and 30 °C (78 hours). HPLC was used for concentration analysis. The half-life for hydrolysis at 25 °C was determined as listed in the table above.

The author of the study indicated that review of the hydrolysis pathway identified four hydrolysis products. However, there was no indication of hydrolysis product identification during the test to verify this conclusion. Steps were not taken to address the potential that test substance may adhere to the test vessel walls or that the co-solvent did not contribute to hydrolysis of the test substance. Therefore, these results should be treated with caution.

Test Facility Harlan (2013a)

Partition Coefficient (n-octanol/water) log Pow = 5.50-5.58

Method OECD TG 117 Partition Coefficient (n-octanol/water).

Remarks HPLC Method. The test substance (an inseparable mixture of the notified chemicals) was dried in a vacuum oven at 65 °C before testing was conducted. The test substance eluted as two isomeric peaks with determined log P_{OW} of 5.50 and 5.58, respectively.

Test Facility Cytec (2012)

Adsorption/Desorption – screening test log K_{OC} = 3.98-4.07

Method HPLC screening method designed to be compatible with OECD TG 121 Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC).

Remarks Acetonitrile was used for preparation of the solutions of the test substance (an inseparable mixture of the notified chemicals). Retention time was determined for all the test and reference solutions at a column temperature of 30 °C. The test substance eluted as two isomeric peaks with determined log K_{OC} values 3.98 and 4.07.

Test Facility Harlan (2013a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Analogue chemical

METHOD: OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure
- Species/Strain: Rat/RccHan:WIST
- Vehicle: Arachis oil BP
- Remarks - Method: No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	1F	300	0/1
2	1F	2000	1/1
3	4F	300	2/4
4	5F	50	0/5

- LD50: > 300 and < 2000 mg/kg bw
- Signs of Toxicity: The mortalities occurred within two days of dosing. Hunched posture, piloerection and lethargy were observed at 300 and 2000 mg/kg bw. Ataxia and decreased respiratory rate were observed in animals treated at 300 mg/kg bw. Surviving animals gained weight throughout the study.
- Effects in Organs: Red lungs, dark liver and kidneys, and a clear yellow liquid present in the stomach, and epithelial sloughing and reddened gastric mucosa and small intestine, were observed in the animal treated at 2000 mg/kg bw. In the mortalities that occurred at 300 mg/kg bw, haemorrhagic or abnormally red lungs, and dark liver and kidneys were observed. A cavity was observed in the right kidney in one surviving animal treated at 300 mg/kg bw. No abnormalities were noted in any animal treated at 50 mg/kg bw.

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

TEST FACILITY: Harlan (2012a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Inseparable mixture of the notified chemicals

METHOD: OECD TG 402 Acute Dermal Toxicity – Limit Test
- Species/Strain: Rat/RccHan:WIST
- Vehicle: None
- Type of dressing: Semi-occlusive
- Remarks - Method: No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	1M + 1F	1000	0/2
2	1M + 1F	2000	0/2
3	4M + 4F	2000	1/8

- LD50: > 2000 mg/kg bw
- Signs of Toxicity - Local: Very slight to well defined erythema and very slight oedema were noted in animals treated at 2000 mg/kg bw. Very slight erythema was observed in the female treated at 1000 mg/kg bw.
- Signs of Toxicity - Systemic: One male treated at 2000 mg/kg bw was found dead on day 4. No clinical

signs of toxicity were observed during the study. Body weight loss was observed in one female treated at 1000 mg/kg bw and one female treated at 2000 mg/kg bw over the first week of the study. One other female treated at 2000 mg/kg bw showed no gain in body weight during the second week of the study.

Effects in Organs: Abnormally red lungs, and dark liver and kidneys was observed in the animal that died during the study. No abnormalities were noted in other animals.

Remarks - Results: The effects in the lung, kidney and liver noted in the animal that died were consistent with the effects noted in the acute oral toxicity study. The mortality is therefore considered to be possibly related to treatment.

CONCLUSION: The inseparable mixture of the notified chemicals is of low toxicity via the dermal route.

TEST FACILITY: Harlan (2012b)

B.3. Irritation – skin

TEST SUBSTANCE: Analogue chemical

METHOD: OECD TG 404 Acute Dermal Irritation/Corrosion

Species/Strain: Rabbit/New Zealand White

Number of Animals: 4

Vehicle: None

Observation Period: 7 days

Type of Dressing: Semi-occlusive

Remarks - Method: The first rabbit was treated on three separate sites with the patches removed after 3 minutes, 1 hour and 4 hours, then subject to observation over 7 days. Subsequent rabbits were exposed on one site for 4 hours then observed over 7 days.

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	2	1	2	2	< 7 days	0
Oedema	1.3	1	1.7	2	< 7 days	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.
**Based on results from the 4 hour exposure duration.

Remarks - Results: One animal was killed *in extremis* due to breathing problems, lethargy and pallor of the extremities observed 24 hours post exposure. It was not determined whether this mortality was attributable to treatment of the test substance. Very slight erythema and oedema was observed in this animal prior to sacrifice.

Skin irritation after 3 minutes and 1 hour in the first animal was similar or slightly decreased in severity and duration, when compared to irritation in the 4 hour exposure test sites.

CONCLUSION: The analogue chemical is irritating to the skin.

TEST FACILITY: Harlan (2012c)

B.4. Irritation – eye

TEST SUBSTANCE: Inseparable mixture of the notified chemicals

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion
- Species/Strain: Rabbit/New Zealand White
- Number of Animals: 3
- Observation Period: 72 hours
- Remarks - Method: No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0.7	1.0	0.3	2	< 72 hours	0
Conjunctiva: chemosis	0.7	0.3	0	2	< 72 hours	0
Conjunctiva: discharge	0.3	0	0	2	< 48 hours	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	1	< 24 hours	0

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

CONCLUSION: The inseparable mixture of the notified chemicals is slightly irritating to the eye.

TEST FACILITY: Harlan (2012d)

B.5. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE: Inseparable mixture of the notified chemicals

METHOD: OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
- Species/Strain: Mouse/CBA/CaOlaHsd
- Vehicle: Acetone:olive oil (4:1)
- Remarks – Method: A screening study was conducted at 10, 25 or 50% concentration with mice (1/concentration) treated daily for three consecutive days. Animals were observed twice daily for the first three days and once daily on days 4 and 5. Ear thickness measurements were taken on day 3 and 6. The main study was conducted at 5, 10 or 25% concentration (5/concentration).

  A concurrent positive control was not conducted. The result of a positive control study with α-hexylcinnamaldehyde, conducted by the laboratory within 6 months of the main study, was provided.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/animal)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	2364	-
5	10019	4.24
10	10065	4.51
25	14827	6.27

- Remarks - Results: The animal treated at 50% in the screening study was killed humanely on day 5 due to the presence of hunched posture, lethargy and ptosis. There were no marked changes in ear thickness from day 1 to day 6. Slight skin irritation was observed in the animals treated at 25 and 50%.

  In the main study, mild redness was observed on the ears of mice treated at

	10 and 25%. Body weights were similar in treated and control groups. The EC3 value is <5%.
CONCLUSION	There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the inseparable mixture of the notified chemicals.
TEST FACILITY	Harlan (2012e)

B.6. Repeat dose toxicity

TEST SUBSTANCE	Inseparable mixture of the notified chemicals
METHOD	Non-guideline range-finding study
Species/Strain	Rat/Wistar Han:RccHan:WIST
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 7 days
Vehicle	Arachis oil BP
Remarks - Method	In a 7 day study, rats (3/sex/dose) were administered the test substance by gavage at 0, 30, 100 or 200/150 mg/kg bw/day. The 200 mg/kg bw/day dose was reduced to 150 mg/kg bw/day after the second dose as a single female was found dead on day 2 and treatment-related effects were observed in surviving animals at this dose level. Clinical observations, body weights, and food and water consumption were recorded. Necropsy was conducted on day 8.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	3M + 3F	0	0/6
low dose	3M + 3F	30	0/6
mid dose	3M + 3F	100	0/6
high dose	3M + 3F	200/150	1/6

Mortality and Time to Death

One female from the high dose group was found dead 5 hours after dosing on day 2. No clinical signs of toxicity were observed prior to death.

Clinical Observations

Increased salivation was observed shortly after dosing in one male and one female treated at 150 mg/kg bw/day, between days 5 and 7.

Body weight losses were observed before the dose was reduced in the 200 mg/kg bw/day treatment group. This group gained weight over the study period following the reduction, although body weight gain in males treated at 200/150 mg/kg bw/day from days 1 to 8 was decreased. Food consumption was decreased in the high dose over days 1 to 3 but recovery was observed following the reduction in dose. Water consumption was increased in animals treated at 200/150 mg/kg bw/day, with increases also observed in low and mid dose females compared to controls.

Effects in Organs

Reddened lungs and distended stomach was observed in the female mortality.

Remarks – Results

A preliminary study was reported with animals (number not specified) at 150 and 300 mg/kg bw/day for three days. One male treated at 300 mg/kg bw/day was killed *in extremis* one hour post dosing on day 2 due to marked adverse clinical signs of toxicity. The other male in this group was found dead on day 2 and macroscopic examination revealed a clear fluid in the thoracic cavity, autolytic changes characterised by enlarged and reddened stomach, darkened liver and abnormally swollen lungs. No response was observed in the females treated at 300 mg/kg bw/day, or in animals treated at 150 mg/kg bw/day.

The study authors considered the changes in feed and water consumption in the main study to be associated with the irritant and unpalatable nature of the test substance.

CONCLUSION
Mortality was observed in animals treated at 200 mg/kg bw/day and above. Based on the limited number of endpoints examined and the small group numbers, which prevented meaningful statistical analyses, a NOAEL could not be established.

TEST FACILITY Harlan (2012f)

B.7. Repeat dose toxicity

TEST SUBSTANCE Inseparable mixture of the notified chemicals

METHOD OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents
Species/Strain Rat/Wistar Han:RccHan:WIST
Route of Administration Oral – gavage
Exposure Information Total exposure days: 28 days
Dose regimen: 7 days per week
Post-exposure observation period: none
Vehicle Arachis oil BP
Remarks - Method No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5M + 5F	0	0/10
low dose	5M + 5F	30	0/10
mid dose	5M + 5F	75	0/10
high dose	5M + 5F	150	0/10

Clinical, Behaviour, Functional and Sensory Observations
Increased salivation was observed in both sexes at all treatment levels immediately after dosing (from the second week onwards). Noisy respiration was observed in two females treated at 150 mg/kg bw/day on days 25 and 26.

There were no treatment-related effects noted in weekly open-field observations.

There were no changes in measured functional performance tests. A statistically significant decrease in overall activity in males treated at 150 mg/kg bw/day and a statistically significant increase in activity during the final 20% of the observation period was noted in females treated at 75 and 150 mg/kg bw/day.

There were no changes in measured sensory reactivity parameters.

Bodyweight, and Food and Water Consumption
There were no statistically significant changes in absolute body weights in males or females although terminal bodyweights in males treated at 150 mg/kg bw/day were slightly decreased (↓8%) compared to controls. There were no body weight gain changes in treated females. There were statistically significant decreases in body weight gain of males in 150 mg/kg bw/day at all weekly observation points. Statistically significant decreased body weight gain over the final week of dosing was observed in males treated at 30 and 75 mg/kg bw/day. Overall bodyweight gain was reduced in males treated at 75 and 150 mg/kg bw/day (↓15% and ↓26%, respectively). The overall bodyweight gain in males treated at 30 mg/kg bw/day was similar to controls. Body weight gain decreases are considered to be treatment related in males treated at 75 and 150 mg/kg bw/day.

There were no changes in absolute feed consumption. There were slight decreases in feed efficacy in males treated at 150 mg/kg bw/day but was not considered of toxicological concern due to the small magnitude of the change. There were no changes in water consumption.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis and Thyroid Hormones

There were statistically significant decreases in mean cell haemoglobin concentration in females treated at 75 and 150 mg/kg bw/day. These decreases were minimal (↓2%) and were within historical values provided by the laboratory for this strain of rat and were therefore not considered to be treatment related. There were no other changes in measured haematology parameters.

There was a slight but statistically significant increase in albumin levels in males treated at 150 mg/kg bw/day (↑11%) and there were statistically significant increases in bilirubin in both sexes treated at 150 mg/kg bw/day (↑85%/47%; males/females) and in females treated at 75 mg/kg bw/day (↑28%). Also in females treated at 150 mg/kg bw/day there were statistically significant increases in inorganic phosphate (↑36%), and decreases in blood urea (↓37%), chloride levels (↓3%) and creatinine concentration (↓24%), with a statistically significant decrease of blood urea in females treated at 75 mg/kg bw/day (↓26%). These changes were mostly within expected normal range for the test strain provided by the laboratory.

Effects in Organs

There were no macroscopic changes noted at necropsy.

There were dose dependent statistically significant increases in absolute and relative liver and kidney weights in males at all treatment levels and in females treated at 75 and 150 mg/kg bw/day. In the liver, centrilobular hypertrophy was observed in both sexes of animals treated at 75 and 150 mg/kg bw/day. The enlarged liver hepatocytes showed increased cytoplasmic eosinophilia and/or a ground glass appearance. In the kidney, tubular hypertrophy was observed in both sexes treated at 75 and 150 mg/kg bw/day throughout the proximal tubules, with prominent hypertrophy in the S3 segment (see following table). Hypertrophy (liver or kidney) was not observed in the 30 mg/kg bw/day or control groups.

	Males (mg/kg bw/day)		*Females (mg/kg bw/day)*	
	75	*150*	*75*	*150*
Liver				
hepatocellular hypertrophy, centrilobular	4 (1.0)	5 (1.0)	4 (1.0)	5 (1.2)
Kidney				
tubular hypertrophy, proximal tubules, diffuse	2 (1.0)	5 (1.6)	1 (1.0)	5 (1.4)

( ), indicates average severity.

There were statistically significant decreases in absolute brain weights in males treated at 75 and 150 mg/kg bw/day but this was accompanied with statistically significant changes in relative brain weights at these treatment levels that were not dose-dependent, thus these brain weight changes are considered incidental.

There were statistically significant increases in absolute and relative pituitary weights in both sexes at all treatment levels. The relevance of this effect is unclear but as there were no associated histopathological observations, this finding is not considered to be of toxicological concern.

There were no treatment related effects on oestrous cycle.

Remarks – Results

The organ weight changes in the liver and kidney were considered to be associated with the hypertrophy. The study authors noted that as there were no associated inflammatory or degenerative changes the observed hypertrophy was considered to be an adaptive effect.

Overall, effects in the liver and kidney at 75 and 150 mg/kg bw/day (increased weights and hypertrophy) are possibly treatment related and may be adverse, but are considered to be of low toxicological concern based on the lack of regenerative or degenerative effects. The kidney and liver weight changes in males treated at 30 mg/kg bw/day are unlikely to be of toxicological significance based on the lack of associated histopathological findings.

CONCLUSION

The NOAEL was established as 30 mg/kg bw/day in this study, based on increased liver and kidney weights, centrilobular hepatocellular hypertrophy and tubular hypertrophy of the kidney in both sexes, and decreased body weight gain in males.

TEST FACILITY Harlan (2013b)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE	Analogue chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test – Plate Incorporation and Pre-incubation Methods
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	Phenobarbitone/β-naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: up to 5000 µg/plate b) Without metabolic activation: up to 5000 µg/plate
Vehicle	Dimethyl sulfoxide
Remarks - Method	No significant protocol deviations.

RESULTS

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

CONCLUSION	The analogue chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Harlan (2012g)

B.9. Genotoxicity – in vivo

TEST SUBSTANCE	Inseparable mixture of the notified chemicals
METHOD	OECD TG 474 Mammalian Erythrocyte Micronucleus Test
Species/Strain	Mouse/Hsd:ICR(CD-1)
Route of Administration	Oral – gavage
Vehicle	Arachis oil
Remarks - Method	A range finding study was conducted at single gavage doses at 300, 320, 400 and 600 mg/kg bw and observed for 2 days. The main study was conducted at up to 300 mg/kg bw.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*	*Mortality*
Range finding study				
1	3M + 1F	300	48 hours	0/4
2	2M	320	48 hours	1/2
3	1M + 1F	400	48 hours	1/2
4	1M + 1F	600	48 hours	2/2
Main study				
vehicle control	7M	0	24 hours	0/7
low dose	7M	75	24 hours	0/7
mid dose	7M	150	24 hours	0/7
high dose	7M	300	24 hours	2/7
high dose	7M	300	48 hours	0/7
positive control, CP	5M	50	24 hours	0/5

CP, cyclophosphamide

RESULTS

Doses Producing Toxicity	Mortality was observed in the study in doses as low as 300 mg/kg bw. Clinical signs observed at 150 mg/kg bw included ptosis and hunched posture. Clinical signs at 400 mg/kg bw included piloerection, splayed gait, ataxia, decreased respiration rate and laboured respiration. The mortalities and clinical signs of toxicity are considered to be evidence of systemic absorption of the test material.
Genotoxic Effects	There were no statistically significant increases in the micronucleated polychromatic erythrocytes in any treatment groups. A positive response was observed in the cyclophosphamide treated group.
Remarks - Results	The mortalities observed in the main study are not considered to affect the sensitivity of the study to detect clastogenic potential of the test substance.

CONCLUSION The inseparable mixture of the notified chemicals was not clastogenic under the conditions of this *in vivo* micronucleus test.

TEST FACILITY Harlan (2012h)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Inseparable mixture of the notified chemicals
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test
Inoculum	Activated sewage sludge
Exposure Period	28 days
Auxiliary Solvent	Not reported
Analytical Monitoring	The amount of CO_2 evolved was collected for the determination of theoretical amount of carbon dioxide ($ThCO_2$).
Remarks - Method	On the basis of pre-study solubility work, it was concluded that the best method of test solution preparation was by ultrasonication. This method formed a cloudy dispersion with no undissolved test item visible. The test was conducted according to the guidelines above, with no other significant deviations from protocol, and with Good Laboratory Practice (GLP) compliance. In a preliminary test at 10 mg C/L of test substance, the toxicity control attained less than 25% biodegradation after 14 days. Therefore, the definitive test was conducted at 5 mg C/L of test substance. A reference control with sodium benzoate and a toxicity control with the test substance and sodium benzoate were conducted.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	0	2	61
14	0	14	76
28	0	28	105

Remarks - Results	All validity criteria were met. Based on the preliminary results of the toxicity control at 10 mg C/L test substance, the test substance would be classed as exhibiting inhibitory effects. However, the toxicity control in the definitive test attained 37% degradation after 14 days, indicating that the test substance was not toxic to microorganisms at 5 mg C/L concentration. This was confirmed by statistical analysis showing that there was no significant difference ($P \geq 0.05$) between the respiration of the microorganisms in the control and test substance vessels. As the test substance attained 0% degradation after 28 days, the test substance is not considered to be readily biodegradable.
CONCLUSION	Under the conditions of this test, the notified chemicals are not ready biodegradable.
TEST FACILITY	Harlan (2012i)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Inseparable mixture of the notified chemicals
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-static EC Council Regulation No 440/2008 C.1 Acute Toxicity for Fish - Semi-static
Species	Rainbow Trout (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	None

Water Hardness	140 mg $CaCO_3$/L
Analytical Monitoring	The test concentrations were analysed by Gas Chromatography (GC) at 0, 24, and 96 hours of the test.
Remarks – Method	Conducted in accordance with the guidelines above with GLP compliance. Following a range finding test, a definitive test was conducted at a nominal loading rate of 1.0, 3.2, 10, 32, and 100 mg/L using water accommodated fractions (WAFs) at 14°C, a dissolved oxygen level of ≥ 9.7 mg/L and a pH range of 7.9 to 8.5. For preparation of the WAFs, mixtures of the test substance and water were prepared at each concentration followed by agitation for 23 hours and standing for 1 hour. A significant amount of dispersed test item was visually observed, therefore, a glass wool plug was used for sampling by mid-depth siphoning (the first 75-100 mL discarded). Microscopic observation of the filtered WAFs showed that no micro-dispersions or particles of the test item were present. Although the test preparations were renewed daily to prevent the build-up of nitrogenous wastes, the media was replaced with the originally prepared WAF solutions. The LL50 was determined by the maximum-likelihood probit method (Finney, 1971) and ToxCalc (ToxCalc, 1999).

RESULTS

Nominal WAF Concentration mg/L	*Actual WAF Concentration (0 hour, 96 hour old media) mg/L*	*Number of Fish*	*Mortality*				
			6h	*24 h*	*48 h*	*72 h*	*96 h*
0	<LOQ	7	0	0	0	0	0
1	0.586, 0.0612	7	0	1	1	1	1
3.2	0.876, 0.0222	7	0	3[a]	4[b]	5	5
10	0.98, -	7	0	4	4	7[e]	7
32	0.92, -	7	0	5	6[c]	7	7
100	0.868, -	7	0	3	3[d]	7	7

LOQ: Limit of quantification;

a: After 22 hours 3 out of 7 were moribund, which were killed and classed as moralities for the following time point;

b: 1 out of 3 exhibited prolonged sub-lethal effects;

c: 1 out of 1 fish exhibited prolonged sub-lethal effects;

d: 4 out of 4 fish exhibited prolonged sub-lethal effects;

e: 3 out of 3 fish exhibited prolonged sub-lethal effects.

LL50	2.49 mg/L (95% CL 1.75-8.53 mg/L) WAF at 96 hours.
NOEL	1 mg/L WAF at 96 hours.
Remarks – Results	All the test validity criteria were met except that the analysed concentrations of the test substance in solution indicated that the test substance was not stable in the test medium. One out of 7 fish was observed dead after 24 hours exposure at 1 mg/L. This is not considered to be due to the toxicity of the test substance given no further moralities were observed at this level. Therefore, the 96 h NOEL was determined to be 1 mg/L WAF loading rate. The 96 h LL50 was determined to be 2.49 mg/L (95% CL 1.75-8.53 mg/L) WAF loading rate. Although the determined LL50 value of 2.5 mg/L is based on the nominal WAF loading rate, the actual concentration of notified chemicals would be expected to be less than or equal to its measured water solubility of 0.7 mg/L. In addition, the test substance is readily hydrolysable with a half-life of ≥27 hours. This half-life is comparable with the agitation time for test solutions' preparation and significant hydrolysis may be expected over this period. However, GC analysis at 0 hours of the fish test show that

measured concentrations (0.58 – 0.98 mg/L) were not dissimilar to the measured water solubility value of 0.7 mg/L.

Over the duration of the test, the test substance concentrations declined. Thus, the observed effects may be from both the test substance and its hydrolysis products. Given that the toxicity cannot be attributed to a single component but to the test substance as a whole, the results are based on nominal loading rates only. Therefore, the test substance and its hydrolysis products are expected to be toxic to fish.

CONCLUSION The notified chemicals and their hydrolysis products are toxic to fish.

TEST FACILITY Harlan (2013c)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Inseparable mixture of the notified chemicals

METHOD OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Static
EC Council Regulation No 440/2008 C.2 Acute Toxicity for Daphnia - Static

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	The test concentrations were analysed by Gas Chromatography (GC) at 0 and 48 hours of the test.
Remarks - Method	Conducted in accordance with the guidelines above with GLP compliance. Following a range finding test, a definitive test was conducted at nominal loading rates of 0.10, 0.18, 0.32, 0.56, and 1.0 mg/L using WAFs at 21°C and a pH range of 7.9 to 8.5. For preparation of WAFs, mixtures of the notified chemical and water were prepared at 1 mg/L by agitation for 23 hours and standing for 1 hour. A significant amount of dispersed test item was visually observed, therefore, a glass wool plug was used for sampling by mid-depth siphoning (the first 75-100 mL discarded). Microscope observation of the filtered WAFs showed that no microdispersions or particles of the test item were present. This 1.0 mg/L WAF preparation was further diluted to give target test level of loading rates. A positive control test was conducted using potassium dichromate as the reference item. The immobilisation data were analysed by the trimmed Spearman-Karber method (Hamilton *et al.*, 1977) for calculation of EL50.

RESULTS

Nominal WAF Concentration mg/L	*Actual WAF Concentration (0 hour, 48 hour old media) mg/L*	*Number of D. magna*	*Number Immobilised*	
			24 h	*48 h*
0	<LOQ	20	0	0
0.10	Not provided	20	0	0
0.18	0.0388, < LOQ	20	0	1
0.32	0.0722, 0.016	20	0	3
0.56	0.122, 0.0482	20	2	2
1.0	0.244, 0.0849	20	20	20

LOQ: Limit of quantification

EL50	0.63 (95% CL 0.65-0.76) mg/L (WAF) at 48 hours
NOEL	0.18 mg/L (WAF) at 48 hours

Remarks - Results	The 24 h and 48 h NOEC for the positive control was determined to be 0.56 mg/L and 0.32 mg/L, respectively. The 24 h and 48 h EC50 for the positive control was determined to be 0.75 (95% CL 0.56-1.0) mg/L and 0.32 (95% CL 0.42-0.48) mg/L, respectively. All the test validity criteria are met except that a decline in the test concentration was observed which indicated that the test substance is not stable in water. The 48 h EL50 was determined to be 0.63 (95% CL 0.65-0.76) mg/L, the 48 h NOEL was determined to be 0.18 mg/L. In addition, the test substance is readily hydrolysable with a half-life of ≥27 hours, which is comparable with the agitation time for test solutions' preparation. Thus, significant hydrolysis may have occurred during the test solution preparation. Over the duration of the test, the test substance concentrations declined. Thus, the observed effects may be from both the test substance and its hydrolysis products. Given that the toxicity cannot be attributed to a single component but to the test substance as a whole, the results are based on nominal loading rates only. Therefore, the test substance and its hydrolysis products are expected to be very toxic to daphnids.
CONCLUSION	The notified chemicals and their hydrolysis products are very toxic to aquatic invertebrates.
TEST FACILITY	Harlan (2013d)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Inseparable mixture of the notified chemicals
METHOD	OECD TG 201 Freshwater Alga and Cyanobacteria, Growth Inhibition Test
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 10, 20, 40, 80, and 160 mg/L Actual: 0.29-1.0 mg/L
Auxiliary Solvent	None
Analytical Monitoring	The test concentrations were analysed by GC at 0 and 72 hours of the test.
Remarks - Method	Following a preliminary test, a definitive test was conducted at a nominal loading rate of 10, 20, 40, 80, and 160 mg/L using WAFs at 21°C and pH range of 7.2 to 8.3. Six replicates were used for the control and four were used for each of the test levels. For preparation of the WAFs, mixtures of the test substance and water at the target concentrations were prepared by agitation for 23 hours followed by standing for 1 hour. The aqueous phase sampled by mid-depth siphoning (the first 75-100 mL discarded) for the test. Microscope observations of the sampled WAFs showed that no micro-dispersions or undissolved test item were present. A positive control test was conducted using potassium dichromate as the reference item. The cell densities were determined using a Coulter® Multisizer Particle Counter.
RESULTS	

Biomass		*Growth*	
EbL50 mg/L at 72 h	*NOEL mg/L at 72 h*	*ErL50 mg/L at 72 h*	*NOEL mg/L at 72 h*
> 160 mg/L (WAF)	10 (WAF)	> 160 mg/L (WAF)	10 (WAF)

Remarks - Results

All the test validity criteria were met. The 72 h E_rL50 and NOEL for the positive control was determined to be 1.4 (95% CL 1.2 -1.7) mg/L and 0.25 mg/L, respectively.

A mean inhibition rate of 3% and 9% was observed for growth and biomass, respectively. The inhibition rate for both growth and biomass are below 50% at the top test loading rate of 160 mg/L. Therefore, the 72 h NOEL was determined to be 10 mg/L, and the EC50 is determined to be > 160 mg/L for both growth and biomass.

Analysis of the test solutions at 0 hours showed test concentrations ranged from 0.29 – 1.0 mg/L. The test concentrations declined to below the limit of quantification of 0.0068 mg/L at 72 hours. The notified chemical is readily hydrolysable with a half-life of ≥27 hours, which is comparable with the agitation time for test solutions' preparation. Thus, significant hydrolysis may have occurred during the test solution preparation and test period.

The observed effects may be from both the test substance and its hydrolysis products. Given that the toxicity cannot be attributed to a single component but to the test substance as a whole, the results are based on nominal loading rates only. Therefore, the test substance and its hydrolysis products are expected to be not harmful to algae.

CONCLUSION

The notified chemicals and their hydrolysis products are not harmful to algae.

TEST FACILITY

Harlan (2013d)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE

Inseparable mixture of the notified chemicals

METHOD

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

Inoculum: Synthetic sewage sludge
Exposure Period: 3 hours
Concentration Range: Nominal: 10, 32, and 100 mg/L mg/L
Actual: Not determined

Remarks – Method

Following a range finding test, a definitive test was conducted at three nominal loading rates for 3 hours at 20°C. Five replicates were used for the control and four were used for each of the test levels. For preparation of the test preparations, mixtures of the test substance and water at the target concentrations were prepared by ultrasonication for 15 minutes followed by magnetic stirring for 24 hours.

A reference control test was conducted using 3,5-dichlorophenol as the reference item at 3.2, 10 and 32 mg/L.

RESULTS

IL50: 100 mg/L
NOEL: 10 mg/L

Remarks – Results

The reference control gave a 3 h IL50 of 7.2 mg/L (95% CL 5.5-9.4mg/L). About 50% of inhibition was reported after 3 hours of exposure to the test

	substance in the definitive test, the IL50 can therefore be determined to be 100 mg/L. No analytical determination of the test concentrations was conducted. Based on the data from the tests on fish and daphnids, the actual concentration of the notified chemical at the end of the test is not expected to be > 1 mg/L. In addition, the notified chemical is readily hydrolysable with a half-life of 27 hours, which is comparable with the agitation time for test solutions' preparation. The observed effects can be from both the test substance and its hydrolysis products. Given that the toxicity cannot be attributed to a single component but to the test substance as a whole, the results are based on nominal loading rates only. Therefore, based on the test results and the above considerations, the test substance and its hydrolysis products are considered to significantly inhibit microbial respiration at concentrations ≤ 100 mg/L.
CONCLUSION	The notified chemicals and their hydrolysis products significantly inhibit microbial respiration at concentrations ≤ 100 mg/L
TEST FACILITY	Harlan (2012j)

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