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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

R11053A

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 Full Public Report may be inspected at our NICNAS office by appointment only at Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

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:

Street Address:	Level 7, 260 Elizabeth Street, SURRY HILLS NSW 2010, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX	+ 61 2 8577 8888
Website:	www.nicnas.gov.au

Director
NICNAS

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
BP Australia Pty Ltd (ABN 53 004 085 616)
132 McCredie Rd
GUILFORD NSW 2161

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 and structural formulae, molecular weight, analytical data, degree of purity, impurities, additives/adjuvants, use details, import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: dissociation constant, acute inhalation toxicity, bioaccumulation.

NOTIFICATION IN OTHER COUNTRIES
USA (2007) (PMN P-08-0087)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
R11053A

MOLECULAR WEIGHT
Mn <1000 Da.

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

3. COMPOSITION

DEGREE OF PURITY Neat

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Pale amber coloured liquid

Property	Value	Data Type/Justification
Pour Point	< -20 ± 3°C	Measured
Boiling Point	Not determined	Decomposes above 222°C at 100.79 kPa
Density	987 kg/m^3 at 20°C	Measured
Vapour Pressure	1.4 x 10^{-4} kPa at 25°C	Measured
Water Solubility	< 1.69 × 10^{-4} g/L at 20°C	Measured
Hydrolysis as a Function of pH	Not determined	The notified chemical belongs to a class of chemicals that hydrolyse in water. However, in the environment it is not expected to hydrolyse significantly due to its low solubility in water.
Partition Coefficient	log Pow > 6.70 at 20°C	Measured

(n-octanol/water)		
Adsorption/Desorption	Not determined	In the case of a spill on land, the notified chemical is expected to partition to soil and sediment due its low solubility and hydrophobic structure.
Dissociation Constant	Not determined	The notified chemical has no dissociable functions
Flash Point	97ºC at 101.325 kPa	Measured
Flammability	Not expected to be flammable	Estimated based on flash point
Autoignition Temperature	> 400°C	Measured
Explosive Properties	Not expected to be explosive	Estimated based on structure

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

Reactivity
The notified chemical is expected to be stable under normal ambient conditions.

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported by sea (neat) or as a component of engine oils at <0.1%.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	1-3	1-3	3-10	3-10	3-10

PORT OF ENTRY
Sydney, Melbourne, Perth and Brisbane.

IDENTITY OF MANUFACTURER/RECIPIENTS
ALMC Pty Ltd (Lytton, QLD; Newport, VIC; Spotswood, VIC; North Fremantle, WA)

BP Australia Pty Ltd (Guildford, NSW)

TRANSPORTATION AND PACKAGING
The notified chemical will be imported by sea (neat) in 200 L drums and transported by road to the notifier's blending facilities in VIC, WA and QLD for reformulation into finished engine oil products. Alternatively, the notified chemical will be imported by sea in 1 or 5 L plastic containers, 20 L tinplate drums, or 205/209 L steel drums as a component of finished engine oil products at <0.1% and transported by road to storage facilities. Some repackaging of the finished engine oil products may occur prior to distribution to various distribution centres, automotive centres and retail outlets for sale to customers or end use.

USE
The notified chemical will be used as a component of engine oil products at <0.1%.

OPERATION DESCRIPTION
Formulation of engine oil products using the notified chemical imported as a neat additive
The notified chemical will be imported in 200 L drums to 3-4 BP sites for storage and blending into passenger car engine oil products.

During formulation, the drums of the notified chemical (neat) will be transferred onto a pallet by forklift from

the warehouse area to the blending area. The drums will be emptied into a blending vessel by a controlled, automated system that weighs and measures the additive, along with all other ingredients. The ingredients will be blended in a closed system. Finished passenger car engine oils will be transferred from blending vessels by hard piping to automated filling machines that will fill various size containers such as: 1 L, 5 L, 20 L and 205 L. These containers will be placed into cartons and then, if appropriate, onto pallets for distribution to distribution centres, retail outlets and automotive service centres for sale and end use in passenger car engines.

Quality control analysis will be performed onsite on the finished product. Blending vessels and filling lines will be cleaned after formulation.

Import of finished engine oils
Engine oil products containing the notified chemical at <0.1% will be imported in package sizes of 1 L, 5 L, 20 L, 205 L and 209 L. Some repackaging of the finished product from larger containers into 1 L and 5 L containers may take place. The repackaging operation will involve transfer of the product using controlled automated systems.

The blending vessels and filling lines will be cleaned after reformulation. Any loss of product is contained within a facility drain pit system that will capture and allow for any lost material to be recycled or disposed of in accordance with regulations.

The finished product will be warehoused prior to transport to distributors, commercial customers and consumers around Australia.

End Use
The finished engine oil containing the notified chemical will be supplied in bulk to car dealerships for 'factory fill' applications. Additionally, it may also be supplied in smaller containers for use in service applications through automotive service centres or sold to the public for DIY use.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration (hrs/day)*	*Exposure Frequency (days/year)*
Transport and storage	30-50	2-4	40-60
Blending			
Blending Operator	10-15	2-4	40-60
Filling Operator	25-30	1-2	40-60
Quality Control	8-10	1-2	40-60
Importation of finished product			
Decant Operator	10-15	1-2	15-20
Warehouse Operator	80-120	4-6	150-200
Product disposal Operator	70-100	1-2	40-60
End use – Commercial	>10,000	2-3	150-200
End user – DIY	>10,000	<1	1-2

EXPOSURE DETAILS
Transport and storage
Transport and storage workers on the docks and in the storage warehouses are not likely to be exposed to the notified chemical except in the case of an accident involving damage to the containers or the wrapped pallets.

Formulation of engine oil products using the notified chemical imported as a neat additive
Exposure to the notified chemical as imported (neat) may be experienced by workers during transfer to blending vessels, residues in lines, and on coupling and occasionally from leaks and spills. Blending of the notified chemical is expected to take place in a closed, fully automated system equipped with ventilation which is expected to result in limited potential for exposure. The main route of exposure is expected to be dermal,

although some ocular exposure is also possible. The notifier states that exposure to aerosols is not likely during formulation considering the high viscosity of the notified chemical and the closed and automated nature of the processes involved.

Dermal and ocular exposure may also occur when sampling the notified chemical for quality control analysis as imported (neat) or as a component of finished engine oil products at <0.1%. Gloves, goggles and a laboratory coat are expected to be worn to minimise exposure during analysis.

Packaging of blended engine oils
Filling and packaging of the finished engine oil product containing the notified chemical (<0.1%) is expected to be performed using a closed, automated system to minimise dermal and ocular exposure to workers.

Cleaning, maintenance and drum disposal workers may encounter dermal and ocular exposure to engine oil residues containing the notified chemical (<0.1%) during cleaning of blending vessels and filling lines and disposal of drums.

Repackaging of imported engine oils
Workers decanting finished engine oils containing the notified chemical (<0.1%) from 205 or 209 L drums into smaller containers are not expected to experience significant exposure given the automated systems in place.

According to the MSDS, all workers involved in the formulation and repackaging of engine oil products are expected to wear personal protective equipment (PPE) such as gloves, goggles and protective clothing to minimise dermal and ocular exposure.

End use
Workers may be exposed to engine oils containing the notified chemical (<0.1%) during use at car dealerships or automotive service centres.

At car dealerships, the finished engine oil containing the notified chemical (<0.1%) will be added to engines using automated systems and exposure is unlikely. However, dermal exposure from drips, spills and splashes as well as from handling equipment contaminated with engine oil is possible. The potential for dermal and ocular exposure may be reduced by wearing gloves, long sleeve shirts and goggles.

At automotive service centres, professional users such as mechanics may experience dermal or ocular exposure to the engine oil products containing the notified chemical (<0.1%) when transferring engine oil to cars. The potential for dermal and ocular exposure may be reduced by wearing gloves, long sleeve shirts and goggles. Overall, exposure to the notified chemical is not expected to be significant, given the low concentration (<0.1%) of the notified chemical in the finished engine oils.

6.1.2. Public exposure

The notified chemical will be used as a component of engine oils at <0.1% concentration. Once engine oil containing the notified chemical is added to the engine, the general public will not be exposed during its use in the engine. DIY users may experience dermal and accidental ocular exposure to engine oils containing <0.1% of the notified chemical when adding engine oil to their vehicles. However, most engine oils are added or replaced by certified mechanics and as such, exposure to the general public during the addition of engine oil is expected to be minimal.

Overall, public exposure to the notified chemical is expected to be limited due to its infrequent use and low concentration (<0.1%) in finished engine oil.

6.2. Human health effects assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity – Fixed dose procedure	LD50 >300 and <2000 mg/kg bw; harmful
Rat, acute dermal toxicity – Limit test	LD50 > 2000 mg/kg bw; low toxicity

Skin irritation – In vitro test (human epidermal model)	irritant
Skin corrosion – In vitro test (human epidermal model)	non corrosive
Eye irritation – Rabbit eye enucleation test	severely irritating
Skin sensitisation – Mouse local lymph node assay	evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days	NOEL = 30 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	equivocal
Genotoxicity – in vitro mammalian chromosome aberration	non genotoxic
Genotoxicity – in vitro mammalian cell gene mutation	non mutagenic
Developmental toxicity	NOAEL = 1000 mg/kg bw/day

Toxicokinetics
The notified chemical is a UVCB with MW <1000 Da. including some species with MW <500 Da. It has low water solubility and high lipophilicity which indicates that it may be absorbed via the gastro-intestinal tract, perhaps by micellular solubilisation. This is supported by the systemic toxicity observed in repeat dose studies conducted on the notified chemical (see Appendix B for details).

Absorption via the dermal and inhalation routes is not expected to be significant due to the low water solubility and high lipophilicity of the notified chemical.

Acute toxicity
The notified chemical was found to be harmful in an acute oral toxicity test in rats with an LD50 in the range >300 and <2000 mg/kg bw. Mortalities were reported in all females (4/4) in the group treated with 2000 mg/kg bw. No signs of systemic toxicity (other than increased salivation) were observed in animals treated at 300 mg/kg bw.

The notified chemical was found to be of low toxicity in an acute dermal toxicity test in rats (LD50 >2000 mg/kg bw). Effects were limited to very slight erythema observed in 5/5 males and 3/5 females, which persisted up to 4-5 days after treatment. In addition, light brown discolouration of the epidermis was observed in 5/5 males and 2/5 females. No mortalities or signs of systemic toxicity were reported.

No acute inhalation toxicity data are provided.

Irritation and Sensitisation
The notified chemical was found to be non-corrosive in an *in vitro* study. Another *in vitro* study showed skin irritation effects to the notified chemical. Also, the very slight erythema observed in rats up to 4-5 days in the acute dermal toxicity study indicates that there is potential for skin irritation.

A Rabbit Enucleated Eye Test (REET) was performed in place of an *in vivo* eye irritation test because the notified chemical was suspected to be strongly irritating and/or corrosive. Treatment of enucleated rabbit eyes with the notified chemical for 10 secs yielded the following effects: corneal opacity, sloughing, corneal swelling and fluorescein staining. Based on these effects the notified chemical was considered to have the potential to cause severe ocular irritation and therefore an *in vivo* study was not performed due to animal welfare concerns. According to the *Manual of Decisions*, the European Chemicals Bureau (ECB) states that a positive result in the REET is sufficient for classification with *R41 Risk of serious damage to eyes* (ECB, 2006).

The notified chemical was found to have the potential for skin sensitisation according to a mouse LLNA study. The stimulation index (SI) was reported to exceed 3 at concentrations of 50 and 100% and the EC3 value was calculated to be 43.5%.

Repeated Dose Toxicity
A 28-day repeat dose oral toxicity study in rats established a NOEL of 30 mg/kg bw/day, based on the presence of adverse effects at higher dose levels. No mortalities were reported in the study. Toxicologically significant effects that were recorded at 300 and 1000 mg/kg bw/day included modified haemotology and blood chemistry parameters and microscopic changes in the liver, thyroid/parathyroid and kidneys.

Further investigations of the effects in the liver, thyroids and kidneys were performed as part of the

developmental toxicity study. Recovery groups (control and high dose) were included to investigate the reversibility of changes observed in these organs in the previous study. Two mortalities from the high dose group (1 male and 1 female) and 1 mortality from the mid dose group (male) were reported during the study (killed *in extremis*). Histopathological examinations revealed the cause of death in males was due to the administration method, causing aspiration pneumonia. The cause of death of the female was not determined, but considered unrelated to treatment. The effects observed in treated animals were similar to those reported in the 28-day repeat dose oral toxicity study. The changes observed in the liver and thyroids in this study were not present in the recovery groups and were considered reversible. Histopathological examination of males treated at 1000 and 300 mg/kg bw/day revealed the following effects in the kidneys: tubular degeneration, tubular simple dilation and granular casts. These effects were also observed in males from the recovery high dose group and were therefore considered irreversible. Upon necropsy, a mottled appearance in the kidneys was observed in high (9/10), mid (2/10) and low (1/10) dose males. This effect was not reported in the 28-day study. It was unclear whether the mottled kidneys observed was an indication of the start of histopathological changes.

Mutagenicity
In the Ames test, a statistically significant increase in the number of revertant colonies in the TA1535 strain was observed at concentrations of 5000 µg/plate in the range finding test with or without S9 and at 4000 and 5000 µg/plate in an additional test without S9. The result was considered equivocal given statistically significant increases were only observed in the TA1535 strain in 2 of the 3 experiments.

The notified chemical was found not to be clastogenic in an *in vitro* mammalian chromosome aberration test in human peripheral lymphocytes.

The notified chemical was found not be mutagenic in an *in vitro* cell gene mutation test performed using mouse lymphoma cells.

Overall, based on the weight of evidence, the notified chemical is considered non-mutagenic and non-clastogenic.

Toxicity for reproduction
A reproductive and developmental toxicity study in rats established a NOAEL for the notified chemical of 1000 mg/kg bw/day for both reproductive and developmental toxicity.

Health hazard classification
Based on the data provided, the notified chemical is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrases:

R22 Harmful if swallowed
R43 May cause sensitisation by skin contact

In vitro irritation/corrosion test methods are not recognised for hazardous classification under the *Approved Criteria for Classifying Hazardous Substances* [NOHSC: 1008 (2004)].

However, the REET was conducted according to Good Laboratory Practices (GLP) and according to the *Manual of Decisions*, the European Chemicals Bureau (ECB) states that a positive result in the REET is sufficient for classification with *R41 Risk of serious damage to eyes* (ECB, 2006).

Since the notified chemical can be reasonably expected to produce severe eye irritation *in vivo*, it should be considered as though classified as:

R41 - Risk of serious damage to eyes

Considering the results of the in vitro skin irritation test, the notified chemical should be considered as though classified as:

R38 – Irritating to skin

6.3. Human health risk characterisation

6.3.1. Occupational health and safety

The notified chemical (neat) has the potential to cause severe eye irritation, skin irritation and skin sensitisation. However, the notified chemical in engine oils at <0.1% is not expected to cause irritation or sensitisation effects.

The workers expected to experience the highest exposure are those handling the notified chemical as imported (neat) at blending sites. Workers involved in blending may experience dermal and ocular exposure to the notified chemical (neat) during transfer of the notified chemical to blending vessels, residues in lines and on coupling as well as from occasional leaks and spills. The blending process is largely enclosed and automated and therefore, significant exposure is not expected to occur and is expected to be further minimised by the use of PPE such as long-sleeved clothing, gloves and goggles and respirators. Given the use of automated and enclosed systems and appropriate use of PPE during handling of the notified chemical as imported (neat), the potential for eye irritation and skin sensitisation is not considered unacceptable.

Dermal and ocular exposure of workers to the notified chemical at <0.1% concentration in engine oil products is possible during repackaging, quality control analysis and cleaning and maintenance. However, the use of automated systems during repackaging, and the use of PPE including gloves, safety glasses and protective clothing during all these activities is expected to minimise the potential for exposure. In addition, the concentration of the notified chemical in the engine oils is very low and therefore, exposure is not expected to result in an unacceptable risk to workers.

Engine oil products containing the notified chemical at <0.1% concentration will be used by workers at car dealerships and automotive service centres. Exposure to these workers may occur frequently during oil changes. The use of PPE is not considered likely. However, the potential for eye irritation and skin sensitisation is not expected to be unacceptable due to the low concentration of the notified chemical in the engine oils (<0.1%).

Overall, the potential for adverse health effects in workers exposed to the notified chemical (neat) is not considered unacceptable given the use of appropriate engineering controls and PPE as described above. The potential for adverse health effects in workers exposed to engine oil products containing the notified chemical at <0.1% is not considered unacceptable given the very low concentration of the notified chemical in these products.

6.3.2. Public health

Engine oil products containing <0.1% notified chemical will be available to the public for DIY use. During engine oil changes dermal and ocular exposure is possible, especially considering that members of the public are not likely to use PPE. However, exposure is not considered to lead to eye irritation and/or skin sensitisation due to the anticipated infrequent use of engine oils and the low concentration of the notified chemical (<0.1%).

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be manufactured overseas and will either be imported in finished lubricants or as a neat additive that will be reformulated locally into end-use products. Local blending or repackaging will be performed in an automated or semi-automated enclosed system. During reformulation it is expected that any significant spillage will be captured in the site drain pit and will be recycled, or disposed of according to State/Territory regulations. The notified chemical is not expected to be released to water drains or site sewer systems. Empty containers may contain up to 0.1% residue of the notified chemical and are expected to be recycled by licensed waste contractors. Minimal environmental release is expected from blending or repackaging.

RELEASE OF CHEMICAL FROM USE

Approximately 45% of the product containing notified chemical will be used by professionals in the commercial sector. Release of the notified chemical to the environment from professional activities is expected to be limited by the requirement for appropriate disposal of waste oil according to State/Territory regulations.
A small amount of notified chemical may be spilled due to oil changes by do-it-yourself (DIY) users.

During use, the finished product containing the notified chemical is contained within the car engine and release or leakage is expected to be low (<1.0 %). It is expected that passenger cars consume up to 20% of engine oil between drain intervals and that loss due to spillage when crankcases are filled is estimated to be <1.5% of the total.

RELEASE OF CHEMICAL FROM DISPOSAL

Products containing the notified chemical are expected to be disposed of in accordance with State/Territory regulations and consequently, the notified chemical is expected to be recycled, re-refined or used as low grade burner fuel. It is likely that the notified chemical will be degraded into simpler compounds during re-refining with any residue partitioning to the heavy fractions such as lubricating oils or asphalt. Similarly, during metal recycling of automotive components with chemical residues, the notified chemical is expected to be completely combusted.

Use of engine oil containing the notified chemical by professionals is expected to be disposed of according to State/Territory regulations. Of the total imported engine oil containing notified chemical to be disposed of by DIY users, approximately 20% will be collected for recycling, 25% will be buried or disposed of in landfill, 5% (i.e. 2.75% of the total import volume) may be disposed of inappropriately into storm water drains and the remaining 50% will be used in treating fence posts, killing grass and weeds or disposed of in other ways.

7.1.2 Environmental fate

Two studies submitted by the notifier indicate the notified chemical is not readily biodegradable. The notified chemical is not expected to be bioaccumulative or bio-available to aquatic organisms due to its low water solubility and anticipated limited release to the aquatic environment. Most of the notified chemical will be either thermally decomposed during use, recycled or re-refined. A small amount of the notified chemical is expected to be sent to landfill as residues in containers or as a component of waste oil. Notified chemical sent to landfill or spilt on the ground is expected to sorb strongly to soil and sediment. It is anticipated to ultimately be degraded into water and oxides of carbon and titanium by thermal decomposition in industrial facilities or by natural processes in landfill.

For the details of the environmental fate studies, refer to Appendix C.

7.1.3 Predicted Environmental Concentration (PEC)

The percentage of the imported quantity of notified chemical inappropriately disposed to stormwater drains is estimated be 2.75%. That is, 55% (fraction collected by DIY users) × 5% (fraction disposed to stormwater). A worst case PEC can be calculated if it is assumed that 2.75% of the notified chemical (maximum 275 kg) is released into stormwater drains in a single metropolitan area with a geographical footprint of 500 km^2 and an average annual rainfall of 500 mm, all of which drains to stormwater. With a maximum annual release into this localised stormwater system of 275 kg and the annual volume of water drained from this region estimated to be 250 × 10^6 m^3, the resultant PEC is approximately 1.10 µg/L. This result reflects a worst-case scenario upper limit, as in reality releases of the notified chemical will be distributed over multiple urban areas. Moreover, the notified chemical will be further diluted if it reaches the ocean.

7.2. Environmental effects assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity (96 h)	LL50 >100 mg/L (WAF)	Not harmful up to the limit of its solubility in water
Daphnia Toxicity (48 h)	EL50 >100 mg/L (WAF)	Not harmful up to the limit of its solubility in water
Algal Toxicity (72 h)	E_rL50 = 47 mg/L (WAF)	Harmful
Inhibition of Bacterial Respiration (3 h)	IL50 >1000 mg/L	Does not inhibit respiration of waste water microorganisms

Under the Globally Harmonised System of Classification and Labelling of Chemicals (United Nations, 2009) the notified chemical is classified as not harmful to fish and aquatic invertebrates up to the limit of its solubility in water but is harmful to algae. Based on the acute toxicity data and biodegradability studies, the notified chemical is classified as harmful to aquatic life with long lasting effects.

7.2.1 Predicted No-Effect Concentration

The lowest endpoint from ecotoxicological studies on the notified chemical was used to calculate the PNEC. An assessment factor of 100 was used as acute toxicity endpoints are available for the effects of the notified chemical on aquatic species from three trophic levels.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
E_rL50 (algae)	47	mg/L
Assessment Factor	100	
PNEC:	470	µg/L

7.3. Environmental risk assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q – River	< 1.10	470	$< 2.3\times10^{-3}$
Q – Ocean	< 1.10	470	$< 2.3\times10^{-3}$

The Risk Quotients (Q = PEC/PNEC) for the worst case discharge scenario have been calculated to be << 1 for the river and ocean compartments. This indicates the notified chemical is not expected to pose an unacceptable risk to the aquatic environment based on its reported use pattern.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

Based on the data provided, the notified chemical is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrases:

R22 Harmful if swallowed
R43 May cause sensitisation by skin contact

In vitro irritation/corrosion test methods are not recognised for hazardous classification under the *Approved Criteria for Classifying Hazardous Substances* [NOHSC: 1008 (2004)]. However, based on the results of the in vitro studies provided, the notified chemical should be considered as though classified as:

R38 – Irritating to skin
R41 - Risk of serious damage to eyes

and

The classification of the notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations 2009) 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***
Acute toxicity	4	Warning: Harmful if swallowed
Skin irritation	2	Warning: Causes skin irritation
Eye irritation	1	Danger: Causes serious eye damage
Sensitisation	1	Warning: May cause an allergic skin reaction
Environment	Acute 3	Harmful to aquatic life
	Chronic 3	Harmful to aquatic life with long lasting effects

Human health risk assessment
Under the conditions of the occupational settings described, the notified chemical is not considered to pose an unacceptable risk to the health of 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 and the reported use pattern, the notified chemical is not expected to pose an unacceptable risk to the environment.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- Safe Work Australia, should consider the following health hazard classifications for the notified chemical:
  - Xn: R22 Harmful if swallowed
  - Xi: R38 Irritating to skin
  - Xi: R41 Risk of serious eye damage
  - Xi: R43 May cause sensitisation by skin contact

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - Conc. ≥25%: R22; R41; R43; R38
  - ≥20% Conc. <25%: R41; R43; R38
  - ≥10% Conc. <20%: R41; R43
  - ≥5% Conc. <10%: R36; R43
  - ≥1% Conc. <5%: R43

Health Surveillance

- As the notified chemical is a skin sensitiser, blending/formulation facility employers should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of sensitisation.

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical as imported (neat):
  - Automated processes, where possible

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced (neat):
  - Avoid contact with skin and eyes

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced (neat):
  - Gloves
  - Goggles
  - 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.

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from a component of engine oils at <0.1%, or is likely to change significantly;
  - the amount of chemical being introduced has increased more than 10 tonnes, or is likely to increase, significantly;
  - the chemical has begun to be manufactured in Australia;
  - additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

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

Material Safety Data Sheet
The MSDS 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 remains the responsibility of the applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point < -20 ± 3ºC pour point

Method	OECD TG 102 Melting Point/Melting Range. EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks	Pour point determined to be < -20º ± 3ºC.
Test Facility	Harlan Laboratories Ltd (2009a)

Boiling Point Decomposes above 222ºC at 100.79 kPa

Method	OECD TG 103 Boiling Point. EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks	Determined by differential scanning calorimeter
Test Facility	Harlan Laboratories Ltd (2009a)

Density 987 kg/m^3 at 20.0 ± 0.5ºC

Method	OECD TG 109 Density of Liquids and Solids. EC Directive 92/69/EEC A.3 Relative Density.
Remarks	Determined by pycnometer method
Test Facility	Harlan Laboratories Ltd (2009a)

Vapour Pressure 1.4 x 10^{-4} kPa at 25ºC

Method	OECD TG 104 Vapour Pressure. EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks	Determined by a vapour pressure balance using linear regression analysis
Test Facility	Harlan Laboratories Ltd (2009a)

Water Solubility < 1.69 × 10^{-4} g/L at 20°C and pH 4.5

Method	OECD TG 105 Water Solubility
Remarks	Flask Method. (The elution method was found to be impractical due to the physical nature of the notified chemical). Based on a preliminary test, approximately 0.1 g of notified chemical was added to three flasks, each containing 100 mL of double distilled water. The flasks were shaken (for 24, 48 and 72 hrs, respectively) at approximately 30°C and after standing at 20°C for a period not less than 24 hrs, the contents of the flasks were centrifuged at 13,500 rpm for 10 min. The pH of each solution was measured. The concentration of notified chemical was determined based on the measured titanium content.
Test Facility	Harlan Laboratories Ltd (2009a)

Partition Coefficient (n-octanol/water) log Pow > 6.70 at 20°C

Method	In-house method
Remarks	The partition coefficient was estimated based on the ratio of the solubilities of notified chemical in water (> 844 g/L) and n-octanol (< 1.69 × 10^{-4} g/L).
Test Facility	Harlan Laboratories Ltd (2009a)

Flash Point 97 ± 2ºC at 101.325 kPa

Method	EC Directive 92/69/EEC A.9 Flash Point.
Remarks	Determined by the closed cup equilibrium method
Test Facility	Harlan Laboratories Ltd (2009b)

Autoignition Temperature > 400ºC

Method	EC Directive 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases). EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Test Facility	Harlan Laboratories Ltd (2009b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure. EC Directive 92/69/EEC B.1 bis Acute toxicity (oral) fixed dose method.
Species/Strain	Rat/Wistar
Vehicle	Arachis oil
Remarks - Method	A sighting study was conducted in which 1 female was administered with 2000 mg/kg bw and another female was administered 300 mg/kg bw. No signs of toxicity were observed in either animal. Based on these results, a group of 4 females was administered with 2000 mg/kg bw and another group of 4 females was administered 300 mg/kg bw.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	4 F	2000	4/4
II	4 F	300	0/4

LD50	>300 and <2000 mg/kg bw
Signs of Toxicity	Signs noted in animals treated at a dose level of 2000 mg/kg were ataxia, lethargy, pilo-erection, splayed gait, decreased respiratory rate and laboured respiration. Four animals treated at 2000 mg/kg bw were comatose 2 hrs after dosing and were humanely killed. One animal treated at 300 mg/kg bw showed signs of increased salivation. No other signs of systemic toxicity were observed in animals treated at 300 mg/kg bw.
Effects in Organs	Yellow liquid was found in the stomach of the animals treated at 2000 mg/kg bw at necropsy.
Remarks - Results	The surviving animal from the sighting study treated at 2000 mg/kg bw appeared normal 1 day after dosing. The acute oral LD50 was determined to be between >300 and <2000 mg/kg bw.
CONCLUSION	The notified chemical is harmful via the oral route.
TEST FACILITY	Harlan Laboratories Ltd (2010a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal) – Limit Test.
Species/Strain	Rat/Wistar
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*
I	5 / sex	0	0/5
II	5 / sex	2000	0/5

LD50	> 2000 mg/kg bw

Signs of Toxicity - Local	Very slight erythema was observed at the test sites of all males and 3 females, persisting in all males and 2 females 4 and 5 days following treatment. Light brown discolouration was observed at the test sites of all males and 2 females. No signs of dermal irritation were reported in 2 females.
Signs of Toxicity - Systemic	No mortalities occurred during the study. No signs of systemic toxicity were observed during the study.
Effects in Organs	No macroscopic findings were observed at necropsy.
Remarks - Results	None
CONCLUSION	The notified chemical is of low acute toxicity via the dermal route.
TEST FACILITY	Harlan Laboratories Ltd (2010d)

B.3. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	Analogous to OECD TG 431: In Vitro Skin Corrosion: Human Skin Model Test A preliminary test was conducted to assess the ability of the notified chemical to reduce MTT (2*H*-tetrazolium, 2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-, bromide (1:1)). The notified chemical did not directly reduce MTT. The irritation potential of the test substance was assessed by applying 10 µL of the notified chemical undiluted onto the surface of 3 EpiSkin reconstructed human epidermis (RHE) units for 15 mins. The notified chemical was then washed from the surface of the EpiSkin units which were incubated for a recovery period of approximately 42 hrs. Triplicate Episkin samples with 10 µL of negative (Phosphate Buffered Saline (PBS)) and positive (Sodium Dodecyl Sulfate (SDS) 5%) controls were treated similarly. After the recovery period, the skin units were placed on a plate shaker for approximately 15 mins to homogenise the released mediators in the maintenance medium. 1.6 mL of the maintenance medium from beneath each tissue was transferred to mircro tubes for frozen storage for the purpose of inflammatory mediator determination in case of the MTT reduction being equivocal. The maintenance medium was removed from the tissues which were then transferred to assay medium containing 0.3 mg/mL MTT and returned to the incubator for approximately 3 hrs. The tissues were dried and examined for MTT staining (qualitatively). Biopsies of the EpiSkin membranes (including the epidermis and collagen matrix) were then removed and added to 500 µL acidic isopropanol and mixed thoroughly in a vortex mixer. After mixing, the biopsies were refrigerated to allow for extraction of formazan crystals. Once the formazan extraction was complete, the samples were homogenised using a vortex mixer and optical density was determined by measuring absorption at 540 nm and the viability of each individual tissue calculated as a percentage of the mean negative control viability.
Remarks - Method	The acceptance criteria for the positive control were met given the tissue viability of tissues treated with the positive control were 8.0% relative to the negative control. Acceptance criteria The assay was deemed acceptable if the following occurred: The mean optical density (OD) value of the 3 negative control tissues was ≥ 0.6 and the standard deviation value (SD) was ≤ 20. The mean % viability of the 3 positive control tissues was ≤ 40% and the

SD was ≤ 20.

RESULTS

Remarks - Results

The notified chemical did not stain the MTT solution blue/purple and therefore, it was concluded that the notified chemical did not reduce MTT.

A qualitative evaluation of the MTT staining of the tissues treated with the notified chemical noted the colour of the tissues treated with the notified chemical were blue/white in colour, indicating semi-viable tissue. Tissues treated with the positive control were white, indicating dead tissue.

Material	*Mean OD_{540} of triplicate tissues*	*± SD of OD_{540}*	*Relative mean viability (%)*
Negative control	0.916	0.05	100*
Positive control	0.073	0.01	8.0
Notified chemical	0.337	0.02	36.8

* The relative viability of tissue treated with the negative control is assigned 100%.

CONCLUSION

The relative mean tissue viability of tissues treated with the notified chemical was 36.8% indicating the notified chemical was a skin irritant.

TEST FACILITY

Harlan Laboratories Ltd (2009c)

B.4. Corrosion – skin

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 431: In Vitro Skin Corrosion: Human Skin Model Test
In vitro human skin model test methods such as EPISKIN™ and EpiDerm™ are accepted internationally via OECD Test Guideline 431.

A preliminary test was conducted to assess the ability of the notified chemical alone to reduce MTT (2*H*-tetrazolium, 2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-, bromide (1:1)). It was found not to directly reduce MTT.

Duplicate tissues of reconstituted human epidermis (consisting of adult human-derived epidermal keratinocytes seeded on a dermal substitute with a collagen type I matrix coated with type IV collagen) were treated with the 50 µL notified chemical for exposure periods of 3, 60 or 240 mins. Duplicate tissues were treated with 50 µL of the positive (glacial acetic acid) and the negative (0.9% sodium chloride) control for 240 mins. The tissues were removed and rinsed with Phosphate Buffered Saline (PBS) with Ca^{++} and Mg^{++} before being placed in 2.2 mL of 0.3 mg/mL MTT solution. The tissues were incubated for 3 hrs at room temperature then dried on absorbent paper. The tissues were examined to evaluate the degree of MTT staining and tissue viability.

After MTT loading a total biopsy of each epidermis was made and placed into micro tubes containing acidified isopropanol for extraction of formazan crystals out of the MTT-loaded tissues. At the end of the formazan extraction period each tube was mixed thoroughly and duplicate 200 µL samples were transferred to the appropriate wells of a pre-labelled 96 -well plate. The optical density was measured at 540 nm and served as the relative tissue viability (MTT reduction in the notified chemical treated

tissues relative to negative control tissues).

Remarks - Method

The acceptance criteria for the positive control were met given the tissue viability of tissues treated with the positive control were 9.5% relative to the negative control.

Acceptance criteria:
The relative mean tissue viability for the positive control treated tissues was 0 to 20% relative to the negative control treated tissues following exposure for 240 mins.

RESULTS

Remarks - Results

The notified chemical did not stain the MTT solution blue/purple and therefore, it was concluded that the notified chemical did not reduce MTT.

A qualitative evaluation of the MTT staining of the tissues treated with the notified chemical noted the colour of the tissues treated with the notified chemical for 3 mins and 60 mins were blue, indicating the tissues were viable. Tissues treated with the notified chemical for 240 mins were blue/white in colour, indicating semi-viable tissue. Tissues treated with the positive control for 240 mins were white, indicating dead tissue.

Material	*Exposure Period (mins)*	*Mean OD_{540} of duplicate tissues*	*Relative mean % viability*
Negative control	240	0.201	100*
Positive control	240	0.019	9.5
Notified chemical	240	0.185	92.0
	60	0.35	174.1
	3	0.268	133.3

* The relative viability of tissue treated with the negative control was assigned 100%.

The relative mean tissue viability of the positive control was <20% of the negative control indicating the acceptability of the assay.

CONCLUSION

The notified chemical was considered to be non-corrosive under the conditions of the test.

TEST FACILITY

Harlan Laboratories Ltd (2009d)

B.5. Irritation – eye

TEST SUBSTANCE

Notified chemical

METHOD

Rabbit Enucleation Eye Test (REET), conducted according to GLP. In-house method as described below. The Rabbit Enucleation Eye Test was conducted in place of the OECD TG 405 Acute Eye Irritation/Corrosion test.

Observation Period

240 mins

Remarks - Method

Five enucleated rabbit eyes were excised and allowed to equilibrate for 30 mins in a perspex clamp placed within a superfusion chamber. Saline solution was allowed to irrigate the surface of the cornea via a saline drip in the rear of the chamber. The eyes were re-examined after approximately 30 mins of equilibration to ensure that no signs of irritation resulted from the excision. Corneal thickness was measured using an ultrasonic pachymeter. Any eyes with corneal swelling greater than 10% of the pre-enucleation measurement or stained with fluorescein were discarded.

After inspection, 3 eyes held by perspex clamps were removed from the superfusion chamber and placed horizontally into a petri dish. 0.1 mL of the notified chemical (undiluted) was applied evenly to the surface of the cornea of 3 eyes. After 10 secs the notified chemical was rinsed off using a minimum 20 mL of saline solution.
The remaining 2 eyes remained untreated (ie. saline solution only) and served as negative controls.

The thickness of the cornea was measured using an ultrasonic pachymeter under a slit-lamp biomicroscope pre-enucleation, post-equilibration and at 60, 120, 180 and 240 mins following treatment. For each enucleated eye a measurement was made at the optical centre, and at four other locations at the apex of the cornea. A mean value for corneal thickness was calculated based on these four measurements. The corneal thickness for each eye 60, 120, 180 and 240 mins following treatment was used to calculate the percentage change compared with the corneal thickness pre-treatment.
The condition of the cornel epithelium was assessed pre-enucleation, post-equilibration and approximately 60, 120, 180 and 240 mins following treatment. Examination of the eye was assessed using a slit-lamp biomicroscope.
The uptake of fluorescein by the corneal epithelium was assessed pre-enucleation, post-equilibration and approximately 240 mins following treatment using a cobalt blue filter of the split-lamp biomicroscope after application of Fluorescein Sodium drops.

RESULTS

Corneal swelling increased in all test eyes at each of the observation points. Corneal thickness of the test eyes was calculated to be between 45.1% and 71.1% greater than control eyes 240 mins following treatment.
Corneal cloudiness (level 1) was observed in all test eyes during the study covering 1-25% up to 75% of the cornea (mean swelling = 58%). Cloudiness appeared from the 120 minute observation point in 2 animals and at the 240 minute observation point in 1 animal.
Sloughing was observed in all test eyes from 120 mins to 240 mins following treatment.

Remarks - Results

Slight fluorescein staining covering up to 50% of the area of the cornea was observed in 2 test eyes 240 mins post-teatment. In the third test eye, moderate fluorescein staining was observed covering up to 75% of the area of the cornea at 240 mins following treatment.

No corneal effects were observed in the control eyes.

Positive control data from in-house validation of the test protocol were presented to confirm the suitability of the study to predict the potential for severe eye irritation.

The results of the test indicated the potential for severe eye irritation. Accordingly, the *in vivo* eye irritation test was considered unnecessary and was not performed in the interests of animal welfare.

CONCLUSION The notified chemical is severely irritating to the eye.

TEST FACILITY Harlan Laboratories Ltd (2009e)

B.6. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)

Species/Strain Mouse/CBA/Ca

Vehicle Acetone/Olive oil (4:1)

Remarks - Method No significant protocol deviations. A preliminary irritation study was conducted in 1 mouse to select the highest concentration of the notified chemical to be used in the main study. In the main study, 3 groups of 4 animals were treated with notified chemical at a different concentration per group. One group of 4 animals was treated with vehicle. A test to confirm the reliability of the positive control substance (Alpha-Hexylcinnamaldehyde (HCA)) to predict the skin sensitisation potential using the LLNA test was conducted within the 6 months prior to the test using the notified chemical.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	1306.72	N/A
25	2193.43	1.68
50	4525.14	3.46
100	4236.83	3.24
Positive Control - HCA		
15	Not provided	3.12

Remarks - Results No signs of systemic toxicity were displayed in any of the animals.
The results indicate that the notified chemical could elicit a Stimulation Index (SI) ≥3. The data failed to show a dose response. The mean DPM/lymph node for animals treated with 100% notified chemical were considered to be an aberration and therefore, the EC3 value was calculated to be 43.5% on the basis of the data for control animals and the animals treated with the notified chemical at 25% and 50%.
The positive control test found HCA to induce a Stimulation Index (SI) of 3.12 at 15% concentration, thus confirming the acceptability of HCA as a reliable positive control substance.

CONCLUSION There was evidence of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY Harlan Laboratories Ltd (2010e)

B.7. Repeat dose toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.

Species/Strain Rat/Wistar

Route of Administration Oral – gavage

Exposure Information Total exposure days: 28 days
Dose regimen: 7 days per week
Post-exposure observation period:

Vehicle Polyethylene glycol 400

Remarks - Method Dosage levels were selected following the effects observed in a preliminary 14-day range-finder study in the rat (250, 500 and 1000 mg/kg bw/day). A recovery group was not included as part of the test.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 M / 5 F	0	0
low dose	5 M / 5 F	30	0
mid dose	5 M / 5 F	300	0
high dose	5 M / 5 F	1000	0

Mortality and Time to Death

No mortalities were reported during the study.

Clinical Observations

No adverse or abnormal effects on behaviour, sensory reactivity, bodyweight or food consumption were reported during the study. Slight differences in food efficiency were observed but were not considered significant. Water consumption was increased slightly in treated animals compared to controls in week 3 but there was no variation observed in week 4 and the observation was not considered to be of toxicological significance.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Males in the high and mid dose groups showed a statistically significant increase in alkaline phosphatase levels when compared to controls. A statistically significant decrease in alanine aminotransferase levels was observed in males in the high dose group when compared to controls. A small statistically significant increase in albumin/globulin ratio ($p<0.05$) observed in males in the high dose group was not considered to be of toxicological significance given all individual values were within the normal expected range for this parameter and there were no significant variations observed in similar parameters such as total protein or albumin levels.

Males and females in the high dose groups displayed a reduction in haemoglobin and haematocrit levels compared to controls (males: $P<0.01$ and females: $P<0.05$). Erythrocyte counts were also reduced in these groups although statistical significance was only observed in males ($P<0.05$) but this was not considered to be toxicologically significant given a lower than normally expected value was observed in one of the control males. In addition, mean cell volume and mean cell haemoglobin were also reduced in these animals but were not statistically significant.

Males and females in the high dose groups displayed a reduction in leucocyte counts, specifically in the neutrophil fraction (males: $P<0.01$ and females: $P<0.05$). Increases in lymphocyte counts were also observed in these animals although statistical significance was only reached in females ($P<0.05$). Statistically significant increases in neutrophil counts were also observed in males in the mid dose groups ($P<0.01$).

Effects in Organs

Increased absolute and relative liver weights were observed in males and females in the high dose groups when compared to controls ($P<0.01$) as well as males and females in the mid dose groups ($P<0.05$). Enlargement of the centrilobular hepatocytes was also observed in males and females of the high dose groups. This finding was considered to be treatment related although it was considered to be an adaptive response rather than an adverse effect. Centrilobular hepatocyte enlargement was also observed in 1 female only from the mid dose group.

Thyroid/parathyroid weights were significantly increased in animals of both sexes in the high dose groups ($P<0.01$) as well as females in the mid dose group ($P<0.05$). This was accompanied by increased levels of follicular cell hypertrophy in males from the high and mid dose groups. However, this effect was considered to be an adaptive response related to hepatocyte hypertrophy.

Males in the high and mid dose groups displayed an increase in kidney weights (absolute and relative compared to controls) ($P<0.01$). This was accompanied by increased levels of globular accumulations of eosinophilic material in the tubular epithelium as well as associated tubular basophilia and tubular necrosis in males from the high and mid dose groups. The increased globular accumulations observed are consistent with the presence of hydrocarbon nephropathy which is caused by the excessive accumulation of α_2-microglobulin in renal proximal tubular epithelial cells. α_2-Microglobulin is found only in the proximal tubular epithelium of adult male rats and is not considered to be toxicologically relevant to humans.

Slight increases in absolute and relative spleen weights were also observed in males in the high ($P<0.01$) and

mid dose (P<0.05) groups when compared to controls.

Females in the high and mid dose groups displayed an increase in absolute and relative pituitary weights when compared to controls (P<0.05). However, in the absence of a clear dose response and histopathological effects, the increases were not considered by the study authors to be related to treatment with the notified chemical.

No abnormal macroscopic findings were reported in treated animals at necropsy. A reddened thymus was observed in 1 male from the control group. A small left kidney was observed in 1 female from the control group.

Effects reported during histopathological examination (except for those described above for the liver, thyroid gland and kidneys) were considered normal for this age and strain of rat and in the absence of a dose response were not considered related to treatment.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was considered to be 300 mg/kg bw/d based on some haematological and organ weight changes observed at this dose level and above. The No Observed Effect Level (NOEL) was 30 mg/kg bw/day in this study.

TEST FACILITY Harlan Laboratories Ltd (2010f)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure/Pre incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100. *E. coli*: WP2uvrA
Metabolic Activation System	Phenobarbitone/β-naphthoflavone-induced rat liver (S9 homogenate)
Concentration Range in Main Test	a) With metabolic activation: 5 – 5000 μg/plate b) Without metabolic activation: 1.5 – 5000 μg/plate
Vehicle	Tetrahydrofuran
Remarks - Method	A range-finding assay was conducted using the plate incorporation method with doses from 50 – 5000 μg/plate. Due to toxicity in the main test (related to the test procedure), the dose range was expanded to 1.5 – 5000 μg/plate. In the main experiment, the pre-incubation method was used in preference to the plate incorporation method. A third experiment was performed according to the plate incorporation method to evaluate whether there was statistical significance in the increase in TA1535 revertant colony frequency (as observed in the range-finding experiment). Vehicle and positive controls were used in parallel with the test material. In the main test, positive controls were as follows: i) without S9: N-ethyl-N'-nitro-N-nitrosoguanidine (TA100, TA1535, WP2uvrA), 9-aminoacridine (TA1537) and 4-nitroquinoline-1-oxide (TA98); ii) with S9: 2-aminoanthracene (TA100, TA1535, TA1537, WP2uvrA) and benzo(a)pyrene (TA98).

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/plate) Resulting in:*

	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1 (range finding)	-	-	≥1500	Positive (TA1535) 5000 only
Test 2	-	5000	≥500	Negative
Test 3*		-	≥3000	Positive (TA1535)
Present				
Test 1 (range finding)	-	-	≥1500	Positive (TA1535) 4000 and 5000
Test 2	-	5000	≥1500	Negative
Test 3*	-	-	≥3000	Negative

* Test 3 conducted using TA1535 only

Remarks - Results

In the range-finding test (performed according to the plate incorporation procedure), no visible reduction in the growth of the bacterial lawn was observed at any dose level. Precipitate was found at concentrations ≥1500 µg/plate. A statistically significant increase in the number of revertant colonies was observed in the TA1535 strain both in the absence and presence of metabolic activation at 5000 µg/plate. A small, statistically significant increase in the WP2uvrA revertant colony frequency was observed in the absence of metabolic activation at 150 µg/plate. The increase was not reproducible in the main experiment and in the absence of a dose response this result was not considered biologically relevant. The increase was only 1.27 times the concurrent vehicle control and within the historical control range for this strain.

In the main study (performed according to the pre-incubation procedure), toxicity was observed in bacterial strains TA100, TA1535 and TA1535 in the absence of metabolic activation and in TA1535 in the presence of metabolic activation at the highest concentration tested (5000 µg/plate). A precipitate was also observed at ≥500 µg/plate in the absence of metabolic activation and ≥1500 µg/plate in the presence of metabolic activation. No statistically significant increases in the number of revertant colonies were observed at any of the concentrations tested.

Due to the findings of the increased number of revertant colonies in the TA1535 strain in the range finding test, another experiment was carried out in the TA1535 strain using concentrations from 1000 – 5000 µg/plate. A small but statistically significant increase in the number of revertant colonies was observed at the 4000 and 5000 µg/plate concentrations in the absence of metabolic activation only. Precipitate was observed at concentrations ≥3000 µg/plate in the absence and presence of metabolic activation.

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

CONCLUSION

The study authors note that TA100 strain contains a plasmid that makes it more sensitive than TA1535, but TA100 did not produce any increase in the number of revertant colonies, compared to TA1535. In addition, the study authors argued that there was no convincing dose response. However, the number of revertant colonies increased to statistical significance at concentrations of 4000 and 5000 µg/plate with the observed increase slightly higher at the highest dose tested (52 and 58 respectively, compared to 138 in the positive control).

Based on the above, the study authors concluded that the chemical is non mutagenic.

TEST FACILITY Harlan Laboratories Ltd (2009f)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian

Species/Strain	Human volunteers
Cell Type/Cell Line	Peripheral lymphocytes
Metabolic Activation System	Phenobarbitone/β-naphthoflavone-induced rat liver (S9 homogenate)
Vehicle	Tetrahydrofuran
Remarks - Method	Due to the cytotoxicity of the solvent at higher concentrations, the highest concentration tested was 2500 µg/mL. No other significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 78.1*, 156.25*, 234.35*, 312.5, 468.75, 625	4 hrs	24 hrs
Test 2	0*, 9.8, 19.5*, 39.1*, 58.6*, 78.1*, 156.25	24 hrs	24 hrs
Present			
Test 1	0*, 19.5, 39.1*, 78.1*, 156.25*, 234.35*, 312.5	4 hrs	24 hrs
Test 2	0*, 19.5*, 39.1*, 78.1*, 156.25*, 234.35, 312.5		

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥234.35	≥234.35	≥78.1	Negative
Test 2	-	≥78.1	≥78.1	Negative
Present				
Test 1	≥234.35	≥234.35	≥39.1	Negative
Test 2	-	≥156.25	≥78.1	Negative

Remarks - Results

In Test 1, the mitotic index was reduced below 50% (to 42%) at 156.25 µg/mL without metabolic activation. The mitotic index was not reduced below 50% for any of the concentrations with metabolic activation. Precipitate was observed at concentrations 78.1 µg/mL and above without metabolic activation and 39.1 µg/mL with metabolic activation. Toxicity at higher doses prevented analysis of plates treated with concentrations >234.35 µg/mL.

In Test 2, the mitotic index was reduced below 50% (to 46%) at 78.1 µg/mL without metabolic activation. The mitotic index was not reduced below 50% for any of the concentrations treated with metabolic activation. Precipitate was observed at concentrations 78.1 µg/mL and above with and without metabolic activation. Toxicity at higher doses prevented analysis of plates treated with >78.1 µg/mL without metabolic activation and >156.25 µg/mL with metabolic activation.

No increase in cells with aberrations or incidence of polyploidy was observed at any of the concentrations tested with or without metabolic activation in either Test 1 or Test 2.
The positive and vehicle controls gave satisfactory responses, confirming

the validity of the test system.

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

TEST FACILITY Harlan Laboratories Ltd (2010g)

B.10. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
Species/Strain Mouse
Cell Type/Cell Line L5178Y TK+/- 3.7.2c Lymphoma
Metabolic Activation System Phenobarbitone/β-naphthoflavone-induced rat liver (S9 homogenate)
Vehicle Tetrahydrofuran
Remarks - Method Ethylmethansulfonate and Cyclophosphamide were used as positive controls. Tetrahydrofuran was used as the negative control. The concentration of S9 mix was reduced from 2% in the first experiment to 1% in the second. Both concentrations are within the concentration range recommended in the OECD test guidelines.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	0*, 9.77, 19.53*, 39.06*, 78.13*, 156.25*, 312.5*, 625*, 1250	4	48	52
Test 2	0*, 20*, 30*, 40*, 60*, 80*, 100*, 120*, 160*	24	48	72
Present				
Test 1	0*, 9.77, 19.53, 39.06*, 78.13*, 156.25*, 312.5*, 625*, 1250*	4	48	52
Test 2	0*, 9.77*, 19.53*, 39.06*, 78.13*, 156.25*, 312.5*, 625*, 1250	4	48	52

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:* *Cytotoxicity* in Preliminary Test*	*Cytotoxicity* in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	None observed	156.25 and ≥625	≥39.06	Negative
Test 2	-	None observed	≥60	Negative
Present				
Test 1	None observed	None observed	≥39.06	Negative
Test 2	-	1250	≥19.53	Negative

* Cytotoxicity = relative suspension growth <20%

Remarks - Results The maximum dose level used in the test was the maximum achievable dose level of 1250 μg/mL for the 4 hr exposure groups in both the absence and presence of metabolic activation, and limited by notified chemical induced toxicity for the 24 hr exposure group in the absence of metabolic activation. It was not possible to undertake microtitre plate counts at 1250 μg/mL for the 4 hr exposure groups due to excessive toxicity both in the presence and absence of metabolic activation.

In the first experiment a precipitate of the notified chemical was observed at concentrations ≥39.06 μg/mL, in both the absence and presence of metabolic activation. In the second experiment a precipitate of notified

chemical was observed at concentrations ≥60 μg/mL in the absence of metabolic activation, and at concentrations ≥19.53 μg/mL in the presence of metabolic activation.

The positive controls induced marked increases in the mutant frequency indicating the suitability of the test system and of the activity of the metabolising system.

In the first experiment, statistically significant increases in the mutant frequency in both the absence and presence of metabolic activation were observed in a dose-dependent manner. However, the global evaluation factor (GEF = 126 x 10^{-6}) was not exceeded and the mutant frequencies observed in the treated cells were within the historical range for controls. Therefore, the increases were not considered to be toxicologically significant.

In the second experiment, statistically significant increases in the mutant frequency in the absence of metabolic activation were observed in a dose-dependent manner. However, statistically significant increases did not exceeded the global evaluation factor (GEF = 126 x 10^{-6}) and the mutant frequencies observed in the treated cells were within the historical range for controls. Therefore, the increases were not considered to be toxicologically significant.

The notified chemical did not induce any toxicologically significant dose-related increases in the mutant frequency, either with or without metabolic activation, in either experiment.

CONCLUSION The notified chemical was not mutagenic to Mouse L5178Y TK+/- 3.7.2c Lymphoma cells treated *in vitro* under the conditions of the test.

TEST FACILITY Harlan Laboratories Ltd (2010h)

B.11. Developmental toxicity

TEST SUBSTANCE Notified chemical

METHOD
- Species/Strain: Rat/Wistar
- Route of Administration: Oral – gavage
- Exposure Information: Females - 42 days (except during parturition where applicable) (5 *days post partum*)
  Males - 42 days

  Dose regimen – 7 days/week (continuous)

  Post-exposure observation period: 14 days
- Vehicle: Polyethylene glycol 400
- Remarks - Method: Histopathological examination of the liver, thyroids and kidneys of animals in the recovery control and recovery high dose groups was undertaken in addition to the requirements of OECD TG 421 to investigate further the effects seen in these organs during a previous 28-day repeat dose oral toxicity study in rats (Harlan Laboratories Ltd, (2010f)).

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	10/sex	0	0

Low dose	10/sex	30	0
Mid dose	10/sex	300	1
High dose	10/sex	1000	2
Recovery control	5/sex	0	0
Recovery High dose	5/sex	1000	0

Mortality and Time to Death

Three treated animals were killed *in extremis* during the study.

Two males; one from the mid dose group and one from the high dose group were killed on Days 2 and 3 of treatment, respectively. Post mortem examination revealed gaseous distention of the gastro-intestinal tract and red discolouration of the lungs. Histopathological investigations indicated the cause of the deaths to be aspiration pneumonia related to administration by gavage.

One female from the high dose group was killed on Day 33 with foetuses *in utero* following the appearance of red fluid around the vagina. Post mortem examination also revealed gaseous distention of the gastro-intestinal tract. The cause of death was unable to be determined but was considered to be unrelated to the toxicity of the notified chemical.

Effects on Parents

Clinical observations reported in surviving animals included: increased salivation immediately after dosing (up to 1 hr) and noisy respiration, mainly in the high dose groups and to a lesser extent in the mid and low dose groups. One male from the high dose group showed signs of laboured and decreased respiration, pilo-erection and hunched posture on Day 15 of the study but no other abnormalities were noted in this animal throughout the remainder of the study. Other abnormal clinical observations were reported in isolation and were not considered related to treatment.

Statistically significant decreases in bodyweight gain were observed in high dose males in week 1 ($P<0.01$) and week 3 ($P<0.05$) when compared to controls. A statistically significant decrease in bodyweight gains was also observed during week 5 of treatment for recovery high dose males ($P<0.05$). Minor variations in bodyweight gains were observed in other groups but statistical significance was not attained.

Statistically significant increases in food intake in high dose females ($P<0.05$) were observed during the final week of gestation ($P<0.05$) compared to controls but this was considered to be caused by the slightly larger litter sizes observed in females in this dose group.

No significant variations in mating, fertility, gestation length, weight or appearance of reproductive organs were observed between treated animals and controls.

Effects in organs

Kidneys

Males from the high dose group showed statistically significant increases in absolute and relative kidney weights ($P<0.01$). However, no increases were reported in males in the recovery high dose group. Upon necropsy, a mottled appearance in the kidneys was reported in 9 males from the high dose group as well as 2 males from the mid dose group and 1 male from the low dose group. This effect was also seen in 1 male from the recovery high dose group. Six males from the high dose group also showed pallor of the kidneys. Histopathological examinations revealed a dose dependent increase in the incidence and severity of tubular basophilia in males from the mid and high dose groups with the observation reported in 4/5 males from the recovery high dose group. Increased incidence and severity of tubular epithelial hyaline droplets and granules was observed in males from the mid and high dose groups with this effect also observed in 1 male in the low dose group. All males in the recovery control group and recovery high dose group also showed this effect. Tubular degeneration was observed in all males from the high dose group and 1 male from the mid dose group as well as 4/5 males from the recovery high dose group. Tubular simple dilation was observed in males from the high dose group. Granular casts were observed in all males in the high dose group and 1 male from the mid dose group and 1 male from the recovery high dose group. Tubular epithelial hypertrophy was observed in males from the high dose group and 2 males from the mid dose group. Interstitial fibrosis was also reported in 1 male from the recovery high dose group.

Females from the high dose group showed statistically significant increases in absolute and relative kidney weights ($P<0.001$ and $P<0.05$, respectively). However, no treatment related changes were reported during

macroscopic or histopathological examinations.

Liver
Males and females from the high dose groups showed statistically significant increases in absolute and relative liver weights. Upon necropsy, enlarged livers were observed in 6 males from the high dose group. A mottled appearance of the liver was also observed in 1 male from the low dose group but not in males from the mid or high dose groups. A portion of the liver protruding through the diaphragm was reported in 1 female from the control group. Histopathological examinations revealed centrilobular/diffuse hepatocellular hypertrophy in all males and 1 female from the high dose groups as well as 2 males from the mid dose group. These findings were not reported in the recovery high dose groups.

Thyroid glands
Statistically significant increases in the absolute and relative weights of the thyroid glands were reported in males from the high and mid dose groups ($P<0.05$). Males from the high dose group showed an increased incidence of follicular cell hypertrophy (5) compared to males and females from the mid dose groups (1 in each). This effect was also reported in 1 male from the recovery high dose group as well as 1 male from the recovery control group.

Effects on foetus
Offspring bodyweights of the litter from the high dose groups were slightly lower than control offspring bodyweights but statistical significance was not reached. The decrease was considered to be due to the slightly larger litter size of offspring from the high dose groups.

No abnormal clinical signs or macroscopic findings related to treatment were observed in any of the offspring from the treated groups.

Remarks – Results (Reproductive/Developmental)
Under the conditions of this study, the test substance elicited no signs of maternal toxicity, had no influence on gestational parameters and induced no signs of developmental toxicity up to and including a dose of 1000 mg/kg bw/day administered to rats.

Remarks – Results (Organs)
Effects were observed in the liver, kidneys and thyroid glands of treated animals which were similar to those observed in the 28-day repeat dose oral toxicity study previously conducted with the notified chemical. The increased organ weights of treated animals were confined to non-recovery treatment groups indicating that these effects were reversible. The tubular basophilia and tubular epithelial hyaline droplets and granules were observed in all male groups (including controls) and the study authors considered these to be consistent with the presence of hydrocarbon nephropathy which is caused by the excessive accumulation of α_2-microglobulin in renal proximal tubular epithelial cells. α_2-Microglobulin is found only in the proximal tubular epithelium of adult male rats and is not considered to be toxicologically relevant to humans. Males from the mid and high dose groups showed signs of tubular degeneration, tubular simple dilation and granular casts which did not resolve in the recovery high dose males indicating these effects were irreversible and were considered treatment related. Therefore, on the basis of the effects observed in the kidneys of males treated at 1000 and 300 mg/kg bw/day, the study authors considered the NOEL as 30 mg/kg bw/day. However, it was unclear whether the mottled kidneys observed in 1 male of the low dose group indicated the beginning of a dose response demonstrated by the increased incidence of mottled kidneys and histopathological changes in the kidneys in males from the mid and high dose groups.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) for reproductive and developmental toxicity was established as 1000 mg/kg bw/day, based on the absence of treatment related reproductive or developmental effects at any dose level.

The NOEL for repeat dose toxicity was established as 30 mg/kg bw/day by the study authors, based on the irreversible, treatment related effects observed in the kidneys of male rats treated at higher doses.

However, NICNAS noted the mottled appearance in kidneys was observed in males at all dose levels with some histopathological changes at mid and high dose groups. Therefore, it is unclear whether this mottled appearance was an indication of the start of histopathological changes in the kidneys.

TEST FACILITY	Harlan Laboratories Ltd (2010i)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability (Study 1)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test. EC Directive 440/2008 C.4-C
Inoculum	Activated sewage sludge from domestic sewage treatment plant
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	CO_2 analysis – TOC analyser
Remarks - Method	The notified chemical (1 g) was dissolved in 5 mL THF and an aliquot (236 µL) was absorbed onto filter paper prior to dispersion in the test medium in order to aid dispersion and to increase the surface area of the test substance exposed to the test organisms. The THF was allowed to evaporate to dryness prior to the addition of the filter paper containing the notified chemical to inoculated culture medium to give a final concentration of 10 mg carbon/L. A positive control, reference sample and toxicity control were run in parallel. The test was carried out in a temperature controlled room at approximately 21°C in darkness.

RESULTS

Test substance		*Sodium Benzoate (positive control)*	
Day	*% degradation*	*Day*	*% degradation*
0	0	0	0
6	7	6	75
10	11	10	76
21	39	21	89
29*	48	29*	92

*Day 29 values corrected to include any carry-over of CO_2 detected in test vessels.

Remarks - Results	All validity criteria for the test were satisfied and no significant deviations from test guidelines were reported. The reference substance, sodium benzoate, reached the pass level by day 14 and thus confirmed the suitability of the inoculum and test conditions. The toxicity control attained 64% degradation after 14 days indicating the notified chemical is non-inhibitory to microorganisms used in the test. The notified chemical did not surpass the pass level of 60% degradation within a 10 day window and is therefore not considered ready biodegradable.
CONCLUSION	The notified chemical is not ready biodegradable
TEST FACILITY	Harlan Laboratories Ltd (2009h)

C.1.2. Ready biodegradability (Study 2)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 310 Ready Biodegradability, CO_2 in sealed vessels (Headspace Test) ISO Guideline No 14593 Water quality – Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium – Method by analysis of inorganic carbon in sealed vessels (CO_2 headspace test)
Inoculum	Activated sewage sludge from domestic sewage treatment plant

Exposure Period	42 days
Auxiliary Solvent	None
Analytical Monitoring	CO_2 analysis – TOC Analyser, HPLC-MS, GC-MS (headspace analysis)
Remarks - Method	The notified chemical was dissolved in THF and absorbed onto filter paper prior to dispersion in the test medium in order to aid dispersion and to increase the surface area of the notified chemical exposed to the test organisms. The THF was allowed to evaporate to dryness prior to the addition of the filter paper containing the notified chemical to inoculated culture medium to give a final concentration of 10 mg carbon/L. The test was carried out in the dark at 20±1.0°C. Two potential breakdown products were also monitored for biodegradation. A sample of breakdown product 1 was added to a vessel containing reference material and inoculum at a concentration of 10 mg carbon/L. Breakdown product 2 was added to sterilised inoculum to serve as a volatility control.

RESULTS

Test substance		*Breakdown product 1 of test substance*		*Sodium Benzoate*	
Day	*% degradation*	*Day*	*% degradation*	*Day*	*% degradation*
0	1	0	1	0	0
3	3	3	2	3	51
6	1	6	-1	6	74
16	3	16	2	16	74
28	5	28	0	28	76
42	9	42	6	42	78

Remarks - Results	All validity criteria for the test were satisfied and no significant deviations from test guidelines were reported. The notified chemical attained 5% degradation after 28 days and 9% degradation after 43 days calculated from the results from inorganic carbon analysis. Breakdown product 1 attained 0% degradation after 28 days and 6% degradation after 42 days calculated from the results from inorganic carbon analysis. No significant losses of breakdown product 2 were observed.
CONCLUSION	The notified chemical is not ready biodegradable
TEST FACILITY	Harlan Laboratories Ltd (2010l)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test - Semi-static EC Directive 440/2008 C.1 Acute Toxicity for Fish
Species	*Oncorhynchus mykiss* (Rainbow trout)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	140 mg/L $CaCO_3$
Analytical Monitoring	HPLC-MS (LOQ = 0.022 mg/L)
Remarks – Method	Based on the results of a preliminary range-finding test the following limit test was performed. Test substance was added to dechlorinated tap water to give a loading rate of 100 mg/L. The solution was then stirred for 23 hrs and allowed to stand for 1 hr. The aqueous phase (water accommodated fraction, WAF) was removed by mid-depth siphoning. Microscopic inspection of the WAF showed no micro-dispersions or undissolved test item to be present. A semi-static test regime was employed with temperature of test and control groups maintained at

14°C.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 hr*	*24 hrs*	*48 hrs*	*72 hrs*	*96 hrs*
0	0	7	0	0	0	0	0
100	14.0 - 23.5*	14	0	0	0	0	0

*Based on the alkyl acid component of notified chemical

LL50	>100 mg/L at 96 h (based on loading rate, WAF)
NOEL	>100 mg/L at 96 h (based on loading rate, WAF)
Remarks – Results	All validity criteria for the test were satisfied and no significant deviations from test guidelines were reported. No mortalities were observed. The results are expressed as nominal loading rates since the notified chemical is a complex UVCB chemical that has limited solubility and toxicity cannot therefore be attributed to a single or combination of constituents in the notified chemical.
CONCLUSION	The notified chemical is not harmful to fish up to the limit of its solubility in water.
TEST FACILITY	Harlan Laboratories Ltd (2010j)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Static EC Directive 440/2008 C.2 Acute Toxicity for Daphnia
Species	*Daphnia magna*
Exposure Period	48 hrs
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	HPLC-MS (LOQ = 0.37 mg/L)
Remarks - Method	Based on the results of a preliminary range-finding test the following limit test was performed. Test substance was added to dechlorinated tap water to give a loading rate of 100 mg/L. The solution was then stirred for 23 hrs and allowed to stand for 1 hr. The aqueous phase (water accommodated fraction, WAF) was removed by mid-depth siphoning. Microscopic inspection of the WAF showed no micro-dispersions or undissolved test item to be present. Twenty daphnia (4 replicates of 5 animals) were exposed to the WAF of the notified chemical, at a single nominal loading rate of 100 mg/L for 48 hrs at a temperature of approximately 21°C under static test conditions. Immobilisation and any adverse reactions to exposure were recorded after 24 and 48 hrs. A positive control test was performed by exposing *daphnia* to potassium dichromate.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 hrs*	*48 hrs*
0	0	20	0	0
100	30.1 – 32.2*	20	0	1

*Based on the alkyl acid component of notified chemical

EL50	>100 mg/L at 48 h (based on loading rate, WAF)
NOEL	>100 mg/L at 48 h (based on loading rate, WAF)
Remarks - Results	All validity criteria for the test were satisfied and no significant deviations from test guidelines were reported. The mean 48 hrs EC50 for the positive control test was 0.78 mg/L (95% CI 0.68 – 0.88 mg/L) which

was within the normal range for the reference material. The results are expressed as nominal loading rates since the notified chemical is a complex UVCB chemical that has limited solubility and toxicity cannot therefore be attributed to a single or combination of constituents in the notified chemical.

CONCLUSION The notified chemical is not harmful to aquatic invertebrates up to the limit of its solubility in water.

TEST FACILITY Harlan Laboratories Ltd (2009g)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test.
EC Directive 440/2008 C.3 Algal Inhibition Test.

Species	*Desmodesmus subspicatus*
Exposure Period	72 hrs
Concentration Range	Nominal: 10 – 160 mg/L Actual: 6.87 – 38.3 mg/L (at 72 hrs)
Auxiliary Solvent	None
Water Hardness	0.15 mmol/L (Ca^{2+} and Mg^{2+})
Analytical Monitoring	HPLC-MS (LOQ = 0.37 mg/L)
Remarks - Method	Based on a preliminary range-finding test, algae cells were exposed to nominal loading rates of 10, 20, 40, 80 and 160 mg/L for a period of 72 hrs under constant illumination and shaking at a temperature of 24±1°C. Each solution was then stirred for 23 hrs and allowed to stand for 1 hr. The aqueous phase (water accommodated fraction, WAF) was removed by mid-depth siphoning. Microscopic inspection of the WAF showed no micro-dispersions or undissolved test item to be present. A positive control test was performed by exposing algae to potassium dichromate under similar conditions to those in the limit test. Statistical analysis of the results was performed using one way analysis of variance incorporating Bartlett's test for homogeneity of variance and Dunnett's multiple comparison procedure.

RESULTS

Biomass		*Growth*	
E_bL50 mg/L at 72 hrs (loading rate WAF)	*NOEL mg/L (loading rate WAF)*	*E_rL50 mg/L at 72 hrs (loading Rate WAF)*	*NOEL mg/L (loading rate WAF)*
29 (95% CI 36 -33)	*20*	*47 (95% CI 44 -51)*	*20*

Remarks - Results All validity criteria for the guideline were satisfied and no significant deviations from the guidelines were reported. The EC50 for the positive control test was 0.79 mg/L based on growth rate and 0.30 mg/L based on yield which were within the normal range for the reference material. The results are expressed as nominal loading rates since the notified chemical is a complex UVCB chemical that has limited solubility and toxicity cannot therefore be attributed to a single or combination of constituents in the notified chemical.

CONCLUSION The notified chemical is harmful to algae

TEST FACILITY Harlan Laboratories Ltd (2010k)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 440/2008 C.11 Biodegradation: Activated Sludge Respiration Inhibition Test. OPPTS 850.6800 (Modified Activated Sludge, Respiration Inhibition Test for Sparingly Soluble Chemicals)
Inoculum	Activated sludge of a predominantly domestic sewage
Exposure Period	3 hrs
Concentration Range	Nominal: 0 - 1000 mg/L Actual: Not determined
Remarks – Method	Activated sewage sludge was exposed to an aqueous dispersion of the test item at loading rates of 10, 32, 100, 320 and 1000 mg/L for 3 hrs under normal laboratory lighting in a temperature controlled room at 21 ± 1°C.
RESULTS	
IL50	>1000 mg/L
NOEL	1000 mg/L
Remarks – Results	All validity criteria for the guideline were satisfied and no significant deviations from the guidelines were reported.
CONCLUSION	The notified chemical does not inhibit respiration of waste water microorganisms
TEST FACILITY	Harlan Laboratories Ltd (2010m)

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