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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Z-97

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

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

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

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Director
NICNAS

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Lubrizol International, Inc. (ABN 52 073 495 603)
28 River St
SILVERWATER NSW 2128

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, CAS Number, Structural Formula, Molecular Weight, Means of Identification, Composition, Use details, Import Volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows:
Hydrolysis as a function of pH, Dissociation Constant, Particle Size Distribution, Flammability Limits.

PREVIOUS NOTIFICATION IN AUSTRALIA
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Z-97 (product containing the notified chemical Z-97 at < 1% concentration)

MOLECULAR WEIGHT
Mn < 500 Da.

ANALYTICAL DATA
Reference NMR, IR, and UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY > 90%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (> 1% by weight)
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Tan coloured waxy solid

Property	Value	Data Source/Justification
Melting Point	85.9-112°C	Measured
Boiling Point	Not determined	As decomposition occurred at 325°C,

		boiling point could not be determined
Density	1040 kg/m^3 at 20°C	Measured
Vapour Pressure	< $1.6x10^{-6}$ kPa at 25°C	Measured
Water Solubility	< 1.3×10^{-4} g/L at 20°C	Measured
Hydrolysis as a Function of pH	Not determined	The notified chemical is not expected to hydrolyse over the environmental pH range (4–9), based on its low solubility in water and the absence of readily hydrolysable functional groups
Partition Coefficient (n-octanol/water)	log Pow = 6.84 at 20°C	Measured
Adsorption/Desorption	log Koc > 5.63 at 40°C	Measured
Dissociation Constant	Not determined	The notified chemical does not contain any functional groups that are expected to dissociate in water.
Particle Size	Not determined	The notified chemical is a waxy liquid.
Flash Point	48 ± 2°C at 101.325 kPa 56.6 ± 2°C at 101.325 kPa	Measured (confirmatory test)
Flammability	Not expected to be flammable	Estimated
Autoignition Temperature	366 ± 5°C	Measured
Oxidising Properties	Not predicted to be an oxidising agent	Estimated based on chemical structure
Explosive Properties	Not predicted to be explosive	Estimated (based on structural indication of explosive properties)

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

Reactivity
Based on the chemical structure, the notified chemical is not predicted to be an oxidising agent.

Dangerous Goods classification
Based on the submitted physical-chemical data (flash point) in the above table, the notified chemical is classified as follows according to the Australian Dangerous Goods Code (NTC, 2007).

Class 3 – Flammable liquids (Packing Group III)

However, the data above does 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 at < 1% as a component of finished engine oil products. In future, the notified chemical may be imported at < 10% as a component of a concentrate for blending into engine oil products.

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

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

PORT OF ENTRY
Melbourne, Brisbane, Perth

IDENTITY OF MANUFACTURER/RECIPIENTS
Lubrizol International, Inc.
28 River St
SILVERWATER NSW 2128

TRANSPORTATION AND PACKAGING
The notified chemical (< 1%) will be imported by sea as a component of finished engine oil products in 20 tonne isotainers. In future, the notified chemical may be imported in a concentrate at < 10% concentration in 20 tonne isotainers or 260 L steel drums for reformulation into engine oil products. Finished engine oil products containing the notified chemical at < 1% will be sold in 1 L or 5 L containers.

USE
The notified chemical is intended for use as an engine oil additive (lubricant) at concentrations of < 1%. Engine oils containing the notified chemical will be used in vehicle manufacturing facilities, mechanical workshops and also by members of the public (do-it-yourself (DIY) use).

OPERATION DESCRIPTION
The notified chemical will be imported as an additive in finished engine oil products at concentrations of < 1%. In future, the notified chemical may be imported at < 10% concentration in a concentrate additive package, which will undergo formulation into engine oil products at customer sites.

A typical formulation operation would involve blending the concentrate containing the notified chemical (< 10%) with oil and possibly other additives. The concentrate containing the notified chemical will be pumped from isotainers (20 tonnes) or decanted from drums (260 L) to tanks where it will be mixed with oil and possibly other additives. After blending, the engine oil which now contains the notified chemical at < 1%, would be packaged into 1 L or 5 L containers for sale. Packaging equipment is expected to be automated and housed within or near the blending operation area. The blending facility is expected to be well ventilated and fully automated.

The finished engine oil containing the notified chemical will be supplied in bulk to vehicle manufacturers for 'factory fill' applications. Additionally, it may also be supplied in smaller containers for use in service applications through garages 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 (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	2-3	1-3	4-6
Plant Operation	2-3	<1	50
Maintain/clean	2-3	2-4	10-20
Plant Operator-Sampling	1	<1	100
End user	1-3	2-4	Typically < 20

EXPOSURE DETAILS
Transport and storage
Transport and storage workers are not likely to be exposed to the notified chemical except in the case of an accident involving damage to the packaging.

Blending of engine oil products
Blending is expected to be fully automated and ventilated with limited potential for exposure. Therefore, exposure to the notified chemical at < 10% may be experienced by workers during transfer to mixing vessels, residues in lines, and on coupling and occasionally from leaks and spills. The main route of exposure is expected to be dermal, although some ocular exposure is also possible. Inhalation exposure may occur if mists are generated during blending processes. Workers are expected to wear nitrile or neoprene gloves, chemical goggles or face shield, and a long sleeve shirt, chemical protective suit or apron to minimise exposure. The notifier anticipates that a full face respirator with a combination of organic vapour and dust/mist cartridge will be used if the recommended exposure limit of 5 mg/m^3 [NOHSC: 3008 (1995)] is exceeded.

Dermal and ocular exposure may also occur when sampling the blended engine oil containing the notified chemical (< 1%). This involves opening a valve to fill a small container. To minimise exposure, gloves, goggles and a long sleeved shirt are expected to be worn to minimise exposure.

The packaging equipment is expected to be automated and housed within or near the blending operation area. An enclosed or open filling system may also be used. Dermal and ocular exposure may occur during packaging, however workers are expected to wear aprons, gloves and safety glasses to minimise exposure.

End use
Workers may be exposed to engine oils containing the notified chemical at less than 1% during use in vehicle manufacturing or mechanical workshops.

At vehicle manufacturers, the finished engine oil 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. Workers are expected to wear PPE such as long sleeve shirts and gloves to minimise dermal exposure. Ocular exposure would be minimised if goggles are worn during transfer of engine oils containing the notified chemical (< 1%).

At mechanical workshops, professional users such as mechanics may experience dermal or ocular exposure to the final product containing the notified chemical at <1% when transferring engine oil to cars and other machinery. The potential for dermal and ocular exposure may be reduced by wearing gloves, long sleeve shirts and goggles. Overall, exposure to the notified chemical will be low, given the low concentration (< 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 < 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 due to closed systems. DIY users may experience dermal and ocular exposure to final products containing < 1% of the notified chemical when adding and/or replacing engine oil in their vehicles. However, most engine oils are added and/or replaced by certified mechanics and as such, exposure during the addition of engine oil to the general public 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 (< 1%) in finished engine oil.

6.2. Human health effects assessment

The results from toxicological investigations conducted on the notified chemical (> 90%) are summarised in the table below. A summary of all the studies is provided in the section below. Further details of some of these studies can be found in Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw low toxicity
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	not a skin sensitiser
Rat, repeat dose oral toxicity (incl. reproductive/developmental screening) – 42 days	NOEL = 50 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* chromosome aberration	non genotoxic
Genotoxicity – *in vitro* mammalian cell gene mutation	non genotoxic

Toxicokinetics
Based on the low molecular weight (< 500 Da) and the lipophilicity of the notified chemical (water solubility $\leq 1.3 \times 10^{-4}$ g/L at 20ºC; log Pow = 4.54 - 7.81), dermal absorption is expected to be limited. Oral absorption may take place via micellular solubilisation given its low water solubility and relatively high estimated log Pow (> 4). The effects seen in the thymus and bone marrow of animals treated with 500 mg/kg bw/day in the repeated

dose oral toxicity study indicate systemic absorption may have occurred.

Acute toxicity
The notified chemical was found to be of low acute oral toxicity in a rat study conducted according to OECD TG 420 (Harlan Laboratories, 2009c). No mortalities or adverse effects were observed during the study. The acute oral LD50 was determined to be > 2000 mg/kg bw.

The notified chemical was found to be of low acute dermal toxicity in a rat study (limit test) conducted according to OECD TG 402 (Harlan Laboratories, 2009d). No mortalities, clinical signs or irritation effects were observed during the study. The acute dermal LD50 was determined to be > 2000 mg/kg bw.

Data on the acute inhalation toxicity of the notified chemical was not provided. The notified chemical has low vapour pressure and is thus unlikely to be inhaled. If inhaled, the notified chemical may be absorbed directly across the respiratory tract epithelium, based on its high partition coefficient.

Irritation and Sensitisation
The notified chemical did not elicit any irritant effects to rabbit skin in a test conducted according to OECD TG 404 (Harlan Laboratories, 2009e) and was found to be slightly irritating to the eyes of rabbits.

The notified chemical does not contain any known structural alerts for skin sensitisation (Barratt et al. 1994). The potential for skin sensitisation was evaluated using a local lymph node assay (LLNA). A lymphocyte proliferative response resulting in a stimulation index (SI) of 3.77 (omitting the outlier at 642 dpm) was observed in animals treated with the notified chemical at 50%. (A SI of 2.98 was reported for animals treated with the notified chemical at 10% concentration and an SI of 2.53 was reported for animals treated with the notified chemical at 25% concentration.) Statistical analysis of the SI for all treated animals found that the lower bounds on the 95% confidence intervals were below 3 at all concentrations. In addition, there was no statistically significant increase in dose-response (ie. SI score) between animals treated with the notified chemical at 10% and animals treated with the notified chemical at 25% and 50% (see Appendix B for further details). Based on the lack of a structural alert; no dose-response relationship and the exceedance of the SI of 3 lacking statistical significance, the notified chemical was not considered to be a skin sensitiser.

Repeated Dose Toxicity
The notified chemical was tested in a combined repeated dose 42-days oral toxicity study (including a reproduction/developmental toxicity screening test) in rats at concentrations of 50, 350 and 500 mg/kg bw/day. The high dose group was initially treated with 1000 mg/kg bw/day. However, due to the early termination of one male from this group on Day 12 and the reported significant bodyweight losses in other animals from the first week of the study, the highest dose was reduced to 750 mg/kg bw/day on Day 8 and then reduced again (due to the persistence of bodyweight losses in the high dose group) on Day 15 to 500 mg/kg bw/day.

Treatment-related effects were observed in both sexes at the high (1000/750/500 mg/kg bw/day) and mid (350 mg/kg bw/day) dose levels. The effects at the high dose included clinically observable signs, decreased bodyweight gains, increased incidence of adipose infiltration of the bone marrow in males, decreased total protein levels in males from the high dose and high dose recovery groups, and increased incidence of lymphoid atrophy of the thymus in females of the high dose group (see Appendix B for details). The effects at the mid dose included clinical observable signs in some animals, slight reduction in body weight development for males, an increase in albumin/globulin ratio for males and lymphoid atrophy in the thymus for three males. The No Observed Effect Level (NOEL) was determined to be 50 mg/kg bw/day, based on the treatment related effects observed at mid and high dose levels.

No treatment related effects were observed in the reproductive parameters measured.

Mutagenicity and Genotoxicity
The notified chemical was not found to be mutagenic using a bacterial reverse mutation test, and not clastogenic to Chinese Hamster Lung (CHL) cells and mouse L5178Y TK+/- 3.7.2c cells *in vitro*.

Toxicity for reproduction
Screening for the reproduction/developmental toxicity potential of the notified chemical was included in the repeated dose oral toxicity test. No adverse effects were reported on the reproductive performance or litters of animals treated at doses up to 500 mg/kg bw/day.

Health hazard classification
Based on the available data the notified chemical is not classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
The notified chemical is considered to have the potential for slight eye irritation.

Workers may experience dermal and ocular exposure to the notified chemical at < 10% concentration during blending of the imported fuel additive package into the finished engine oil. The blending process is largely enclosed and automated and 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 safety goggles and respirators. Therefore, the potential for eye irritation is not considered to be unacceptable.

Dermal and ocular exposure of workers to the notified chemical at < 1% concentration in engine oil products is possible during factory-fill operations at vehicle manufacturers. However, the use of fully automated systems should minimise the potential for exposure. In addition, workers are expected to wear PPE including gloves, safety glasses and coveralls and under these circumstances the risk of eye irritation is not expected.

Engine oil products containing the notified chemical at < 1% concentration will also be used by mechanics in workshops and garages. Exposure to mechanics may occur frequently during oil changes. The use of PPE is not considered likely. However, the risk of eye irritation is not expected due to the low concentration of the notified chemical in the engine oils (< 1%).

6.3.2. Public health
Engine oil products containing < 1% notified chemical will be available to the public for DIY use. During engine oil changes dermal and ocular exposure is likely, especially considering that members of the public are not likely to use PPE. The risk of eye irritation is not expected due to the low concentration of the notified chemical in engine oil products (< 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 imported in isotainers and drums as finished engine oil and, initially, no manufacturing or blending will occur in Australia.

In the future, if local blending is undertaken, release during the highly automated blending process is not expected. The equipment used will typically be cleaned with oil, with these washings used in the formulation of the next batch or another oil blend. Accidental spills, and residue of the notified chemical remaining in import containers (<1% of the contents), are expected to be contained and disposed to landfill or recycled.

RELEASE OF CHEMICAL FROM USE
Minor spills may occur during addition, and removal, of oil to machines. For vehicle applications the majority (~86%) of oil changes take place in specialised automotive service centres, where release of the product containing the notified chemical from professional activities is expected to be disposed of appropriately in landfill, by thermal decomposition or recycling (AIP, 1995). The majority of recycled oil will be reused as burner oil (e.g. in kilns, furnaces and industrial burners). The "do-it-yourself" (DIY) proportion (~14%) of oil changes could potentially lead to improper disposal of approximately half the used oil to soils, sediments and storm water drains. Approximately 25% of engine oil (and thus notified chemical) is consumed during operation, and < 0.5% of engine oil is estimated to be lost from leaks in seals and gaskets.

RELEASE OF CHEMICAL FROM DISPOSAL
Isotainers and drums are anticipated to be sent for cleaning and reconditioning by a licensed company. The resultant washings from such companies are typically passed to an on-site waste treatment facility and any

waste sludge is likely to be sent to landfill.

Used oil drained from crankcases at specialised automotive service centres (approximately 75%) is expected to be disposed of to oil recycling centres.

Only around 20% of used oil removed by DIY enthusiasts is collected for recycling. Approximately 25% is buried or disposed of to landfill, 5% is disposed of into storm water drains and the remaining 50% is used in treating fence posts, killing grass and weeds or disposed of in other ways (Snow, 1997). In a worst case scenario, whereby DIY enthusiasts dispose of oil to sewer, up to 0.7% (i.e. 5% x 14%) of the total import volume could potentially be released to the aquatic environment.

7.1.2 Environmental fate

Most of the notified chemical will be thermally decomposed during engine operation or through re-use as an energy source. Smaller amounts may be consigned to landfill, or disposed of inappropriately to land or stormwater. Disposal to land or landfill would result in its immobilisation because of the strong sorption to soil organic carbon. If disposed of to water, the notified chemical is likely to adsorb to suspended solids and sediment. Either in landfill or through thermal decomposition, the notified chemical will decompose into water and oxides of carbon and nitrogen. Although the notified chemical has a log Pow of 6.84, is not readily biodegradable and does not contain readily hydrolysable functional groups, it is not expected to bioaccumulate in aquatic organisms due to its low potential for aquatic exposure.

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

7.1.3 Predicted Environmental Concentration (PEC)

A worst case PEC might be calculated if it is assumed that 0.7% of the introduction volume of the notified chemical (maximum 70 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 700 kg and the annual volume of water drained from this region estimated to be approximately 250 x 10^6 m^3, the resultant PEC < 0.3 µg/L. It should be stressed that this result reflects a worst case scenario, as in reality releases of the notified chemical would be more diffuse and at lower levels.

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	LC50 (96 h) >100 mg/L	Not harmful to fish up to the limit of solubility
Daphnia Toxicity – Acute	EL50 (48 h) >100 mg/L	Not harmful to aquatic invertebrates up to the limit of solubility
Daphnia Toxicity – Chronic	EL50 (21 d) = 16 mg/L NOEL (21 d) = 1 mg/L	Toxic to aquatic life with long lasting effects up to the limit of solubility
Algal Toxicity	EL50 (72 h) >100 mg/L NOEL (72 h) = 100 mg/L	Not harmful to algae up to the limit of solubility
Inhibition of Bacterial Respiration	IC50 (3 h) >1000 mg/L	Not harmful to bacterial respiration.

Under the Globally Harmonised System of Classification and Labelling of Chemicals (United Nations, 2009) the notified chemical is not harmful to fish, algae, and bacteria. To aquatic invertebrates the notified chemical exhibits no acute toxicity, yet has long lasting toxic effects. The endpoints of the studies are based on nominal loading rates due to the low water solubility of the notified chemical. The actual concentration of the notified chemical in the studies ranged from less than the limit of quantification (LOQ = 0.010 mg/L) to 0.15 mg/L (determined by HPLC), and therefore these values should be treated with caution. Details of these studies can be found in Appendix C.

7.2.1 Predicted No-Effect Concentration

The PNEC has been calculated using the daphnia chronic toxicity endpoint NOEL = 1 mg/L and an assessment factor of 50, given a total of three acute endpoints and two chronic endpoints are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
NOEL (Daphnia, chronic)	1	mg/L
Assessment Factor	50	
PNEC:	20	µg/L

7.3. Environmental risk assessment

The risk quotients (Q = PEC/PNEC) are tabulated below.

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	<0.3	20	**< 0.015**
Q - Ocean	<0.03	20	**< 0.002**

A calculated RQ value < 1 indicates that the notified chemical is not expected to pose any unacceptable risk to aquatic life, even under the conservative exposure assumptions outlined above. The PEC overestimates the likely level of exposure, as it reflects a worst case scenario with no consideration of the hydrophobicity of the notified chemical, which would favour sorption to sediment rather than dissolution in the water column. The notified chemical is not expected to pose a risk to the environment when it is used as proposed in engine oils.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

Based on the available data the notified chemical is not classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

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

	Hazard category	***Hazard statement***
Aquatic Toxicity	Chronic Category 2	Toxic to aquatic life with long lasting effects

Human health risk assessment

Under the conditions of the occupational settings described, the notified chemical is not considered to pose an 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 the health of general public.

Environmental risk assessment

On the basis of the PEC/PNEC ratio calculated, the very low water solubility and the reported use pattern, the notified chemical is not expected to pose a risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical when imported at ≥ 1% during formulation:
  - Avoid contact with eyes.

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical when imported at ≥ 1% during formulation:

- Safety glasses

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 NOHSC *Approved Criteria for Classifying Hazardous Substances*, 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 removal.

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 additive in engine oil (lubricants), or is likely to change significantly;
- the amount of chemical being introduced has increased, 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 a product 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

All physical and chemical properties were conducted on a product containing the notified chemical at > 100%.

Melting Point 85.9 – 112 ± 0.5ºC

Method	OECD TG 102 Melting Point/Melting Range. ASTM E537-86
Remarks	Determined by differential scanning calorimetry
Test Facility	Harlan Laboratories Ltd (2009a)

Boiling Point Could not be determined.

Method	OECD TG 103 Boiling Point. ASTM E537-86
Remarks	Determined by differential scanning calorimetry. As the test material decomposed from 325 ± 0.5ºC at 102.38 kPa, no value for point could be determined.
Test Facility	Harlan Laboratories Ltd (2009a)

Density 1040 kg/m^3 at 20.5ºC ± 0.5ºC

Method	OECD TG 109 Density of Liquids and Solids.
Remarks	Determined by pycnometer
Test Facility	Harlan Laboratories Ltd (2009a)

Vapour Pressure < $1x10^{-6}$ kPa at 25ºC

Method	OECD TG 104 Vapour Pressure.
Remarks	Determined by vapour pressure balance
Test Facility	Harlan Laboratories Ltd (2009b)

Water Solubility <$1.3x10^{-4}$ g/L at 20ºC

Method	OECD TG 105 Water Solubility.
Remarks	Determination by visual assessment, based on the flask method OECD TG 105. Double-deionised water (1000 mL) was added to definitive amounts of test material. Excess test material was visible at the lowest concentration tested ($1.3x10^{-4}$ g/L) after allowing to shake for 72 h (30ºC) and stand for 24 h (20ºC). Using chemical estimation software WSKOWWIN, version 1.41, © 2000 US Environmental Protection Agency, the test material has a predicted water solubility of 5.77 x 10^{-6} g/L.
Test Facility	Harlan Laboratories Ltd (2009a)

Hydrolysis as a Function of pH Not determined

Method	Test not conducted
Remarks	A hydrolysis test could not be conducted according to OECD TG 111 due to the very low water solubility of the notified chemical. The notified chemical contains functional groups that are expected to be hydrolytically stable over the environmental pH range (4–9).

Partition Coefficient (n-octanol/water) log Pow = 6.84 at 40ºC

Method	OECD TG 117 Partition Coefficient (n-octanol/water).
Remarks	Determination of the partition coefficient of the notified chemical by HPLC method found the range to be log Pow 4.54 to 7.81 with the major peak corresponding to a log Pow of 6.84. Variation to the method was made to decrease the percentage of water in the mobile phase (to 15%v/v cf ≥ 25%) to allow for reasonable retention times of standards

with higher partition coefficients than DDT. This deviation is expected to have an insignificant effect to the outcome of the test. High Pow is expected according to the low water solubility of the notified chemical.

Test Facility Harlan Laboratories Ltd (2009a)

Adsorption/Desorption – screening test log Koc > 5.63 at 40°C

Method OECD TG 121 Estimation of the Adsorption Coefficient (Koc) on Soil and Sewage Sludge using High Performance Liquid Chromatography (HPLC).

Remarks Determination of the adsorption coefficient of the notified chemical was determined by HPLC method. Testing was carried out at neutral pH due to the absence of any possible dissociating functional groups in the notified chemical. The notified chemical was found to be retained longer than the DDT standard, thus was determined to have log Koc > 5.63. The mobile phase was increased to 100% methanol after the DDT had eluted to wash off the highly retained notified chemical. A high Koc is expected from the high log Pow and the low water solubility of the notified chemical.

Test Facility Harlan Laboratories Ltd (2009a)

Dissociation Constant Not determined

Method Test not conducted

Remarks The notified chemical does not contain any functional groups that are expected to dissociate in water.

Flash Point 48 ± 2°C at 101.325 kPa
56.6 ± 2°C at 101.325 kPa (confirmatory test)

Method EC Directive 92/69/EEC A.9 Flash Point.

Remarks Determined by closed cup equilibrium method

Test Facility Harlan Laboratories Ltd (2009b)

Autoignition Temperature 366 ± 5°C

Method EC Directive 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).

Test Facility Harlan Laboratories Ltd (2009b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Irritation – eye

TEST SUBSTANCE: Notified chemical at > 90%

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion.

EC Directive 2004/73/EC B.5 Acute Toxicity (Eye Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 males
Observation Period	72 hours
Remarks - Method	No significant protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1	1	1	2	<72 hours	0
Conjunctiva: chemosis	1	1	0.67	2	<72 hours	0
Conjunctiva: discharge	1	1	0.33	2	<72 hours	0
Corneal opacity	0	0	0	0	-	-
Iridial inflammation	0	0	0	0	-	-

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

Remarks - Results: Redness, chemosis and discharge were observed in the conjunctiva of all 3 animals at 24 and 48 hours. These resolved by 72 hours.

CONCLUSION: The notified chemical is slightly irritating to the eye.

TEST FACILITY: Harlan Laboratories (2009f)

B.2. Repeat dose toxicity

TEST SUBSTANCE: Notified chemical at > 90%

METHOD: OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test

Species/Strain	Rat/Wistar Han
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 42 days Dose regimen: 7 days per week
Vehicle	Arachis oil
Remarks - Method	The highest dose level was reduced to 750 mg/kg bw/day on Day 8 following significant actual bodyweight losses observed in the groups treated with 1000 mg/kg bw/day in the first week. Subsequently, males treated with 750 mg/kg bw/day continued to display bodyweight losses and the highest dose was further reduced to 500 mg/kg bw/day on Day 15 in the high dose group. On Day 15, animals within each non-recovery dose group were paired. The females were allowed to litter and rear their offspring to Day 5 of lactation. Functional observations were conducted on 5 selected males from each non-recovery dose group after the completion of mating and on 5 selected parental females from each non-recovery dose group on Day 4 *post partum*.

Two recovery groups, each containing 5 males and 5 females were treated with the high dose (1000/750/500 mg/kg bw/day) for 42 consecutive days and then observed untreated for an additional 14 days before termination.

A control group of ten males and females was dosed with vehicle alone (arachis oil).

Surviving non-recovery males were terminated on Day 43. All non-recovery females and offspring were terminated on Day 5 *post partum*.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10 M/F	0	0
low dose	10 M/F	50	0
mid dose	10 M/F	350	0
high dose	10 M/F	1000/750/500	1
control recovery	5 M/F	0	0
high dose recovery	5 M/F	1000/750/500	0

Mortality and Time to Death

One male from the high dose group was killed *in extremis* on Day 12.

Clinical Observations

Animals of either sex treated at the high dose level, all males and four females treated with 350 mg/kg bw/day and three males and one female treated with 50 mg/kg bw/day showed episodes of increased salivation immediately or one hour post-treatment throughout the treatment period.

Noisy respiration was reported in 1 male at the high dose level on Day 8. A further male from this high dose group displayed tiptoe gait on Day 15 and another male displayed hunched posture from Days 15 to 21. Instances of generalised staining around the mouth, snout and eyes were reported in both sexes of the high dose groups and in five males of the mid dose group throughout the study. Fur loss was reported in females of the high dose group only and in two females from the 350 or 50 mg/kg bw/day groups.

The high-dose male killed *in extremis* on Day 12 developed hunched posture and was dehydrated from Days 9-12. Increased salivation was observed in this male on Days 10 and 11, pilo-erection on Days 11 and 12 and was found emaciated with staining around the mouth and snout on Day 12.

Males from the high dose group displayed statistically significant reductions in bodyweight gains during the first 2 weeks of treatment. In 3 of these males, significant actual bodyweight losses were reported during the first 2 weeks. The high dose level was 1000 mg/kg bw/day from Days 1-8 and 750 mg/kg bw/day from Days 9-14. The subsequent cumulative bodyweight gains were statistically significantly decreased for high dose males throughout the remainder of the treatment period. Females from the high dose group also displayed a statistically significant reduction in bodyweight gain in the first week of treatment with actual bodyweight losses also observed in these animals. Males from the mid dose group displayed a statistically significant decrease in bodyweight gain during week 2 and a decreased bodyweight gain throughout the treatment period. No such effects were detected in females treated with 350 mg/kg bw/day or animals of either sex treated with 50 mg/kg bw/day.

Females and males from the high dose group showed a reduction in food consumption and food efficiency during the first 1 week and first 2 weeks of treatment, respectively, when they were treated with 1000 and 750 mg/kg bw/day. No such effects were detected in animals of either sex treated with 50 or 350 mg/kg bw/day.

There were no treatment-related changes in the behavioural assessments, functional performance tests or sensory reactivity assessments.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Males from the high dose group displayed a statistically significant increase in albumin/globulin ration and a statistically significant decrease in total protein levels at both the Day 14 and Day 42 evaluations. Total protein

levels remained decreased following examination of high-dose recovery males. Females from the high dose group also displayed a statistically significant decrease in total protein levels at the Day 14 evaluation but the decrease was not observed at Day 4 *post partum* or in females of the high dose recovery group. No such effects were detected in animals of either sex treated with 50 or 350 mg/kg bw/day or recovery females following fourteen days without treatment.

Males from the high dose group displayed a statistically significant increase in alanine aminotransferase and aspartate aminotransferase as well as a statistically significant decrease in cholesterol, phosphorus and alkaline phosphatase at the Day 14 evaluation. None of these statistically significant variances were observed at the Day 42 evaluation but a statistically significant increase in phosphorus was observed in high dose recovery males. Females from the high dose group displayed a statistically significant increase in alanine aminotransferase and aspartate aminotransferase as well as a statistically significant decrease in albumin and cholesterol at the Day 14 evaluation. None of these variances were observed at the Day 4 *post partum* evaluation. A statistically significant decrease in plasma bilirubin was observed in females from all treatment groups at the Day 4 *post partum* evaluation. In the absence of a true dose-related response, these intergroup differences were considered of no toxicological significance.

There were no significant findings in the haematology parameters measured and from urinalysis in any dose group.

Effects in Organs

A statistically significant decrease in absolute thymus weight was reported in males from the high dose group. No such effects were detected in females at the high dose level, animals of either sex treated with 50 or 350 mg/kg bw/day or recovery animals following fourteen days without treatment.

Examination of bone marrow revealed an increase in the severity of adipose infiltration of the marrow, indicative of marrow hypoplasia in males from the high dose group only. There was no evidence of regression of the condition among recovery high dose animals following an additional fourteen days without treatment.

In 4 females of the high dose group, lymphoid atrophy of the thymus was observed. Three females from the mid dose group were also considered to have displayed atrophy. Two males (one of them killed *in extremis*) from the high dose group also displayed atrophy in the thymus. Thymic atrophy was also observed sporadically in *post partum* and lactating female rats.

No other significant macroscopic, histopathological or morphological abnormalities were reported in the study.

Effects on Reproduction

There was no significant difference in mating performance, fertility, gestation length or litter response between treated and control animals with evidence of mating observed in the majority of animals within 4 days of pairing.

Effects on offspring

No abnormalities were reported in the mean numbers of corpora lutea, litter size at Day 1 or Day 4 in treated groups.

No significant variations were observed in the bodyweight, bodyweight gain, or clinical signs of offspring of treated animals. Litters of the low dose group displayed a statistically significant decrease in the number of offspring passing surface righting reflex on Day 1. However, this was not observed in the offspring of any other treated groups.

Remarks – Results

Findings of toxicological significance in males of the high dose group were considered to be: reduced bodyweight gains, a statistically significant decrease in total protein levels which persisted in males from the high dose recovery group as well as an increased incidence of higher grade adipose infiltration of the bone marrow which persisted in high dose recovery males. The decreased weight of the thymus in males of the high dose group was not considered to be an adverse effect given the thymus weights in the high-dose recovery males were comparable to controls. The reduced bodyweight observed in males from the mid dose group was not considered to be an adverse effect.

In females, treatment-related adverse effects were limited to lymphoid atrophy of the thymus in 4 animals in the high-dose group. Similar observations were made in 3 females from the mid dose group.

There were no treatment-related effects on reproductive parameters.

Litters of the low dose group displayed a statistically significant decrease in the number of offspring passing surface righting reflex on Day 1 but in the absence of a dose-response in higher dose groups, this effect was not considered to be of toxicological significance.

CONCLUSION
NICNAS noted that there are some statistically significant decreases in the number of offspring from the group treated with 50 mg/kg bw/day passing surface righting reflex on Day 1. In the absence of a dose-related response this was considered of no toxicological importance.

The No Observed Effect Level (NOEL) was established as 50 mg/kg bw/day in this study, based on the lack of adverse effects seen at high and mid dose levels.

TEST FACILITY Harlan Laboratories (2009h)

B.3. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical at > 90%

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.
Plate incorporation procedure

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA$^-$
Metabolic Activation System	S9 fraction from phenobarbitone/β-naphthoflavone-induced rat liver.
Concentration Range in Main Test	a) With metabolic activation: 50-5000 µg/plate b) Without metabolic activation: 50-5000 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 µg/plate	>5000 µg/plate	≥ 1500 µg/plate	Negative
Test 2	-	-	≥ 1500 µg/plate	Negative
Present				
Test 1	>5000 µg/plate	>5000 µg/plate	≥ 1500 µg/plate	Negative
Test 2	-	-	≥ 1500 µg/plate	Negative

Remarks - Results
The notified chemical did not induce an increase in revertant colonies.

Precipitation was observed at concentrations of ≥ 1500 µg/plate in the presence and absence of metabolic activation in both tests but did not prevent scoring.

There was no evidence of cytotoxicity or reduced growth of the background lawn.

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

TEST FACILITY Harlan Laboratories (2009i)

B.4. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical at > 90%

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Species/Strain Chinese Hamster
Cell Type/Cell Line CHL
Metabolic Activation System S9 fraction from phenobarbitone/β-naphthoflavone-induced rat liver.
Vehicle Dimethyl sulfoxide (DMSO)
Remarks - Method The notified chemical was diluted to 5% in DMSO in Test 1 and 2% in DMSO in Test 2. No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 2.5, 5, 10*, 20*, 30*, 40 MMC 0.1*^	6 hours	24 hours
Test 2	0*, 1.25, 2.5, 5.0*, 7.5*, 10*, 15 CP 5*^	24 hours	24 hours
Present			
Test 1	0*, 10, 20, 40, 60*, 80*, 120* MMC 0.05^	6 hours	24 hours
Test 2	0*, 20*, 40*, 60*, 80, 120, 160 CP 5^	6 hours	24 hours

*Cultures selected for metaphase analysis.
MMC = Mitomycin, CP = Cyclophosphamide
^ Positive Controls

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*		
	Cytotoxicity in Main Test	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	30	≥ 30	Negative
Test 2	10	-	Negative
Present			
Test 1	120	≥ 80	Negative
Test 2	80	≥ 80	Negative

Remarks - Results

A dose range test was conducted at concentrations ranging from 19.5 to 5000 µg/mL. Cytotoxicity was observed and dose ranges of 0.63 to 40 µg/mL in the absence of metabolic activation and 2.5 to 120 µg/mL in the presence of metabolic activation were selected.

In Experiment 1, 20% mitotic inhibition was observed at 30 µg/mL and there were no metaphases present at 40 µg/mL in the absence of metabolic activation. In the presence of metabolic activation, 39% mitotic inhibition was observed at 120 µg/mL.

In Experiment 2, 32% and 70% mitotic inhibition was observed at 10 and 15 µg/mL respectively in the absence of metabolic activation. 72% mitotic inhibition was observed at 80 µg/mL in the presence of metabolic activation.

Mitomycin was dosed at 0.1 and 0.05 µg/mL for cultures exposed for 6 (18) or 24 hours in the absence of metabolic activation.

Cyclophosphamide was dosed at 5 µg/mL for cultures exposed for 6(18) hours in the presence of metabolic activation. Both positive controls confirmed the sensitivity of the test system.

The test material did not induce any statistically significant increases in the frequency of cells with aberrations, or in the numbers of polyploid cells.

CONCLUSION The notified chemical was not clastogenic to CHL cells treated *in vitro* under the conditions of the test.

TEST FACILITY Harlan Laboratories (2009j)

B.5. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical at > 90%

METHOD OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
EC Directive 2000/32/EC B.17 Mutagenicity - In vitro Mammalian Cell Gene Mutation Test.

Species/Strain	Mouse
Cell Type/Cell Line	L5178Y TK+/- 3.7.2c
Metabolic Activation System	Rat S9 fraction from phenobarbitone/β-napthoflavone induced rat liver
Vehicle	Dimethyl sulfoxide
Remarks - Method	Due to the cytotoxicity of the notified chemical observed in the Chromosome Aberration test, the maximum concentration tested was 1000 µg/mL. The selective agent used was Trifluorothymidine. The selection time was not recorded.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*
Absent			
Test 1	0, 1.25, 2.5, 5, 10, 15, 20, 25, 30	4 hours	2 days
Test 2	0, 1.25, 2.5, 5, 10, 15, 20, 25, 30	24 hours	2 days
Present			
Test 1	0, 2.5, 5, 10, 20, 30, 40, 50, 60	4 hours	2 days
Test 2	0, 1.25, 2.5, 5, 10, 20, 30, 40, 50	4 hours	2 days

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 20	> 20	None	Negative
Test 2	-	-	None	Negative
Present				
Test 1	> 40	> 40	None	Negative
Test 2	-	-	None	Negative

Remarks - Results The vehicle controls had acceptable mutant frequency values that were within the normal range for the L5178Y cell line at the TK +/- locus. The positive control materials induced marked increases in the mutant frequency indicating the satisfactory performance of the test and of the activity of the metabolising system.

The test material did not induce any toxicologically significant or dose-related increases in the mutant frequency at any dose level, either with or without metabolic activation, in either the first or the second experiment

using a dose range where the maximum dose level was limited by cytotoxicity of the notified chemical to the cell line.

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

TEST FACILITY Harlan Laboratories (2009k)

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

TEST SUBSTANCE Notified chemical at > 90%

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 DMSO
Remarks - Method No significant protocol deviations.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	1676.38	1
10	4999.67	2.98
25	4242.02	2.53
50	5181.11*	3.09*

Remarks - Results *The DPM for 1 animal in the group treated with the notified chemical at 50% was 642.43 (less than half the DPM for that of control animals). This was considered to be an outlier and when omitted the mean DPM for the group administered 50% of the notified chemical was determined to be 6315.78 which is equivalent to an SI = 3.77.

A SI score greater than 3 is considered to indicate a potential for skin sensitisation. However, statistical analysis in accordance with the methods of Hothorn and Vohr (2010) (cited by the notifier) found that none of the lower bound Stimulation Indices were greater than 3 for any of the concentrations tested.

In addition, a Dunnett's comparison of the DPM measured for animals treated with 25% and 50% notified chemical found that there was no statistically significant increase when compared to the DPM reported in the animals treated with 10% notified chemical.

A positive control was not tested concurrently as part of this test. However, a previous test using α-Hexylcinnamaldehyde at 15% concentration in DMSO produced a stimulation index of 5.73 confirming the sensitivity of the assay to predict sensitising potential.

CONCLUSION A lymphocyte proliferative response resulting in a stimulation index slightly greater than 3 was observed in animals treated with the notified chemical at 50% concentration. However, statistical analysis demonstrated the increase was not statistically significant nor was there a dose-response relationship. Therefore, the notified chemical was not considered to have demonstrated a potential for skin sensitisation under the conditions of the test.

TEST FACILITY Harlan Laboratories (2009g)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE: Notified chemical

METHOD
- Inoculum: Activated sewage sludge micro-organisms from a municipal STP
- Exposure Period: 28 days
- Auxiliary Solvent: None
- Analytical Monitoring: Dissolved organic carbon determination
- Remarks - Method: The test material and reference substance (sodium benzoate) were added at nominal levels of 10 mg total organic carbon/L to inoculated mineral medium. The test material was dissolved in a tetrahydrofuran stock solution and adsorbed onto silica gel prior to dispersion in culture medium (to aid the dispersion of the test material in the test medium and to increase the exposure area to the test organisms). The solvent was removed before use. Silica gel was also added to the control and standard material vessels, and the toxicity control, in order to maintain consistency between all materials. All tests were conducted in duplicate except for the toxicity control where only a single flask was used.
  The test vessels were incubated at 21°C in darkness for 28 days. Degradation was determined by measuring the amount of CO_2 produced, corrected with the blank inoculum, and expressed as % of theoretical amount of CO_2 ($ThCO_2$).

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
6	0	6	60
14	0	14	86
21	4	21	91
28	5	28	95

Remarks - Results: The test material attained 5% degradation after 28 days and therefore cannot be considered to be readily biodegradable under the conditions of OECD Guideline 301B. All validation criteria given in the OECD Test Guideline were satisfied, thus validating the test.

The toxicity control test attained 25% degradation by day 14 and the notified chemical is thus considered to be non-inhibitory. Sodium benzoate attained 95% degradation after 28 days thereby confirming the suitability of the inoculum and test conditions, and > 60% degradation in a 10-day window thereby validating the test.

CONCLUSION: The notified chemical is not readily biodegradable

TEST FACILITY: Harlan Laboratories Ltd (2009p)

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

Species	Juvenile rainbow trout (*Onchorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	140 mg $CaCO_3$/L
Analytical Monitoring	HPLC was used for determination of the concentration of the notified chemical.
Remarks – Method	After a range-finding test was conducted, two separate pilot tests were conducted with rainbow trout (7 for each test) in water accommodated fractions (WAFs), due to the low water solubility of the notified chemical, at a nominal loading rate of 100 mg/L. The notified chemical (2100 g) was added to the surface of dechlorinated tap water (21 L) to achieve the loading rate of 100 mg/L. The test medium was stirred for 23 h and allowed to stand for 1 h. The WAF was removed by mid-depth siphoning (discarding the first 75–100 mL). Microscopic inspection of the WAF showed no micro-dispersions or undissolved test material to be present. The fish, introduced to the WAF and maintained at 14°C under semi-static conditions for 4 days (pH 7.6–8.0, 9.3–10.1 mg O_2/L), were observed for mortality and sub-lethal effects.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Loading rates	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	0	7	0	0	0	0	0
100	≤ 0.128	14	0	0	0	0	0

LC50	> 100 mg/L at 96 hours (based on loading rates).
NOEL	100 mg/L at 96 hours (based on loading rates).
Remarks – Results	There were no sub-lethal effects of exposure observed in 14 fish exposed to a 100 mg/L loading rate WAF for a period of 4 days. The actual concentrations of the notified chemical in the duplicate test vessels were determined by HPLC to be 0.046 mg/L and 0.128 mg/L at 0 h, 0.039 mg/L and 0.109 mg/L at 24 h, and less than the limit of quantification (LOQ = 0.010 mg/L) at 76 and 96 h. The measured concentrations at 0 h and 24 h were considered to possibly be due to micro-emulsions of the test material present, despite the use of a glass wool plug when siphoning off the WAF. Therefore, these concentration values should be treated with caution.

CONCLUSION	The notified chemical is not harmful to fish up to the limit of its solubility in water
TEST FACILITY	Harlan Laboratories Ltd (2009l)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE

METHOD	OECD TG 202 *Daphnia* sp., Acute Immobilisation Test – semi-static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	HPLC was used for determination of the concentration of the notified chemical
Remarks - Method	A limit test was conducted at a single loading rate of 100 mg/L (WAF, as per C.2.1. Acute toxicity to fish), under semi-static conditions. Four replicates of 250 mL WAFs and the control were prepared, and each had 5 daphnia added. Microscopic inspection of the WAF showed no micro-

dispersions or undissolved test material to be present. The daphnia were observed for immobilisation over two days (test conditions: artificial light dark cycle of 16 to 8 hours, 21–22°C, pH 8–8.1, 8.6–9.6 mg O_2/L). Daphnia unable to swim within 15 seconds of gentle agitation were considered to be immobile. The probit method was used to analyse the positive control's (potassium dichromate) data.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Loading rates	*Actual*		*24 h*	*48 h*
0	0	5	0	0
100	LOQ–0.0241	20	0	2

EL50 > 100 mg/L at 48 hours. Based on loading rates.
NOEL 100 mg/L at 48 hours. Based on loading rates.
Remarks - Results The immobilisation of 2/20 daphnids observed at 48 h is within the ≤ 10% limit accepted by the guidelines. There were no immobilised daphnia in the control group and the dissolved oxygen in the control group and test vessels were ≥ 3 mg/L, thus validating the test.

The actual concentrations of the notified chemical in the test vessels were determined by HPLC to range between 0.0.024 mg/L and 0.012 mg/L from 0 h to 24 h, and less than the limit of quantification (LOQ = 0.010 mg/L) at 48 h. These concentrations are just above the LOQ and were only just quantifiable from the baseline noise, and therefore these results should be treated with caution. The EC50 of 0.78 mg/L and NOEC of 0.32 mg/L for the positive control were within the normal range for this reference material.

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

TEST FACILITY Harlan Laboratories Ltd (2009m)

C.2.3. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 211 *Daphnia magna*, Reproduction test – semi-static
Species *Daphnia magna*
Exposure Period 21 d
Auxiliary Solvent None
Water Hardness Total hardness 122–152 mg $CaCO_3$/L
Analytical Monitoring HPLC was used for determination of the concentration of the notified chemical
Remarks - Method *Daphnia magna* (10 replicates of a single daphnid per group) were exposed to the test substance over a range of nominal loading rates of 1.0, 3.2, 10, 32, and 100 mg/L (WAF, as per C.2.1. Acute toxicity to fish) for a period of 21 days, under semi-static conditions (test conditions: artificial light dark cycle of 16 to 8 hours, 20–22°C, pH 7.7–8.9, 8.0–9.7 mg O_2/L). Microscopic inspection of the WAF showed no micro-dispersions or undissolved test material to be present. The daphnia were fed with algal suspension and numbers of live and dead (adult and young) were monitored daily. The EL50 (reproduction) value was calculated by the maximum-likelihood probit method.

RESULTS

Day 21				
Concentration (mg/L)		Mean Percent Adult Survival	Mean Number of Living Offspring Produced per female – cumulative (SD)a	Mean Total Body Length in mm (SD)
Nominal	Actual			
Control	< LOQ	90	78 (9.1)	4.4 (0.2)
1.0	< LOQ	90	68 (12)	4.1 (0.2)
3.2	< LOQ–0.015	90	31* (12)	3.8* (0.3)
10	< LOQ	90	47* (15)	4.1 (0.3)
32	< LOQ–0.027	80	31* (8.6)	3.9* (0.3)
100	< LOQ–0.018	60	29* (7.0)	4.1 (0.3)

*Denotes a significant difference (P ≥ 0.05) from the control.
[a]SD = standard deviation

EL50 (reproduction)	16 mg/L at 21 d. (based on loading rates).
LOEL (reproduction)	3.2 mg/L at 21 d. (based on loading rates).
NOEL (reproduction)	1.0 mg/L at 21 d .(based on loading rates).
Remarks - Results	A statistically significant impairment of reproduction was observed at the loading rates greater than 3.2 mg/L after 21 days. There were no other sub-lethal effects (size and colour) observed at any of the concentrations tested when compared to the control. In the control, the mortality of the parent animals was 10% and the mean number of live offspring produced per surviving adult was 78, thus validating the test. Over all the test concentrations, the actual concentrations of the notified chemical were determined by HPLC to be less than the limit of quantification (LOQ = 0.010 mg/L) – to a maximum of 0.027 mg/L. These concentrations are just above the LOQ and were only just quantifiable from the baseline noise and, therefore, the values should be treated with caution.
CONCLUSION	The notified chemical is toxic to aquatic life with long lasting effects up to the limit of its solubility in water
TEST FACILITY	Harlan Laboratories Ltd (2009n)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test
Species	*Desmodesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 100 mg/L (WAF) Actual: 0.11–0.15 mg/L
Auxiliary Solvent	None
Water Hardness	0.15 mmol Ca^{2+} & Mg^{2+}/L
Analytical Monitoring	HPLC was used for determination of the concentration of the notified chemical
Remarks - Method	After a range-finding test, algae with a density of 4.18×10^3 cells per mL were exposed to a WAF of the test material at a single nominal loading rate of 100 mg/L (6 replicates). Microscopic inspection of the WAF showed no micro-dispersions or undissolved test material to be present. The test mixtures were irradiated 24 h/day at pH 7.0–8.0 and 24 ± 1°C for a period of 72 hours. The positive control was provided by potassium dichromate (0.0625–1.0 mg/L). A student's t-test incorporating Bartlett's test for homogeneity of variance was carried out on the data to determine

any statistically significant differences between test and control groups.

RESULTS

Biomass		*Growth*	
E_bL_{50} mg/L at 72 h	*NOEL mg/L*	*E_rL_{50} mg/L at 72 h*	*NOEL mg/L*
> 100	100	> 100	100

Remarks - Results

Under the same conditions as for the test substance, the E_bC_{50} and E_rC_{50} values for the positive control were 0.30 mg/L (95% CI: 0.26–0.34 mg/L) and 0.52 mg/L (95% CI: 0.43–0.62) respectively, which were within the normal range for this reference material. Cell growth of the control increased 94-fold after 72 h, thus validating the test.

The actual concentrations of the notified chemical in the test medium were determined by HPLC to be less than the limit of quantification (LOQ = 0.010 mg/L) at 0 h, and in the range of 0.11–0.15 mg/L at 72 h. Therefore, these values should be treated with caution.

CONCLUSION

The notified chemical is not harmful to algae up to the limit of its solubility in water

TEST FACILITY

Harlan Laboratories Ltd (2009o)

C.2.5. Inhibition of microbial activity

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 209 Activated Sludge, Respiration Inhibition Test.

Inoculum

Activated sewage sludge from a municipal STP

Exposure Period

3 hours

Concentration Range

Nominal: 1000 mg/L

Actual: 1000 mg/L

Remarks – Method

After a range-finding test was conducted, tests (in triplicate) were conducted by exposing activated sewage sludge to 1000 mg/L dispersion of the test substance and synthetic sewage for a period of 3 h at 21°C. Reference material (3,5–dichlorophenol) at concentrations of 3.2, 10, and 32 mg/L was used in order to confirm the suitability of the inoculum. The test water had a total hardness of 140 mg $CaCO_3$/L.

RESULTS

IC50

> 1000 mg/L

NOEC

1000 mg/L

Remarks – Results

Variation in respiration rates of control after 3 h contact time was ± 2%, and the IC_{50} (3- hour contact time) for reference substance 3,5-dichlorophenol was 7.8 mg/L, thus validating the test.

CONCLUSION

The notified chemical is not expected to be harmful to microbial respiration.

TEST FACILITY

Harlan Laboratories Ltd (2009q)

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