File No: STD/1585

June 2016

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Z-148

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (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 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.

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1585	Lubrizol International Inc.	Z-148	Yes	≤ 100 tonnes per annum	Component of automatic transmission fluid

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

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

Hazard classification	*Hazard statement*
Skin Sensitisation (Category 1)	H317 – May cause an allergic skin reaction

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase(s):
R43: May cause sensitisation by skin contact

Human health risk assessment
Under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

When used in the proposed manner, with labelling of products regarding the potential for skin sensitisation during use, the notified chemical is not considered to pose an unreasonable risk to public health.

Environmental risk assessment
On the basis of the reported use pattern and the low expected aquatic exposure, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Skin Sensitisation (Category 1): H317 – May cause an allergic skin reaction

The above should be used for products/mixtures containing the notified chemical, if applicable, based on the concentration of the notified chemical present and the intended use/exposure scenario.

Health Surveillance

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

(Material) Safety Data Sheet

- The (M)SDS provided by the notifier should be amended to include relevant hazard classification, label elements and personal protection information for skin sensitisation.

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical during reformulation and/or repackaging processes:
  - Enclosed, automated processes, where possible
  - Use of well ventilated environments

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical during reformulation and/or repackaging processes:
  - Avoid contact with skin and eyes

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical:
  - 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 (M)SDS should be easily accessible to employees.

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

Public Health

- Products available to the public containing the notified chemical should be labelled with appropriate warnings due to the potential of the chemical to cause skin sensitisation including:
  - Avoid contact with skin
  - Wear protective gloves when mixing or using
  - Wash hands after use

Disposal

- Where reuse or recycling are not appropriate, dispose of the notified chemical in an environmentally sound manner in accordance with relevant Commonwealth, state, territory and local government legislation.

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 component of automatic transmission fluid, 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.

No additional secondary notification conditions are stipulated.

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Lubrizol International, Inc. (ABN: 52 073 495 603)
28 River Street
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, other names, CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, use details, manufacture/import volume and site of manufacture/reformulation.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
USA, China and New Zealand

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Z-148

MOLECULAR WEIGHT
> 1,000 Da

3. COMPOSITION

DEGREE OF PURITY
100 %

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (> 1% BY WEIGHT)
None

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Light brown, viscous liquid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	< -20 ± 3 °C (<253 ± 3 K)	Measured
Boiling Point	281 °C (554 K) at 101.3 kPa	Measured
Density	898 kg/m^3 at 20 ± 0.5 °C	Measured
Vapour Pressure	2.4×10^{-5} kPa at 25 °C 2.1×10^{-3} kPa at 100 °C	Measured
Water Solubility	< 2.04×10^{-6} g/L at 20 °C	Measured
Hydrolysis as a Function of	Not determined	Contains hydrolysable functionalities;

pH		however, not expected to rapidly hydrolyse under environmental conditions (pH 4–9)
Partition Coefficient (n-octanol/water)	log Pow > 10.0	Measured
Adsorption/Desorption	log K_{oc} > 5.63	Measured
Dissociation Constant	Not determined	Expected to be ionised under environmental conditions (pH 4–9)
Flash Point	210 ± 2 °C	Measured
Autoignition Temperature	386 ± 5 °C	Measured
Explosive Properties	Predicted negative	Contains no functional groups that would imply explosive properties
Oxidising Properties	Predicted negative	Contains no functional groups that would imply oxidising properties

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

Reactivity
The notified chemical is normally stable at moderately elevated temperatures and pressures. The notified chemical is not compatible with strong oxidising agents.

Physical hazard classification

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. The notified chemical will be imported as a component of transmission fluid additives (at ≤17% concentration) for reformulation into end use products at ≤1.5% concentration.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	30–40	55–65	70–80	85–95	90–100

PORT OF ENTRY
Western Australia, Queensland and Victoria

IDENTITY OF MANUFACTURER/RECIPIENTS
Lubrizol International Inc.

TRANSPORTATION AND PACKAGING
The notified chemical will be imported into Australia and transported via intermediate bulk container (~1,250 L). Smaller quantities will be transported in steel drums (~208 L).

USE
The notified chemical is a friction modifier for automatic transmission fluids (ATFs), which helps fully formulated ATFs to improve lubricating qualities by reducing the surface friction of the lubricated parts of the machine (automobile). It will be formulated with other chemicals to produce a 'concentrate' or 'additive package'. The notified chemical will be imported in products at ≤17% concentration. Customers of the notifier will reformulate the concentrate containing the notified chemical by diluting it with mineral oil and possibly other components to make end-use products containing the notified chemical at ≤1.5% concentration.

OPERATION DESCRIPTION
The concentrate/additive package containing the notified chemical (at ≤17% concentration) will be reformulated after importation.

Reformulation
After importation, it is expected that the additive packages containing the notified chemical at ≤17% concentration will be transferred into blending tanks (containing mineral oil and other additives) using automated, well ventilated and enclosed processes. After blending, it is expected that the end-use product containing the notified chemical at ≤1.5% concentration will be packaged using automated processes. The resulting ATFs containing the notified chemical at ≤1.5% concentration may be supplied in bulk for industrial users or in smaller containers for use in commercial service applications or do-it-yourself (DIY) users.

End use
ATFs containing ≤1.5% of the notified chemical will be supplied in bulk to automobile manufacturers who will use it for factory fill applications. The notifier states that the notified chemical containing lubricants will also be used by commercial automotive and industrial engine service outlets and to a lesser extent by the public. Use by the public will involve the transmission fluids being manually decanted into the transmission fluid tank.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment
Transport and storage workers may come into contact with the notified chemical at ≤17% concentration only in the event of accidental rupture of containers.

Reformulation
Dermal and ocular exposure of workers to the notified chemical at ≤17% concentration may occur during reformulation when connecting and disconnecting hoses and during sample testing. The blending process and packaging is expected to be automated and within a closed system.

Dermal and ocular exposure to workers should be mitigated through the use of personal protective equipment (PPE) including protective clothing, impervious gloves, goggles and respiratory protection. Inhalation exposure is not expected given the enclosed systems and low vapour pressure of the notified chemical. However, respiratory protection is expected for area in poorly ventilated areas or where dusts/mists are generated and released.

End-use
At automotive service centres, professional users such as mechanics may experience dermal or ocular exposure to the transmission fluids containing the notified chemical at ≤1.5% concentration when transferring transmission fluids to vehicles. The potential for dermal and ocular exposure may be mitigated through the use of PPE (e.g. gloves, protective clothing, and goggles).

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Workers involved in blending operations	1–3	4–5
Workers involved in packaging operations	2–5	1–3
Distribution	0–2	100–225

EXPOSURE DETAILS

Transport and storage
Transport and warehouse workers will only be exposed to the notified chemical in the event of an accident.

Reformulation
Dermal and ocular exposure to the notified chemical (≤17% concentration) is possible when blending operators are connecting and disconnecting pump lines to storage tanks or blending vessels. The blending facilities are expected to be largely automatic and enclosed systems with ventilation and control systems in place for

accidental spills and wastewater treatment. Dermal exposure is possible when cleaning up spills or leaks and during maintenance of the blending equipment. The use of personal protective equipment (PPE) such as coveralls, safety glasses, impervious gloves and respirators by the workers, and a high degree of automation will minimise the worker's exposure to the notified chemical.

Transfer of the finished transmission fluids containing the notified chemical at ≤1.5% concentration to packaging will mainly be performed by automated processes; hence, exposure to workers is expected to be minimal. Inhalation exposure is expected to be low given the low vapour pressure of the notified chemical (2.4×10^{-5} kPa at 25 °C), unless aerosols or mists are generated.

Quality assurance sampling
At reformulation facilities samples will be taken from blending vessels for quality assurance testing. Dermal exposure to the notified chemical (≤17% concentration) may occur during sampling. To minimise exposure, staff are expected to wear gloves, eye protection and long sleeved coats.

End use
Operators at the garage/automobile workshops and DIY users may come into contact with the notified chemical at ≤1.5% concentration. The exposure may occur during the manual transfer of the transmission fluid from the product container into the transmission fluid tank or during the cleaning and maintenance of equipment. It is expected that at the professional end-use site the processes will be mostly enclosed or supplied engineering controls such good general ventilation to reduce exposure from splashes, mists and vapours (if generated). Exposure will be minimised by the use of personal protective equipment (PPE) such as gloves, goggles and protective clothing.

6.1.2. Public Exposure
Dermal and ocular exposure to the notified chemical may occur to members of the public when adding ATFs containing the notified chemical at ≤1.5% concentration to vehicles. Given the low concentration (≤1.5%) of the notified chemical in the transmission fluid and that transmission fluid is changed mostly at the commercial garages by professional mechanics, potential for exposure to the notified chemical is expected to be low. If PPE is used, exposure of the public is expected to be of a similar or lesser extent than that experienced by workers using products containing the notified chemical.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical are summarised in the following table. For full details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rabbit, eye irritation	mildly irritating
Rabbit, acute dermal irritation	mildly irritating
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
Rat, repeated dose toxicity	NOAEL 1,000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* (chromosome aberration, cultured human lymphocyte)	non clastogenic

Toxicokinetics, metabolism and distribution
For dermal absorption, molecular weights below 100 Da. are favourable for absorption and molecular weights above 500 Da do not favour absorption (ECHA, 2014). In substances with log P values above 6, the rate of transfer between the stratum corneum and the epidermis will be slow and will limit absorption across the skin (ECHA, 2014). The notified polymer is of high molecular weight (> 1,000 Da), and partition coefficient (log Pow > 10 at 20 °C) and low water solubility < 2.04×10^{-6} g/L at 20 °C). Therefore absorption across biological membranes is expected to be low.

Acute toxicity
The notified chemical was found to have low acute oral and dermal toxicity in rats.

Irritation and sensitisation
Based on studies conducted in rabbits, the notified chemical was considered to be slightly irritating to the skin and eyes. No acute dermal toxicity was observed in rats. The notified chemical was a skin sensitiser in a local lymph node assay (LLNA) in mice, with reported stimulation indices of 7.47, 13.16, and 13.05 at 25, 50 and 100% concentration, respectively. An EC3 value was not determined by the study authors; however, based on the available data it is <25%.

Repeated dose toxicity
A 28 day repeat dose study by oral gavage was conducted in rats with the notified chemical at dose levels of 0, 100, 400 and 1,000 mg/kg bw/day. No toxicologically significant findings could be attributed to the notified chemical.The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1,000 mg /kg bw/day.

Mutagenicity/Genotoxicity
The notified chemical was not considered to be mutagenic in a bacterial reverse mutation study and was not considered to be clastogenic in an *in vitro* mammalian (human lymphocyte, cultured) chromosome aberration test.

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

Hazard classification	***Hazard statement***
Skin Sensitisation (Category 1)	H317 – May cause an allergic skin reaction

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase(s):
R43: May cause sensitisation by skin contact

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Based on the information available the notified chemical has potential to be a slight eye irritant. The critical health effect of the notified chemical is as a skin sensitiser.

During reformulation, workers may be exposed to the notified chemical at ≤17% concentration. As the EC3 is <25% there is a potential risk of sensitising effects at the concentrations workers are exposed to during reformulation. The notifier states that engineering controls such as enclosed and automated processes and local ventilation will be implemented where possible and appropriate PPE (coveralls, impervious gloves, eye protection and respiratory protection) will be used to limit workers' exposure.

During end-use professional workers may be exposed to the notified chemical at ≤1.5% concentration when manually decanting the notified chemical containing transmission fluid into the transmission fluid tank. At this end-use concentration, the potential risk of skin sensitisation cannot be ruled out. Appropriate PPE (coveralls, impervious gloves, eye protection) will be used to limit workers' exposure.

Therefore, under the occupational settings described, the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

6.3.2. Public Health

The public (DIY users) will be exposed to the notified chemical at low concentration (≤1.5%) and on an infrequent basis. The risk of skin sensitisation from use of the notified chemical has not been quantitatively determined; however, as the EC3 is <25%, the potential for skin sensitisation cannot be ruled out.

Warnings on the product labels regarding the potential for skin sensitisation would reduce the exposure and risk to the public. Therefore, based on the information available and provided the products containing the notified chemical are labelled appropriately, the risk to the public associated with use of the notified chemical at ≤ 1.5% in automatic transmission fluids is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported into Australia as a component of lubricant additive packages for reformulation into automotive transmission fluids. No significant release of the notified chemical is expected from transportation and storage except in the unlikely event of accidental spills or leaks.

Local blending and repackaging of the additive containing the notified chemical into automotive transmission fluids is expected to occur within enclosed automated systems. Blending tanks and equipment are expected to be cleaned with mineral oil, which is expected to be recycled during subsequent blending. Accidental spills and leaks during normal blending and packaging procedures will be contained and collected for recycling where appropriate, or disposed of in accordance with local government regulations, most likely to landfill.

RELEASE OF CHEMICAL FROM USE

The finished products containing the notified chemical will be used as a component of automotive transmission fluids. Release during use may arise from spills when pouring lubricants into automotive vehicles or from vehicle leaks, and is expected to be very low.

RELEASE OF CHEMICAL FROM DISPOSAL

After reformulation, empty import containers containing residues of the notified chemical are expected to be sent to a container recycling facility for reconditioning. Empty containers will be washed with mineral oil and the wastes containing the notified chemical collected for disposal in accordance with local government regulations, most likely to landfill. Therefore, the release of the notified chemical to surface waters from the cleaning of empty containers is expected to be limited.

The major release of the notified chemical to the environment is expected from inappropriate disposal of waste or used lubricants. Lubricant products containing the notified chemical will be poured into automotive vehicles at automotive service centres or by do-it-yourself (DIY) consumers. A survey by the Australian Institute of Petroleum (AIP, 1995) indicates that of the annual sales of engine oils in Australia, 60% of oils are potentially recoverable (i.e. not burnt in the engines during use). This report also indicates that around 86% of oil changes take place in specialised automotive service centres, where old oil drained from crankcases is disposed of responsibly (e.g. oil recycling). Assuming this is the case, negligible release of the notified chemical should result from these professional activities. The remaining 14% of oil is used by DIY consumers. In these cases, some of the used oil would either be left at transfer stations where it is likely to be recycled, or deposited into landfill.

According to a survey tracing the fate of used lubricating oil in Australia (Snow, 1997), approximately 20% of oil used by DIY consumers is collected for recycling, approximately 25% is buried or disposed of to landfill, 5% is disposed of into stormwater drains, and the remaining 50% is used in treating fence posts, killing grass and weeds or disposed of in other ways. In a worst case scenario involving the 14% of oil used by DIY consumers, up to 0.7% (14% × 5% stormwater disposal) of the total import volume of the notified chemical (or 700 kg) may enter the aquatic environment via disposal to stormwater drains. Since the use of the engine oils will occur throughout Australia, all releases resulting from use or disposal of used oil will be very diffuse. Release of the notified chemical in neat concentrations is unlikely except as a result of transport accidents.

7.1.2. Environmental Fate

Based on the results of a biodegradability study, the notified chemical is not expected to be readily biodegradable (16% in 28 days). For details of the environmental fate study, please refer to Appendix C. The notified chemical, however, is not expected to be bioaccumulative based on its high molecular weight and ultimate biodegradability.

The majority of the notified chemical will be thermally decomposed during use, or collected for recycling and re-refined. Up to 0.7% of annual import volume of the notified chemical (or 700 kg) may be released to stormwater drains from incorrect disposal of wastes and used engine oils by DIY consumers. In surface waters, the majority of the notified chemical is expected to partition to soil and sediment due to its high

adsorption/desorption coefficient (log K_{OC} > 5.63). In landfill and in soil and sediment, the notified chemical is expected to eventually degrade by biotic and abiotic processes to form water and oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

For the worst case scenario, the percentage of the imported quantity of notified chemical inappropriately disposed to stormwater drains is estimated to be 0.7%. That is, 14% (fraction collected by DIY users) × 5% (fraction disposed to stormwater). The release of the notified chemical may be up to 700 kg/year (= 100 tonnes/year × 0.7%). In this worst case scenario, it is assumed that the release goes 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 250 × 10^6 m^3, the calculated PEC will be up to 2.80 µg/L. This result reflects a worst-case scenario upper limit, as in reality releases of the notified chemical will be distributed over multiple regions and it 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 to fish up to water solubility limit
Daphnia Toxicity	48 h EL50 > 100 mg/L (WAF*)	Not harmful to aquatic invertebrates up to water solubility limit
Algal Toxicity	72 h EL50 > 1 mg/L (WAF*)	Not harmful to algae up to water solubility limit
Inhibition of Bacterial Respiration	3 h IC50 > 1,000 mg/L	Not inhibitory to microbial respiration

*Water accommodated fraction

Based on the above ecotoxicological endpoints, the notified chemical is not expected to be harmful to aquatic life up to the limit of its water solubility. Therefore, the notified chemical is not formally classified under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009) for acute and chronic toxicities.

7.2.1. Predicted No-Effect Concentration

A predicted no-effect concentration (PNEC) for the aquatic compartment has not been calculated since the notified chemical is not considered to be harmful to aquatic organisms up to the limit of its solubility in water. Also no significant release of the notified chemical to the aquatic environment is expected.

7.3. Environmental Risk Assessment

The Risk Quotient (Q = PEC/PNEC) of the notified chemical has not been calculated as a PNEC is not available due to the low potential for release to the aquatic compartment based on its assessed use pattern in engine oils. Although the notified chemical is not readily biodegradable, it is not expected to bioaccumulate due to low water solubility. On the basis of the maximum annual importation volume, low expected aquatic exposure and assessed use pattern in engine oils, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point < -20 ± 3 °C (<253 ± 3 K)

Method	OECD TG 102 Melting Point/Melting Range. EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks	The experiments were carried out by the pour point method.
Test Facility	Harlan (2015a)

Boiling Point 281 °C (554 K) at 101.3 kPa

Method	OECD TG 103 Boiling Point. EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks	The differential scanning calorimetry (thermal analysis) method was used. The test item had a broad boiling range with an extrapolated onset of approximately 400 °C (673K).
Test Facility	Harlan (2015a)

Density 0.898×10^3 kg/m^3 at 20.0 °C ± 0.5 °C

Method	OECD TG 109 Density of Liquids and Solids. EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks	The density was determined using a glass pycnometer. The test result is mean value of two independent tests.
Test Facility	Harlan (2015a)

Vapour Pressure 2.4×10^{-5} kPa at 25 °C
2.1×10^{-3} kPa at 100 °C

Method	OECD TG 104 Vapour Pressure. EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks	Determined using the vapour pressure balance method
Test Facility	Harlan (2014a)

Water Solubility < 2.04×10^{-6} g/L at 20 °C

Method	OECD TG 105 Water Solubility. EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks	Flask Method
Test Facility	Harlan (2015a)

Partition Coefficient (n-octanol/water) log Pow > 10.0

Method	OECD TG 117 Partition Coefficient (n-octanol/water). EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	HPLC Method
Test Facility	Harlan (2015a)

Adsorption/Desorption log K_{oc} > 5.63

Method	OECD TG 121 Estimation of the Adsorption Coefficient (KOC) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC).
Remarks	HPLC Screening Method
Test Facility	Harlan (2015b)

Flash Point 210 ± 2°C at 101 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks	Closed cup equilibrium method.
Test Facility	Harlan (2014m)

Autoignition Temperature 386 ± 5 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Test Facility	Harlan (2014m)

Explosive Properties

Method	EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks	No structural alerts within the chemical structure of the notified chemical
Test Facility	Harlan (2014m)

Oxidizing Properties

Method	EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids).
Remarks	No structural alerts within the chemical structure of the notified chemical
Test Facility	Harlan (2014m)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure. Method B1 bis Acute Toxicity (Oral) of Commission Regulation (EC) No. 440/2008
Species/Strain	Rat Wistar (RccHan™:WIST)
Vehicle	None
Remarks - Method	GLP Certificate. A group of four animals (F) were administered a single 2,000 mg/kg oral dose of test substance and then were observed for acute toxicity for 14 days. Dosing was performed through gavage, wherein a metal cannula attached to a graduated syringe was affixed. At the end of the observation period all animals were sacrificed by cervical dislocation and subjected to macroscopic necroscopy examination.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
Preliminary	1F	2,000	None
Main	4F	2,000	None

LD50	>2,000 mg/kg bw
Signs of Toxicity	No evidence
Effects in Organs	No effect
Remarks - Results	*Preliminary test* (1 test animal): A 2,000 mg/kg bw dose was administered to one female test animal and was observed for 14 days. No clinical symptoms or necroscopy-related abnormalities were observed. *Main experiment:* No deaths or signs of systemic toxicity were observed. All animals showed expected gains in the bodyweight over the study period and no abnormalities were noted at necroscopy.
CONCLUSION	The notified chemical is of low acute toxicity by the oral route.
TEST FACILITY	Harlan (2014e)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test. EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal) – Limit Test.
Species/Strain	RatWistar (RccHan™:WIST)
Vehicle	None
Type of dressing	Semi-occlusive.
Remarks - Method	GLP Certificate. A single dose volume of 2.24 mL/kg of the test substance was applied to closely-clipped skin using a graduated syringe and a piece of surgical gauze was placed over the treatment area. Dressings were removed after 24 hour contact period and observed at 0.5, 1, 2 and 4 hours post dosing for deaths or overt signs of toxicity and subsequently once daily for 14 days. Animals were observed for erythema and eschar formation, oedema formation and for presence of any other lesion.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
Main	5M, 5F	2,000	0

Remarks - Results: There were no deaths, signs of systemic toxicity, cutaneous reactions, change in body weight or necroscopy-related abnormalities noted in test animals.

CONCLUSION: The notified chemical is of low acute toxicity by the dermal route.

TEST FACILITY: Harlan (2014f)

B.3. Irritation – skin

TEST SUBSTANCE

METHOD: OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Council Regulation No 440/2008 B.4 Acute Toxicity (Skin Irritation).

Species/Strain: Rabbit/New Zealand White
Number of Animals: 2F
Vehicle: None
Observation Period: 72 Hours
Type of Dressing: Semi-occlusive.
Remarks - Method: GLP Certificate.
A single dose volume of 0.5 mL of the test substance was applied directly to closely-clipped skin of one flank of the rabbit for 4 hours under a piece of cotton gauze patch (semi-occluded). Cutaneous reactions were observed approximately 1, 24, 48 and 72 hours after removal of the dressing. Animals were observed for erythema and eschar formation, and for oedema formation. Grading of irritancy was done according to the scheme devised by Draize, J. H. (1959).

RESULTS

Lesion	*Mean Score* Animal No.*		*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2			
Erythema/Eschar	1	1	1	>72	1
Oedema	0	0	0	–	0

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

Remarks - Results: Very slight erythema was noted in one treated skin site 1 hour after patch removal and at both the treated skin sites od test animals at 24, 48 and 72 hour observations. No other cutaneous reactions were observed during the study and erythema was reversed by day 7.

CONCLUSION: The notified chemical is a mild irritant to the skin.

TEST FACILITY: Harlan (2014g)

B.4. Irritation – eye

TEST SUBSTANCE

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion.
EC Council Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation).

Species/Strain: Rabbit/New Zealand White

Number of Animals	2F
Observation Period	72 hours. An additional observation was made on day 7 to assess the reversibility of the ocular effects.
Remarks - Method	GLP Certificate. The test substance (0.1 mL) was applied to conjunctival sac of right eye. Untreated left eye served as control. Eyes were not rinsed after administration of the test item. Animals were examined at 1, 24, 48 and 72 hours post administration and were scored according to degree of positive response. These scores were then used for calculating the respective mean values. Ocular irritancy potential of the notified chemical was measured and interpreted by numerical evaluation.

RESULTS

Lesion	*Mean Score* Animal No.*		*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2			
Conjunctiva: redness (A)	2	2	2	≥72 h	2
Conjunctiva: chemosis (B)	1	1	1	≥72 h	1
Conjunctiva: discharge (C)	0.3	0.6	1	48 h	0
Corneal opacity (E,F)	0	0	0	–	0
Iridial inflammation (D)	0	0	0	–	0

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

Remarks - Results	Both test animals demonstrated an ocular reaction to the test substance in varying degree and the observed clinical changes were reversible under the test condition. *Score for Cornea:* corneal opacity was measured and scored both for affected area (F) and intensity of cloudiness (E) separately. Corneal opacity was measured as (E × F) × 5. During 72 hour observation period, no change in corneal opacity was observed. *Score for Iris:* Iridial inflammation was measured as D × 5 and during 72 hour observation period no signs of iridial inflammation was observed. *Score for conjunctivae:* Conjunctival redness (A), chemosis (B) and discharge (C) was measured separately and score for conjunctivae was measured as (A + B + C) × 2. *Redness:* Both animals were positive for conjunctival redness, which was reversed by day 7. At the end of 72 hour observation period, both the test animals demonstrated a 2 score for the criterion. *Chemosis:* Both animals were positive for conjunctival chemosis, which was reversed by day 7. At the end of 72 hour observation period, both the test animals demonstrated a 1 score for the criterion. *Discharge*: Both animals were positive for conjunctival discharge, which was reversed by day 3. The notified chemical produced a maximum group score of 14 which according to modified Kay and Calandra system is a mild irritant.
CONCLUSION	The notified chemical is mildly irritating to the eye according to modified Kay and Calandra system.
TEST FACILITY	Harlan (2014h)

B.5. 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 (female)
Vehicle	Butanone
Preliminary study	Yes
Positive control	Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using α-Hexylcinnamaldehyde (HCA), 15% v/v concentration in butanone.
Remarks - Method	GLP Certificate. On days 1, 2 and 3, a dose-volume of 25 µL of the control or dosage form preparations were applied to the dorsal surface of both ears. Mice were checked for clinical signs, morbidity and mortality every day. Body weight was measured at day 1 and 6. Thickness of ear was measured on Day 1 (pre dose), 3 and 6; and irritation reaction was checked in parallel. At day 6, animals were given a single intravenous injection of 20 µCi dose of ^{3}H-TdR, 5 hours prior to they were sacrificed by carbon dioxide affixation. Single cell suspension from auricular lymph nodes were prepared and proliferative response was measured.

RESULTS

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
25% (in vehicle)	5F	12035.69**	7.47
50% (in vehicle)	5F	21194.12***	13.16
100%	5F	21015.39***	13.05
0 (vehicle control)	5F	1,610.55	–
Positive Control	5F	–	11.92

** = Significantly different from vehicle control group $p < 0.01$
*** = Significantly different from vehicle control group $p < 0.001$

EC3	
Remarks - Results	There were no signs of systemic toxicity or irritation (i.e., ≥25% increase in mean ear thickness) noted in the test or contro animals during the test. Very slight erythema on ears and persisted in two animals on day 2. An EC3 value could not be interpolated as all the test substance concentrations resulted in a stimulation index greater than 3. In addition, the EC3 could not be extrapolated as the lowest stimulation index is not near the EC3 and there is not a linear relation between the stimulation indices of the three tested concentrations.

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

TEST FACILITY Harlan (2013i)

B.6. Repeat dose toxicity

TEST SUBSTANCE

METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	RatWistar (RccHan™:WIST)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week
Vehicle	Arachis oil BP
Remarks - Method	No significant deviations from the OECD guidelines. Four treatment groups of 5 male and 5 female test animals were administered the test substance at respective dose levels of 0 (vehicle alone, negative control), 100, 400 and 1,000 mg/kg/day by the oral gavage for 28 consecutive days.

Two recovery groups each of five males and five females, were treated with the 1,000 mg/kg/day dose or the vehicle alone for 28 consecutive days and then maintained without treatment for 14 days. All animals were subjected to gross necrosopy and histopathological examination of the selected tissues. Following observations were made during the 28-day regimen:
Detailed clinical observations: once daily. All animals were examined for overt signs of toxicity, ill-health or behavioral change immediately before dosing, up to thirty minutes post dosing and one hour after dosing;
Functional observations: Prior to the start of treatment and on Days 7, 14, 21 and 25 of their dosing.
Body weights and food consumption: in weekly intervals and at the beginning and termination of the experiment.
Necropsy examinations, organ weights and microscopic examination of tissues: at study termination

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5M, 5F	0	0
low dose	5M, 5F	100	0
mid dose	5M, 5F	400	0
high dose	5M, 5F	1,000	0
control recovery	5M, 5F	0	0
high dose recovery	5M, 5F	1,000	0

Remarks – Results

Mortality and Time to Death

There were no deaths on the study

Clinical Observations

No clinical signs were considered to be related to the toxicity of the test item

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No toxicologically significant findings could be attributed to the notified chemical. Males given notified chemical at all dose levels showed marginally but statistically significantly lower haemoglobin levels when compared with controls ($p<0.01$ at 1,000 mg/kg bw/day and $p<0.05$ for 100 or 400 mg/kg bw/day). The mean cell haemoglobin concentrations in these males were also marginally lower than controls. The study authors mentioned that, although a dose-relationship was noticed for the mean cell haemoglobin and the mean cell haemoglobin concentration, all individual values were within the historical control background data ranges.

The males in the high dose group also showed statistically significantly lower mean neutrophil count when compared with controls ($p<0.05$); however, there was no dose-relationship and all individual values were within the historical background data ranges.

In blood chemistry analysis it was noticed that at all dose levels, non-recovery males receiving the test item showed statistically significant lower plasma levels of triglycerides when compared with controls ($p<0.01$ at 400 or 1,000 mg/kg bw/day and $p<0.05$ at 100 mg/kg bw/day). Non-recovery females from the high dose group also showed slightly lower triglyceride levels than controls but the difference was not statistically significant. Overall, there were no blood chemistry related findings considered to be toxicologically significant.

The histopathological analysis of the tissues from all animals of either sex did not indicate any treatment-related findings likely to be of any toxicological significance. The above-mentioned obsevations, thus, according to study authors were considered to be incidental.

Effects in Organs

The histopathological examination of organs did not reveal any significant treatment-related finding.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1,000 mg/kg bw/day in this study, based on the in-life results and histopathology findings.

TEST FACILITY Harlan (2015j)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE

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 from Phenobarbitone (PB)/ β-napthoflavone (NF) induced induced rat liver

Concentration Range in Main Test a) With metabolic activation: 50–5,000 µg/plate
b) Without metabolic activation: 50–5,000 µg/plate

Vehicle None

Remarks - Method No significant deviations from the OECD guidelines. There were a preliminary dose finding experiment (1.5–5,000 µg/plate) and a main experiment both in absence and presence of metabolic activation.
Positive controls:
With metabolic activation: 2-aminoanthracene (all strains except TA98), Benzo(a)pyrene (TA98)
Without metabolic activation: *N*-ethyl-*N*'-nitro-*N*-nitrosoguanidine (WP2uvrA , TA100, TA1535) 9-aminoacridine (TA1537), 4-Nitroquinoline-1-oxide (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	–	>5,000	–	negative
Present				
Test 1	–	>5,000	Y	negative

Remarks - Results No signs of toxicity were noted at any dose level. Precipitation was observed in a 1,500 and another 5,000 µg/plate concentration plate, which according to the study conductors, did not affect colony count. The number of revertant colonies in the control was within the normal range, and the positive controls were all mutagenic in their appropriate tester strain, confirming the validity of the test.

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

TEST FACILITY Harlan (2014k)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.

Species/Strain *Homo sapiens*

Cell Type/Cell Line Lymphocytes from whole blood samples (primary cell culture)

Metabolic Activation System	S9 from Phenobarbitone (PB)/ β-napthoflavone (NF) induced induced rat liver
Vehicle	Dimethyl sulfoxide
Remarks - Method	GLP Cerificate The notified chemical was tested in three independent sets of experiment (preliminary toxicity test, Experiment 1 and 2 (main experiment). There were 3 conditions in preliminary toxicity test: a. without metabolic activation (exposed to notified chemical for 4 hours), b. with metabolic activation (exposed to notified chemical for 4 hours) and c. 24 hour continuous exposure to the to the notified chemical without metabolic activation. The preliminary test was conducted to select the dose level for the main experiment (dose range tested 0.49–125 µg/ml of the notified chemical). In Expreiment 1, two exposure groups were used which are 4 hours exposure to notified chemical in a. presence and b. absence of metabolic activation, followed by 20 hour exposure of treatment-free media. In Experiment 2 there were two conditions which are: a. 24 hour continuous exposure to notified chemical without metabolic activation and b. 4 hour continuous exposure to notified chemical with metabolic activation followed by 20 hour exposure of treatment-free media. For 'without S9 mix' media, Mitomycin C (MMC) was added as positive control, whereas cyclophosphamide (CP) was added in 'with S9 mix' media for the same purpose.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time (media without test substance)*
Absent			
Test 1	0*, 3.91, 7.81, 15.63, 31.25*, 62.5*, 125*, MMC 0.4*	4 h	20 h
Test 2	0*, 3.91, 7.81, 15.63, 31.25*, 62.5*, 125*, MMC 0.2*	24 h	0 h
Present			
Test 1- S9 2%	0*, 3.91, 7.81, 15.63, 31.25*, 62.5*, 125*, CP 5*	4 h	20 h
Test 2- S9 1%	0*, 3.91, 7.81, 15.63, 31.25*, 62.5*, 125*, CP 5*	4 h	20 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*		
	Cytotoxicity in Main Test	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	>125	–	Negative
Test 2	>125	–	Negative
Present			
Test 1	>125	–	Negative
Test 2	>125	–	Negative

Remarks - Results	In both absence and presence of metabolic activation, the maximum dose level for suitable metaphase scoring was 125 µg/mL.The mitotic index data for the main experiment corroborates with the historical data of the testing lab and confirms the dose-related inhibition pattern in mitotic index (MI) both in presence and absence of S9. Cells were further assessed for chromosome aberration by metaphase analysis. No statistically significant chromosome or chromatid aberrations were observed that could be attributed to the notified chemical. The test item did not induce statistically significant number of polyploidy cells at any dose level.
CONCLUSION	The notified chemical was not clastogenic to primary human lymphocytes treated *in vitro* under the conditions of the test.

TEST FACILITY	Harlan (2015l)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO2 Evolution Test.
Inoculum	Activated sewage sludge
Exposure Period	29 days (corrected for last gas wash)
Auxiliary Solvent	None
Analytical Monitoring	Theoretical Carbon Dioxide ($ThCO_2$)
Remarks - Method	The test was conducted in accordance with the test guideline above, with no significant deviation in protocol reported.

RESULTS

Test substance		*Toxicity control*		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*	*Day*	*% Degradation*
6	9	6	50	6	76
14	13	14	55	14	83
21	10	21	47	21	76
29*	16	29*	48	29*	88

* Corrected for the last gas wash

Remarks - Results	All validity criteria for the test were satisfied. The percentage degradation of the reference compound surpassed the threshold level of 60% by 2 days (73%), and attained 88% degradation in 29 days. Therefore, the test indicates the suitability of the inoculums. The percentage degradation of the toxicity control surpassed the threshold level of 25% by 2 days (42%; 48% in 29 days), showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the test substance after 28 days was 16%.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	Harlan (2014b)

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	*Oncorhynchus mykiss* (rainbow trout)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	140 mg $CaCO_3$/L
Analytical Monitoring	HPLC-MS
Remarks – Method	The test was conducted in accordance with the test guideline with no significant deviation in protocol reported. The test substance was prepared as water accommodated fraction (WAF) due to its low water solubility. A stock solution with a nominal loading rate of 100 mg/L was prepared by stirring the test substance in water for 1 day, and any undissolved material was removed by siphoning.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality (%)*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	Control	7	0	0	0	0	0
100	<0.00057	7	0	0	0	0	0

LL50	>100 mg/L (WAF) at 96 hours.
NOEL	100 mg/L (WAF) at 96 hours.
Remarks – Results	All validity criteria for the test were satisfied. The test solutions were renewed every 24 hours during the 96 h test period. The actual concentrations of the test substance were measured every 24 hours during the 96 h test period. The 96 h LL50 and NOEL for fish were determined to be >100 mg/L and 100 mg/L (WAF), respectively, based on nominal loading concentrations.
CONCLUSION	The notified chemical is not considered to be harmful to fish up to the limit of its water solubility.
TEST FACILITY	Harlan (2015c)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	None reported
Remarks – Method	The test was conducted in accordance with the test guideline above, with no significant deviation in protocol reported. The test substance was prepared as water accommodated fraction (WAF) due to its low water solubility. A stock solution with a nominal loading rate of 100 mg/L was prepared by stirring the test substance in water for 1 day, and any undissolved material was removed by siphoning. A total of 20 daphnids were used.

RESULTS

Concentration mg/L		*Number of D. magna*	*Cumulative Immobilised (%)*	
Nominal	*Actual*		*24 h*	*48 h*
Control	Control	20	0	0
100	Not determined	20	0	0

EL50	>100 mg/L (WAF) at 48 hours
NOEL	100 mg/L (WAF) at 48 hours
Remarks - Results	All validity criteria for the test were satisfied. The test solutions were not renewed during the 48 h test period. The actual concentrations of the test substance were not measured as no effects were observed at the highest concentration tested. The 48 h EL50 and NOEL for daphnids were determined to be >100 mg/L and 100 mg/L (WAF), respectively, based on nominal loading concentrations.
CONCLUSION	The notified chemical is not considered to be harmful to aquatic invertebrates up to the limit of its water solubility.

TEST FACILITY Harlan (2013)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Freshwater Alga and Cyanobacteria, Growth Inhibition Test.

Species	*Pseudokirchneriella subcapitata* (green alga)
Exposure Period	72 hours
Concentration Range	Nominal: 0.1–1 mg/L Actual: <0.0009 mg/L
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	HPLC-MS
Remarks - Method	The test was conducted in accordance with the test guideline above, with no significant deviation in protocol reported. The test substance was prepared as water accommodated fraction (WAF) due to its low water solubility. A stock solution with a nominal loading rate of 1 mg/L was prepared by stirring the test substance in water for 1 day, and any undissolved material was removed by siphoning.

RESULTS

Biomass		*Growth*	
EL50 *mg/L at 72 h*	*NOEL* *mg/L*	*EL50* *mg/L at 72 h*	*NOEL* *mg/L*
>1	1	>1	1

Remarks - Results All validity criteria for the test were satisfied. The test solutions were not renewed during the 72 h test period. The actual concentrations of the test substance were measured at the start and end of the 72 h test period. The 72 h EL50 was determined to be >1 mg/L (WAF), respectively, based on nominal concentration. The 72 h NOEL was determined to be 1 mg/L (WAF).

CONCLUSION The notified chemical is not considered to be harmful to algae up to the limit of its water solubility.

TEST FACILITY Harlan (2015d)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 209 Activated Sludge, Respiration Inhibition Test.

Inoculum	Activated sewage sludge
Exposure Period	3 hours
Concentration Range	Nominal: 10–1,000 mg/L Actual: Not determined
Remarks – Method	The test was conducted in accordance with the test guideline above, with no significant deviation in protocol reported. 3,5-Dichlorophenol was used as the reference control. The respiration rate was determined by measurement of Biological Oxygen Demand during the test after 3 hours of exposure.

RESULTS

IC50 >1,000 mg/L at 3 hours

NOEC	1,000 mg/L at 3 hours
Remarks – Results	All validity criteria for the test were satisfied. No significant inhibition of respiration rates were observed at 1,000 mg/L. The 3 h IC50 was determined to be >1,000 mg/L, based on nominal concentrations.
CONCLUSION	The notified chemical is not inhibitory to microbial activity.
TEST FACILITY	Harlan (2014c)

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Appendix B - Updated ICCVAM-Recommended Protocol: The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products