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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Chemical in Z–159

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.

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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/1581	Lubrizol International Inc.	Chemical in Z–159	Yes	≤ 100 tonnes per annum	Component of engine oils

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 1B)	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, the notified chemical is not considered to pose an unreasonable risk to public health.

Environmental risk assessment

On the basis of the maximum import volume, low expected aquatic exposure and the reported use pattern, 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 1B): 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.

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 during reformulation and/or repackaging processes:
  - 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.

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 containment, physical collection and subsequent safe disposal.

Regulatory Obligations

Secondary Notification

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

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

(1) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component of engine oils, 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
US (2015)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Z–159

3. COMPOSITION

DEGREE OF PURITY
100%

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

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

Property	Value	Data Source/Justification
Melting Point/Freezing Point	< -20 °C	Measured
Boiling Point	Decomposed from ~243 °C at 101 kPa	Measured
Density	0.972×10^3 kg/m^3 at 20 ± 0.5 °C	Measured
Vapour Pressure	3.1×10^{-3} kPa at 25 °C 14.9×10^{-3} kPa at 100 °C	Measured
Water Solubility	5.59×10^{-5} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	$t_{½}$ > 1 year at pH 4 $t_{½}$ = 117 hours at pH 7 $t_{½}$ = 96.9 hours at pH 9	Measured
Partition Coefficient (n-octanol/water)	log Pow = 6.56	Measured
Adsorption/Desorption	log K_{oc} > 4.21	Measured
Dissociation Constant	Not determined	Expected to be ionised under environmental conditions (pH 4–9)
Flash Point	102 ± 2°C at 101.3 kPa	Measured

Autoignition Temperature	338 ± 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 into Australia as a component of engine oils at ≤ 10% concentration and will be reformulated.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	45–55	60–75	75–85	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 tank container (~20,000 L) or intermediate bulk container (~1,250 L). Smaller quantities will be transported in steel drums (~208 L).

USE
The notified chemical will be used as a component of engine oils at ≤1.0% concentration for automotive use (primarily for heavy duty diesel engines).

OPERATION DESCRIPTION
The concentrate/additive package containing the notified chemical (at ≤ 10% concentration) will be reformulated after importation.

Reformulation
After importation, it is expected that the additive packages containing the notified chemical at ≤ 10% 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% concentration will be packaged using automated processes. The resulting engine oil products containing the notified chemical at ≤ 1% concentration may be supplied in bulk for industrial users or smaller containers for use in commercial service applications or do-it-yourself (DIY) users.

End use
Engine oil products containing ≤ 1% of the notified chemical will primarily 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 engine oils being manually decanted into automobile engines, while at industrial sites the engine oils are expected to be pumped from the drums.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment
Transport and storage workers may come into contact with the notified chemical at ≤ 10% concentration only in the event of accidental rupture of containers.

Reformulation
Dermal and ocular exposure of workers to the notified chemical at ≤ 10% 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 and goggles. Inhalation exposure is not expected given the enclosed systems and low vapour pressure of the notified chemical.

End-use
At automotive service centres, professional users such as mechanics may experience dermal or ocular exposure to the engine oil products containing the notified chemical at ≤ 1% concentration when transferring engine oils 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 (≤ 10% 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 along with proper training in handling of the notified polymer as anticipated by the notifier and a high degree of automation should minimise the workers' exposure to the notified chemical.

Transfer of the finished lubricant containing the notified chemical at ≤ 1% 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 (3.1×10^{-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 (≤ 10% 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 stores and DIY users may come into contact with the notified chemical at ≤ 1% concentration. The exposure may occur during the manual transfer of the final lubricant from the product container into the engine oil tank or during the cleaning and maintenance of equipment. The notifier states that processes will be mostly enclosed or supplied engineering controls such as shielding and good general ventilation to reduce exposure from splashes, mists and vapours will be present. 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 engine oil containing the notified chemical at ≤ 1% concentration to vehicles. Given the low concentration (≤ 1%) of the notified chemical in the engine oil and that engine oil is changed infrequently, 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
Rat, repeated dose toxicity	NOAEL male: 1,000 mg/kg bw/day NOAEL female: 400 mg/kg bw/day
Rabbit, eye irritation	moderately irritating
Rabbit, acute dermal irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro (chromosome aberration, cultured human lymphocyte)	non clastogenic

Toxicokinetics, metabolism and distribution
Based on the molecular weight (~ 500 Da) and relatively low water solubility (5.59×10^{-5} g/L at 20 °C) of the notified chemical, the possibility of dermal absorption cannot be ruled out.

Acute toxicity
The notified chemical was found to have low acute oral and dermal toxicity in rats. No acute inhalation toxicity studies were provided.

Irritation and sensitisation
Based on studies conducted in rabbits, the notified chemical was considered to be slightly irritating to the skin and moderately irritating to eyes; however, the effects were insufficient to warrant classification of the chemical. 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 2.14, 1.99, and 4.89 at 1, 10 and 100% concentration, respectively. An EC3 value was not determined by the study authors.

Repeated dose toxicity
A 28 day repeat dose study by oral gavage was conducted in rats with the notified at dose levels of 0, 100, 300, 400 and 1,000 mg/kg bw/day. A range of clinical and laboratory observations were noted, including high reticulocyte counts and organ weight variations in the liver and kidneys. Most non-recovery females in the high-dose group showed minimal or mild periportal fat vacuolation with the latter considered to be adverse. One female in the high-dose recovery group showed mild periportal fat vacuolation at the end of the tretament-free period indicating partial reversibility.The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1,000 mg /kg bw/day for males and 400 mg/kg bw/day for females.

Mutagenicity/Genotoxicity
The notified polymer 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 1B)	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
The critical health effects of the notified chemical are as a skin irritant and a weak sensitiser.

During reformulation workers may be exposed to the notified chemical at ≤ 10% concentration. At these concentrations, the potential risk of irritating and sensitising effects is expected to be low. Furthermore, this risk is expected to be further minimised by the expected use of personal protective equipment including protective clothing, imperious gloves and goggles, and largely automated and enclosed processes limiting exposure.

During end-use workers may be exposed to the notified chemical at ≤ 1% concentration when changing or topping-up engine oil. At these low end-use concentrations, the potential risk of irritating and sensitising effects is not expected.

Given the lower end-use concentration and stated controls in place to minimise exposure during reformulation, the risk to the health of workers is not considered unreasonable.

6.3.2. Public Health
Given the public will only be exposed to the notified chemical at low concentrations (≤ 1%) and on an infrequent basis, the risk to public health from use of the notified chemical is not considered 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 engine oils. 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 engine oils 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. Release of the notified chemical to the environment during transport and normal blending and packaging procedures are expected to be limited to accidental spills or leaks. The notified chemical 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 engine oils. Release during use may arise from spills when pouring lubricants into engines or from engine leaks, and is expected to be very low.

RELEASE OF CHEMICAL FROM DISPOSAL

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

The major release of the notified chemical to the environment will come from inappropriate disposal of waste or used oils. Oil products containing the notified chemical will be poured into engines by 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 or incineration). Assuming this is the case, negligible release of the notified chemical should result from these professional activities. The remaining 14% of oil is removed 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 used oil removed 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 used oil removed 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, and 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 biodegradable (0% 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 rapid hydrolysability (117 hours at pH 7, and 96.9 hours at pH 9).

The majority of the notified chemical will be thermally decomposed during use, collected for recycling, or 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 low water solubility and high adsorption/desorption coefficient (log K_{OC} > 4.21). 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 > 1 mg/L (WAF*)	Not harmful to aquatic invertebrates up to water solubility limit
Algal Toxicity	72 h E_rL50 = 0.51 mg/L (WAF*) 72 h E_bL50 = 0.34 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).

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 and no significant release of the notified polymers 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, and due to the low potential for release to the aquatic compartment based on its assessed use pattern in engine oils. Whilst the notified chemical is not biodegradable, it is rapidly hydrolysable and is therefore not expected to bioaccumulate. 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 °C (<253 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 crystallisation method, using a procedure compatible with above-mentioned methods. The test result is an approximation based on observation of two independent tests.
Test Facility Harlan (2015b)

Boiling Point 243 °C (516 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. Onset of boiling was observed at 246.88 °C and 243.44 °C in two separate experiments. The test items boiled over the approximate range from 243 °C to 365 °C.
Test Facility Harlan (2015b)

Density 0.972×10^3 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 (2015b)

Vapour Pressure 3.1×10^{-3} kPa at 25 °C
14.9×10^{-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 (2015a)

Water Solubility 5.59×10^{-5} 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 (2015b)

Hydrolysis as a Function of pH $t_{½} > 1$ year at pH 4
$t_{½} = 117$ hours at pH 7
$t_{½} = 96.9$ hours at pH 9

Method OECD TG 111 Hydrolysis as a Function of pH.
EC Council Regulation No 440/2008 C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$*
4	25	> 1 year
7	25	117 hours
9	25	96.9 hours

Remarks After 5 days under the accelerated conditions of 50 °C the rate of hydrolysis of was less than 10% at pH 4; this equates to a half-life at 25 °C of $t0.5_{25\ °C} > 1$ year. Therefore, it can be concluded that under the conditions of the test, the notified chemical is hydrolytically stable at pH 4.

After 5 days under the accelerated conditions of 50 °C the rate of hydrolysis of was greater than 90% at pH 7 and 9. Further testing at 40–70 °C indicated that the half-life of the notified chemical was ≤ 31.7 hours at pH 7 and ≤ 29.2 at pH 9 under accelerated conditions, which equates to $t0.5_{25\ °C}$ = 117 hours at pH 7, and $t0.5_{25\ °C}$ = 96.9 hours at pH 9. Therefore, it can be concluded that under the conditions of the test, the notified chemical is hydrolysable at pH 7 and 9.

Test Facility Envigo (2015a)

Partition Coefficient (n-octanol/water) log Pow = 6.56

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 (2015b)

Adsorption/Desorption log K_{oc} > 4.21

Method OECD TG 121 Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC).
EC Council Regulation No 440/2008 C.19 Adsorption - Desorption.
Remarks HPLC Screening Method
Test Facility Harlan (2015c)

Flash Point 102 °C ± 2 °C at 101.3 kPa

Method EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks Closed cup equilibrium method.
Test Facility Harlan (2015h)

Autoignition Temperature 338 ± 5 °C

Method EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Test Facility Harlan (2015h)

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	Arachis Oil BP
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*
1	5F	2,000	None

LD50	>2,000 mg/kg bw
Signs of Toxicity	No evidence
Effects in Organs	No effect
Remarks - Results	*Preliminary test* (2 female test animals): 300 and 2,000 mg/kg bw doses were administered to two female test animals and were 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 (2015i)

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 2.07 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*
Preliminary	1M, 1F	2,000	0
Main	4M, 4F	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 (2015j)

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: 3M
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, oedema formation and for presence of any other lesion. 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	3			
Erythema/Eschar	0	1.6	0	2	>72	2
Oedema	0	0.6	0	0	≥72	1

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

Remarks - Results: Very slight erythema was noted in 1/3 animal at hour 1. Well defined erythema and very slight oedema were noted in this animal at 48 and 72-hour observations. No other cutaneous reactions were observed during the study.

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

TEST FACILITY: Harlan (2015l)

B.4. Irritation – eye

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion.

	EC Council Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation). EC Directive 2004/73/EC B.5 Acute Toxicity (Eye Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	2M
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 right 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 according to modified Kay and Calandra system.

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)	1.6	1.3	1.6	≥72 h	1
Conjunctiva: chemosis (B)	1.3	1	1.3	≥72 h	1
Conjunctiva: discharge (C)	0.6	0.3	0.6	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 1 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, test animals demonstrated a 0 and a 1 score for the criterion. *Discharge*: Both animals were positive for conjunctival discharge, which was reversed by day 3.
CONCLUSION	The notified chemical is moderately irritating to the eye according to modified Kay and Calandra system.
TEST FACILITY	Harlan (2015k)

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	Acetone-olive oil (4:1 v/v)
Preliminary study	Yes
Positive control	α-Hexylcinnamaldehyde (HCA), 25% v/v concentration in vehicle.
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 first measured one day before the application and then once daily for 6 days post application; 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/v)	*Number and sex of animals*	*Proliferative response (mean DPM/Animal•)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	5F	2,269.72	-
1% (in vehicle)	5F	4,847.82	2.14
10% (in vehicle)	5F	4,516.67	1.99
100%	5F	11,089.66♦	4.89*
		14,051.29♦♦	6.19**
Positive Control	5F	23,134.76♦♦	10.19

• = Total number if lymph node per animal is 2.
* = Result based on 4F animals due to exclusion of outlier value.
** = Result with inclusion of outlier value.
♦ = Significantly different from vehicle control group $p < 0.05$
♦♦ = Significantly different from vehicle control group $p < 0.01$

Remarks - Results	Group mean values for disintegration per minute and standard deviation were first calculated and then assessed for suitability by analysis of normality and homogeneity of variance. Parametric one way analysis of variance (ANOVA) and Dunnett's multiple comparison procedure were used to determine statistical significance. Note that mean value proliferative response result in negative control has a very large variance; and p value of positive control and notified chemical at 100% concentration when compared with negative control is large (i.e. confidence level for this set of data is relatively low).
CONCLUSION	There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	Harlan (2015n)

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	GLP Certificate. No significant deviations from the OECD guidelines. Five 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, 300, 400 and 1,000 mg/kg/day by the oral gavage for 28 consecutive days. All animals were maintained without treatment for a further fourteen days and then were subjected to necrosopy. 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
control (recovery)	5M, 5F	0	0
Low	5M, 5F	100	1M
mid (I)	5M, 5F	300	0
mid (II)	5M, 5F	400	0
High	5M, 5F	1,000	0
high (recovery)	5M, 5F	1,000	0

Remarks – Results

Mortality and Time to Death

One male test animal in the low-dose group was found dead during the blood sampling procedure on Day 28 of dosing. No clinical signs for this animal prior to death was observed. Microscopic examination of the selected tissues from this male revealed liver congestion but a reason for death could not be established. The study conductors concluded the premature death could have resulted due to stress associated with the blood sampling procedure and the death was unrelated to treatment regime.

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 test substance. Male animals receiving the test item in the mid (II) and high dose groups, and females from all dose groups showed statistically significantly higher reticulocyte counts when compared with the respective controls ($p < 0.01$ for females treated with 400 or 1,000 mg/kg bw/day and $p < 0.05$ for the remaining instances). In the absence of any treatment-related changes in any associated parameters, these intergroup differences were considered to be of no toxicological significance.

Mean plasma levels of cholesterol in males treated low, mid (I) and high dose group were statistically significantly higher than controls ($p < 0.05$ at 300 mg/kg bw/day and $p < 0.01$ for the remaining dose groups) in a dose-related manner. The microscopic examination of the liver indicated minimal centrilobular hypertrophy in most non-recovery males in the high dose group. The study authors concluded that variations in this plasma marker and hepatic changes are likely to be indicative in fluctuations in metabolic processes associated with test item detoxification were not considred to represent an adverse effect of treatment.

Effects in Organs

The group mean absolute and body weight-related kidney weights in males given mid and high dose; and liver weights in animals of either sex from these dose groups; as well as males receiving low dose were statistically

significantly higher than controls ($p < 0.01$ in all instances). Dose-relationships were established and intergroup differences were particularly noticeable for the high dose group animals.Males in the high dose recovery group had statistically significant higher group mean absolute and body weight-related liver and kidney weights compared with controls ($p < 0.05$). The study authors considered it likely that these changes would be fully reversible over time.

Histopathological observation revealed that changes considered to be associated with the oral (gavage) administration of the notified chemical were noticeable in the kidneys of males and the liver and thyroid gland of both sexes. In males in the intermediate and high dose groups there were findings consistent with α-2μ-globulin nephropathy which is not clinically relevant to humans. The incidence and/or severity of minimal or mild diffuse hypertrophy of the follicular epithelium was greater in non-recovery animals of either sex in the mid (I and II) and high dose groups compared with controls. The finding was considered to be non-adverse due to the higher sensitivity of the thyroid in rats compared with humans. Most non-recovery females in the high-dose group showed minimal or mild periportal fat vacuolation with the latter considered to be adverse. One female in the high-dose recovery group showed mild periportal fat vacuolation at the end of the tretament-free period indicating partial reversibility.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1,000 mg/kg bw/day for males and 400 mg/kg bw/day for females in this study, based on periportal fat vacuolation in females, the in-life results and histopathology findings.

TEST FACILITY Envigo (2015b)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

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

Species/Strain	*S. typhimurium*: TA1538, TA1535, TA1537, TA98 & TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 microsomal fraction (in house preparation)
Concentration Range in Main Test	a) With metabolic activation: 15–5,000 μg/plate μg/plate b) Without metabolic activation: 15–5,000 μg/plate μg/plate
Vehicle	Dimethyl sulfoxide
Remarks -	No significant deviation from OECD guideline. Positive controls: Without metabolic activation: N-ethyl-N'-nitro-N-nitrosoguanidine (WP2*uvrA*), (TA100) and (TA1535); 9-Aminoacridine (TA1537); 4-Nitroquinoline-1-oxide (TA98). With metabolic activation: 2-Aminoanthracene (TA100), (WP2*uvrA*), (TA1535) & (TA1537); Benzo(a)pyrene (TA98)

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	>5,000	>5,000	Yes	negative
Present	>5,000	>5,000	Yes	negative

Remarks - Results No signs of toxicity were noted at any dose level. A precipitate was observed at the highest concentration but did not prevent the scoring of revertant colonies. The number of revertant colonies in the vehicle-treated 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 (2015m)

B.8. Genotoxicity – *in vitro*

Test Substance	Notified chemical
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 microsomal fraction (in house preparation)
Vehicle	Dimethyl sulfoxide
Remarks - Method	GLP Certificate. The notified chemical was tested in two independent sets of experiment (preliminary and main) and in both sets of experiment there were 3 conditions: *a.* **without** metabolic activation (exposed to notified chemical for 4 hours), *b.* **without** metabolic activation (exposed to notified chemical for 20 hours) and *c*. **with** metabolic activation (exposed to notified chemical for 4 hours). The preliminary test was conducted to select the dose level for the main experiment (dose range tested 19.53–5,000 µg/ml of the notified chemical). 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 (without S9)			
Test 1	0*, 4.88, 9.75, 19.5*, 29.25*, 39*, 58.5*, 78, MMC 0.4*	4 h	20 h
Test 2	0*, 4.88, 9.75, 19.5, 39*, 58.5*, 78, MMC 0.2*	24 h	0
Present (with 2% S9)			
Test 1	0*, 9.75, 19.5*, 39*, 78*, 117*, 156, 312,CP 5*	4 h	20 h

*Cultures selected for metaphase analysis.

Results

Metabolic Activation	*Test Substance Concentration (mg/mL) Resulting in:* *Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	>78	-	Negative
Test 2	>78	-	Negative
Present			
Test 1	>312	Yes	Negative

Remarks - Results

In absence of metabolic activation, the maximum dose level for suitable metaphase scoring was 78µg/mL whereas in presence of metabolic activation the corresponding concentration was 312 µg/mL. Precipitate observations were made only in 312 µg/mL exposure group (+S9) amongst all treatment conditions of the main experiment.

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 (2015o)

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 the last gas wash)
Auxiliary Solvent	None
Analytical Monitoring	Theoretical Carbon Dioxide ($ThCO_2$)
Remarks - Method	No significant deviation in protocol was reported.

RESULTS

Test substance		*Toxicity control*		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*	*Day*	*% Degradation*
6	0	6	45	6	73
14	0	14	52	14	88
21	0	21	57	21	83
29*	0	29*	63	29*	78

* 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 (67%), and attained 78% degradation in 29 days. Therefore, the tests indicate the suitability of the inoculums. The percentage degradation of the toxicity control surpassed the threshold level of 25% by 6 days (45%; 63% 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 0%. Therefore, the test substance is not considered to be readily biodegradable according to the OECD (301 B) guideline.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	Harlan (2015g)

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 substance was prepared as a 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 23 hours, and the aqueous phase was removed by mid-depth siphoning. No significant deviation in protocol was reported.

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	0.0714	7	0	0	0	0	0

LL50 > 100 mg/L at 96 hours (WAF).
NOEL 100 mg/L at 96 hours (WAF).
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 at 0 and 96 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 (2015e)

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 HPLC-MS
Remarks - Method The test substance was prepared as a 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 23 hours, and the aqueous phase was removed by mid-depth siphoning. A total of 20 daphnids were used. No significant deviation in protocol was reported.

RESULTS

Concentration mg/L		*Number of D. magna*	*Cumulative Immobilised (%)*	
Nominal	*Actual*		*24 h*	*48 h*
Control	Control	20	0	0
1	0.0989	20	0	0

EL50 > 1 mg/L at 48 hours (WAF)
NOEL 1 mg/L at 48 hours (WAF)
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 measured at 0 and 48 hours during the 48 h test period. The 48 h EL50 and NOEL for daphnids were determined to be > 1 mg/L and 1 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 (2015c)

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: 0.014-0.14 mg/L
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	HPLC-MS
Remarks - Method	The test substance was prepared as a 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 23 hours, and the aqueous phase was removed by mid-depth siphoning. No significant deviation in protocol was reported.

RESULTS

Biomass		*Growth*	
E_bL50 mg/L at 72 h	*NOEL mg/L*	*E_rL50 mg/L at 72 h*	*NOEL mg/L*
0.34	0.1	0.51	0.1

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 0 and 72 hours during the 72 h test period. The 72 h E_bL50 and E_rL50 were determined to be 0.34 mg/L and 0.51 mg/L (WAF), respectively, based on nominal concentrations. 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 sludge from a domestic wastewater treatment plant
Exposure Period	3 hours
Concentration Range	Nominal: 10–1,000 mg/L Actual: Not determined
Remarks – Method	No significant deviation in protocol was reported. 3,5-Dichlorophenol was used as the reference control. The respiration rate was determined by measurement of Biochemical Oxygen Demand during the test after 3 hours of exposure.
RESULTS	
IC50	> 1,000 mg/L at 3 hours
NOEC	100-1,000 mg/L at 3 hours
Remarks – Results	All validity criteria for the test were satisfied. No significant inhibition of respiration rates were observed at 1000 mg/L. The 3 h IC50 was determined to be > 1000 mg/L, based on nominal concentrations.
CONCLUSION	The notified chemical is not inhibitory to microbial activity.

TEST FACILITY	Harlan (2015f)

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