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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Z-161

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 and Energy.

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/1611	Lubrizol International, Inc.	Z-161	Yes	≤ 100 tonnes per annum	Additive in gear 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*
Sensitisation, Skin (Category 1B)	H317 – May cause an allergic skin reaction
Irritation, eye (Category 2A)	H319 – Causes serious eye irritation

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

Hazard classification	*Hazard statement*
Acute (Category 3)	H402 - Harmful to aquatic life
Chronic Aquatic Toxicity (Category 3)	H412 – Harmful to aquatic life with long lasting effects

Human health risk assessment
Under the conditions of the occupational settings described, the notified chemical is not considered to pose an 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:
  - Sensitisation, Skin (Category 1): H317 – May cause an allergic skin reaction
  - Irritation, eye (Category 2A): H319 – Causes serious eye irritation

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:
  - Enclosed, automated processes, where possible
  - Adequate local exhaust ventilation

- 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:
  - Avoid contact with skin and eyes
  - Avoid inhalation

- 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:
  - Coveralls
  - Impervious gloves
  - Eye protection
  - Respiratory protection, if inhalation exposure may occur

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

- A copy of the 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 of 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 physical containment, collection and subsequent safe disposal.

Regulatory Obligations

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 an additive in gear 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.

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Lubrizol International lnc. (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, use details and import volume.

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, EU, China, Canada, New Zealand, Korea, and Taiwan

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Z-161

MOLECULAR WEIGHT
< 1,000 Da

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

3. COMPOSITION

DEGREE OF PURITY
100%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Pale, hazy, viscous liquid

Property	Value	Data Source/Justification
Pour Point	31°C ± 3 °C	Measured
Boiling Point	198°C at 103 kPa	Measured
Density	994 kg/m^3 at 20 ± 0.5°C	Measured
Vapour Pressure	0.254 Pa at 25 °C 0.687 Pa at 80 °C	Measured
Water Solubility	6.21×10^{-3} – 2.97 g/L at 20 ± 0.5 °C	Measured
Hydrolysis as a Function of pH	$t_{½}$ > 1 year at 25°C at pH 4, 7 and 9	Measured
Partition Coefficient (n-octanol/water)	log Pow = -9.88×10^{-2} – 2.68 at 23.5 ± 0.5 °C	Measured
Surface Tension	40.0 mN/m at 21.8 °C	Measured
Adsorption/Desorption	log K_{oc} < 1.25	Measured
Dissociation Constant	Not determined	Expected to be ionised under

		environmental conditions (pH 4-9)
Flash Point	99 ± 2°C at 102.6 kPa	Measured
Flammability	Not determined	Not expected to be highly flammable, based on the flash point results
Autoignition Temperature	296 ± 5°C	Measured
Explosive Properties	Predicted negative	Estimated
Oxidising Properties	Predicted negative	Estimated

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

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

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. It will be imported as an additive package ≤ 20% 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 as a concentrate or additive package and transported via isotainer or 330 gallon (~1,250 L) intermediate bulk container (IBC). Smaller quantities will be transported in 55 gallon (~208 L) drums.

USE
The notified chemical will be used as an antiwear additive in gear oils at concentrations of ≤ 1.5%.

OPERATION DESCRIPTION
The notified chemical as a concentrate/additive package will be reformulated after importation.

Reformulation
At the reformulation facility, it is expected that the additive package containing the notified chemical will be transferred into blending tanks (containing mineral oil and other additives) using automated, 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.

End use
Gear oil products containing ≤ 1.5% of the notified chemical will primarily be used by commercial automotive and industrial engine service outlets. The gear oils are expected to be pumped from the drums.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

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

EXPOSURE DETAILS

Transport and storage

Transport and storage workers may come into contact with the notified chemical at ≤ 20% concentration only in the event of accidental rupture of containers.

Reformulation

Dermal and ocular exposure of workers to the notified chemical at ≤ 20% concentration may occur during reformulation when connecting and disconnecting hoses and during sample testing. The blending and packaging processes are expected to be automated and in an enclosed systems with ventilation in place. Dermal exposure will also be possible when cleaning up spills or leaks and during maintenance of the blending equipment.

Dermal and ocular exposure of workers to the notified chemical will be reduced through the use of personal protective equipment (PPE) including protective clothing, safety glasses, impervious gloves, and respiratory protection. Inhalation exposure is not expected given the enclosed systems and low vapour pressure of the notified chemical, unless aerosols or mists are generated.

Transfer of the finished lubricant 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.

End-use

Dermal and ocular exposure of workers at automotive service centres may occur when handling the products containing the notified chemical at ≤ 1.5% concentration. It is expected that at automotive service centres processes will be mostly enclosed or supplied with engineering controls and good general ventilation to reduce exposure from splashes, mists and vapours (if generated). Exposure may be mitigated through the use of PPE (including goggles, face shield, gloves, protective clothing).

6.1.2. Public Exposure

The products containing the notified chemical will be for industrial use only. Therefore, exposure of the general public to the notified chemical is not expected.

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 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	severely irritating
Guinea pig, skin sensitisation – Buehler test.	evidence of sensitisation
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
Rat, repeat dose oral (gavage) toxicity – 7 & 28 days.	NOAEL = 35 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro Chromosome Aberration test in	non genotoxic

Human Lymphocytes

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). Dermal absorption will be limited for substances with log P values < 0 due to poor lipophilicity, however, log P values between 1 and 4 favour dermal absorption, particularly if water solubility is high (ECHA, 2014). The notified chemical is a mixture with components having molecular weights are up to 1000 Da, a water solubility range of 6.21 x 10^{-3} – 2.97 g/L, and partition coefficients between -9.88 × 10-2 and 2.68. Thus, absorption across biological membranes may occur, particularly for lower molecular weight components (< 500 Da).

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 severely irritating to the eyes.

The notified chemical was a skin sensitiser in both a Buehler test in guinea pig and in a local lymph node assay (LLNA) in mice. The EC3 value was determined by the study authors to be 9%.

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, 35, 350 and 600 mg/kg bw/day. At a dose level of 600 mg/kg bw/day, there were a number of treatment related deaths along with decreased bodyweight gains, respiratory distress, and adverse changes in the forestomach, trachea, liver, thyroid, kidneys and adrenal glands. Decreased bodyweight gains, reddened lungs, and adverse effects in the liver, kidney and thyroid were also seen in animals dosed at 350 mg/kg bw/day, although to a lesser extent than at the higher dose. The study authors attributed the effects to the irritant properties of the test substance or rat specific toxicology, and therefore considered the No Observed Adverse Effect Level (NOAEL) for systemic toxicity to be equal to the maximum dose tested of 600 mg/kg bw/day. However, when all effects are considered the NOAEL would be the lowest dose tested, 35 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* chromosome aberration test in human lymphocytes.

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***
Sensitisation, Skin (Category 1B)	H317 – May cause an allergic skin reaction
Irritation, eye (Category 2A)	H319 – Causes serious eye irritation

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the information available the notified chemical is expected to be a severe eye irritant and a skin sensitiser.

During reformulation, workers may be exposed to the notified chemical at ≤ 20% concentration and therefore there is a potential risk of sensitising and irritation effects. The use of engineering controls such as enclosed and automated processes and local exhaust ventilation along with appropriate PPE (protective clothing, safety glasses, impervious gloves, and respiratory protection), as recommended by the notifier, would be expected to limit worker exposure.

During end-use, workers may be exposed to the notified chemical at ≤ 1.5% concentration. At this end-use concentration, the potential risk of skin sensitisation and eye and skin irritation cannot be ruled out. However,

the use of appropriate PPE (including goggles, face shield, gloves, protective clothing) is expected to limit worker 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

As the products containing the notified chemical are for industrial use only and will not be available for the general public, exposure of the general public to the notified chemical is not expected, therefore the risk to the general public health is considered negligible.

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 gear 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 gear 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. Accidental spills and leaks during 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 gear oils in industrial settings only. No 'do-it-yourself' (DIY) applications of gear oils containing the notified chemical are intended. Release during use may arise from spills when pouring lubricants into automotive vehicles or from vehicle leaks. It is expected that these wastes are disposed of as waste fluids according to State/Territory regulations.

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. Therefore, the release of the notified chemical to surface waters from the cleaning of empty containers is expected to be limited.

7.1.2. Environmental Fate

Based on the results of a biodegradability study, the notified chemical is not expected to be readily biodegradable (43% in 28 days). For details of the environmental fate study, please refer to Appendix C.

The majority of the notified chemical will be thermally decomposed during use, or collected for recycling and re-refined. Most of the notified chemical is expected be recycled or reused for the calorific value at the end of its useful life. The notified chemical is expected to be either thermally decomposed during the recycling or to be reused for the calorific value as a component of the reused oil. In either case, the notified chemical is expected to be decomposed into water, oxides of carbon and phosphorus.

The notified chemical has a low adsorption/desorption coefficient (log Koc $\leq$ 1.25) which indicates that it will have low likelihood of partitioning to sediments, organic matter in soil or sludge in the sewer system and release to surface waters is expected. However, based on the assessed use pattern release to surface waters is expected to be minimal.

A small amount of the notified chemical may be sent to landfill as residues in empty containers, leaks or spills.
In water or landfill, the notified chemical is expected to undergo abiotic and biotic degradation processes, forming water, oxides of carbon and phosphorus.

7.1.3. Predicted Environmental Concentration (PEC)

The notified chemical is not anticipated to be released to surface waters. Therefore, the predicted environmental concentration (PEC) in aquatic environment has not been calculated.

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 LC50 > 100 mg/L	Not harmful to fish
Daphnia Toxicity	48 h LC50 = 20 mg/L	Harmful to aquatic invertebrates
Algal Toxicity	72 h E_rC50 = 95 mg/L	Harmful to algae
Inhibition of Bacterial Respiration	3 h NOEC = 160 mg/L	Not inhibitory to microbial respiration

Based on the above ecotoxicological endpoints for the notified chemical, it is expected to be harmful to aquatic invertebrates and algae, but is not expected to be harmful to fish. Therefore, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009), the notified chemical is formally classified as "Acute Category 3: Harmful to aquatic life". Based on the acute toxicity and lack of ready biodegradability of the notified chemical, it is formally classified as "Chronic Category 3; Harmful to aquatic life with long lasting effects" under the GHS.

7.2.1. Predicted No-Effect Concentration

The predicted no-effects concentration (PNEC) has been calculated from the most sensitive endpoint for aquatic invertebrates. A safety factor of 100 was used given acute endpoints for three trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50 (Daphnia, 48 h)	20	mg/L
Assessment Factor	100	
Mitigation Factor	1.00	
PNEC:	200	µg/L

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 gear oils. The notified chemical is not expected to be readily biodegradable or bioaccumulate in the environment. On the basis of the maximum annual importation volume, low expected aquatic exposure and assessed use pattern in gear oils, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Pour Point 31°C ± 3 °C

Method OECD TG 102 Melting Point/Melting Range.
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks Modified pour point method
Test Facility Envigo (2016a)

Boiling Point 198 °C at 101.3 kPa

Method OECD TG 103 Boiling Point.
EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks Differential scanning calorimetry method
Test Facility Envigo (2016a)

Density 994 kg/m^3 at 20.0 °C

Method OECD TG 109 Density of Liquids and Solids.
EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks Gas comparison pycnometer method
Test Facility Envigo (2016a)

Vapour Pressure 0.254 Pa at 25 °C
0.687 Pa at 80 °C

Method OECD TG 104 Vapour Pressure.
EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks Vapour pressure was determined at two temperatures using the vapour pressure balance method
Test Facility Envigo (2016b)

Water Solubility 6.21 x 10^{-3} – 2.97 g/L at 20 ± 0.5 °C (the notified chemical is a mixture)

Method OECD TG 105 Water Solubility.
EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks Flask Method. The water solubility of the test item was determined using a nominal loading rate of 10%. The samples were analysed for four main components. The mean sample results were corrected for the percentage composition of the component using an approximate area percentage value obtained from the standards of the definitive test analysis. The results were taken as approximate because there was variation in the area percentage during the analysis.
Test Facility Envigo (2016c)

Hydrolysis as a Function of pH $t_{½}$ > 1 year at 25°C at pH 4, 7 and 9

Method 92/69/EEC C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$ (years)*
4	25	> 1
7	25	> 1
9	25	> 1

Remarks Sample solutions were prepared in glass flasks at nominal concentration of 250 mg/L in three buffer solutions with the aid of sonication. The stock solutions were split into individual glass vessels and sealed with minimal headspace for each data point. The concentration of test item in the sample solutions was determined by high performance liquid chromatography – mass spectrometry (HPLC-MS).
Less than 10% hydrolysis was observed after 5 days at 50°C at pH 4, 7 and 9 and therefore

the estimated half-life at 25°C is > 1 year.
Test Facility Envigo (2016d)

Partition Coefficient (n-octanol/water) log Pow = -9.88 x 10^{-2} – 2.68 at 23.5 ± 0.5 °C (the notified chemical is a mixture of components)

Method OECD TG 107 Partition Coefficient (n-octanol/water): Shake Flask Method
EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks Flask Method. The samples were analysed for four main components.
Test Facility Envigo (2016c)

Surface Tension 40.0 mN/m at 21.8 °C

Method OECD TG 115 Surface Tension of Aqueous Solutions.
EC Council Regulation No 440/2008 A.5 Surface Tension.
Remarks Concentration: 1 g/L
Test Facility Envigo (2016a)

Adsorption/Desorption log K_{oc} = <1.25

Method OECD TG 121 Estimation of the Adsorption Coefficient (K_{oc}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)
Remarks The test was performed with an approximately neutral pH mobile phase as the test item was an inorganic salt. The sample solution was analysed for four main anionic components: monoalkyl-phosphate, dialkyl-pyrophosphate, dialkyl-phosphate and trialkyl-pyrophosphate. The sample results were calculated based on these four main components.
Test Facility Envigo (2016d)

Flash Point 99 ± 2°C °C at 102.6 kPa

Method EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks Closed cup equilibrium method
Test Facility Envigo (2016e)

Autoignition Temperature 296 ± 5 °C

Method EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks Measured using a carbolite flask heater use
Test Facility Envigo (2016e)

Explosive Properties Predicted negative

Method EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks Based on the chemical structure of the test item
Test Facility Envigo (2016e)

Oxidizing Properties Predicted negative

Method EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids).
Remarks Based on the chemical structure of the test item
Test Facility Envigo (2016e)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified Chemical

METHOD: OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure.
EC Council Regulation No 440/2008 B.1 bis Acute toxicity (oral) fixed dose method.

Species/Strain	Rat/ Female Wistar (RccHan™:WIST)
Vehicle	Arachis oil BP
Remarks - Method	No deviations from protocol.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity	There were no unscheduled deaths during the study. Noisy respiration was noted in the initial animal, treated at a dose level of 2000 mg/kg, 13 and 14 days after dosing. No other signs of systemic toxicity were noted.
Effects in Organs	There were no abnormalities noted at necroscopy.
Remarks - Results	Animals showed expected gains in body weight over the observation period, except for one animal treated at a dose level of 2000 mg/kg which showed expected gain in body weight during the first week but body weight loss during the second week.

CONCLUSION: The notified chemical is of low toxicity via the oral route.

TEST FACILITY: Envigo (2016f)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 402 Acute Dermal Toxicity.
EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal).

Species/Strain	Rat/ Wistar (RccHan™:WIST)
Vehicle	None
Type of dressing	Semi-occlusive.
Remarks - Method	No deviations from protocol

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 M, 5 F	2000	0/10

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Very slight to well-defined erythema, very slight oedema, crust formation, desquamation and small superficial scattered scabs were noted in treated animals. Treated skin sites of males appeared normal 7 days after dosing and the treated skin sites of four females appeared normal 7 or 14 days after treatment. Small superficial scattered scabs were still present at the treatment site of one female at the end of the observation period on Day 14.

Signs of Toxicity - Systemic	None
Effects in Organs	All animals showed expected gains in body weight.
Remarks - Results	There were no deaths or abnormalities were noted at necropsy.

CONCLUSION The notified chemical of low toxicity via the dermal route.

TEST FACILITY Envigo (2016g)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

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 (Hsdlf:NZW)
Number of Animals	3
Vehicle	None
Observation Period	72 hours
Type of Dressing	Semi-occlusive.
Remarks - Method	No deviations from protocol

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	2.0	1.0	0.7	2	> 7 days	0
Oedema	1.7	1.0	0.3	2	< 7 days	0

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

Remarks - Results Very slight to well-defined erythema and very slight to slight oedema was observed in all three rabbits after application of the test substance to the intact skin. A light brown discoloration of the epidermis, loss of skin elasticity, slight desquamation and glossy skin were also observed. One treated skin site appeared normal at the 72-hour observation and one other treated skin site appeared normal at the 7-day observation. In the remaining rabbit glossy skin and slight desquamation was present at the 7-day observation. No corrosive effects were noted.

CONCLUSION The notified chemical is slightly-irritating to the skin.

TEST FACILITY Envigo (2016h)

B.4. Irritation – eye (in vivo)

TEST SUBSTANCE Notified chemical

METHOD Evaluation of Ocular Irritancy Potential (Rabbit Enucleated Eye Test (REET))

Vehicle	none
Remarks - Method	Method for Evaluation of Ocular Irritation by Slit-Lamp Biomicroscopic Examination - McDonald - Shadduck Score System The test item was applied onto the cornea of each of three enucleated eyes which had been maintained at a temperature of 32 ±1.5 °C within the superfusion chamber. A further two enucleated eyes remained untreated for control purposes.

RESULTS

Corneal Opacity	Fluorescein Uptake	Mean Corneal Swelling (%)						Condition of Corneal Epithelium
		Test Eyes[a]			Control Eyes[b]			
Corneal cloudiness × Area	Intensity of fluorescein uptake × Area	60 min	120 min	240 min	60 min	120 min	240 min	
3	4+	31.3 +	54.7 +	90.0 +	7.9	9.0	7.8	Sloughing +

a = For each time point the swelling recorded is the mean of three eyes
b = For each time point the swelling recorded is the mean of two eyes
+ = Meets or exceeds cut-off value indicating a severe ocular irritant

Remarks - Results

Some loss of transparency was noted in all eyes tested with the test substance. No corneal effects were noted in the control eyes during the study period.
Corneal swelling of the eyes treated with the test substance was considerably greater than that observed in the control eyes over the same period and exceeded the 25% cut-off value.
Sloughing of the corneal epithelium was noted in the eyes tested with the test substance. The condition of the corneal epithelium of the control eyes appeared normal during the study.
Fluorescein uptake was noted in the eyes tested with the test substance.

CONCLUSION

The notified chemical was considered to cause severe ocular irritancy *in vivo* to the eye under the conditions of the test.

TEST FACILITY

Envigo (2016i)

B.5. Skin sensitisation

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 406 Skin Sensitisation - Modified Buehler test.

Species/Strain

Guinea pig/Hartley-derived albino

PRELIMINARY STUDY

Maximum Non-irritating Concentration:
topical: 20%

MAIN STUDY

Number of Animals

Test Group: 25M, 25F Control Group: 10M, 10F

Vehicle

Mineral oil

Positive control

Conducted in parallel with the test substance

INDUCTION PHASE

Induction Concentration:
topical: 100%

Signs of Irritation

Yes

CHALLENGE PHASE

1st challenge

topical: 25% and 15%

2nd challenge

topical: 1.5%

Remarks - Method

α-Hexylcinnamaldehyde (HCA) was used as positive control (5% in ethanol for induction and 2.5% and 1.0% in acetone for challenge).
A dose level of 100, 75, 50 and 25% was used in the Experimental Design-1st Range-Finding Study, and 20, 12.5, 5 and 1% in the Experimental Design- 2nd Range-Finding Phase.
None of the protocol deviations was considered to have impacted the overall integrity of the study or the interpretation of the study results and conclusions.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st challenge		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	25%	5/20	15/20		
	15%			10/20	18/20
(Rechallenge)	1.5%			15/20	16/20
Control Group	25%	0/10	0/10		
	15%	0/10	0/10		

Remarks - Results: The test substance was considered to be a contact sensitiser in guinea pigs. The criterion for sensitisation (dermal scores ≥ 1 in at least 15% of the test animals) was met. The results of the HCA positive control study demonstrated that a valid test was performed and indicated that the test design would detect potential contact sensitizers.

CONCLUSION: There was evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.

TEST FACILITY: Charles River (2016)

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

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 429 Skin Sensitisation: Local Lymph Node Assay

Species/Strain: Mouse/ CBA/Ca

Vehicle: Acetone/olive oil 4:1

Preliminary study: Yes

Positive control: Conducted in parallel with the test substance. α-Hexylcinnamaldehyde, tech., 85%, at a concentration of 25% v/v in acetone/olive oil 4:1.

Remarks - Method: No significant deviations from protocol.
Preliminary Screening Test: No signs of systemic toxicity, visual local skin irritation or irritation indicated by an equal to or greater than 25% increase in mean ear thickness were noted.
Based on this information the dose levels selected for the main test were 50%, 15% and 1.5% w/w in acetone/olive oil 4: 1.

RESULTS

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	5	2920.91	NA
1.5%	5	2229.08	0.76
15%	5	14062.02*	4.81
50%	5	23889.00*	8.18
Positive Control			
25%	5	15487.97*	5.30

*Significantly different from control group $p < 0.01$

EC3: 9%

Remarks - Results: No deaths were noted. No signs of systemic toxicity or local irritation were observed in the test or control animals during the test.

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

TEST FACILITY: Envigo (2016j)

B.7. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain	Rat/Wistar Han™:RccHan™:WIST
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Arachis oil BP
Remarks - Method	Based on the results of a Seven Day Repeated Dose Oral (Gavage) Range-Finding Toxicity Study in the Rat which was performed at doses up to 1000 mg/kg bw/day. A high dose level of 600 mg/kg bw/day was considered suitable for use in the twenty-eight day repeated dose oral gavage toxicity study based on reduced body weight gains at 1000 mg/kg bw/day.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5M, 5F	0	0/10
low dose	5M, 5F	35	0/10
mid dose	5M, 5F	350	0/10
high dose	5M, 5F	600	3/10
control recovery	5M, 5F	0	0/10
high dose recovery	5M, 5F	600	0/10

Mortality and Time to Death

Three unscheduled deaths during the study were noted in the 600 mg/kg bw/day dose group related to the irritant properties of the test item and/or gavage-related reflux. One Female was found dead on day 14 and the other two females were killed for welfare reasons on Days 27 and 23 due to signs of respiratory distress along with poor clinical condition and body weight losses.

Clinical Observations

Sporadic episodes of respiratory signs were seen in surviving animals of both sexes of the 600 mg/kg bw/day dose group during the treatment period. This was related to microscopic lesions in the trachea, which included moderate ulceration and/or areas of epithelial degeneration/regeneration in some animals.

Instances of increased post-dose salivation were observed in animals of both sexes in the 350, and 600 mg/kg bw/day dose groups, and was considered by the study authors to be due to an irritant nature of the test item.

Functional performance test and behavioural and sensory reactivity assessments did not identify any effect from treatment with the test item at any dose level.

A decrease in body weight gains in animals of both sexes in the 600 mg/kg bw/day dose group at the end of the treatment period was noted when compared to the control group (approximately 29% lower in males and 34% lower in females).

Marginally low food intake in 600 mg/kg bw/day dose group (both sexes) and 350 mg/kg bw/d male dose group were observed compared to the control group.

Compared to the control group, sporadic instances of slightly higher water intake in 600 mg/kg bw/d both sexes animal group and 350 mg/kg bw/day male dose group from week two of treatment were noted as well as in the 350 mg/kg bw/d female dose group during the last week of treatment.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No toxicologically significant effects were detected at any dose level in animals of both sexes from haematology, blood chemistry or urinalysis findings.

Effects in Organs

In the 600 mg/kg bw/day dose group all males and 3 out of 4 surviving females had sloughing of the non-glandular region of the stomach, with the remaining female at this dose having sloughing of the glandular region of the stomach. One male in the 350 mg/kg bw/day dose group had reddened lungs from multifocal alveolar haemorrhages. Another male in the 35 mg/kg bw/day dose group had small testes and epididymides with bilateral testicular tubular degeneration/atrophy and reduced luminal sperm in the epididymides. There were no treatment related macroscopic effects in the recovery animals.

There were statistically significant increases in liver and kidney weights in male animals in the 350 and 600 mg/kg bw/day dose groups. Females in the 600 mg/kg bw/day dose group showed increases in body weight/brain weight ratios and decreases in body weight/pituitary weight ratios. Male animals in the 35 mg/kg bw/day dose group and the recovery group dosed at 600 mg/kg bw/day had higher thyroid/parathyroid weights.

Minimal to slight, epithelial hyperplasia and hyperkeratosis of the forestomach was observed in animals dosed at 600 mg/kg bw/day along with epithelial degeneration/regeneration of the trachea with or without associated inflammation and a moderate ulceration of the respiratory epithelium, with underlying inflammation in one male. Minimal to slight centrilobular hepatocellular hypertrophy in the liver was seen in most males in the 350 and 600 mg/kg bw/day dose groups along with some females in the 600 mg/kg bw/day dose group. Minimal hypertrophy of the follicular epithelium of the thyroid glands in some males at 350 mg/kg bw/day and male and female animals in the 600 mg/kg bw/day dose groups. Minimal hypertrophy of the zona glomerulosa of the adrenal glands was seen in some males at 600 mg/kg bw/day dose group. An increased incidence and severity of accumulation of hyaline droplets in cortical tubules of the kidneys in association with tubular basophilia and granular casts was observed in males in the 350 and 600 mg/kg bw/day dose groups.

Remarks – Results	At a dose level of 600 mg/kg bw/day there were a number of treatment related deaths along with decreased bodyweight gains, respiratory distress, and adverse changes in the forestomach, trachea, liver, thyroid, kidneys and adrenal glands. Decreased bodyweight gains, reddened lungs, and adverse effects in the liver, kidney and thyroid were also seen in animals dosed at 350 mg/kg bw/day, although to a lesser extent than at the higher dose. The study authors attributed the effects to the irritant properties of the test substance or rat specific toxicology, and therefore considered the No Observed Adverse Effect Level (NOAEL) for systemic toxicity to be equal to the maximum dose tested of 600 mg/kg bw/day. However, when all effects are considered the NOAEL would be the lowest dose tested, 35 mg/kg bw/day.
CONCLUSION	The NOAEL was established as 35 mg/kg bw/day in this study, based on the adverse effects seen at 350 and 600 mg/kg bw/day.
TEST FACILITY	Envigo (2016k & 2016l)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, *E. coli*: WP2uvrA
Metabolic Activation System	S9 Microsomal fraction from male rats induced with Phenobarbitone/β-Naphthoflavone
Concentration Range in Main Test	a) With metabolic activation: 1.5 to 5000 µg/plate b) Without metabolic activation: 5 to 5000 µg/plate
Vehicle	Acetone

Remarks - Method

The positive control items used in the series of plates without S9-mix were as follows:
N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG): for WP2uvrA, TA100 and TA1535. 9-Aminoacridine (9AA): for TA1537 and 4-Nitroquinoline-1-oxide (4NQO): for TA98

In addition, 2-Aminoanthracene (2AA) and Benzo(a)pyrene (BP), which are non-mutagenic in the absence of metabolizing enzymes, were used in the series of plates with S9-mix:
2-Aminoanthracene (2AA): for TA100, TA1535, TA1537 and WP2uvrA
Benzo(a)pyrene (BP): for 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		≥ 1500	≥ 5000	negative
Test 2		≥ 5000	≥ 5000	negative
Present				
Test 1		≥ 5000	≥ 5000	negative
Test 2		> 5000	≥ 5000	negative

Remarks - Results

There were no significant increases in the frequency of revertant colonies recorded for any of the bacterial strains, with any dose of the test item, either with or without metabolic activation (S9-mix) in test 1and in test 2.

All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with or without metabolic activation. Thus, the sensitivity of the assay and the efficacy of the S9-mix were validated.

CONCLUSION

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

TEST FACILITY

Envigo (2016m)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Species/Strain: Rat/Sprague-Dawley
Cell Type/Cell Line: Human Lymphocytes
Metabolic Activation System: S9 Microsomal fraction from male rats induced with Phenobarbitone/β-Naphthoflavone
Vehicle: Acetone

Remarks - Method

No deviations from protocol
The positive control items were as follows: Mitomycin C (MMC) (without S9-mix, and Cyclophosphamide (CP) (with S9-mix.
The dose levels used in the Main Experiment were selected using data from the Cell Growth Inhibition Test (Preliminary Toxicity Test) where the results indicated that the maximum concentration was limited on precipitate and toxicity.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*

Absent			
Test 1	0*, 40, 80*, 160*, 320*, 640*, 1280	4h	24
Test 2	0*, 20*, 40*, 80*, 160*, 240, 320, 640	24h	24
Present			
Test 1	0*, 40, 80*, 160*, 320*, 640*, 1280	4h	24
Test 2			

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 312.5	> 640	> 640	negative
Test 2		≥ 80	> 160	negative
Present				
Test 1	≥ 1250	> 640	> 640	negative

Remarks - Results

The test item induced marked evidence of toxicity in all three of the exposure groups. There was no statistically significant increase in chromosome or numerical aberrations in any of the exposure groups when comparing the notified chemical to the control.

All vehicle (Acetone) controls had frequencies of cells with aberrations within the range expected for normal human lymphocytes.

All of the positive control chemicals used in the test induced statistically significant increases in the frequency of cells, with aberrations indicating that the sensitivity of the assay and the efficacy of the S9-mix were validated either with or without metabolic activation.

CONCLUSION

The notified chemical was not clastogenic to Human Lymphocytes treated in vitro under the conditions of the test.

TEST FACILITY

Envigo (2016n)

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: CO_2 Evolution.
Inoculum	Activated sewage sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Inorganic carbon (IC) analysis
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	23	6	63	6	39
14	31	14	65	14	70
21	43	21	78	21	72
28	49	28	81	28	73

Remarks - Results

All validity criteria for the test were satisfied.
The reference item (sodium benzoate) attained 70% biodegradation after 14 days and 86% biodegradation after 28 days thereby confirming the suitability of the inoculums and test conditions.

The toxicity control attained 65% degradation after 14 days and 77% biodegradation after 28 days thereby confirming that the test material was not toxic to the sewage treatment micro-organisms used in the study.
The test material attained 43% degradation after 28 days and, therefore, cannot be considered as readily biodegradable under the conditions of OECD Guideline 301B.

CONCLUSION: The notified chemical is not readily biodegradable.

TEST FACILITY: Envigo (2015a)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi static. EC Council Regulation No 440/2008 C.1 Acute Toxicity for Fish – Semi static.
Species	*Oncorhynchus mykiss* (Rainbow trout)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	140 mg $CaCO_3$/L
Analytical Monitoring	High performance liquid chromatography with mass spectrometry (HPLC-MS)
Remarks – Method	The test was conducted in accordance with the test guideline above, with no significant deviation in protocol reported. A nominal amount of 2200mg of test item was weighed out, diluted and

sonicated for 90-120 minutes to give the 100 mg/L test concentration. Temperature and dissolved oxygen of the test solution after sonication was checked and adjusted before exposure.

RESULTS

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

LC50	> 100 mg/L at 96 hours
NOEC	100 mg/L at 96 hours
Remarks – Results	All validity criteria for the test were satisfied. The test solutions were renewed daily. Analysis of the fresh test preparations at 0 and 72 hours and old media at 24 and 96 hours showed measured test concentrations to be similar to nominal concentration. Thus, the results were based on nominal concentrations. The 96 h LC50 and NOEC for fish were determined to be > 100 mg/L and 100 mg/L, respectively, based on nominal concentrations.
CONCLUSION	The notified chemical is not considered to be harmful to fish.
TEST FACILITY	Envigo (2015b)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test – Static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	High performance liquid chromatography with mass spectrometry (HPLC-MS)
Remarks - Method	The test was conducted in accordance with the test guideline above, with no significant deviation in protocol reported. A nominal amount of test item (200 mg) was dissolved in test water with the aid of ultrasonication for approximately 1 hour. The volume was adjusted to 2 litres to give 100 mg/L test concentration from which a series of dilutions was made to give further test concentrations of 6.2, 12.5, 25, and 50 mg/L. Each prepared concentration was inverted several times to ensure adequate mixing and homogeneity.

RESULTS

Concentration mg/L *Nominal*	*Number of D. magna*	*Cumulative Immobilised (%)* *24 h*	*48 h*
Control	20	0	0
6.2	20	0	0
12.5	20	0	20
25.0	20	10	65
50.0	20	20	95
100.0	20	100	100

LC50	20 mg/L (95% CI 17-25 mg/L) at 48 hours
NOEC	12.5 mg/L 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. Analysis of the test preparations at 0 and 48 hours showed measured test concentrations to be similar to nominal concentration. Thus, the results were based on nominal concentrations. The 48 h EC50 and NOEC for daphnids were determined to be 20 mg/L (95% CI 17-25 mg/L) and 12.5 mg/L, respectively, based on nominal concentrations.
CONCLUSION	The notified chemical is considered to be harmful to aquatic invertebrates.
TEST FACILITY	Envigo (2016o)

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: 1.0, 3.2, 10, 32 and 100 mg/L Actual: Not reported
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	High performance liquid chromatography with mass spectrometry (HPLC-MS)
Remarks - Method	The test was conducted in accordance with the test guideline above, with no significant deviation in protocol reported. A nominal amount of test item (100 mg) was dissolved in culture medium with the aid of ultrasonication for approximately 15 minutes and the volume adjusted to 1 litre to give the 100 mg/L stock solutions. A series of dilutions was made from this stock solutions to give further stock solutions of 32, 10, 3.2 and 1.0 mg/L. An aliquot (500 mL) of each of the stock solutions was separately inoculated with algal suspension (2.6 mL) to give the required test concentrations of 1.0, 3.2, 10, 32 and 100 mg/L.

RESULTS

Biomass		*Growth*	
E_bC50 *mg/L at 72 h*	*NOE_bC* *mg/L*	*E_rC50* *mg/L at 72 h*	*NOE_rC* *mg/L*
22 (95% CI 18-27)	10	95	10

Remarks - Results	All validity criteria for the test were satisfied. Renewal of the test solutions was not specified. Analysis of the test preparations at 0 showed measured test concentrations to range from 80% to 97% of nominal. All measured concentrations at 72 hours were within ±20% of the nominal concentrations with the exception of the 100 mg/L test sample where a measured concentration of 79% of nominal was attained. However, given that the overall concentration to which the algae were exposed to was 88% of nominal, this was considered insignificant. Thus, all results were calculated based on nominal test concentrations. The 72 h E_rC50 and NOEC for algae were determined to be 95 mg/L and 10 mg/L, respectively, based on nominal concentrations.
CONCLUSION	The notified chemical is considered to be harmful to algae.

TEST FACILITY	Envigo (2016p)

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: 56, 100 and 180 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 substance. Following a preliminary range-finding test, activated sewage sludge was exposed to an aqueous dispersion of the test item at concentrations of 56, 100 and 180 mg/L (5 replicates) for a period of 3 hours at temperatures of between 21 to 22 °C with the addition of a synthetic sewage as a respiratory substrate.
RESULTS	
IC50	Not reported
NOEC	180 mg/L at 3 hours
Remarks – Results	All validity criteria for the test were satisfied. The 3 h NOEC was determined to be 180 mg/L, based on nominal concentrations.
CONCLUSION	The notified chemical is not inhibitory to microbial respiration.
TEST FACILITY	Envigo (2016q)

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