File No.: STD/1727

February 2021

AUSTRALIAN INDUSTRIAL CHEMICALS INTRODUCTION SCHEME (AICIS)

PUBLIC REPORT

Z-190

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals Act 2019 (the IC Act) and Industrial Chemicals (General) Rules 2019 (the IC Rules) by following the Industrial Chemicals (Consequential Amendments and Transitional Provisions) Act 2019 (the Transitional Act) and Industrial Chemicals (Consequential Amendments and Transitional Provisions) Rules 2019 (the Transitional Rules)*. The legislations are Acts of the Commonwealth of Australia. The Australian Industrial Chemicals Introduction Scheme (AICIS) is administered by the Department of Health, and conducts the risk assessment for human health. The assessment of environmental risk is conducted by the Department of Agriculture, Water and the Environment.

This Public Report is available for viewing and downloading from the AICIS website. For enquiries please contact AICIS at:

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

Executive Director
AICIS

TABLE OF CONTENTS

SUMMARY

The following details will be published on the AICIS website:

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1727	Lubrizol International, Inc.	Z-190	Yes	< 1,000 tonnes per annum	Industrial lubricant additive

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard Classification	*Hazard Statement*
Flammable Liquids (Category 4)	H227 – Combustible liquid

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 2	H401 – Toxic to aquatic life
Chronic Category 2	H411 – Toxic to aquatic life with long lasting effects

Human Health Risk Assessment
Under the conditions of the occupational settings described, the assessed chemical is not considered to pose an unreasonable risk to the health of workers.

When used in the proposed manner, the assessed chemical is not considered to pose an unreasonable risk to public health.

Environmental Risk Assessment
On the basis of the reported use pattern, the assessed chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The assessed chemical should be classified as follows:
  - Flammable Liquid (Category 4): H227 – Combustible liquid

  The above should be used for products/mixtures containing the assessed chemical, if applicable, based on the concentration of the assessed chemical present.

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 assessed chemical during reformulation:
  - Automated processes
  - Enclosed processes if possible
  - Adequate general ventilation

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during reformulation:
  - 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 assessed chemical during reformulation:
  - Impervious gloves
  - Safety glasses or goggles
  - Protective clothing

  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 assessed 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.

Storage

- The handling and storage of the assessed chemical should be in accordance with the Safe Work Australia Code of Practice for *Managing Risks of Hazardous Chemicals in the Workplace* (SWA, 2012) or relevant State or Territory Code of Practice.

Emergency procedures

- Spills or accidental release of the assessed chemical should be handled by containment and subsequent safe disposal.

Disposal

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

Regulatory Obligations

Specific Requirements to Provide Information
This risk assessment is based on the information available at the time of the application. The Executive Director may initiate an evaluation of the chemical based on changes in certain circumstances. Under Section 101 of the IC Act the applicant of the assessed chemical has post-assessment regulatory obligations to provide information to AICIS when any of these circumstances change. These obligations apply even when the assessed chemical is listed on the Australian Inventory of Industrial Chemicals (the Inventory).

Therefore, the Executive Director of AICIS must be notified in writing within 20 working days by the applicant or other introducers if:

- the function or use of the chemical has changed from an industrial lubricant additive, 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 on the skin sensitisation of the assessed chemical;
- additional information has become available to the person as to an adverse effect of the chemical on human health, or the environment.

The Executive Director will then decide whether an evaluation of the introduction is required.

Safety Data Sheet
The SDS of the assessed chemical provided by the applicant was reviewed by AICIS. The accuracy of the information on the SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND APPLICATION DETAILS

APPLICANT(S)
Lubrizol International, Inc. (ABN: 52 073 495 603)
28 River Street
Silverwater NSW 2128

APPLICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne per year)

PROTECTED INFORMATION (SECTION 38 OF THE TRANSITIONAL ACT)
Data items and details taken to be protected information include: chemical name, other name(s), CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, impurities, additives/adjuvants and use details.

VARIATION OF DATA REQUIREMENTS (SECTION 6 OF THE TRANSITIONAL RULES)
Schedule data requirements are not varied.

PREVIOUS APPLICATION IN AUSTRALIA BY APPLICANT(S)
None

APPLICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Z-190

MOLECULAR WEIGHT
< 2,000 g/mol

ANALYTICAL DATA
Reference NMR, FTIR, TGA, APC, ESI-MS, and UV-Vis spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: light-yellow liquid

Property	*Value*	*Data Source/Justification*
Pour Point	-33 °C	Measured
Boiling Point	178.7 °C	Measured
Density	1,097 kg/m^3 at 20 °C	Measured
Vapour Pressure	2.96 × 10^{-4} kPa at 25 °C	Measured
Water Solubility	12.6 mg/L at 20 °C (TOC Analysis) 26.3 mg/L at 20 °C (Phosphorus Analysis)	Measured
Hydrolysis as a Function of pH	Not determined	Chemical consists of a complex mixture having most species containing hydrolysable functional groups; however, hydrolysis is not expected to occur due to low water solubility.

Property	*Value*	*Data Source/Justification*
Partition Coefficient (n-octanol/water)	log Pow < 1 - > 6.5 at 20 °C	Measured
Surface Tension	55.0 ± 0.8 Dynes/cm at 20 °C	Measured
Adsorption/Desorption	log Koc < 0.5 - 4.92	Measured
Dissociation Constant	Not determined	Chemical consists of a complex mixture having some species containing potentially dissociable functional groups.
Flash Point	106 °C	Measured
Flash Point	88.1 °C	Measured
Flammability	Combustible liquid	SDS
Autoignition Temperature	287 °C	Measured
Explosive Properties	Not explosive	Measured
Oxidising Properties	Not an oxidising liquid	Measured

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

Reactivity
The assessed 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 assessed chemical is recommended for physical 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*
Flammable Liquids (Category 4)	H227 – Combustible liquid

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF ASSESSED CHEMICAL (100%) OVER NEXT 5 YEARS
The assessed chemical will not be manufactured in Australia. The assessed chemical will be imported into Australia at 5-10% concentration for reformulation into lubricant products at a concentration of ≤ 1%.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 10	< 100	< 100	< 1,000	< 1,000

PORT OF ENTRY
Western Australia, Queensland and Victoria

IDENTITY OF RECIPIENTS
Lubrizol International Inc.

TRANSPORTATION AND PACKAGING
The assessed chemical will be transported via isotainer or 249 L IBC containers or in 208 L drums and as finished lubricant products within Australia by road.

USE
The assessed chemical will be used as a lubricant additive at ≤ 1% concentrations.

OPERATION DESCRIPTION
The imported product containing the assessed chemical at 5-10% concentration will be added to a blending vessel using an automated transfer steps. The assessed chemical will be mixed with other ingredients. Once mixing is complete, the finished lubricant product containing ≤ 1% of the assessed chemical will be transferred into containers of various sizes. Packaging equipment is expected to be automated and housed within or near the

blending operation area. The blending and packaging facilities are expected to be well ventilated and fully automated. No Do-it-Yourself (DIY) use is expected.

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)*
Blending operations	1-3	3-4
Packaging operations	2-4	1-3
Distribution	0-2	100-225
End use	0-2	100-225

EXPOSURE DETAILS

Transport and storage

Transport and storage workers are not likely to be exposed to the assessed chemical except in the case of an accident involving breach of containers.

Reformulation

During reformulation, dermal and ocular exposure of workers to the assessed chemical at ≤ 10% concentration may occur during transfer stages, blending, packaging, and cleaning and maintenance of equipment. The applicant states that exposure is expected to be minimised through the use of personal protective equipment (PPE) such as protective clothing, safety glasses and gloves. Given the low vapour pressure of the chemical, inhalation exposure is not expected and the blend facility is also expected to be well ventilated.

The packaging equipment is expected to be automated and housed within or near the blending operation area. An enclosed or open filling system may be used. Dermal contact would be the main route of occupational exposure and the packaging workers are expected to wear aprons, gloves and safety glasses to minimise exposure.

End use

Workers may be exposed to lubricants containing the chemical at ≤ 1% concentration during use as an anti-wear additive in transmissions and drive train oil for off highway applications.

Given the low vapour pressure of the chemical, inhalation exposure is not expected; however dermal and ocular exposure is possible. According to the applicant, dermal and ocular exposure will be mitigated through the use of appropriate PPE, including protective clothing, safety goggles, and nitrile or neoprene gloves.

6.1.2. Public Exposure

The assessed chemical will not be sold or made available to the general public. Therefore, the general public is not expected to be exposed to the assessed chemical.

6.2. Human Health Effects Assessment

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

Endpoint	***Result and Assessment Conclusion***
Acute oral toxicity – rat	LD50 > 2,000 mg/kg bw; low toxicity
Acute dermal toxicity – rat	LD50 > 2,000 mg/kg bw; low toxicity
Skin irritation – *in vitro* Epiderm Skin Corrosion Test	non-corrosive
Skin irritation – *in vitro* EpiDermTM Reconstructed Human Epidermis Model	non-irritating
Skin irritation – rabbit	slightly irritating
Eye irritation – *in vitro* Bovine Corneal Opacity and Permeability (BCOP) Test	no prediction was made
Eye irritation – Reconstructed Human Cornea-Like Epithelium (RhCE) test method EpiOcular Test	non-irritating

Endpoint	*Result and Assessment Conclusion*
Eye irritation – rabbit	slightly irritating
Skin sensitisation – guinea pig, Buehler Test	no evidence of sensitisation
Skin sensitisation – *in vitro* ARE-Nrf2 luciferase test	positive
Repeat dose Oral toxicity – rat, 28 days	NOAEL = 100 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* Mammalian Chromosome Aberration Test in Human Lymphocytes	non genotoxic
Genotoxicity – *in vivo* L5178Y TK +/- Mouse Lymphoma Assay	non genotoxic
Reproductive and developmental toxicity – rat	NOAEL = 600 mg/kg bw/day for reproductive and developmental toxicity

Toxicokinetics, Metabolism and Distribution
No toxicokinetic data on the assessed chemical were submitted. Based on the water solubility (12.6 – 26.3 mg/L at 20 °C), molecular weight and partition coefficient (log Pow < 1 - > 6.5 at 20 °C) of the assessed chemical, there is potential for the chemical to cross biological membranes.

Acute Toxicity
The assessed chemical is of low acute oral and dermal toxicity based on studies conducted in rats. No acute inhalation toxicity data on the assessed chemical was submitted.

Irritation
According to the results of the *in vitro* corrosion/irritation assays and studies conducted in rabbits, the assessed chemical was found to be only slightly irritating to the skin and eyes.

Sensitisation
The assessed chemical was not considered a skin sensitiser based on a Buehler test in guinea pigs.

In a skin sensitisation Buehler test, 20 guinea pigs were induced with the assessed chemical applied three times topically, with one week apart. Irritation responses were observed after topical challenge with the assessed chemical at 100% concentration in both the test and control (acetone) groups. The study report concluded that the incidence and severity of the irritation in the test group and the control group were similar during challenge phase. Therefore, the assessed chemical was not considered a skin sensitiser.

Only the second Key Event assay on the adverse outcome pathway (AOP) for skin sensitisation, Keratinocyte Activation ARE-Nrf2 Luciferase test, was provided. The ARE-Nrf2 Luciferase Assay investigates keratinocyte activation by measuring the expression of a report luciferase gene under the control of a promoter from the antioxidant response element (ARE), a responding gene known to be upregulated by contact sensitisers. The assessed chemical showed positive responses in the *in vitro* ARE-Nrf2 Luciferase test, suggesting keratinocyte activation.

Although the assessed chemical is not classified as a skin sensitiser, skin sensitisation potential of the assessed chemical cannot be fully ruled out.

Repeated Dose Toxicity
In a 14-day repeated dose oral range finding study in rats no mortalities or treatment-related adverse effects were observed up to 1,000 mg/kg bw/day.

In a 28-day repeated dose oral toxicity study (with a 14-day recovery period), rats were administered with the assessed chemical at 0, 100, 350 and 1,000 mg/kg bw/day. No mortality or morbidity was observed in animals, throughout the study period. However, liver toxicity was observed at doses of 350 mg/kg bw/day and 1,000 mg/kg bw/day. Instances of salivation in some animals and a decreased activity in week 4 of two male animals, treated at 1,000 mg/kg bw/day were also noted. A reduction of weight gain was observed at 350 mg/kg bw/day in females (-9.7%) and at 1,000 mg/kg bw/day in both sexes (-14.2% in males and -11.7% in females) after 4 weeks of treatment. Adjusted kidney weights were statistically significantly high for males in the 350 mg/kg/day group and for both sexes in the 1,000 mg/kg/day group. Kidney weights were slightly high without statistical significance for males in the 100 mg/kg/day group. Liver cell necrosis was observed in the 350 (with less severity) and 1,000 mg/kg bw/day groups. The No Observed Adverse Effect Level (NOAEL) was established as 100 mg/kg bw/day in this study.

Mutagenicity/Genotoxicity
The assessed chemical was not mutagenic in a bacterial reverse mutation test. The assessed chemical was not clastogenic in an *in vitro* mammalian chromosome aberration test in human Lymphocytes and in an *in vitro* mammalian cell gene mutation L5178YTK+1- Mouse Lymphoma test.

Toxicity for Reproduction/Development
In a reproductive/development toxicity screening study, rats were administered oral gavage doses of the assessed chemical at 0, 100, 150, 350 and 600 mg/kg bw/day. All F0 males and females survived to the scheduled necropsies at up to 600 mg/kg bw/day. There were no effects reported on reproductive performance, offspring survival or development at 600 mg/kg bw/day. The NOAEL was established as 600 mg/kg bw/day for reproductive performance of adult animals and the survival, growth and development of their offspring.

Health Hazard Classification
Based on the available information, the assessed chemical is not recommended for classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The assessed chemical may cause slight skin and eye irritation. Repeated systemic exposure at high doses may cause adverse health effects.

Potential dermal and ocular exposure of workers to the assessed chemical at ≤ 10% concentration during reformulation and to the chemical at ≤ 1% concentration during end use may occur. However, the exposure during reformulation will be minimised by the use of the control measures in place and PPE according to the applicant. In addition, occupational exposure during end use will be mitigated by use of PPE by workers. Hence, no high exposure is expected during reformulation and end use to cause systemic effects.

During reformulation process, or when adding the industrial lubricant additive containing the assessed chemical, workers may be at risk of slight skin and eye irritation effects. However, the concentrations handled by workers (≤ 10% during reformulation and ≤ 1% during end-use applications) are relatively low and no irritation effects are expected at those concentrations. This risk should be reduced through the control measures in place to minimise worker exposure under industrial settings, including the use of automated processes and use of PPE (such as protective clothing, safety glasses and gloves), according to the applicant.

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

6.3.2. Public Health
The assessed chemical will not be made available to the public. It will be for industrial use only, hence public exposure is not expected. Therefore, when used in the proposed manner, the assessed chemical is not considered to pose an unreasonable risk to public health.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The assessed chemical will be imported as a component of a formulation for reformulation into the finished products. In general, the reformulation process is expected to involve blending operations that will be highly automated in closed systems, followed by automated filling of the reformulated products into containers. Waste generated from the reformulation process and accidental spills containing the assessed chemical are expected to be collected and either re-used to the extent practicable or disposed of to landfill, in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE

The assessed chemical will be used for lubricating various machinery and equipment with no DIY use expected. No significant environmental release is expected. Wastes containing the assessed chemical generated from operations such as cleaning machinery or equipment are expected to be collected and disposed of in accordance with local government regulations. Any accidental spills of the assessed chemical during use are to be collected and disposed of in accordance with local government regulations. Used oil containing the assessed chemical will be collected for disposal.

RELEASE OF CHEMICAL FROM DISPOSAL

Residues of the assessed chemical are expected to remain in the empty drums and containers. Empty drums and commercial containers are expected to be disposed of to landfill. Used oil containing the assessed chemical is expected to be collected and mixed with waste oils which are to be recycled, re-refined, possibly used as low-grade burner fuel or disposed of by approved waste management contractors, in accordance with local government regulations.

7.1.2. Environmental Fate

Most of the assessed chemical is expected to be disposed of by approved waste management contractors as a part of the waste oil recycling process. It is likely that the assessed chemical will be degraded into simpler compounds during refining. A minority of the assessed chemical may be released from accidental spills and leaks. In the environment, some components of the assessed chemical are expected to sorb to soil, with some components expected to be mobile based on wide log Koc range (log Koc < 0.5 – 4.92). The assessed chemical is not expected to bioaccumulate based on the low biomagnification factor (measured BMF = 9.3×10^{-6}). Two ready biodegradation studies were provided which were both conducted according to OECD TG 301B. Both studies indicated the assessed chemical is not readily biodegradable (3.3% and 6.3% degradation after 28 days, respectively). An inherent biodegradability study indicated that the chemical is considered to be inherently primarily degradable (26.6% ultimate degradation after 28 days based on BOD/COD and 78.3% primary degradation after 28 days based on chemical analysis; OECD TG 302C). The assessed chemical is expected to eventually be degraded via biotic and abiotic processes to form water and oxides of carbon, sulfur and phosphorus. For the details of the environmental fate studies refer to Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)

A predicted Environmental Concentration (PEC) was not calculated as the assessed chemical is not expected to be released into the aquatic environment in significant quantities under the proposed use pattern.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on the assessed 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 = 14 mg/L	Harmful to fish
Acute Daphnia Toxicity	48 h EL50 = 2.0 mg/L	Toxic to aquatic invertebrates
Daphnia reproductive toxicity	21 d NOEL = 0.6 mg/L 21 d EL50 = 1.7 mg/L	Toxic to aquatic invertebrate reproduction
Algal Toxicity	72 h EL50 > 100 mg/L NOEL = 10 mg/L	Not harmful to algal growth
Inhibition of Bacterial Respiration	3 h IC50 > 1,000 mg/L	Not harmful to bacterial respiration

Based on the above ecotoxicological endpoints, the assessed chemical is expected to be acutely very toxic to aquatic invertebrates and harmful to fish. Therefore, based on the acute ecotoxicity endpoint for daphnia, the assessed chemical is formally classified as Acute Category 2 (H401) – Toxic to aquatic life according to the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009).

Since there are only two adequate chronic endpoints, the long-term hazard is determined by comparing the long-term hazard obtained from both the acute and chronic endpoints and the most stringent outcome is used. This would result in a Chronic Category 1 classification based on the acute daphnia endpoint. However, according to Annex 9 of the GHS (section A9.3.3.2.3), the reproductive ecotoxicity study should be used to determine the chronic classification as the study was conducted on the same species as the most sensitive acute ecotoxicity endpoint (*Daphnia magna*). Based on the biodegradation data the assessed chemical is not rapidly biodegradable for the purposes of GHS classification. Therefore, based on the available data the assessed chemical is formally classified under the GHS as Chronic Category 2 (H411) – Toxic to aquatic life with long lasting effects.

7.2.1. Predicted No-Effect Concentration

A Predicted No-Effect Concentration (PNEC) was calculated based on the most sensitive chronic endpoint for daphnia (21-d NOEL = 0.6 mg/L) using an assessment factor of 50 as three acute endpoints and two chronic endpoints are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
21-d NOEL (invertebrates)	0.6	mg/L
Assessment Factor	50	
Mitigation Factor	1	
PNEC:	12.0	µg/L

7.3. Environmental Risk Assessment

A risk quotient (Q = PEC/PNEC) was not calculated as significant aquatic release of the assessed chemical is not expected based on the proposed use pattern. On the basis of the low environmental exposure from the reported use pattern, the assessed chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Pour Point -33 °C

Method	ASTM D97-15 (2015)
Test Facility	Lubrizol (2019)

Boiling Point 178.7 ± 0.5 °C

Method	OECD TG 103 Boiling Point
Remarks	A modification of the Siwoloboff procedure (a heated metal block instead of a liquid bath) was used. The boiling point testing showed an initial emergence of bubbles (at a temperature of 174.9 ± 1.5 °C. The boiling range was 174.9 ± 1.5 °C to 178.7 ± 0.5 °C. Thermal decomposition appeared to occur at 180 ºC.
Test Facility	Lubrizol (2019)

Density 1,097.1 ± 0.6 kg/m^3 at 20 ± 0.1 °C

Method	OECD TG 109 Density of Liquids and Solid
Remarks	Hubbard specific gravity bottles were used. Overall mean density result for the three samples were reported.
Test Facility	Lubrizol (2019)

Vapour Pressure 2.96×10^{-4} kPa at 25 °C
1.68×10^{-3} kPa at 65 °C
2.35×10^{-3} kPa at 85 °C

Method	OECD TG 104 Vapour Pressure (2006) EC Council Regulation No 440/2008 A.4 Vapour Pressure
Remarks	The balance method was used.
Test Facility	Covance (2019a)

Water Solubility 12.6 mg/L at 20 °C (TOC Analysis)
26.3 mg/L at 20 °C (Phosphorus Analysis)

Method	OECD TG 105 Water Solubility EC Council Regulation No 440/2008 A.6 Water Solubility
Remarks	Slow stirring flask method
Test Facility	Eurofins (2019a)

Partition Coefficient (n-octanol/water) log Pow (peak 1) < 1
log Pow (peak 2) = 1.69
log Pow (peak 3) = 5.35
log Pow (peaks 4 -7) > 6.5

Method	OECD TG 117 Partition Coefficient (n-octanol/water). EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	HPLC Method. Seven peaks were identified under the test conditions representing a complex mixture. The test substance was the assessed chemical.
Test Facility	Eurofins (2020a)

Surface Tension 55.0 ± 0.8 Dynes/cm at 20 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions
Remarks	Determined using a surface tensiometer with test substance concentration of 23.6 mg/L.
Test Facility	Dekra (2019)

Adsorption/Desorption log Koc (peaks 1 – 3) < 0.5
log Koc (peak 4) = 4.92

Method	OECD TG 121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)
Remarks	HPLC Method. Four peaks were identified under the test conditions representing a complex mixture.
Test Facility	Eurofins (2019b)

Flash Point 106 °C

Method	ASTM D93
Remarks	Pensky-Martens closed cup tester was used.
Test Facility	Lubrizol (2019)

Flash Point 88.1 °C

Method	ASTM D93
Remarks	Pensky-Martens closed cup tester was used. Average of the flash point results of two samples of the test substance (89.1 & 87.0 °C).
Test Facility	Lubrizol (2019)

Autoignition Temperature 287 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases)
Remarks	No cool flames were observed. Ignition produced an orange flame.
Test Facility	Dekra (2019)

Explosive Properties Not explosive

Method	EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks	The chemical did not exhibit an explosion during any of the two tests using BAM Fallhammer and Koenen Tube.
Test Facility	Dekra (2019)

Oxidizing Properties Not an oxidising liquid

Method	EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids)
Remarks	The chemical was found to have a mean pressure rise time greater than that observed for the reference: nitric acid.
Test Facility	Dekra (2019)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute Oral Toxicity – Rat

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure (2001)
Species/Strain	Rat/RccHan™:WIST albino
Vehicle	Corn oil
Remarks – Method	No protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	1 F	300	0/1
2	1 F	2,000	0/1
3	4 F	2,000	0/4

LD50	LD50 > 2,000 mg/kg bw
Signs of Toxicity	Piloerection (4 in 5 animals) and hunched posture (2 in 5 animals) were noted in animals dosed at 2,000 mg/kg bw and the effects disappeared by day 2. No clinical signs were noted in the animal dosed at 300 mg/kg bw or the remaining animal dosed at 2,000 mg/kg bw.
Effects in Organs	No abnormalities were observed in any animal at the macroscopic examination at study termination on day 15.
Remarks – Results	All animals showed expected gains in body weight.
CONCLUSION	The assessed chemical is of low acute toxicity via the oral route.
TEST FACILITY	Covance (2019b)

B.2. Acute Dermal Toxicity – Rat

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 402 Acute Dermal Toxicity (2017)
Species/Strain	Rat/Sprague-Dawley derived, albino
Vehicle	None
Type of dressing	Occlusive
Remarks – Method	No protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	5 per sex	2,000	0/10

LD50	> 2,000 mg/kg bw
Signs of Toxicity – Local	Dermal irritation was observed at the dose sites of six animals on day l and effects disappeared by day 2.
Signs of Toxicity – Systemic	No systemic toxicity was observed.
Effects in Organs	No gross abnormalities were observed for any of the animals at necropsy on day 14 (end of the observation period).
Remarks – Results	All treated animals survived and gained body weight during the study period.
CONCLUSION	The assessed chemical is of low acute toxicity via the dermal route.
TEST FACILITY	Product Safety Labs (2020a)

B.3. Skin Irritation – *In Vitro* Epiderm Skin Corrosion Test

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 431 *In vitro* Skin Corrosion – Human Reconstructed EpiDermis (RHE) Test (2016)
Vehicle	None
Remarks – Method	No protocol deviations. Negative control: sterile distilled water Positive Control: 8.0 N potassium hydroxide

RESULTS

Test Material	*Mean OD_{570} of Triplicate Tissues*	*Relative Mean Viability (%)*	*SD of Relative Mean Viability*
Negative control	2.269/2.362	100/100	0.196/0.004
Test substance	2.123/1.110	93.6/47.0	0.042/0.011
Positive control	0.076/0.059	3.3/2.5	0.024/0.008

OD = optical density; SD = standard deviation
Values are for exposure periods (3 minutes/60minutes)

Remarks – Results	The (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazoliumbromide) (MTT) solution containing the test substance turned brown rather than blue/purple. Therefore, an additional procedure using freeze-killed tissues was performed to obtain the true amount of MTT reduction that reflects metabolic conversion and avoid a false negative result. The interference by the test substance relative to the corresponding negative control was 0.0% after 3 minutes exposure and 3.7% after 60 minutes exposure (< 30%). Hence the direct reduction was considered acceptable. The solution containing the test substance did not become coloured, showing that it did not cause colour interference. Based on the mean tissue viability of ≥ 50% after 3 min exposure and ≥ 15% after 60 min exposure, the test substance is not classified as a skin corrosive according to the test guidelines, using the GHS criteria. The positive and negative controls performed as expected, confirming the validity of the test system.
CONCLUSION	The assessed chemical was considered non-corrosive to the skin under the conditions of the test.
TEST FACILITY	Covance (2019c)

B.4. Skin Irritation – *In Vitro* EpiDerm™ Reconstructed Human Epidermis Model

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method (2015)
Vehicle	None
Remarks – Method	Minor deviations from the study plan were not considered to have affected the outcome of the study: 1. An assessment showed that the test substance could directly reduce MTT. Therefore, an additional procedure using water-killed tissues was run. However, the results showed no interference due to direct reduction of MTT. It was therefore

decided unnecessary to use the results of the water-killed tissues for quantitative correction of results or for reporting purposes.
2. The study was repeated due to a quality control failure affecting the positive control group in the initial run. The repeated run succeeded. The study report was based on the repeated run rather than from the initial run for reporting purposes.

Negative control: Dulbecco's Phosphate Buffered Saline (DPBS) with Ca^{2+} and Mg^{2+}
Positive control: sodium dodecyl sulphate

RESULTS

Test Material	*Mean OD_{570} of Triplicate Tissues*	*Relative Mean Viability (%)*	*SD of Relative Mean Viability*
Negative control	0.738 ± 0.019	100	2.6
Test substance	0.416 ± 0.054	56.4	7.3
Positive control	0.040 ± 0.020	5.4	2.7

OD = optical density; SD = standard deviation

Remarks – Results

The MTT solution containing the test substance turned brown rather than blue/purple. Therefore, an additional procedure using freeze-killed tissues was performed to avoid a false negative result.

The solution containing the test substance was colourless, indicating unnecessary to perform colour correction tissues.

It was unnecessary to run IL-1α analysis as the results of the MTT test were inconclusive.

The positive and negative controls performed as expected and the standard deviation calculated from individual tissue viabilities of the test substance-treated tissues was within acceptable range, confirming the validity of the test system.

Based on the mean tissue viability of > 50%, the test substance is not classified as a skin irritant according to the GHS criteria.

CONCLUSION

The assessed chemical was considered non-irritating to skin.

TEST FACILITY

Covance (2019d)

B.5. Skin Irritation – Rabbit

TEST SUBSTANCE

Assessed chemical

METHOD

OECD TG 404 Acute Dermal Irritation/Corrosion (2015)
EC Council Regulation No 440/2008 B.4 Acute Toxicity (Skin Irritation)

Species/Strain
Rabbit/New Zealand White

Number of Animals
3 F

Vehicle
None

Observation Period
Up to 10 days

Type of Dressing
Semi-occlusive

Remarks – Method
No protocol deviations. One animal was tested for 3 minute, 1-hour and 4-hour exposure and other two animals were only tested for 4-hour exposure.

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	1	1.7	1.7	2	< 10 d	0
Oedema	0	0.3	0	1	48 h	0

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

Remarks – Results

Within 30-60 minutes of patch removal (3-minute exposure), very slight erythema and very slight oedema was observed at the exposure site and the effects disappeared by 24 hours.

Within 30-60 minutes of patch removal (1-hour exposure), very slight erythema was observed at the exposure site and the effects disappeared by 48 hours.

Within 24 hours of patch removal (4 hours exposure), all three treated animal sites showed well defined erythema and/or very slight oedema. The overall incidence and severity of irritation decreased gradually with time. Due to desquamation observed at the dose sites on day 7, scoring continued until day 10 (study termination).

CONCLUSION

The assessed chemical is slightly irritating to the skin.

TEST FACILITY

Product Safety Labs (2020b)

B.6. Eye Irritation – *In Vitro* Bovine Corneal Opacity and Permeability Test

TEST SUBSTANCE

Assessed chemical

METHOD

OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants (2017)

Vehicle

None

Remarks – Method

No protocol deviations.

Negative control: Sodium chloride 0.9% w/v
Positive control: Ethanol

RESULTS

Test Material	*Mean Opacities of Triplicate Tissues (SD)*	*Mean Permeabilities of Triplicate Tissues (SD)*	*IVIS (SD)*
Vehicle control	0.0	0.008	0.1
*Test substance**	9.3	0.060	10.2
*Positive control**	31.0	1.659	55.9

SD = Standard deviation; IVIS = *in vitro* irritancy score
* Corrected for background values

Remarks – Results

The corneas treated with the test substance and positive control were cloudy post treatment and post incubation. The corneas treated with the negative control were clear post treatment and post incubation.

The negative and positive controls gave satisfactory results confirming the validity of the test system.

CONCLUSION

The IVIS for the test substance was > 3 and ≤ 55, indicating no prediction can be made for the test substance according to the test guideline.

TEST FACILITY

Covance (2019e)

B.7. Eye Irritation – *Reconstructed Human Cornea-Like Epithelium (RhCE) test method EpiOcular™*

TEST SUBSTANCE	Assessed chemical
METHOD	OECD Test Guideline 492 Reconstructed human Cornea-like Epithelium (RhCE) test method
Vehicle	None
Remarks – Method	No protocol deviations. Negative control: sterile water Positive control: methyl acetate

RESULTS

Test Material	*Mean OD_{570} of Duplicate Tissues*	*Relative Mean Viability (%)*
Negative Control	2.200	100.0
Test Substance	2.054	93.4
Positive Control	0.937	42.6

OD = optical density

Remarks – Results	The MTT solution containing the test substance turned brown rather than blue/purple. Therefore, an additional procedure using freeze-killed tissues was performed to obtain the true amount of MTT reduction that reflected metabolic conversion and avoid a false negative result. As the test substance has a light yellow colour, it was treated as a non-coloured substance. As the water and isopropanol solutions were colourless, it was not required to run colour correction tissues. The relative mean viability after correction for the effects of MTT reduction was > 60% for the test substance. The positive and negative controls performed as expected.
CONCLUSION	The assessed chemical was considered non-irritating to the eye under the conditions of the test.
TEST FACILITY	Covance (2020a)

B.8. Eye Irritation – Rabbit

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion (2017)
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 F
Observation Period	72 hours
Remarks – Method	No protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva – Redness	0.67	0.67	0.67	1	< 72 h	0
Conjunctiva – Chemosis	0.67	0.67	0.67	2	< 72 h	0
Conjunctiva – Discharge	0.33	0.33	0.67	2	< 48 h	0
Corneal Opacity	0	0	0.67	1	< 72 h	0
Iridial Inflammation	0	0	0	0	-	0

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

Remarks – Results	All animals were active and healthy and gained body weight. No other signs of gross toxicity, adverse clinical effects, or abnormal behaviour were observed other than eye irritation. Within one hour after test substance application, all three treated eyes showed conjunctiva effects. Corneal opacity was in one treated eye by 48 hours. No iritis was recorded. The incidence and severity of irritation were reduced with time with all effects being resolved by 72 hours (study termination).
CONCLUSION	The assessed chemical is slightly irritating to the eye.
TEST FACILITY	Product Safety Labs (2020b)

B.9. Skin Sensitisation – Guinea Pig Buehler Test

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 406 Skin Sensitisation – Buehler Test (1992)
Species/Strain	Guinea pig/Hartley albino
PRELIMINARY STUDY	Maximum non-irritating concentration: 100% Topical: 25, 50, 75 and 100%
MAIN STUDY	
Number of Animals	Test Group: 20 (males) Control Group: 10 (males)
Vehicle	Acetone
Positive Control	Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using Hexyl Cinnamic Aldehyde (HCA).
INDUCTION PHASE	Induction concentration: Topical: 100%
Signs of Irritation	Very faint to faint erytherna (0.5-1) was noted at all test sites during the induction phase.
CHALLENGE PHASE	
Challenge	Topical: 100%
Remarks – Method	No protocol deviations.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after Challenge*	
		24 h	*48 h*
Test Group	100%	0/20	0/20
Control Group	100%	0/10	0/10

Remarks – Results	No dermal irritation was observed at any naive control or test site during the challenge phase.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the assessed chemical under the conditions of the test.
TEST FACILITY	Product Safe Labs (2020c)

B.10. Skin Sensitisation – *In Vitro* ARE-Nrf2 Luciferase Test

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 442d *In Vitro* Skin Sensitisation Assays Addressing the AOP Key Event on Keratinocyte Activation (2018) - The ARE-Nrf2 luciferase KeratinoSens™ test method (Appendix IA)

Vehicle	Dimethylsulphoxide (DMSO)
Positive Control	Cinnamic aldehyde
Remarks – Method	Minor deviations from the study plan were not considered to have affected the outcome of the study: • The incubation with DMSO end time was omitted. However, the time between the start of incubation and the plate read was longer than what was in the study plan and the control wells gave the expected response. • Study plan stated that the cells would be subcultured when they had reached 80-90% confluence. The cells only reached 60% confluence in the study, however, the cells were actively growing and there were sufficient cells to perform the assay.

RESULTS

Sample	*Concentration (µM)*	*Cell viability*	*Luciferase Induction*
Test substance	0.98	137.25/115.22	0.87/1.00
	1.95	122.93/105.80	1.02/0.98
	3.91	102.25/101.45	1.17/1.51
	7.81	118.38/111.84	2.16/2.23
	15.63	68.73/81.82	3.32/4.28
	31.25	2.41/100.30	3.35/5.98
	62.5	26.83/40.95	1.75/3.38
	125	0.94/8.08	0.03/0.01
	250	0.27/2.04	0.01/0.00
	500	1.20/1.69	0.01/0.00
	1,000	4.35/1.78	0.00/0.00
	2,000	1.47/1.69	0.00/0.01
Positive control - Cinnamic Aldehyde	4	95.36/97.01	1.35/1.26
	8	97.77/98.87	1.41/1.41
	16	91.81/91.95	1.64/1.65
	32	113.36/99.32	2.26/2.55
	64	115.30/123.04	3.65/2.18

Test 1 values/Test 2 values

Remarks – Results	The I_{max} (the maximal average fold induction of luciferase activity) for both tests (3.35 in test 1 and 5.98 in test 2) was > 1.5 fold and statistically significant compared with the vehicle control. The $EC_{1.5}$ (the concentration for which induction of luciferase activity is above the 1.5 fold threshold, was achieved) was 5.22 µM and 3.88 µM for tests 1 and 2. The IC_{30} value was 15.43 µM in test 1 and 47.20 µM in test 2 and the IC_{50} values were 20.04 µM and 57.74 µM in tests 1 and 2 (the IC_{50} and IC_{30} are concentrations for 50% and 30% reduction of cellular viability). There was overall dose-response for luciferase induction. The luciferase activity induction obtained with the positive control was statistically significant above the threshold of 1.5 in at least one of the tested concentrations (4 to 64 µM) in both tests. The KeratinoSens™ prediction was positive. The study was reported to have passed the assay acceptance criteria.
CONCLUSION	The test substance was positive in the second key event (keratinocytes response) of the adverse outcome pathway (AOP) for skin sensitisation as defined in the test guideline.
TEST FACILITY	Covance (2020b)

B.11. Preliminary Toxicity Study by Oral Gavage Administration –Rats

TEST SUBSTANCE	Assessed chemical
METHOD	Preliminary Toxicity Study by Oral Gavage Administration to Han Wistar Rats for 14 Days
Species/Strain	Rats/RccHan®:WIST (Han Wistar)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 14 days Dose regimen: 7 days per week
Vehicle	Corn oil
Remarks – Method	No protocol deviations. The study was used to select a suitable high dose for a subsequent OECD 407 repeated dose 28-day toxicity study and an OECD 421 screening study.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	3 per sex	0	0/6
Low Dose	3 per sex	100	0/6
Mid Dose	3 per sex	300	0/6
High Dose	3 per sex	1,000	0/6

Mortality and Time to Death

No unscheduled mortality was observed in the study.

Clinical Observations

There were no clinical signs and no effects on body weight, body weight rain or food consumption related to treatment.

Effects in Organs

There was a dose-related increase in liver weight in the 300 and 1,000 mg/kg bw/day groups. However, the study author considered the change in liver weight was attributed to an adaptive response and was not considered toxicologically significant. Lower adrenal (× 0.87) and higher thymus (× 1.3) weights were observed in 1,000 mg/kg bw/day males. There were no treatment related macroscopic findings.

CONCLUSION

It was concluded that 1,000 mg/kg bw/day was a suitable high dose for further longer duration toxicity studies. No NOAEL was established in this study.

TEST FACILITY Covance (2020c)

B.12. Repeat Dose Oral Gavage Toxicity –Rats

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents (2008)
Species/Strain	Rat/RccHan®: WIST (Han Wistar)
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	Corn oil
Remarks – Method	Minor deviations from the study plan were not considered to have affected the outcome of the study: -The animals body weights which require to be performed on day 29 for the main test and on day 15 for the recovery phase (before necropsy) were not taken in error and the body weights of the animals taken prior to dispatching the animals to necropsy were used instead for reporting with

annotation that the animals were deprived of food for clinical pathology investigations (this could be a major issue if setting NOAEL based on body weights).
-Investigations for prothrombin time and activated partial thromboplastin time as part of the haematology parameters investigation were performed and reported despite that they were not required by the study plan for recovery week 2.
-Microscopy of urine sediment was not performed on some animals due to lack of initial preparation. This error was noted only after the animals were moribund. Therefore, no repeat samples could be taken. However, sufficient samples volume for the remaining animals were adequate for microscopy. There were no significant findings for microscopy of urine sediment using samples from at least one animal in each group.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	5 per sex	0	0/10
Low Dose	5 per sex	100	0/10
Mid Dose	5 per sex	350	0/10
High Dose	5 per sex	1,000	0/10
Control Recovery	5 per sex	0	0/10
High Dose Recovery	5 per sex	1,000	0/10

Mortality and Time to Death
No unscheduled mortality was observed in animals in the study.

Clinical Observations
The general appearance and behaviour of the animals and sensory activity, grip strength assessment and motor activity did not indicate any adverse effect of treatment.

A statistically significant reduction of weight gain was observed compared to the control groups in 350 mg/kg bw/day group females (-9.7%) and in both sexes (-14.2% in males and -11.7% in females) in the 1,000 mg/kg bw/day group after 4 weeks of treatment. There was no treatment related effect on food consumption, however, water consumption was high for males and females in the 1,000 mg/kg/day group for weeks 3 and 4.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
For males in the 350 and 1,000 mg/kg/day group, there were statistically significant increases in erythrocyte counts after the treatment. These males had lower mean cell volume (-4.4% and –7.5%) and mean cell haemoglobin (-5.1% and –8.2%) without statistical significance for mean cell haemoglobin in the 350 mg/kg/day group. For females in the 1,000 mg/kg/day group, there was statistically significant increase in mean reticulocyte count (+35.4%) after treatment with no clear related effect of treatment on other erythrocyte parameters. After the recovery, mean cell haemoglobin (-6.6%) and mean cell volume (-4.1%) were statistically significantly lower for males treated with 1,000 mg/kg/day, although mean erythrocyte counts were comparable to the controls.

Total leucocyte counts after treatment, for both sexes in the 1,000 mg/kg/day group, were statistically significantly higher than controls (+67.3% for males and +30.2% for females). For these males, differential leucocyte count showed increased neutrophils, lymphocytes, eosinophils, basophils, monocytes and large unstained cells (+68.7%, +67.3%, +40.0%, +250.0%, +45.5%, and +113.0% respectively), although the increase for monocytes did not achieve statistical significance. For the females, differential leucocyte count revealed increased neutrophils, lymphocytes, basophils, monocytes and large unstained cells (+80.4%, +22.7%, +200.0%, +140.0%, and +57.1% respectively), although the increase for lymphocytes did not achieve statistical significance.

There was significant decrease in prothrombin time for females in all treatment groups (8.5%, 15.4% and 11.9% decrease compared to the control group) while males were not affected. Prothrombin time after recovery was comparable to controls.

For both sexes in the 1,000 mg/kg/day group, biochemical examination of blood plasma showed statistically significant high alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase activity (+142.6%, +445.2% and +175.6% in males and +162.5%, +233.3%, and +112.1% in females respectively), suggesting the liver damage. After recovery, alkaline phosphatase activity were statistically significantly higher in both sexes treated with 1,000 mg/kg/day (+49.4% and +65.5% for males and females respectively), with alanine aminotransferase and aspartate aminotransferase activity (+106.7% and +32.0% respectively) being statistically significantly higher than controls for males. However, as the values after recovery seemed closer to the controls, partial recovery was considered to be achieved.

There were statistically significant increases in total bilirubin for both sexes (+400% for males and +100% for females) and bile acid concentrations (+122.4%) for females in the 1,000 mg/kg/day group. Bile acid concentrations were higher for males treated with 1,000 mg/kg/day (+39.3%), despite the mean male control value being adversely influenced by an uncommon high value for one control male (166 compared to average of 35 µmol/L for other bile acid concentrations controls). After recovery, total bilirubin and bile acid concentration averages for both sexes treated with 1,000 mg/kg/day were similar to control.

Blood glucose concentrations were slightly low with statistical significance in both sexes treated with 1,000 mg/kg/day (-37.5% for males and -26.0% for females) compared to control groups. There was statistical significance for slightly high total cholesterol concentrations for females (+62.3%) and low triglyceride for males and females (-51.0% and - 33.8% respectively) at this dose level. Plasma urea concentrations for females treated with 1,000 mg/kg/day was slightly lower with statistical significance (-22.7%). After recovery, blood glucose, total cholesterol, triglyceride and plasma urea levels were similar to the controls.

Slightly low total protein concentrations (-7.8% for males and -8.6% for females), possibly associated with a slight decrease in albumin concentration (-8.1% for males and -14.6% for females), were noted for both sexes treated with 1,000 mg/kg/day with statistical significance. For females, this resulted in a slight decrease to albumin:globulin ratio with statistical significance. Total protein concentrations were similar to the controls after recovery. Although statistically significant low albumin concentrations and albumin:globulin ratio remained for females treated with 1,000 mg/kg/day (-14.6 and -15.6% respectively), it was less severe than observed after treatment, showing partial recovery (-7.0% and -5.4% respectively). For males treated with 1,000 mg/kg/day, albumin concentration and albumin:globulin ratio were similar to the controls after recovery.

There was a statistically significant decrease in measured total protein (-42.8%) and protein concentration (-55.3%) in the urine for males treated with 1,000 mg/kg/day. For females, measured total protein at all dose levels was statistically significant low (-39.2%, -49.0% and -31.6%) with no dose-relationship and for females treated with 1,000 mg/kg/day, protein concentration was also low (-29.4%) without statistical significance.

Effects in Organs

Liver weights were statistically significant high for both sexes (+48.7% in males and +55.8% in females) in the 1,000 mg/kg/day treatment group. There were statistically significant increases in adjusted liver weight for both sexes in the 100 (+17.6% in males and +16.9% in females) or 350 (+15.0% in males and +27.7% in females) mg/kg/day groups, however there was no dose relationship in values for males and the mean value for females in the 100 mg/kg/day group was slightly higher than control.

Adjusted kidney weights were statistically significantly high for males in the 350 mg/kg/day group + 10.9%) and for both sexes in the 1,000 mg/kg/day group (+16.0% in males and +18.5% in females). The increase in kidney weight was not considered as an adverse effect by study authors as the effect was not associated with any histopathological change. Kidney weights were slightly high without statistical significance (+6.8%) for males in the 100 mg/kg/day group. There were no supporting histopathological changes.

Adjusted adrenal weights for males in the 1,000 mg/kg/day treatment group were low without statistical significance (-9.2%). This finding was considered by study authors non-adverse even with minor histopathological change as no evidence of impaired adrenal function was observed. After 2 weeks of recovery, there was statistically significantly low adjusted mean adrenal weight (21.3% reduction compared to control group) for males treated at 1,000 mg/kg/day. As partial recovery was noted during the recovery, this finding was not considered adverse.

Necropsy examination showed macroscopic changes for the non-glandular stomach of 4 males (including dark areas, depressions and thickening) and 3 females (thickening) in the 1.000 mg/kg/day group.

There were moderate to marked hepatocyte karocytomegaly and slight to moderate single cell necrosis in the liver of all animals in the 1,000 mg/kg/day group. Majority of animals (2 males and 2 females) in the 350 mg/kg/day group had these effects with a lesser severity. There was minimal to moderate micro/macrovesicular vacuolation in all animals in the 1,000 mg/kg/day group but only two females treated at 350 mg/kg/day showed this effect at minimal severity. There was minimal to slight Kupffer cell pigmentation in one male and one female in the 1,000 mg/kg/day group and in one female (with minimal severity) in the 350 mg/kg/day group. There were moderate to marked hepatocyte karyocytomegaly and minimal micro/macrovesicular vacuolation in the liver of all animals in the 1,000 mg/kg/day group after the recovery. There was also minimal single cell necrosis in two males and three females and minimal pigmented Kupffer cells in all males and three females from this recovery group.

There were microscopic changes, indicating a local irritant effect, for the non-glandular mucosa of the stomach in the 1,000 mg/kg/day group animals, including minimal to moderate hyperplasia and minimal to slight hyperkeratosis in all males and two females, minimal to slight oedema for two males and two females, minimal ulceration in one female and minimal erosion in one male, accompanied by minimal to slight mixed inflammatory cells infiltrate. There were microscopic changes to the stomach for animals in the 350 mg/kg/day group (2 females). One female had slight hyperplasia, slight hyperkeratosis, minimal erosion, slight oedema and minimal mixed inflammatory cells infiltrate and another female exhibited slight oedema and slight mixed inflammatory cells infiltrate. Complete recovery of the microscopic stomach findings for animals in the 1,000 mg/kg/day group was noted after the recovery period.

Remarks – Results
The study authors commented that the liver is a target organ for toxicity.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 100 mg/kg bw/day in this study based on adverse effects observed mainly in the liver and kidneys in the 350 and 1,000 mg/kg bw/day groups.

TEST FACILITY Covance (2020d)

B.13. Genotoxicity – Bacteria

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test (1997) Test 1: Plate incorporation procedure/Test 2: Pre incubation procedure
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100 *Escherichia coli*: WP2uvrA
Metabolic Activation System	S9 fraction was prepared from rat liver homogenate metabolising system (10% liver S9 in standard co-factors).
Concentration Range in Main Test	With and without metabolic activation in test 1: 0, 1.5, 5, 15, 50, 150, 500, 1,500, 5,000 µg/plate With and without metabolic activation in test 2: 0, 15, 50, 150, 500, 1,500, 5,000 µg/plate)
Vehicle	Acetone
Remarks – Method	No protocol deviations. There was no preliminary test. Positive controls: Without metabolic activation: N-ethyl-N'-nitro-N-nitrosoguanidine (ENNO) for TA1535, TA100 and WP2uvrA; 9-Aminoacridine (9AA) for TA1537; 4-Nitroquinoline-1-oxide (4NQO) for TA98; With metabolic activation: 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 Main Test	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	> 5,000	≥ 5,000	Negative
Test 2	> 5,000	≥ 5,000	Negative

Present			
Test 1	> 5,000	≥ 5,000	Negative
Test 2	> 5,000	≥ 5,000	Negative

Remarks – Results

There was no visible reduction in the growth of the bacterial background lawn at any dose level, either in the presence or absence of metabolic activation in both tests.

A test substance precipitate (globular in appearance) was noted at 5,000 µg/plate in both the presence and absence of metabolic activation (S9-mix) in both tests. It did not prevent the scoring of revertant colonies.

No significant increases in the frequency of revertant colonies either with or without metabolic activation in both tests.

Positive and negative controls performed as expected in both tests.

CONCLUSION

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

TEST FACILITY

Covance (2019f)

B.14. Genotoxicity – *In Vitro* Mammalian Chromosome Aberration Test in Human Lymphocytes

TEST SUBSTANCE

Assessed chemical

METHOD

OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test (2016)

Species/Strain: Human

Cell Type/Cell Line: Lymphocytes

Metabolic Activation System: S9 mix prepared from phenobarbital and β-naphtha flavone induced rat liver homogenate

Vehicle: Acetone

Remarks – Method: Minor deviations from the study plan were not considered to have affected the outcome of the study: the source of S9 microsomal fraction used in the study was changed. However, the protein content was adjusted to 20 mg/mL to maintain consistency with the processes in the General Study Plan, all other processes and preparation remained the same and the purchased S9 responded to the positive controls well as expected and results fell in line with the in-house historical data.

Positive controls:
With metabolic activation: cyclophosphamide
Without metabolic activation: Mitomycin C.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 10, 20, 40*, 60*, 80*, 160	4	20
Test 2	0*, 10, 20, 40*, 60*, 80*, 160	24	-
Present			
Test 1	0*, 10, 20, 40*, 60*, 80*, 160	4	20

*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	≥ 40	> 80	≥ 80	negative
Test 2	-	> 80	≥ 80	negative
Present				

Test 1	≥ 80	> 80	≥ 80	negative

Remarks – Results: The results of the mitotic indices showed that no dose-related inhibition of mitotic index was observed in the 4(20)-hour exposure group in the presence of S9 or in the 24-hour exposure group. In the absence of S9, a dose-related inhibition of mitotic index was observed at 60 µg/mL and 80 µg/mL with 39% and 47% mitotic inhibition and the toxicity was consistent with the lowest precipitating dose level in the preliminary test.

The test substance did not induce any statistically significant increases in the frequency of cells with chromosomal aberrations or in the numbers of polyploid cells in either the absence or presence of S9. No endoreduplication was observed.

Positive and negative controls performed as expected in both tests.

CONCLUSION: The assessed chemical was not clastogenic to Human Lymphocytes treated *in vitro* under the conditions of the test.

TEST FACILITY: Covance (2019g)

B.15. Genotoxicity – *In Vitro* L5178YTK+1- Mouse Lymphoma Assay

TEST SUBSTANCE: Assessed chemical

METHOD: OECD TG 490 *In vitro* Mammalian Cell Gene Mutation Test Using the Thymidine Kinase Gene (2016)

Species/Strain: Mouse

Cell Type/Cell Line: Thymidine kinase, TK +/-, locus of the L5178Y mouse lymphoma cells (heterozygous at the thymidine kinase locus)

Metabolic Activation System: S9 mix prepared from phenobarbital-5,6 benzoflavon induced rat liver homogenate

Vehicle: Acetone

Remarks – Method: No protocol deviations.
Positive control:
Without S9 mix: ethylmethane sulfonate
With S9 mix: cyclophosphamide

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 1.25, 2.5, 5*, 10*, 20*, 40*, 60*, 80*	4 hours	48 hours
Test 2	0*, 1.25, 2.5*, 5*, 10*, 20*, 40*, 60*, 80	24 hours	48 hours
Present			
Test 1	0*, 1.25, 2.5, 5*, 10*, 20*, 40*, 60*, 80*	4 hours	48 hours

*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	> 10	> 80	> 80	Negative
Test 2	-	> 60	≥ 60	Negative
Present				
Test 1	> 80	> 80	> 80	Negative

Remarks – Results: The test substance did not induce any toxicologically significant increases in the mutant frequency at any of the dose levels in the main tests, using a dose range including the lowest precipitating dose level in all three exposure groups (optimum levels of toxicity were observed at the lowest

	precipitating dose level in the 24-hour exposure group), as recommended by the test guideline. The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.
CONCLUSION	The test substance was not clastogenic to lymphoma treated *in vitro* under the conditions of the test.
TEST FACILITY	Covance (2019h)

B.16. Reproductive/Development Toxicity Screening Test

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 421 Reproductive/Development Toxicity Screening Test (2016)
Species/Strain	Rats/RccHan®: WIST (Han Wistar)
Route of Administration	Oral – gavage
Exposure Information	F0 females received 14 doses daily prior to pairing and were dosed through lactation day 12. F0 males received 14 doses daily prior to mating. Males were dosed throughout the mating period up to necropsy after a minimum of five consecutive weeks. Exposure to the F1 generation was in utero or *via* the milk.
Vehicle	Corn oil
Remarks – Method	Minor deviations from the study plan were not considered to have affected the outcome of the study: 1. No results are presented in the study report on all samples from week 1 or week 2 as the samples were not analysed within the known stability period and the system acceptability criteria were not met; 2. One control animal was dosed in gestation phase from the pairing cage and then this animal was dosed a second time in the treatment phase. However, the total volume of dose administered to this animal was within the Project License and the extra corn oil is not considered to have adversely affected the animal; 3. As the recorded food consumption data for males after pairing (not required by the study plan) did not impact the assessment, the data were not reported.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
1	10 per sex	0	0/20
2	10 per sex	100	0/20
3	10 per sex	350	0/20
4	10 per sex	600	0/20
5	10 per sex	150*	0/20

*A 15% dilution (with mineral oil for which the composition was not reported) of the assessed chemical used at 1,000 mg/kg bw/day, as commercially used in industry.

Mortality and Time to Death

All F0 males and females survived to the scheduled necropsies.

Effects on Parental (P) animals:

There were no treatment related clinical signs observed in relationship with dosing for males during treatment, females prior to pairing, gestation or during lactation.

There was no treatment related adverse effect on mean body weight gain of males throughout the study or of females prior to pairing, gestation or lactation phases of the study.

Food consumption for all groups of males during treatment and for all treated females during the period prior to pairing, gestation or lactation food intake was generally similar to control. However, there was a slightly lower food intake value for males dosed at 600 mg/kg bw/day in the first two weeks of treatment. This difference was slight and not statistically significant and did not adversely impact male body weight and was therefore considered not adverse.

Females treated at 600 mg/kg/day had slightly higher water intake for 5 days during week 2. There was no clear difference noted in any other treated female groups or treated males.

Oestrous cycles, pre-coital interval, mating performance, fertility, gestation length and gestation index were not affected by treatment. At termination, on day 13 of lactation, all females were confirmed to be in dioestrus.

There was a statistically significant increase in body weight-adjusted liver weights in animals of both sexes, treated at 350 (+24.3% or +9.6% for males or females, respectively) or 600 mg/kg/day (+32.7% or +17.8% for males or females, respectively). There was a statistically significant increase in body weight-adjusted liver weights for males treated at 150 mg/kg/day (+12%).

The body weight-adjusted adrenal gland weights in females of all treated groups were lower, compared with the control (-10.7% without statistical significance at 100 mg/kg bw/day and –10.7%, -17.9% and –14.3% with statistical significance at 150, 300 and 600 mg/kg bw/day), however, no clear relationship to dose or not such a similar trend in males was observed.

The body weight-adjusted prostate weight in males treated at 600 mg/kg/day was statistically significantly lower (-17.6%) compared with the control, however, there were no differences in any other treated groups or corresponding effects on male reproduction.

There was minimal to slight hypertrophy/vacuolation of interstitial cells within the ovaries of 6 females treated at 600 mg/kg bw/day and 3 females treated at 150 mg/kg bw/day. The effect was no seen in the control animals.

Statistical significance serum T4 mean concentration decreased in adult males dosed at 150, 350, 600 mg/kg bw/day (-18.6%, -22.3%, -17.2% respectively), although the decrease was minimal with no dose response. There was no clear effect of treatment on T4 serum concentrations obtained from Thyroid Hormone Analysis for F0 adult males. Besides, the mean values were within the 90th percentile range of historical control data.

Effects on 1st Filial Generation (F1)

There were no treatment related clinical signs observed in F1 litter.

Offspring body weight on day 1 was unaffected by parental treatment at any dose. When parental treatment was 600 mg/kg bw/day, offspring mean body weights were lower compared to controls on day 13 (-16.4% or -21.8% for males and females, respectively) and mean body weight gain of offspring was lower (-20.2% or -26.4% of control for males and females, respectively), however, offspring body weight or body weight gain at other doses was unaffected by parental treatment.

There was no clear effect of treatment on T4 serum concentrations obtained from Thyroid Hormone Analysis from day 13 of age offspring.

Ano-genital distance for both male and female offspring was unaffected by parental treatment.

The mean number of implantations for females treated with 600 mg/kg/day was slightly low compared with controls, hence subsequent litter size was slightly low. However, the group mean total litter size on day 1 appeared lower than anticipated due to a slightly lower mean post-implantation survival index, compared with the control, being largely influenced by one female which produced only 1 viable foetus/pup from 9 implantations. Subsequent post-natal survival of the offspring was not affected by parental treatment.

Litter size or other birth indexes such as post-implantation survival, live birth or viability were unaffected at other dose groups. The mean sex ratio was similar in all groups showing there was no selective effect of either treatment on the survival of either sex to day 13 of age.

Offspring macroscopic examination of either died prematurely or at scheduled termination did not show any findings that could be related to parental treatment.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 600 mg/kg bw/day, for reproductive performance of adult animals and the survival, growth and development of their offspring.

TEST FACILITY Covance (2020e)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready Biodegradability (Study 1)

TEST SUBSTANCE	Assessed Chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biochemical Oxygen Demand (BOD)
Remarks – Method	Sodium benzoate was used as a reference substance.

RESULTS

Test Substance		*Sodium benzoate*		*Toxicity control*	
Day	*% Degradation*	*Day*	*% Degradation*	*Day*	*% Degradation*
5	4.9	5	51.2	5	27.9
13	8.1	13	80.1	13	41.1
22	8.8	22	87.4	22	43.3
29	3.3	29	88.1	29	50.3

Remarks – Results	All validity criteria were met. The difference in extremes were less than 20% at the end of the test, the reference compound reached 80% degradation by day 13, the inoculum blank reached a maximum of 26 mg CO_2/L and the inorganic carbon in the test suspension was < 5% of total carbon. The test substance is not considered toxic to the inoculum as the toxicity control reached 27.9% by day 5.
CONCLUSION	The test substance is not readily biodegradable.
TEST FACILITY	Eurofins (2019c)

C.1.2. Ready Biodegradability (Study 2)

TEST SUBSTANCE	Assessed Chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Titration
Remarks – Method	Sodium benzoate was used as a reference substance.

RESULTS

Test Substance		*Sodium benzoate*		*Toxicity control*	
Day	*% Degradation*	*Day*	*% Degradation*	*Day*	*% Degradation*
4	1.6	4	57.9	4	31.8
12	4.7	12	87.8	12	46.9
24	6.3	24	94.2	24	48.0
29	6.3	29	98.9	29	50.3

Remarks – Results	All validity criteria were met. The difference in extremes were less than 20% at the end of the test, the reference compound reached 87.8% degradation by day 12, the inoculum blank reached a maximum of 27.9 mg

	CO_2/L and the inorganic carbon in the test suspension was < 5% of total carbon. The test substance is not considered toxic to the inoculum as the toxicity control reached 31.8% by day 4.
CONCLUSION	The test substance is not readily biodegradable.
TEST FACILITY	CTI (2019)

C.1.3. Inherent Biodegradability

TEST SUBSTANCE	Assessed Chemical
METHOD	OECD TG 302 C Inherent Biodegradability: Modified MITI Test (II)
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	BOD and LC-MS/MS
Remarks – Method	Aniline was used as a reference substance.

RESULTS

Test Substance		*Aniline*	
Day	*% Degradation (BOD/COD)*	*Day*	*% Degradation*
7	0.0	7	61.2
14	14.6	14	71.6
21	26.4	21	78.0
28	26.6	28	79.2

Remarks – Results	All validity criteria were met. The reference substance reached 61.2% degradation after 7 days and 71.6% degradation after 14 days and the recovery rate of the test substance in the inoculum blank sample was 111%. Degradation of the test substance was determined using the ratio of BOD and COD values and a direct chemical analysis. Based on the BOD/COD values the mean biodegradation percentage was 26.6% after 28 days indicating inherent primary biodegradation. The direct chemical method, which only indicates primary degradation of the test substance, showed 72.8% degradation of the test substance after 28 days.
CONCLUSION	The test substance is primarily inherently degradable.
TEST FACILITY	CTI (2020a)

C.1.4. Bioaccumulation

TEST SUBSTANCE	Assessed Chemical
METHOD	OECD TG 305-III Dietary Exposure Bioaccumulation Fish Test
Species	*Gobiocypris rarus*
Exposure Period	Exposure: 28 days Depuration: 14 days
Auxiliary Solvent	N/A
Concentration Range	Nominal: 1,000 mg/kg
Analytical Monitoring	Liquid Chromatography – Tandem Mass Spectrometry (LC-MS/MS)
Remarks – Method	The only deviation from OECD TG 305-III is the test species used. This is not expected to adversely affect the validity of the study.

RESULTS

Biomagnification Factor	9.3×10^{-6}
Remarks – Results	All validity criteria were met. The temperature was maintained at 23.8-24.5°C and the dissolved oxygen was maintained at ≥ 97.5% saturation. Concentration of the test substance was maintained between 101.9 – 111.5% of the nominal. The homogeneity relative standard deviations were between 1.4 – 8.3% and test substance was not detected in the control food samples. No adverse effects or mortality were observed in the control groups.
CONCLUSION	The test substance is not bioaccumulative.
TEST FACILITY	CTI (2020)

C.2. Ecotoxicological Investigations

C.1.5. **Acute Toxicity to Fish**

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-static
Species	*Pimephales promelas* (Fathead minnow)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	132 mg $CaCO_3$/L
Analytical Monitoring	Inductively Coupled Plasma with Optical Emission Spectrometry (ICP/OES)
Remarks – Method	Based on a range finding study, water accommodated fractions (WAFs) were prepared at the test concentrations (detailed below). The test medium was renewed every 24 hours.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*4.5 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	N/A	10	0	0	0	0	0
1.0	< LOD	10	0	0	0	0	0
3.0	1.0	10	0	0	0	0	0
10	4.1	10	0	0	0	0	0
30	8.7	10	0	0	0	0	0
100	31	10	0	4	10	10	10

LL50	14 mg/L at 96 hours
Remarks – Results	All validity criteria were met. The dissolved oxygen content was maintained at ≥ 89% of the air saturation value and the concentration of the test substance was analysed. The LL50 value was calculated based on the measured test concentrations.
CONCLUSION	The test substance is harmful to fish.
TEST FACILITY	Eurofins (2020b)

C.2.1. **Acute Toxicity to Aquatic Invertebrates**

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Semi-static
Species	*Daphnia magna*

Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	136 mg $CaCO_3$/L
Analytical Monitoring	ICP/OES
Remarks – Method	Based on a range finding study, water accommodated fractions (WAFs) were prepared at the test concentrations (detailed below). The test medium was renewed every 24 hours.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	N/A	20	0	0
0.3	< LOD	20	0	0
1.0	0.36	20	0	0
3.0	0.75	20	0	2
10	2.4	20	5	7
30	16	20	9	10

EL50	2.0 mg/L at 48 hours (based on nominal loading rates)
Remarks – Results	The EL50 value was calculated based on the nominal loading rates of the test substance. All validity criteria were met. Dissolved oxygen was maintained at ≥ 7.6 mg/L, pH was maintained between 8.1 and 8.8 and temperature was maintained between 19.8 and 20.8°C.

CONCLUSION The test substance is toxic to daphnia.

TEST FACILITY Eurofins (2020c)

C.2.2. **Algal Growth Inhibition Test**

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test
EC Council Regulation No 440/2008 C.3 Algal Inhibition Test

Species	*Raphidocelis subcapitata*
Exposure Period	96 hours
Concentration Range	Nominal: 1.0, 3.0, 10, 30,100 mg/L
Auxiliary Solvent	None
Analytical Monitoring	Total Organic Carbon
Remarks – Method	Based on a range finding study, water accommodated fractions (WAFs) were prepared at the test concentrations (detailed below). Due to difficulties in measuring concentration of the test samples, results were calculated based on nominal concentrations.

RESULTS

Growth rate		*Yield*	
ErL50 (mg/L at 72 h)	*NOEL (mg/L)*	*EyL50 (mg/L at 72 h)*	*NOEL (mg/L)*
> 100	10	33	10

Remarks – Results	All validity criteria were met. The control cell density increased by a factor of 237, the mean coefficient of variation for section-by-section specific growth was 6.22% and the coefficient of variation for the average specific growth rates was 2.54%.

CONCLUSION The test substance is not harmful to algal growth.

TEST FACILITY Eurofins (2020d)

C.2.3. Chronic Toxicity to Aquatic Invertebrates

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 211 *Daphnia magna* Reproduction test – Semi-static
Species *Daphnia magna*
Exposure Period 21 days
Auxiliary Solvent None
Analytical Monitoring LC-MS/MS
Remarks – Method Based on a range finding study, water accommodated fractions (WAFs) were prepared. The following deviation was noted: due to an oversight in the laboratory, the sample for nominal concentration 2.4 mg/L in the range finding test was not filtered during the WAF preparation. This is not expected to have affected the validity of the study as all adult daphnids at this loading rate survived to Day 10. Due to difficulties in measuring the concentration of the test substance, results were calculated based on nominal concentrations.

Test substance loading rate WAF (mg/L)	Survival (% parental generation)	Mean no. offspring per female
Control	100	140
0.15	90	150
0.3	90	142
0.6	100	151
1.2	100	113
2.4	0	28

NOEL (reproduction) 0.6 mg/L at 21 days (based on loading rate WAF)
EL50 (reproduction) 1.7 mg/L at 21 days (based on loading rate WAF)
Remarks – Results The number of dead young and unhatched eggs were not reported. All validity criteria were met. Mortality of the parent population in the control group was < 20% and the amount of living offspring in the control group was > 60.

The study results show that there are fewer than three concentration groups showing a response, and the overall concentration-response relationship is non-monotonic. In this situation the effect concentrations (e.g. EL10) are not considered reliable (OECD 2003) for regulatory purposes. Therefore, the reproduction NOEL has been selected as the chronic endpoint from this study.

CONCLUSION The test substance is toxic to invertebrate reproduction.

TEST FACILITY Covance (2020i)

C.2.4. **Inhibition of Microbial Activity**

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 209 Activated Sludge, Respiration Inhibition Test
Inoculum Activated sludge
Exposure Period 3 hours
Concentration Range Nominal: 10, 100, 1,000 mg/L
Remarks – Method A reference test was conducted using 3,5 dichlorophenol.

RESULTS
IC50 > 1,000 mg/L

NOEC	1,000 mg/L
Remarks – Results	The results from all test samples indicated negative inhibition values for microbial respiration. The reference tests showed 3,5 dichlorophenol IC50 values from 11.5-17 mg/L which is within the expected range of 2 – 25 mg/L. All validity criteria were met. The oxygen uptake of the controls was 27 mg/g/h and the coefficient of variation between replicates was 30%.
CONCLUSION	The test substance is not inhibitory to microbial respiration.
TEST FACILITY	Eurofins (2020e)

BIBLIOGRAPHY

Covance (2019a) Determination of Vapour Pressure (Study Number: CF89KB, November, 2019) Shardlow Business Park, London Road, Shardlow, Derbyshire DE72 2GD UK, Covance CRS Rsearch Limited (Unpublished report submitted by the applicant)

Covance (2019b) Acute Oral Toxicity Study in the Rat- Fixed Dose Method (Study number: FT06LF, September, 2019) Woolley Road Alconbury Huntingdon Cambridgeshire PE28 4HS UK, Covance (Unpublished report submitted by the applicant)

Covance (2019c) *In vitro* Epiderm Skin Corrosion Test (Study Number: CT28TD, September, 2019) Shardlow Business Park, London Road, Shardlow, Derbyshire DE72 2GD UK, Covance CRS Rsearch Limited (Unpublished report submitted by the applicant)

Covance (2019d) Determination of Skin Irritation Potential using the EPISKIN™ Reconstructed Human Epidermis Model (Study number: DP92LX, December, 2019) Shardlow Business Park London Road Shardlow Derbyshire DE72 2GD UK, Covance CRS Research Limited (Unpublished report submitted by the applicant)

Covance (2019e) The Bovine Corneal Opacity and Permeability (BCOP) Assay (Study number: JS38VC, September, 2019) Shardlow Business Park London Road Shardlow Derbyshire DE72 2GD UK, Covance CRS Research Limited (Unpublished report submitted by the applicant)

Covance (2019f) Reverse Mutation Assay 'Ames Test' using Salmonella typhimurium and Escherichia coli (Study number: JQ51QW, August, 2019) Shardlow Business Park London Road Shardlow Derbyshire DE72 2GD UK, Covance CRS Research Limited (Unpublished report submitted by the applicant)

Covance (2019g) Chromosome Aberration Test in Human Lymphocytes *in vitro* (Study number: HP09FR, October, 2019) Shardlow Business Park London Road Shardlow Derbyshire DE72 2GD UK, Covance CRS Research Limited (Unpublished report submitted by the applicant)

Covance (2019h) L5178Y TK +/- Mouse Lymphoma Assay (Study number: KM39LJ, November, 2019) Shardlow Business Park London Road Shardlow Derbyshire DE72 2GD UK, Covance CRS Research Limited (Unpublished report submitted by the applicant)

Covance (2019i) Daphnia magna Reproduction Test (Study Number: HC74TJ, November, 2020) Shardlow Business Park, London Road, Shardlow, Derbyshire DE72 2GD, UK, Covance CRS Research Limited (Unpublished report submitted by the applicant).

Covance (2020a) Reconstructed Human Cornea-Like Epithelium (RhCE) test method EpiOcular™ in accordance with OECD Test Guideline 492 (Study number: SN40CP, January, 2020) Shardlow Business Park London Road Shardlow Derbyshire DE72 2GD UK, Covance CRS Research Limited (Unpublished report submitted by the applicant)

Covance (2020b) ARE-Nrf2 Luciferase Test (KeratinoSensTM) (Study number: RH14RT, January, 2020) Woolley Road Alconbury Huntingdon Cambridgeshire PE28 4HS UK, Covance CRS Research Limited (Unpublished report submitted by the applicant)

Covance (2020c) Preliminary Toxicity Study by Oral Gavage Administration to Han Wistar Rats for 14 Days (Study number: YJ41SD, January, 2020) Eye, Suffolk IP23 7PX, UK, Covance CRS Limited (Unpublished report submitted by the applicant)

Covance (2020d) Toxicity Study by Oral Administration to Han Wistar Rats for 4 Weeks Followed by a 2 Week Recovery Period (Study number: CQ47HP, July, 2020) Eye, Suffolk IP23 7PX, UK, Covance CRS Limited (Unpublished report submitted by the applicant)

Covance (2020e) Reproductive/Development Toxicity Screening Study in the Han Wistar Rat by Oral Gavage Administration (Study number: SD02FD, September, 2020) Eye, Suffolk IP23 7PX, UK, Covance CRS Limited (Unpublished report submitted by the applicant)

CTI (2019) Ready Biodegradability Test of [Assessed Chemical] (CO_2 Evolution Test) (Study Number: G219301007A, July, 2019), Suzhou, China, CTI-Safety Evaluation Technical Service Co., Ltd. (Unpublished report submitted by the applicant)

CTI (2020a) [Assessed Chemical] Inherent Biodegradability Test (302C Modified MITI Test (II) (Study Number: G20302001A, July, 2020), Suzhou, China, CTI-Safety Evaluation Technical Service Co., Ltd. (Unpublished report submitted by the applicant)

CTI (2020b) A Dietary Bioaccumulation Test in Fish (*Gobiocypris rarus)* (Study Number: G20305001A, August, 2020), Suzhou, China, CTI-Safety Evaluation Technical Service Co., Ltd. (Unpublished report submitted by the applicant)

Dekra (2019) Physico-Chemical Testing on a Sample of the chemical (Study Number: GLP/3016005920, August 2019) Southampton SO 16 7NS United Kingdom, Chiworth Technology Ltd, Process Safety Laboratory (Unpublished report submitted by the applicant)

Eurofins (2019a) Determination of Water Solubility Using the Slow Stir Method (Study Number: 331K-120, October, 2019), Maryland USA, Eurofins EAG Agroscience, LLC (Unpublished report submitted by the applicant)

Eurofins (2019b) Estimation of Adsorption Coefficient Using High Performance Liquid Chromatography (Study Number: 331K-125, September, 2019), Maryland USA, Eurofins EAG Agroscience, LLC (Unpublished report submitted by the applicant)

Eurofins (2019c) Ready Biodegradability by the Carbon Dioxide Evolution Test Method (Study Number: 331E-149, August, 2019), Maryland USA, Eurofins EAG Agroscience, LLC (Unpublished report submitted by the applicant)

Eurofins (2020a) Estimation of n-Octanol/ Water Partition Coefficient Using High Performance Liquid Chromatography (Study Number: 331K-116, January, 2020), Maryland USA, Eurofins EAG Agroscience, LLC (Unpublished report submitted by the applicant)

Eurofins (2020b) A 96-Hour Static-Renewal Acute Toxicity Test with the Fathead Minnow (Pimephales promelas) (Study Number: 331A-176, February, 2020), Maryland USA, Eurofins EAG Agroscience, LLC (Unpublished report submitted by the applicant)

Eurofins (2020c) A 48-Hour Static-Renewal Acute Toxicity Test with the Cladoceran (Daphnia magna) (Study Number: 331A-175, February, 2020), Maryland USA, Eurofins EAG Agroscience, LLC (Unpublished report submitted by the applicant)

Eurofins (2020d) A 96-Hour Toxicity Test with Freshwater Alga (Raphidocelis subcapitata) (Study Number: 331P-104, February, 2020), Maryland USA, Eurofins EAG Agroscience, LLC (Unpublished report submitted by the applicant)

Eurofins (2020e) An Activated Sludge Respiration Inhibition Test (Study Number: 331E-147, January, 2020), Maryland USA, Eurofins EAG Agroscience, LLC (Unpublished report submitted by the applicant)

Lubrizol (2019) Determination of the Physico-chemical Properties on the Chemical (Study Number: 331K-114, September, 2019) 8598 Commerce Drive EASTON, Maryland 21601 USA, Eurofins EAG Agroscience, LLC (Unpublished report submitted by the applicant)

NTC (2018) Australian Code for the Transport of Dangerous Goods by Road and Rail (ADG code), Edition 7.6, National Transport Commission, Commonwealth of Australia

OECD (2003) Draft Guidance Document on the Statistical Analysis of Ecotoxicity Data, Organisation for Economic Co-operation and Development, Paris, 2003.

Product Safety Labs (2020a) Acute Dermal Toxicity in Rats (Study Number 51951, January 2020) 2394 US Highway 130 Dayton, NJ 08810 USA Product Safety Labs (Unpublished report submitted by the applicant).

Product Safety Labs (2020b) Primary Eye Irritation in Rabbits (Study Number 51952, January 2020) 2394 US Highway 130 Dayton, NJ 08810 USA Product Safety Labs (Unpublished report submitted by the applicant).

Product Safety Labs (2020c) Dermal Sensitization Test in Guinea Pigs ~ Buehler Method (Study Number 51954, January 2020) 2394 US Highway 130 Dayton, NJ 08810 USA Product Safety Labs (Unpublished report submitted by the applicant).

SWA (2012) Code of Practice: Managing Risks of Hazardous Chemicals in the Workplace, Safe Work Australia, https://www.safeworkaustralia.gov.au/doc/model-code-practice-managing-risks-hazardous-chemicals-workplace

United Nations (2009) Globally Harmonised System of Classification and Labelling of Chemicals (GHS), 3rd revised edition. United Nations Economic Commission for Europe (UN/ECE), <http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html