File No.: STD/1687

September 2019

**NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)**

# PUBLIC REPORT

## Chemical in ADDITIN RC 4801

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:


| | |
|---|---|
| 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 |
| FAX: | + 61 2 8577 8888 |
| Website: | www.nicnas.gov.au |


**Director**
**NICNAS**

# TABLE OF CONTENTS

# 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/1687 | Lanxess Pty Ltd | Chemical in ADDITIN RC 4801 | Yes | ≤ 10 tonnes per annum | A component of industrial oils |

# CONCLUSIONS AND REGULATORY OBLIGATIONS

**Hazard Classification**
Based on the available information, the notified 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 notified chemical is presented in the following table.

| *Hazard Classification* | *Hazard Statement* |
|---|---|
| Acute Toxicity (Category 4) | H302 - Harmful if swallowed |
| Eye Damage / Irritation (Category 1) | H318 - Causes serious eye damage |
| Skin Corrosion / Irritation (Category 2) | H315 - Causes skin irritation |

**Human Health Risk Assessment**
Provided that the recommended controls are being adhered to, under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

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

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

**Recommendations**

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Acute Toxicity (Category 4): H302 - Harmful if swallowed
  - Eye Damage / Irritation (Category 1): H318 - Causes serious eye damage
  - Skin Corrosion / Irritation (Category 2): H315 - Causes skin irritation

  The above should be used for products/mixtures containing the notified chemical, if applicable, based on the concentration of the notified 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 notified chemical during reformulation:
  - Enclosed/automated processes

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

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical during reformulation and end use:
  - Impervious gloves
  - Safety glasses or goggles
  - Protective clothing
  - Respiratory protection if inhalation is expected

  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.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by physical containment, collection and subsequent safe disposal.

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.

**Regulatory Obligations**

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

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

(1) Under Section 64(1) of the Act; if
  - products containing the notified chemical are made available to the public for DIY use;

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from a component of industrial 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.

*Safety Data Sheet*
The SDS of the notified chemical and products containing the notified chemical provided by the notifier were 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)
Lanxess Pty Ltd (ABN: 58 071 919 116)
Unit 1, 2D Factory Street
GRANVILLE NSW 2142

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details exempt from publication include: chemical name, CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, use details, and import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Schedule data requirements are varied for Hydrolysis as a Function of pH, Dissociation Constant, Flammability Limits, Explosive Properties, Oxidising Properties, Reactivity, *In vivo* Genotoxicity and Acute Inhalation Toxicity

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
China (2013), Taiwan (2015) and ECHA (2018).

## 2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
ADDITIN RC 4801 (Containing the notified chemical at < 70% concentration)

MOLECULAR WEIGHT
> 500 g/mol

ANALYTICAL DATA
Reference NMR, FT-IR, UV-Vis and MS spectra were provided.

## 3. COMPOSITION

DEGREE OF PURITY
< 50%

## 4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Yellow liquid*

| *Property* | *Value* | *Data Source/Justification* |
|---|---|---|
| Glass transition temperature | -18 °C | Measured* |
| Boiling Point | 173 °C at 101.3 kPa | Measured (Decomposition of the test substance began from this temperature)* |
| Relative Density | 1,048 kg/m$^3$ at 20 °C | Measured* |
| Vapour Pressure | $5.79 \times 10^{-6}$ kPa at 20 °C<br>$6.48 \times 10^{-6}$ kPa at 25 °C | Measured* |
| Water Solubility | $< 2 \times 10^{-7}$ g/L – $5.5 \times 10^{-5}$ g/L at 20 °C, based on the three main constituents of the UVCB mixture. | Measured* |

| *Property* | *Value* | *Data Source/Justification* |
|---|---|---|
| Hydrolysis as a Function of pH | Not determined | Contains hydrolysable functional groups. However, significant hydrolysis is not expected in the environmental pH range of 4 – 9. |
| Partition Coefficient (n-octanol/water) | log Pow = 1.15 – 2.36 at 25 °C | Measured* |
| Surface Tension | 34.65 mN/m at 20 °C | Measured* |
| Adsorption/Desorption | log Koc = 7.17 | Calculated |
| Dissociation Constant | pKa = 4.55, 5.24 | Calculated |
| Flash Point | > 110.7 °C at 101 kPa | Measured* |
| Flammability | Not determined | Not expected to be flammable based on flash point. |
| Autoignition Temperature | 364 ± 4 °C | Measured* |
| Explosive Properties | Not determined | Contains no functional groups that would imply explosive properties. |
| Oxidising Properties | Not determined | Contains no functional groups that would imply oxidising properties. |

* Mixture containing the notified chemical at < 50% concentration

DISCUSSION OF PROPERTIES
For 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 at a concentration of < 70% in mineral oil.

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

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

PORT OF ENTRY
Melbourne and Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
Lanxess Pty Ltd

TRANSPORTATION AND PACKAGING
The imported product containing the notified chemical at < 70% will be in 205 L drums and the end-use products with the notified chemical at up to 10% will be packaged in 205 L drums or small containers (1, 4 or 20L plastic bottles oil or tubs for grease). Transportation will be by road or rail within Australia.

USE
The notified chemical will be used as an additive in lubricant oils, greases and rust preventative oils at a concentration of up to 10%, which will be used for industrial applications.

OPERATION DESCRIPTION

*Reformulation*
The imported product containing the notified chemical (at < 70% concentration) will be formulated into end-use products. The reformulation procedure will likely vary depending on the nature of the formulated products, and may involve both automated and manual transfer steps. However, in general, it is expected that the reformulation processes will involve blending operations that will be highly automated and use closed systems with adequate ventilation, followed by automated filling of the reformulated products into containers of various sizes. Samples of the blended oils will be taken by laboratory staff for quality control testing.

*End use*
The finished products (lubricant oils, greases and rust preventative oils) containing the notified chemical at concentrations up to 10% will be used at industrial sites for general lubricating and metal-working applications. The finished products will be added to machinery either manually through closed systems. The rust preventative application will be by a dipping process in an enclosed system.

## 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)* |
|---|---|---|
| Transport and storage workers | 2-4 | 50 |
| Reformulation plant workers | 4-8 | 50 |
| QA Staff | 1-2 | 50 |
| Industrial site workers | 2-8 | 250 |

EXPOSURE DETAILS
*Transport and storage*
Transport, storage and warehouse workers may come into contact with the notified chemical at up to 70% concentration in industrial oil products in sealed containers, only in the event of accidental rupture of containers.

*Reformulation*
During reformulation, dermal, ocular and perhaps inhalation exposure of workers to the notified chemical at < 70% concentration may occur during handling of drums, during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. The notifier states that exposure is expected to be minimised through the use of ventilation and/or enclosed systems, and through the use of personal protective equipment (PPE) such as protective clothing, eye protection and suitable gloves.

*End-use*
Dermal and ocular exposure to the notified chemical at a concentration of up to10% may occur during the transfer from the storage containers into the machinery reservoirs, and during cleaning and maintenance of equipment. Exposure is expected to be minimised by the use of PPE as stated by the notifier.

#### 6.1.2. Public Exposure
Imported products and finished formulated products containing the notified chemical at up 70% concentration are intended for industrial use only and will not be available to the public.

### 6.2. Human Health Effects Assessment
The results from toxicological investigations conducted on an analogue of the notified 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 > 300 and < 2000 mg/kg bw; harmful |
| Acute dermal toxicity – rat | LD50 > 2000 mg/kg bw; low toxicity |
| Skin irritation – *in vitro* EPIDERM | non-corrosive |
| Skin irritation – *in vitro* EPISKIN | irritating |

| *Endpoint* | *Result and Assessment Conclusion* |
|---|---|
| Eye irritation – BCOP Assay | irritating |
| Skin sensitisation – guinea pig, Buehler Assay | no evidence of sensitisation |
| Repeat dose oral toxicity – rat, 28 days | NOAEL = 300 mg/kg bw/day |
| Mutagenicity – bacterial reverse mutation | non mutagenic |
| Genotoxicity – *in vitro* (L15178Y mouse lymphoma cells TK +/- locus mutation assay) | non genotoxic |
| Genotoxicity – *in vivo* (Chromosome aberration in Human lymphocytes) | non genotoxic |
| Reproductive and developmental toxicity – rat | NOAEL = 300 mg/kg bw/day |

*Toxicokinetics, Metabolism and Distribution*
No information on toxicokinetics of the notified chemical was provided. Based on the very low water solubility ($< 2 \times 10^{-7}$ g/L – $5.5 \times 10^{-5}$ g/L at 20 °C), partition coefficient (log Pow = 1.15 – 2.36 at 25 °C) and relatively high molecular weight (> 500 g/mol) of the notified chemical, absorption across biological membranes may not be expected.

*Acute toxicity*
The analogue was found to be harmful to rats via the oral route, with LD50 determined to be between 300 to 2,000 mg/kg bw in rats. The analogue was found to be of low acute toxicity to rats via the dermal route. No information on inhalation toxicity was submitted. However, no inhalation is expected as the notified chemical has a very low vapour pressure.

*Irritation and sensitisation*
Based on studies conducted, the analogue was not corrosive but shown to cause serious damage to the eyes in an *in vitro* bovine corneal opacity and permeability test (BCOP Test). It was also shown to be irritating to the skin in an *in vitro* human skin model test using the EPISKIN model. The analogue showed no evidence of sensitisation in a guinea pig skin sensitisation test.

*Repeated dose toxicity*
A 28 day repeated dose oral toxicity study was conducted on the analogue with dose levels of 30, 100 and 300 mg/kg bw/day. Under the conditions of this study, the NOAEL for local effects was considered to be 100 mg/kg bw/day for males and 300 mg/kg bw/day for females, due to the treatment-related effects seen in the stomach. All other changes were considered non-adverse and the NOAEL for systemic toxicity under the conditions of this study is considered to be 300 mg/kg bw/day for both sexes.

*Mutagenicity/Genotoxicity*
The analogue was not mutagenic in a bacterial reverse mutation test and an in vitro mammalian cell gene mutation test using the Thymidine Kinase Gene. The reaction product was not clastogenic in an in vitro mammalian chromosome aberration test.

*Toxicity for reproduction*
A reproduction/developmental toxicity test was conducted on the analogue at the dose levels of 30, 100, and 300 mg/kg bw/day. No parental, reproduction or developmental toxicity effects were observed in any of the dose levels tested. Therefore, the NOAEL is considered as 300 mg/kg bw/day in rats, for reproduction/developmental toxicity.

***Health Hazard Classification***
Based on the available information, the notified 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 notified chemical is presented in the following table.

| *Hazard Classification* | *Hazard Statement* |
|---|---|
| Acute Toxicity (Category 4) | H302 - Harmful if swallowed |
| Eye Damage / Irritation (Category 1) | H318 - Causes serious eye damage |
| Skin Corrosion / Irritation (Category 2) | H315 - Causes skin irritation |

### 6.3. Human Health Risk Characterisation

#### 6.3.1. Occupational Health and Safety

Based on toxicological data provided on an analogue chemical, the critical health effect of the notified chemical is expected to be skin irritation, and severe irritation to the eyes.

Reformulation workers, professional end-users and transport, storage and warehouse workers may be exposed to the notified chemical at < 70% concentration during reformulation, packaging, end-use, transport, storage and warehouse processes and handling of the chemical. The proposed use of PPE including impervious gloves, coveralls and goggles and largely enclosed, automated processes during reformulation is expected to minimise dermal and accidental ocular exposure.

Provided that the recommended controls are being adhered to, under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

#### 6.3.2. Public Health

The notified chemical is only intended for use in industrial settings, and hence public exposure is not expected. Therefore, when used in the proposed manner, the notified 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 notified chemical will be imported as a constituent of a mineral oil which will be reformulated into finished products. At reformulation sites, the major source of release is expected to be spills which will be contained within concrete bunds and either reclaimed or sent to on-site waste-water treatment facilities where residual hydrocarbon based products will be separated from the aqueous stream by the American Petroleum Industry (API) process, with a claimed removal of greater than 90%. Before being released to the sewerage system, the aqueous waste undergoes further treatment involving biological treatment and biodisk filtration. The remaining oily waste will be incinerated. Therefore, no significant release of the notified chemical is expected during reformulation.

RELEASE OF CHEMICAL FROM USE

Lubricant oils containing the notified chemical will be added to equipment/machinery reservoirs and are expected to remain within these closed systems. Releases during use may come from spills when pouring lubricants into the machinery reservoirs or leaks from the machinery, however these are expected to be negligible. Waste oil from industrial sites will be collected for disposal via liquid waste facility, where the wastes may be recycled or disposed of by incineration. Releases of the notified chemical during its use in lubricating oils are not expected to be significant (OECD, 2014).

Grease and rust prevention products containing the notified chemical are designed to be applied to metal articles and last for the lifetime of the metal parts to which they have been applied. Release from this use application is also expected to be minimal.

RELEASE OF CHEMICAL FROM DISPOSAL

At the end of their useful lives, the products containing the notified chemical will be drained from the machinery for disposal. The main method of disposal will be by recycling or thermal decomposition. The finished products containing the notified chemical are not intended for sale to the public and therefore no release is expected from incorrect disposal by DIY users. Some of the residual fluid within the machinery will have the same fate as the machinery which may be recycled as scrap metal or disposed of to landfill. Notified chemical which is used in grease products or rust prevention oils will likewise share the fate of the metal articles to which it has been applied and be recycled or disposed of to landfill. During metal recycling the notified chemical will be incinerated to produce combustion products.

#### 7.1.2. Environmental Fate

A biodegradability test conducted on a mixture containing the notified chemical shows no evidence of biodegradability (0% degraded over 28 days in an OECD 301 B test - Appendix C).

Each application of the notified chemical is expected to be associated with minimal aquatic release. Used lubricant oils and fluids containing the notified chemical are expected to be recycled, re-refined or disposed of by approved waste management facilities. Greases containing the notified chemical are expected to remain attached to the articles to which they are applied. The majority of the notified chemical is therefore expected to be degraded by incineration or decomposed during metal recycling processes or ultimately, to end up in landfill along with the articles to which it has been applied. In landfill the notified chemical is expected to be immobile based on its very slight water solubility and its strong affinity for organic carbon in soil (log Koc = 7.17). The notified chemical in the environment is expected to eventually degrade into water and oxides of carbon via biotic and abiotic pathways.

### 7.1.3. Predicted Environmental Concentration

The Predicted Environmental Concentration (PEC) has not been calculated as release of the notified chemical to the aquatic environment will be limited based on its reported use pattern.

## 7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on a test substance containing 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 = 26.3 mg/L | Harmful to fish |
| Daphnia Toxicity | 48 h EC50 = 84.91 mg/L | Harmful to aquatic invertebrates |
| Algal Toxicity | 72 h EC50 > solubility limit | Not harmful to algae |
| Inhibition of bacterial respiration | 3 h EC50 > 1,000 mg/L | Not inhibitory to microorganisms |

The tests do not permit determination of a hazard class for the notified chemical because they were conducted using mixtures which contained the notified chemical as well as other constituents. The effects of the other constituents were not resolved from the effect of the notified chemical.

### 7.2.1. Predicted No-Effect Concentration

The Predicted No-Effect Concentration (PNEC) has not been calculated because the ecotoxocity endpoints were determined using mixtures containing the notified chemical as well as other constituents and the hazard of the notified chemical could not be resolved from these tests.

## 7.3. Environmental Risk Assessment

The risk quotient (Q = PEC/PNEC) for the notified chemical has not been calculated as release of the notified chemical to the aquatic environment in ecotoxicologically significant concentrations is not expected based on its reported use pattern. Therefore, on the basis of this assessed use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

## APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

**Melting Point/Freezing Point** -18 °C

| | |
|---|---|
| Method | OECD TG 102 Melting Point/Melting Range |
| Remarks | Differential scanning calorimeter (DSC) method<br>No melting or freezing point was observed, the glass transition point is reported instead. |
| Test Facility | Smithers Viscient (ESG) Ltd (2018) |

**Boiling Point** > 175 °C at 101.3 kPa

| | |
|---|---|
| Method | OECD TG 103 Boiling Point |
| Remarks | The boiling was at 173°C (mean of 171.4 and 173.8°C).<br>The test substance decomposed at this temperature. |
| Test Facility | Smithers Viscient (ESG) Ltd (2018) |

**Density** 1,048 kg/$m^3$ at 20 °C

| | |
|---|---|
| Method | OECD TG 109 Density of Liquids and Solids |
| Remarks | Determined by a gas comparison pycnometer method |
| Test Facility | Smithers Viscient (ESG) Ltd (2018) |

**Vapour Pressure** $5.790 \times 10^{-6}$ kPa at 20 °C<br>$6.479 \times 10^{-6}$ kPa at 25 °C

| | |
|---|---|
| Method | OECD TG 104 Vapour Pressure |
| Remarks | Knudsen cell effusion method. Results were extrapolated from measurements made between 51 °C and 91 °C. The measured vapour pressure is the combined pressure from each volatile constituent in the UVCB. |
| Test Facility | Smithers Viscient (ESG) Ltd (2018) |

**Water Solubility** $< 2 \times 10^{-7}$ g/L – $5.5 \times 10^{-5}$ g/L at 20 °C

| | |
|---|---|
| Method | OECD TG 105 Water Solubility |
| Remarks | Flask Method. Solubilities for each of the three main constituents of the mixture containing the notified chemical were determined by LC-MS. The solubility of the least soluble constituent (the notified chemical) ranged from < 0.02195 µg/mL (LOQ) to 0.2009 µg/mL at pH 3.8. Due to the uncertainty, the highest solubility was selected (0.2 µg/L). The large uncertainty is outside the range recommended by the test guidelines (15%), but the upper limit for solubility is considered appropriate for assessment purposes. The individual constituents had solubilities of $< 2 \times 10^{-7}$ g/L, $1.3 \times 10^{-5}$ g/L and $5.5 \times 10^{-5}$ g/L. |
| Test Facility | Smithers Viscient (ESG) Ltd (2018) |

**Partition Coefficient (n-octanol/water)** log Pow = 1.15 – 2.36 at 25 °C

| | |
|---|---|
| Method | OECD TG 117 Partition Coefficient (n-octanol/water). |
| Remarks | HPLC Method. The individual partition coefficients for the three constituents of the mixture containing the notified chemical were determined as 2.36, 1.99 and 1.15. These were not prescribed to the specific constituents. |
| Test Facility | Smithers Viscient (ESG) Ltd (2018) |

**Surface Tension** 34.65 mN/m at 20 °C

| | |
|---|---|
| Method | OECD TG 115 Surface Tension of Aqueous Solutions<br>EC Council Regulation No 440/2008 A.5 Surface Tension |
| Remarks | Concentration: 90% of saturated solution (90% of 59.37 µg/mL) |
| Test Facility | Smithers Viscient (ESG) Ltd (2018) |

**Adsorption Coefficient** log Koc = 7.17

| | |
|---|---|
| Method | KOCWIN v2.00 (MCI method; US EPA 2012) |
| Remarks | Adsorption coefficients were calculated for the each of the three major constituents of the mixture containing the notified chemical in their neutral (protonated) forms. The adsorption coefficient (log Koc) of the notified chemical was determined to be 7.17. At environmentally relevant pH (4 – 9) the notified chemical is expected to be deprotonated (negatively charged) which will decrease the adsorption coefficient. |

**Dissociation Constant** pKa = 4.55, 5.24

| | |
|---|---|
| Method | ACD/pKa |
| Remarks | Calculated for a suitable analogue for determining the pKa of the notified chemical. |

**Flash Point** > 110.7 °C at 101 kPa

| | |
|---|---|
| Method | EC Council Regulation No 440/2008 A.9 Flash Point |
| Remarks | Closed cup equilibrium method.<br>Corrected for atmospheric pressure, 98.8 kPa on the day of testing. |
| Test Facility | Smithers Viscient (ESG) Ltd (2018) |

**Autoignition Temperature** 364 ± 4 °C

| | |
|---|---|
| Method | EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases) |
| Remarks | The experimental procedure was based on ASTM-E 659-78 |
| Test Facility | Smithers Viscient (ESG) Ltd (2018) |

# APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

## B.1. Acute Oral Toxicity – Rat

| | |
|---|---|
| TEST SUBSTANCE | Analogue |
| METHOD | OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure<br>EC Council Regulation No 440/2008 B.1 bis Acute toxicity (oral) fixed dose method |
| Species/Strain | Rat/ Wistar (RccHan™:WIST) |
| Vehicle | Arachis oil BP |
| Remarks – Method | No significant protocol deviations |

RESULTS

| *Group* | *Number and Sex of Animals* | *Dose (mg/kg bw)* | *Mortality* |
|---|---|---|---|
| 1 | 1F | 2000 | 1/1 |
| 2 | 1F+ 4F | 300 | 0/5 |

| | |
|---|---|
| LD50 | > 300 and < 2000 mg/kg bw |
| Signs of Toxicity | At 2000 mg/kg bw, noisy respiration, hunched posture, pilo-erection, diarrhoea, dehydration, lethargy, hypothermia and tiptoe gait were noted.<br>In animals treated at a dose of 300 mg/kg bw, hunched posture was noted. |
| Effects in Organs | In animals treated at a dose of 2000 mg/kg bw, dark liver, dark kidneys, gaseous stomach and haemorrhage of the gastric mucosa and non-glandular epithelium of the stomach were noted.<br>In animals treated at a dose of 300 mg/kg bw, no abnormalities were noted. |
| Remarks – Results | All animals in group 2 showed expected gains in body weight over the observation period. |
| CONCLUSION | The test item is harmful via the oral route. |
| TEST FACILITY | Envigo (2017a) |

## B.2. Acute Dermal Toxicity – Rat

| | |
|---|---|
| TEST SUBSTANCE | Analogue |
| METHOD | OECD TG 402 Acute Dermal Toxicity<br>EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal) Limit Test |
| Species/Strain | Rat/Wistar (RccHan™:WIST) |
| Vehicle | Arachis oil BP |
| Type of dressing | Semi-occlusive. |
| Remarks – Method | No significant protocol deviations |

RESULTS

| *Group* | *Number and Sex of Animals* | *Dose (mg/kg bw)* | *Mortality* |
|---|---|---|---|
| 1 | 5 M | 2,000 | 0/5 |
| 2 | 5 F | 2,000 | 0/5 |

| | |
|---|---|
| LD50 | > 2000 mg/kg bw |
| Signs of Toxicity – Local | Signs of dermal irritation included very slight to well-defined erythema, very slight to slight oedema, haemorrhage of dermal capillaries, light brown discoloration of the epidermis, crust formation, loss of skin elasticity and flexibility, hardened light brown coloured scab, scab |

| | |
|---|---|
| | cracking, scab lifting to reveal glossy skin, moderate desquamation and glossy skin. |
| Signs of Toxicity – Systemic | There were no signs of systemic toxicity |
| Effects in Organs | No abnormalities were noted at necropsy. |
| Remarks – Results | All animals showed expected gains in body weight, except for one female which showed no gain in body weight during the first week but gained weight during the second week. |

CONCLUSION The test item is of low acute toxicity via the dermal route.

TEST FACILITY Envigo (2016a)

### B.3. Skin Irritation – *In Vitro* Skin Corrosion in the EPIDERM™ Human Skin Model

TEST SUBSTANCE Analogue

METHOD OECD TG 431 *In vitro* Skin Corrosion – Human Skin Model Test
EC Council Regulation No 440/2008 B.40 BIS. *In vitro* Skin Corrosion – Human Skin Model Test

Remarks – Method The purpose of this test is to evaluate the corrosivity potential of the test item using the EpiDerm™ Human Skin Model after treatment periods of 3 and 60 minutes.

Corrosion is directly related to cytotoxicity in the EpiDerm™ tissue. Cytotoxicity is determined by the reduction of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) to formazan by viable cells in the test item treated tissues relative to the corresponding negative control. The results are used to make a prediction of the corrosivity potential of the test item.

Duplicate tissues were treated with the test item for exposure periods of 3 and 60 minutes. Negative (Sterile distilled water) and positive (8.0N Potassium Hydroxide) control groups were treated for each exposure period.

Classification of corrosivity potential is based on relative viabilities for both exposure times according to the test guideline.

RESULTS

| *Test Material* | *Mean $OD_{562}$ of Duplicate Tissues* | | *Relative Mean Viability (%)* | |
|---|---|---|---|---|
| | 3 min | 60 min | 3 min | 60 min |
| *Negative control* | 1.854 | 1.908 | 100 | 100 |
| *Test substance* | 1.277 | 1.246 | 68.9 | 65.3 |
| *Positive control* | 0.087 | 0.050 | 4.7 | 2.6 |

OD = optical density; SD = standard deviation
Results after treatment of 3 min or 60 min

Remarks – Results The mean viability of the negative control tissues is set at 100%.

The quality criteria required for acceptance of results in the test were satisfied.

The test item was considered to be non-corrosive to the skin.

CONCLUSION The test item was considered non-corrosive to the skin under the conditions of the test.

TEST FACILITY Envigo (2016b)

### B.4. Skin Irritation – *In Vitro* EPISKIN

| | |
|---|---|
| TEST SUBSTANCE | Analogue |
| METHOD | OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method<br>EC Council Regulation No 440/2008 B.40 BIS. *In vitro* Skin Corrosion – Human Skin Model Test |
| Remarks – Method | The principle of the assay was based on the measurement of cytotoxicity in reconstructed human epidermal cultures following topical exposure to the test item by means of the colorimetric MTT reduction assay.<br><br>Classification of irritation potential is based upon relative mean tissue viability following the 15-Minute exposure period followed by the 42-hour post-exposure incubation period according to the test guideline. |

RESULTS

| *Test Material* | *Mean $OD_{562}$ of Triplicate Tissues* | *Relative Mean Viability (%)* | *SD of Relative Mean Viability* |
|---|---|---|---|
| *Negative control* | 0.908 | 100 | 3.5 |
| *Test substance* | 0.085 | 9.4 | 1.7 |
| *Positive control* | 0.052 | 5.7 | 0.5 |

OD = optical density; SD = standard deviation

| | |
|---|---|
| Remarks – Results | The quality criteria for the negative and positive controls required for acceptance of results in the test were satisfied.<br><br>The relative mean viability of the test item treated tissues was 9.4% after the 15-Minute exposure period and 42-Hours post-exposure incubation period. |
| CONCLUSION | Based on the mean tissue viability of ≤ 50%, the test item should be classified for skin irritation (Category 2) according to the GHS criteria. |
| TEST FACILITY | Envigo (2016c) |

### B.5. Eye Irritation – *In Vitro* Bovine Corneal Opacity and Permeability Test

| | |
|---|---|
| TEST SUBSTANCE | Analogue |
| METHOD | OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage |
| Vehicle | Eagle's Minimum Essential Medium |
| Remarks – Method | No significant protocol deviations. |

RESULTS

| *Test Material* | *Mean Opacities of Triplicate Tissues (SD)* | *Mean Permeabilities of Triplicate Tissues (SD)* | *IVIS (SD)* |
|---|---|---|---|
| *Vehicle control* | ~0.3 | ~0.006 | 0.4 |
| *Test substance** | 59.3 | 0.461 | 66.2 |
| *Positive control** | 30.7 | 1.114 | 47.4 |

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

| | |
|---|---|
| Remarks – Results | The corneas treated with the test item or the positive control item were cloudy post treatment and post incubation. The corneas treated with the negative control item were clear post treatment and post incubation.<br><br>The positive control and negative controls meet the acceptability criteria. |

| | |
|---|---|
| CONCLUSION | The test item (with an IVIS of > 55) was considered a Cat.1 eye irritant according to the test guideline. |
| TEST FACILITY | Envigo (2016d) |

**B.6. Skin Sensitisation – Guinea Pig Buehler test**

| | |
|---|---|
| TEST SUBSTANCE | Analogue |
| METHOD | OECD TG 406 Skin Sensitisation – Buehler test<br>EC Directive 96/54/EC B.6 Skin Sensitisation – Buehler test |
| Species/Strain | Guinea pig/Albino Dunkin-Hartley |
| PRELIMINARY STUDY | Maximum non-irritating concentration:<br>Topical: 10% |
| MAIN STUDY | |
| Number of Animals | Test Group: (20 females) Control Group: (10 females) |
| Vehicle | Paraffin |
| Positive Control | Not conducted in parallel with the test substance, previously conducted in the test laboratory using α-hexylcinnamaldehyde (CAS No. 101-86-0). |
| INDUCTION PHASE | Induction concentration:<br>Topical: 10% |
| Signs of Irritation | Discrete to moderate erythema was noted in all animals in the test group. |
| CHALLENGE PHASE | |
| Challenge Phase | Topical: Occlusive dressing for 6 hours, consisted of a single topical application of the test item diluted at 10% in liquid paraffin and of a negative control (liquid paraffin). |
| Remarks – Method | The concentration selected for the induction phase and the challenge was based on the result of three pre-tests.<br>Readings were performed 24 and 48 hours after removal of the patches. |

RESULTS

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

| | |
|---|---|
| Remarks – Results | No unscheduled deaths occurred during the study.<br><br>No abnormalities, and no differences in the body weight between the control and the treated group were observed. No adverse clinical signs were observed during the challenge.<br><br>No irritation was recorded in animals from the treated and control groups after the challenge phase. |
| CONCLUSION | There was no evidence of reactions indicative of skin sensitisation to the test item under the conditions of the test. |
| TEST FACILITY | Phycher (2016) |

**B.7. Repeat Dose Oral-Gavage Toxicity – Repeated Dose 28-day Oral Toxicity Study in Rodents Followed by a 2-Week Recovery Period**

| | |
|---|---|
| TEST SUBSTANCE | Analogue |
| METHOD | OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents Followed by a 2-Week Recovery Period |

| | |
|---|---|
| | EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral) |
| Species/Strain | Rat/RccHan™:WIST |
| Route of Administration | Oral – gavage |
| Exposure Information | Total exposure days: 28 days<br>Dose regimen: 7 days per week<br>Post-exposure observation period: 14 days |
| Vehicle | Corn oil |
| Remarks – Method | No significant protocol deviations.<br>The dose levels selected for this study were based on the results of a 14-day dose-range finding study (where doses of 500 and 1000 mg/kg/day were not tolerated (Envigo, 2016e). Tests were terminated on Day 5 (for the 500 mg/kg/day group) and Day 2 (for the 1000 mg/kg/day group) for welfare reasons and severity of the signs observed respectively. |

RESULTS

| *Group* | *Number and Sex of Animals* | *Dose (mg/kg bw/day)* | *Mortality* |
|---|---|---|---|
| Control | 5 M, 5 F | 0 | 0/10 |
| Low Dose | 5 M, 5 F | 30 | 0/10 |
| Mid Dose | 5 M, 5 F | 100 | 0/10 |
| High Dose | 5 M, 5 F | 300 | 0/10 |
| Control Recovery | 5 M, 5 F | 0 | 0/10 |
| High Dose Recovery | 5 M, 5 F | 300 | 0/10 |

*Mortality and Time to Death*

All animals survived the scheduled treatment or recovery periods.

*Clinical Observations*

In the 300 mg/kg/day dose group salivation was observed in 5/10 male and 6/10 female animals, and chin rubbing was observed in 1/10 male and 8/10 female animals. Salivation was also observed in 2/5 females at 100 mg/kg/day.

No significant sensory or motor activities or grip strength were affected by the treatment during week 4 or during week 2 of recovery.

There were no statistically significant differences in food or water consumption.

Body weight gain was unaffected by treatment for female animals. For all treated male animals, body weight gains were slightly (14.9 – 10.4%) lower (statistically significant) than the control groups during the treatment period. During the recovery period, the overall body weight for male animals treated at 300 mg/kg bw/day was similar to the control groups.

*Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis*

Low haematocrit, haemoglobin concentration and erythrocyte count for female animals treated at 300 mg/kg bw/day. The erythrocyte count was also low for female animals in the 30 and 100 mg/kg bw/day dose groups. Slightly high neutrophil counts were observed for male animals treated at 300 mg/kg bw/day. None of the above effects were present in the recovery groups, although male recovery animals had decreased mean cell haemoglobin and mean cell volume and female animals in the recovery group had an increased total leucocyte count including; lymphocytes, monocytes and large unstained cells.

The biochemical examination of the blood revealed slight increases in total bilirubin in males dosed at 30 or 100 mg/kg bw/day and slight increases in chloride in all dosed males. All effects in males were not present in the recovery group. There were no statistically significant changes seen in the blood chemistry of female animals at the end of the treatment period, however glucose levels were decreased and the triglyceride levels increased in the recovery animals.

Urinalysis examination after 4 weeks of treatment showed slightly low urinary pH for males at 30 or 300 mg/kg bw/day (but not at 100 mg/kg bw/day) and slightly high specific gravity also for males at 30 or 300 mg/kg bw/day (but not at 100 mg/kg bw/day), associated with reduced urine volume in these animals. The low urinary pH and specific gravity changes were not apparent after the two week of recovery period in male animals,

however an increase in total protein was seen in the recovery group. No statistically significant changes were observed in the urinalysis parameters of female animals in any of the treatment groups.

*Effects in Organs*

Absolute body weight-adjusted liver weights were not statistically significant at all doses in both sexes. No associated histopathological changes were noted, and the finding showed complete recovery. The relative ovary and spleen weights were decreased and increased respectively in female animals in the recovery group, these changes were not noted in the females at the end of the four week treatment. No other statistically significant changes in organ weights were observed.

After four weeks of treatment, macroscopic examination showed dark areas and/or depressions in the non-glandular region of the stomach of 3/5 males treated at 300 mg/kg bw/day with dark areas in one male treated at 30 mg/kg bw/day. Thickening of the stomach was seen in 3/5 males and 2/5 females treated at 300 mg/kg bw/day and one male treated at 100 mg/kg bw/day. The macroscopic examination performed after two weeks of recovery revealed no test item related lesions.

Histopathological changes related to treatment were observed in the stomach. These included hyperplasia and hyperkeratosis of the epithelium of the non-glandular region in male and female animals treated at 300 mg/kg bw/day and degeneration/vacuolation of the epithelium of the non-glandular region seen in males treated at 300 mg/kg bw/day. Ulceration was found in one male treated at 300 mg/kg bw/day. No changes related to treatment after the two week recovery period were noted.

Remarks – Results

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established for local effects as 100 mg/kg bw/day in this study, based on irritant effects on stomach such as ulceration and degeneration findings in male animals treated at 300 mg/kg bw/day. However, the NOAEL for systemic toxicity was established as 300 mg/kg/day by the study authors.

TEST FACILITY Envigo (2017b)

### B.8. Genotoxicity – Bacteria

| | |
|---|---|
| TEST SUBSTANCE | Analogue |
| METHOD | OECD TG 471 Bacterial Reverse Mutation Test<br>EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria<br>Plate incorporation procedure and Pre incubation procedure |
| Species/Strain | *Salmonella typhimurium*: TA1535, TA1537, TA98, TA100, *Escherichia coli*: WP2uvrA |
| Metabolic Activation System | S9 mix from phenobarbital/β-naphthoflavone induced rat liver |
| Concentration Range in Main Test | a) With metabolic activation: 1.5 to 5000 µg/plate µg/plate<br>b) Without metabolic activation: 1.5 to 5000 µg/plate µg/plate |
| Vehicle | Dimethyl Sulphoxide (DMSO) |
| Remarks – Method | No significant protocol deviations. Standard plate (Test 1) and pre-incubation (Test 2) methods were used.<br>Positive control used in the absence of S9-mix:<br>N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) (used for WP2uvrA, TA100 & TA1535);<br>9-Aminoacridine (9AA) (used for TA1537); and<br>4-Nitroquinoline-1-oxide (4NQO) (used for TA98).<br>Positive control used in the presence of S9-mix:<br>2-Aminoanthracene (2AA) (used for WP2uvrA, TA100 & TA1535 and TA1537); and<br>Benzo(a)pyrene (BP) (used 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 | > 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

The vehicle (DMSO) control was within the normal range, and the positive control confirmed the sensitivity of the test system.

No visible reduction in the growth of the bacterial background lawn at any dose level, either in the presence or absence of metabolic activation (S9-mix) was noted.

No significant increases in the frequency of revertant colonies was recorded for any of the bacterial strains, either with or without metabolic activation.

No test item precipitate was observed on the plates at any of the doses tested in both tests and in either the presence or absence of S9-mix.

CONCLUSION

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

TEST FACILITY

Envigo (2016f)

### B.9. Genotoxicity – *In Vitro* Mammalian Chromosome Aberration Test

TEST SUBSTANCE

Analogue

METHOD

OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test
EC Directive 2000/32/EC B.10 Mutagenicity – *In vitro* Mammalian Chromosome Aberration Test

Cell Type/Cell Line: Human lymphocytes
Metabolic Activation System: S9 mix from phenobarbital/β-naphthoflavone induced rat liver
Vehicle: Acetone
Remarks – Method: No significant protocol deviations.
The positive control items were:
In the presence of S9-mix: Cyclophosphamide (CP)
In the absence of S9-mix: Mitomycin C (MMC)

| *Metabolic Activation* | *Test Substance Concentration (µg/mL)* | *Exposure Period* | *Harvest Time* |
|---|---|---|---|
| *Absent* | | | |
| Test 1 | 0*, 10, 20, 40*, 80*, 120*, 160*, 240, MMC 0.2* | 4 h | 24 h |
| Test 2 | 0*,10, 20*, 40*, 60*, 80*, 120, 160, MMC 0.1* | 24 h | 24 h |
| *Present* | | | |
| Test 1 | 0*, 10, 20, 40, 60*, 80*, 120*, 160*, CP 2* | 4 h | 24 h |

* 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 | ≥ 156.25 | ≥ 160 | ≥ 312.5 | Negative |

| | | | | |
|---|---|---|---|---|
| Test 2 | ≥ 78.13 | ≥ 80 | ≥ 312.5 | Negative |
| *Present* | | | | |
| Test 1 | ≥ 156.25 | > 160 | ≥ 625 | Negative |

Remarks – Results

The test item demonstrated marked cytotoxicity in all three exposure groups.

There were no statistically significant increases in the frequency of cells with aberrations in the 4 h exposure group in the presence of metabolic activation or in the 24-hour exposure group.
In the 4 h exposure group in the absence of metabolic activation there was a small but statistically significant ($p < 0.05$) increase in the frequency of aberrations at 160 µg/mL. This was considered to be of no biological relevance by the study authors as this dose exceeded acceptable toxicity (mitotic index 33%) and it was therefore considered that the response was as a result of cytotoxicity.

The concurrent positive and negative controls produced satisfactory responses, thus confirming the validity of the test.

CONCLUSION

The test item was not clastogenic to human lymphocytes treated *in vitro* under the conditions of the test.

TEST FACILITY

Envigo (2016g)

**B.10. Genotoxicity – *In Vitro* Mammalian Cell Gene Mutation Test**

TEST SUBSTANCE

Analogue

METHOD

OECD TG 490 In Vitro Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene
EC Directive 440/2008 B.17 Mutagenicity – In Vitro Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene

Cell Type/Cell Line: Mouse lymphoma cells / L5178Y TK +/- 3.7.2c
Metabolic Activation System: S9 mix from phenobarbital/β-naphthoflavone induced rat liver
Vehicle: Acetone
Remarks – Method: No significant protocol deviations.

| *Metabolic Activation* | *Test Substance Concentration (µg/mL)* | *Exposure Period* | *Expression Time* |
|---|---|---|---|
| *Absent* | | | |
| Test 1 | 0*, 2.5, 5, 10*, 20*, 30*, 40*, 60*, 80* | 4 h | 48 h |
| Test 2 | 0*, 2.5*, 5*, 10*, 20*, 40*, 60*, 80, 100 | 24 h | 48 h |
| *Present* | | | |
| Test 1 | 0*, 2.5, 5*, 10*, 20*, 40*, 60*, 80*, 100 | 4 h | 48 h |

*Cultures selected for 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 | ≥ 39.06 | ≥ 60 | > 80 | Negative |
| Test 2 | ≥ 39.06 | ≥ 40 | > 100 | Negative |
| *Present* | | | | |
| Test 3 | ≥ 78.13 | ≥ 60 | > 100 | Negative |

Remarks – Results

In the preliminary cytotoxicity test a precipitate was observed at ≥ 156.25 µg/mL in the 4 hour exposure group in the absence of metabolic

| | |
|---|---|
| | activation, at 312.5 µg/mL in the 4-hour exposure group in the presence of metabolic activation and at 625 µg/mL in the 24-hour exposure group.<br><br>No statistically or biologically significant increases in the mutant frequency at the TK +/- locus, were recorded for any cultures treated with the test substance in either the presence or absence of metabolic activation. Positive controls confirmed the sensitivity of the test system. |
| CONCLUSION | The test item was not mutagenic under the conditions of this i*n Vitro L5178Y TK +/- Mouse Lymphoma Assay* |
| TEST FACILITY | Envigo (2016h) |

**B.11. Reproductive/Developmental Toxicity – Oral Gavage - One Generation Study**

| | |
|---|---|
| TEST SUBSTANCE | Analogue |
| METHOD | OECD TG 421 Reproductive/Developmental Toxicity Screening Study in Han Wistar Rats by Oral Gavage Administration |
| Species/Strain | Rat/ RccHan™;WIST |
| Route of Administration | Oral – gavage |
| Exposure Information | Exposure period – female: 15 days before pairing prior to mating then 5 weeks and 7 days of lactation (until necropsy)<br>Exposure period – male: 15 days before pairing prior to mating then 5 weeks (until necropsy) |
| Vehicle | Corn oil |
| Remarks – Method | No significant protocol deviations. |

RESULTS

| *Group* | *Number and Sex of Animals* | *Dose (mg/kg bw/day)* | *Mortality* |
|---|---|---|---|
| 1 | 10 M, 10 F | 0 | 0/20 |
| 2 | 10 M, 10 F | 30 | 0/20 |
| 3 | 10 M, 10 F | 100 | 0/20 |
| 4 | 10 M, 10 F | 300 | 0/20 |

*Mortality and Time to Death*
There were no unscheduled deaths

*Effects on Parental (P) animals:*
Chin rubbing was observed in 1/10 male animals in the 300 mg/kg bw/day dose group and 1/10 females in the 100 mg/kg bw/day dose group and 6/10 females in the 300 mg/kg bw/day dose group. Salivation was observed in 8/10 males and 9/10 females in the 300 mg/kg bw/day dose group as well as 4/10 females in the 100 mg/kg bw/day dose group. Piloerection was observed in 2/10 females in the 300 mg/kg bw/day dose group.

There were no treatment related effects upon body weight or food consumption of the adult male or female animals at any of the treated dose level.

Treatment up to 300 mg/kg/day also had no effect upon mating performance, fertility, and gestation length or gestation index.

*Effects on 1st Filial Generation (F1)*
One female animal treated at 100 mg/kg bw/day failed to litter. This animal was classified as "total litter resorption" as it had only one uterine implantation, which had resorbed. This female was excluded from group mean gestation body weight and food consumption data as this was not a typical pregnancy and it is not uncommon for animals with a very low number of implantations to show total litter resorption. All other females reared a live litter to Day 7 of lactation.

No treatment effect upon mean sex ratio was observed at any of treated dose levels. The mean corpora lutea count was unaffected by treatment.

No findings related to parent treatment observed in a necropsy of offspring dying prematurely or in those killed at scheduled termination.

Remarks – Results

At 300 or 100 mg/kg/day the mean body weights of male and female offspring on Day 1 of age were slightly lower than in Controls. However, the number of offspring born, their subsequent survival, growth and clinical condition to Day 7 of age were unaffected by treatment at dose levels up to 300 mg/kg/day.

No macroscopic findings in offspring related to parental treatment were observed at any of treated dose levels.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) for systemic, reproductive or developmental toxicity was established as 300 mg/kg bw/day in this study.

TEST FACILITY Envigo (2017c)

# APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

## C.1. Environmental Fate

### C.1.1. Ready Biodegradability

| | |
|---|---|
| TEST SUBSTANCE | Analogue |
| METHOD | OECD TG 301 B Ready Biodegradability: $CO_2$ Evolution Test |
| Inoculum | Activated sludge from a treatment plant which receives primarily domestic sewage. |
| Exposure Period | 28 days |
| Auxiliary Solvent | Acetone |
| Analytical Monitoring | None |
| Remarks – Method | The concentrations of each constituent of the notified substance were accurately determined before running the test which was required to determine the ThOD. The notified substance was adsorbed to silica by dissolving it in acetone, adding silica, and removing the solvent. Treated silica was added directly to the test medium to give a test substance concentration equivalent to 15 mg organic carbon/L. The media were inoculated with microorganisms (30 mg/L). The control and reference vessels contained silica which was treated with acetone but no test substance. Sodium benzoate was used as the reference substance but it is unclear whether or not this was adsorbed to silica first or added directly to the media. |

RESULTS

| *Test Substance* | | *Sodium Benzoate* | |
|---|---|---|---|
| *Day* | *% Degradation* | *Day* | *% Degradation* |
| 1 | 0 | 1 | 16 |
| 9 | 0 | 9 | 73 |
| 28 | 0 | 28 | 93 |

| | |
|---|---|
| Remarks – Results | The test did not satisfy two of the validity criteria. The mean $CO_2$ production in the blank control vessels was 71.6 mg/L at the end of the test (day 28) which exceeds the validity criterion (40 - 70 mg/L). Degradation of residual acetone attached to the silica may explain this result. The effect of this deviation on the outcomes of the study is unknown. The IC content of the test medium exceeded 5% of the TOC at the beginning of the test (5.8%). This was not considered to affect the validity of the test because the excess IC would be present in both the control and test vessels and is therefore taken into account in the calculations.<br><br>The toxicity control exceeded 25% biodegradation after 14 days showing that toxicity was not a factor in inhibiting the biodegradability of the test substance.<br><br>The test substance is a UVCB containing a compound which has previously been shown to be biodegradable (ECHA, 2019) - albeit using an inoculum which had been adapted to the test material for 14 days. The test substance would therefore be expected to show some biodegradability within the 28-day period. The fact that no biodegradability is observed in the current test may be because the test substance is strongly adsorbed to silica and is not bioavailable. This will have significantly affected the results of the study but this was not considered in the report. |
| CONCLUSION | The test substance shows no evidence of ready biodegradability. |
| TEST FACILITY | Smithers Viscient (ESG) Ltd (2017) |

## C.2. Ecotoxicological Investigations

### C.2.1. Acute Toxicity to Fish

| | |
|---|---|
| TEST SUBSTANCE | Analogue |
| METHOD | OECD TG 203: Fish, Acute Toxicity Test – Semi-static |
| Species | *Oncorhynchus mykiss* (Rainbow trout) |
| Exposure Period | 96 hours |
| Auxiliary Solvent | None |
| Water Hardness | 70 mg $CaCO_3$/L |
| Analytical Monitoring | HPLC-TOF, MS/MS |
| Remarks – Method | A saturated stock solution of the test substance was prepared by stirring a measured quantity of the solid test substance in water for 24 hours and filtering through a 0.45 µm filter. Test media were prepared by diluting the stock solution to the desired concentration. Based on the results of a range finding test, solutions for the definitive test contained the stock solution at nominal concentrations of 6.25 % to 100 %. Semi-static conditions were used with media replaced daily. Concentrations of the test item were measured in fresh media (at 0 hours, and 72 hours) and aged media (immediately before replacement at 24 hours and 96 hours). The measured concentrations were not within the range of 80 – 120 % of the nominal concentrations. Therefore, the arithmetic mean of the measured concentrations was used to determine LC50 and NOEC. No negative control was run. |

RESULTS

| *Concentration (mg/L)* | | *Number of Fish* | *Mortality* | | | | |
|---|---|---|---|---|---|---|---|
| *Nominal** | *Time Weighted Mean Measured* | | *4 h* | *24 h* | *48 h* | *72 h* | *96 h* |
| Control | - | 7 | 0 | 0 | 0 | 0 | 0 |
| 6.25 | 2.54 | 7 | 0 | 0 | 0 | 0 | 0 |
| 12.5 | 4.55 | 7 | 0 | 0 | 0 | 0 | 0 |
| 25 | 10.6 | 7 | 0 | 0 | 0 | 0 | 0 |
| 50 | 17.3 | 7 | 0 | 0 | 0 | 0 | 0 |
| 100 | 39.6 | 7 | 0 | 0 | 0 | 6 | 7 |

* % of saturated stock solution.

| | |
|---|---|
| LC50 | 26.3 mg/L at 96 hours (based on time weighted mean measured concentrations) |
| NOEC | 17.3 mg/L at 96 hours (based on time weighted mean measured concentrations) |
| Remarks – Results | All validity criteria were satisfied. The dissolved oxygen concentration in the test and control solutions was ≥ 80 % at 15 (± 2) °C. Statistical analysis was performed with CETIS v 1.8.6.8. The LC50 values were estimated using linear interpolation. The measured concentrations of the test substance showed large variability, particularly in the more concentrated samples. For example, the 100% stock solution had a mean measured concentration of 39.6 mg/L with a standard deviation of ± 21 (52%). Thus the measured LC50 has a large associated error. |
| CONCLUSION | The test substance is harmful to fish. |
| TEST FACILITY | Smithers Viscient (ESG) Ltd (2017) |

### C.2.2. Acute Toxicity to Aquatic Invertebrates

| | |
|---|---|
| TEST SUBSTANCE | Notified chemical |

| | |
|---|---|
| METHOD | OECD TG 202: *Daphnia sp*. Acute Immobilisation Test – Static conditions |
| Species | *Daphnia magna* |
| Exposure Period | 48 hours |
| Auxiliary Solvent | None |
| Water Hardness | Not determined |
| Analytical Monitoring | LC-MS/MS, TOF |
| Remarks – Method | A saturated stock solution of the test substance was prepared by stirring a measured quantity of the solid test substance in water for 20 hours and filtering through a 0.45 µm filter. Test media were prepared by diluting the stock solution to the desired concentration. Based on the results of a range finding test, solutions for the definitive test contained the stock solution at nominal concentrations of 6.25 % to 100 %. S Measured concentrations of the test substance at 0 hours (fresh media) and 48 hours (aged media) were within 80 – 120 % of the nominal concentrations. Therefore the results are based on nominal concentrations.<br><br>A positive control was also run as a separate test using potassium dichromate. |

RESULTS

| *Concentration* | | *Number of* | *Number Immobilised* | |
|---|---|---|---|---|
| *Nominal (% saturated stock)* | *Measured Geometric Mean (mg/L)* | *D. magna* | *24 h* | *48 h* |
| Control | 0 | 20 | 0 | 0 |
| 6.25 | 5.8 | 20 | 0 | 0 |
| 12.5 | 11.2 | 20 | 0 | 1 |
| 25 | 26.4 | 20 | 0 | 0 |
| 50 | 51.2 | 20 | 0 | 0 |
| 100 | 103.6 | 20 | 2 | 11 |

| | |
|---|---|
| EC50 | 84.91 mg/L at 48 hours (based on nominal concentrations) |
| NOEC | 50 mg/L at 48 hours (based on nominal concentrations) |
| Remarks – Results | All validity criteria were satisfied. The dissolved oxygen concentration in the test and control solutions was ≥ 73 % at 19 °C. The temperature variation which was ± 2 °C instead of the recommended ± 1 °C. However, all temperatures were maintained within the 19 – 22 °C window so these fluctuations are not considered to have significantly impacted the study outcomes. The 24 h EC50 of the positive control experiment was 0.75 mg/L which is within the range for the test to be considered valid (0.6 – 2.1 mg/L). Statistical analysis was performed with CETIS v 1.8.6.8. The EC50 values were estimated using linear interpolation. |
| CONCLUSION | The test substance is harmful to aquatic invertebrates. |
| TEST FACILITY | Smithers Viscient (ESG) Ltd (2017) |

**C.2.3. Algal Growth Inhibition Test**

| | |
|---|---|
| TEST SUBSTANCE | Notified chemical |
| METHOD | OECD TG 201: Alga, Growth Inhibition Test |
| Species | *Pseudokirchneriella subcapitata* |
| Exposure Period | 72 hours |
| Concentration Range | Nominal: 1.0, 3.2, 10, 32 and 100% saturated solution<br>Geometric mean measured: 0.76, 2.29, 5.24, 22.6 and 59.6 mg/L |
| Auxiliary Solvent | None |
| Water Hardness | Not determined |
| Analytical Monitoring | LC-MS/MS TOF |
| Remarks – Method | A saturated stock solution of the test chemical was prepared by stirring an excess of test substance (100 mg/L) in water for 24 hours. After the |

stirring period any undissolved test substance was removed by filtration (through a 0.45 µm filter) to produce a 100% v/v saturated solution of the test substance. Based on the results of the range finding test, solutions for the definitive test were prepared by diluting the 100% v/v saturated stock solution to 1.0, 3.2, 10, 32 and 100% v/v. Concentrations are reported as the geometric mean of the measured concentrations at 0 h and 72 h. Temperature was maintained at 22 ± 1°C. A positive control experiment was performed separately using potassium dichromate.

RESULTS

| *Biomass* | | *Growth* | |
|---|---|---|---|
| $E_yC_{50}$ *mg/L at 72 h* | *NOEC mg/L* | $E_rC_{50}$ *mg/L at 72 h* | *NOEC mg/L* |
| > 59.6 | 59.6 | > 59.6 | 59.6 |

Remarks – Results: All validity criteria were satisfied. Statistical analysis was performed using CETIS v 1.8.6.8. The cell growth was 83-fold in the controls. The 72 h ErC50 for the positive control was 1.6 mg/L which is within the expected range. The pH in the control increased by a maximum of 3.0 units, which exceeds the recommended study guideline requirement (≤ 1.5 units). However, the test solutions also showed a large drift in pH, and in both the control and test media, algae growth was not inhibited. Therefore this is not considered to have affected the validity of the study. Test media all had higher average yields and growth rates than the controls. The notified chemical appears to promote algal growth.

CONCLUSION: The test substance is not harmful to algae.

TEST FACILITY: Smithers Viscient (ESG) Ltd (2017)

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

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 209 Activated Sludge, Respiration Inhibition Test
- Inoculum: Activated sludge
- Exposure Period: 3 hours
- Concentration Range: Nominal: 9.8, 31.3, 100, 320, 1000 mg/L
- Remarks – Method: Test media were prepared by combining synthetic sewage, activated sewage sludge and the solid test substance. Nominal concentrations of the test substance between 9.8 – 1000 mg/L were used based on the results of a range finding test. Temperature was held at 20 (± 0.2) °C. A positive control test was performed separately with 3,5-dichlorophenol at concentrations of 0.1, 2.0 and 40 mg/L. The nitrification inhibitor, N-allylthiourea (ATU), was added to appropriate test and reference vessels to give a final concentration of 11.7 mg/L in the test system.

RESULTS
- 3 h EC50: > 1000 mg/L
- NOEC: 1000 mg/L
- Remarks – Results: All validity criteria were satisfied. The inhibition data are unusual, suggesting that the average rate of respiration is inhibited with low concentrations of the test substance (54% inhibition with 9.8 mg/L) but gradually returning to the levels of the control as the concentration of the test substance increases (34% with 31.3 mg/L, 21% with 100 mg/L, 5.1% with 320 mg/L and 1.8% with 1000 mg/L). However, large variability in the raw data (*e.g.* respiration rate = 6.6 – 86.9 mg/L with 9.8 mg/L test substance) leads the authors conclude that respiration is not statistically inhibited across the series. The 3 h EC50 for 3,5-dichlorophenol was

| | |
|---|---|
| | 13.5 mg/L (total respiration) which is within the guidelines for the test to be considered valid (2 – 25 mg/L). |
| CONCLUSION | The test substance is not inhibitory to microbial organisms |
| TEST FACILITY | Smithers Viscient (ESG) Ltd (2016) |

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