File No: STD/1666

March 2019

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Alkenyl bis-succinimide in mineral oil

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	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1666	Cintox Australia Pty Ltd	Alkenyl bis-succinimide in mineral oil	No	≤ 20 tonnes per annum	Component of automotive lubricating oil

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified 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.

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

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

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

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- No specific engineering controls, work practices or personal protective equipment are required for the safe use of the notified chemical itself. However, these should be selected on the basis of all ingredients in the formulation.

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

- A copy of the SDS should be easily accessible to employees.

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

Disposal

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

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component of automotive lubricating oil, or is likely to change significantly;
- the amount of chemical being introduced has increased, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

No additional secondary notification conditions are stipulated.

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT
Cintox Australia Pty Ltd (ABN: 63 122 874 613)
38-40 George Street
PARRAMATTA NSW 2150

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: chemical name, other names, CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, impurities, additives/adjuvants, and import volume.

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
USA (2016)
Canada (2017)

2. IDENTITY OF CHEMICAL

MARKETING NAME
Alkenyl bis-succinimide in mineral oil

MOLECULAR WEIGHT
> 500 g/mol

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

3. COMPOSITION

DEGREE OF PURITY
> 70%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Amber liquid

Property	Value	Data Source/Justification
Pour Point	-22 °C	Measured
Boiling Point	325-490 °C	Measured
Density	942.0 kg/m^3 at 20 ± 0.5°C	Measured
Vapour Pressure	4×10^{-6} kPa at 25 °C	Measured
Water Solubility	29×10^{-6} g/L at 25 °C	Measured
Hydrolysis as a Function of pH	$T_{1/2}$ > 1 year (pH 4, 7, 9 at 50 °C)	Measured
Partition Coefficient (n-octanol/water)	log Pow = 8.77	Measured
Adsorption/Desorption	log K_{oc} = 6.8 – 8.1 at 25 °C	Measured
Dissociation Constant	Not determined	Contains potential cationic functionalities but dissociation in the environmental pH

		range (4-9) is expected to be limited due to low water solubility
Flash Point	192 °C	SDS
Flammability	Not expected to be flammable	Based on flash point
Autoignition Temperature	386 °C	SDS
Explosive Properties	Not explosive	Contains no functional groups that would imply explosive properties
Oxidising Properties	Not oxidising	Contains no functional groups that would imply oxidising properties

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

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. It will be imported as a component of lubricant additive packages at ≤ 10% concentration for reformulation into finished engine oils, or as a component of finished engine oils at ≤ 1% concentration.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤ 20	≤ 20	≤ 20	≤ 20	≤ 20

PORT OF ENTRY
Typically Sydney, Melbourne, Perth and Brisbane

IDENTITY OF RECIPIENTS
Cintox Australia Pty Ltd

TRANSPORTATION AND PACKAGING
The additive packages containing the notified chemical at ≤ 10% concentration will be imported by ship, contained in either 20,000 L isotanks or in 205 L steel drums. The isotanks will be offloaded to tank trucks or rail cars at the port for distribution to lubricant manufacturing customers, while the 205 L steel drums will be shipped directly. The finished oils containing the notified chemical at ≤ 1% concentration will be packaged in 205 L drums or 1-5 L plastic bottles for distribution to service stations and end-use customers.

USE
The notified chemical will be used as a component of automotive engine oil at ≤ 1% concentration.

OPERATION DESCRIPTION

Reformulation
At the reformulation sites, the additive package containing the notified chemical at ≤ 10% concentration will be transferred from isotanks on rail cars and tank trucks into storage tanks through 10 cm hosing and pumping equipment. From the storage tanks, the additive package containing the notified chemical will be transferred to blending tanks through a computer controlled automated valve process and fixed lines. The product will be blended using a typical liquid blending process in a closed system with other components into finished oil products containing ≤ 1% of the notified chemical. The finished oil will be transferred back to the storage tanks where it will be filled into 205 L drums or 1-5 L plastic bottles.

End-use
Motor mechanics
Motor mechanics may pump or manually transfer the finished oil containing the notified chemical at ≤ 1% concentration from the 205 L drums or 1-5 L bottles to the vehicle oil reservoir. The motor mechanic will also manually drain spent oil containing the notified chemical from the engine during servicing.

Do-It-Yourself (DIY) users
DIY users will manually transfer the finished oil containing the notified chemical at ≤ 1% concentration from the 1-5 L bottles into the vehicle oil reservoir. They will also manually drain spent oil containing the notified chemical from the engine during servicing.

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)*
Unloading isotanks and drums of additive package	0.5	30
Sampling and analysing additive package	0.1	220
Unloading isotanks and drums of finished oil	0.5	30
Sampling and analysing finished oil	0.1	220
Loading oil into tank trucks	0.5	220
Service stations and workshop	0.5	220

EXPOSURE DETAILS
Transport and Storage
Transport and storage workers may come into contact with the notified chemical at ≤ 10% concentration only in the unlikely event of accidental rupture of containers.

Reformulation
The blending process is expected to be automated in a closed system; however, plant operators may be exposed (dermal and ocular) to the notified chemical at ≤ 10% concentration during opening of containers and connection/disconnection of transfer lines. Workers may also come into contact with the notified chemical during maintenance, cleaning, and sampling.

Dermal and ocular exposure to workers should be mitigated through engineering controls such as the use of a special air back flush system to prevent spillage during transfer and the use of personal protective equipment (PPE) including coveralls, safety glasses and gloves. Inhalation exposure is not expected given the low vapour pressure of the notified chemical.

End-use
At automotive service centres, professional users such as mechanics may experience dermal or ocular exposure to the engine oil products containing the notified chemical at ≤ 1% concentration when topping up or changing engine oil. The potential for dermal and ocular exposure may be mitigated through the use of PPE (e.g. coveralls, safety glasses and gloves).

6.1.2. Public Exposure
Dermal and ocular exposure to the notified chemical at ≤ 1% concentration may occur to DIY users when topping up or changing engine oils. Given engine oil is topped up or changed infrequently and the low concentration of the notified chemical in the finished products, public exposure to the notified chemical is expected to be low.

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation – non-adjuvant test	no evidence of sensitisation
Rat, combined repeated dose oral toxicity with reproduction/developmental screening	NOAEL (parental and repro/develop) = 1,000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* chromosome aberration test in human lymphocytes	non clastogenic
Genotoxicity – *in vivo* mouse micronucleus test	non clastogenic

Toxicokinetics
Based on the relatively high molecular weight (> 500 g/mol), low water solubility (29×10^{-6} g/L at 25 °C) and high partition coefficient (log Pow = 8.77) of the notified chemical, dermal absorption is expected to be limited.

Due to the low vapour pressure (4×10^{-6} kPa at 25 °C) of the notified chemical inhalation exposure is expected to be limited.

Acute toxicity
The notified chemical was found to be of low acute oral and dermal toxicity in studies conducted in rats.

Irritation and sensitisation
Based on studies conducted in rabbits, the notified chemical is slightly irritating to the skin and eyes.

In the skin irritation study, very slight erythema was noted up to 7 days after treatment. At the 14-day observation, all signs of irritation were resolved. No oedema was noted during the study.

In the eye irritation study, slight conjunctival irritation was observed in all treated eyes up to the 48 hour observation. All signs of conjunctival irritation were resolved at the 72 hour observation. One animal displayed minor corneal opacity up to Day 4.

In a guinea pig non-adjuvant test, the notified chemical was determined not to be a skin sensitiser.

Repeated dose toxicity
In a combined repeated dose (gavage) toxicity study with the reproduction/developmental toxicity screening test in rats, the No Observed Adverse Effect Level (NOAEL) for parental and reproductive and developmental toxicity was established as 1,000 mg/kg bw/day based on the absence of adverse effects at the highest dose tested.

Mutagenicity/Genotoxicity
The notified chemical tested negative in a bacterial reverse mutation assay, an *in vitro* mammalian cell chromosome aberration test with human lymphocytes and in an *in vivo* mouse micronucleus test.

Health hazard classification
Based on the available information, the notified 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
Based on the available information, the notified chemical is of low hazard presenting only as a slight skin and eye irritant. However, reformulation workers and end-use professionals (such as mechanics) are not considered to be at risk of skin or eye irritation on account of the low introduction and end-use concentrations of the notified chemical (≤ 10% and ≤ 1%, respectively). The notifier anticipates that worker exposure will be limited through the use of engineering controls such as enclosed systems, automated processes and local exhaust ventilation.

Therefore, based its low hazard and reported use (as a component of automotive engine oil), the notified chemical is not considered to pose an unreasonable risk to the health of workers.

6.3.2. Public Health

Based on the available information, the notified chemical is of low hazard presenting only as a slight skin and eye irritant.

Dermal and ocular exposure to the notified chemical at ≤ 1% concentration may occur to DIY users when topping up or changing engine oils. Given engine oil is topped up or changed infrequently and the low concentration of the notified chemical in the finished products, public exposure to the notified chemical is expected to be low.

Therefore, based its low hazard and reported use (as a component of automotive engine oil), 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 into Australia as a component of finished engine oil, or a component of lubricant additive packs for reformulation into finished engine oil. The reformulation process involves automatic blending operations in closed systems, followed by automatic filling of the reformulated products into end-use containers. Any waste generated from the reformulation process is expected to be disposed of by an approved waste management facility. Accidental spills of the products containing notified chemical during import, transport, reformulation or storage are expected to be collected for recycling or disposal of in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE

The finished engine oil containing the notified chemical will be available to motor mechanics and public consumers. Motor mechanics may pump or manually transfer the finished oil containing the notified chemical from the 205 L drums or 1-5 L bottles to the vehicle oil reservoir. DIY users will manually transfer the finished oil containing the notified chemical from the 1-5 L bottles into the vehicle oil reservoir. Both motor mechanics and DIY users will manually drain spent oil containing the notified chemical from the engine during servicing.

In a recent Australian survey it was found that only 4% of households disposed of motor oil and approximately 70% of this motor oil was correctly disposed (Aither, 2013). Some vehicle lubricating oil is consumed during use but the amount consumed is highly variable (0 - 99%) depending on the type and use of oil. Although there is some uncertainty, it may be estimated based on this data that approximately 1% (0.04 × 0.3) of all motor oil sold could be incorrectly disposed by DIY users. Accordingly, about 1% of the notified chemical in used oil may be disposed of incorrectly. Release during use may arise from drips during manual oil addition to tanks, but it is expected to be minimal.

RELEASE OF CHEMICAL FROM DISPOSAL

Empty drums containing residues of the notified chemical will be steam cleaned. The notifier estimated that up to 2 kg/year of residual notified chemical from this process may be sent to on-site wastewater treatment facilities and be effectively removed before potential release to sewers or waterways. The used oil containing the notified chemical is expected to be collected and recycled, re-refined or disposed of by approved waste management contractors, in accordance with local government regulations.

7.1.2. Environmental Fate

The biodegradability and bioaccumulation studies conducted on the notified chemical shows that it is not readily biodegradable (5% biodegradation in 28 days) and does not significantly bioaccumulate in fish. For details of these environmental fate studies, refer to Appendix C.

The used oil containing the notified chemical is expected to be recycled, re-refined or disposed of by approved waste management contractors. It is likely that the notified chemical will be degraded into simpler compounds during refining. As estimated by the notifier, up to 2 kg/year of residual notified chemical from empty drums

may be released to on-site wastewater treatment facilities where the notified chemical is expected to be effectively removed by adsorption to sludge based on its high log Pow (8.77). A proportion of this may be applied to land when sludge from wastewater treatment facilities is used for soil remediation, or disposed of to landfill. Minor amounts of the notified chemical may also be disposed of to landfill as collected spills. Based on its low water solubility and high log Koc (6.8 – 8.1), the notified chemical is expected to sorb strongly to soil. The notified chemical in the environment is expected to eventually degrade into water, oxides of carbon and nitrogen via biotic and abiotic pathways.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has not been calculated. It is expected that approximately 1% (0.2 tonne per annum) may be incorrectly disposed of. However, this is expected to be dispersed and not all of it will reach waterways. Therefore, the concentration in the aquatic environment is expected to be limited.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96h LL50 > 100 mg WAF*/L	Not harmful to fish up to its water solubility limit
Daphnia Toxicity	48h EL50 > 100 mg WAF*/L	Not harmful to aquatic invertebrates up to its water solubility limit
	ChV NOEL = 100 mg WAF*/L	Reproduction of aquatic invertebrates not affected up to its water solubility limit
Algal Toxicity	72h EC50 > 100 mg WAF*/L	Not harmful to alga up to its water solubility limit

*WAF: Water Accommodated Fraction

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

7.2.1. Predicted No-Effect Concentration

The predicted no-effects concentration (PNEC) has not been calculated as the notified chemical is not expected to be harmful to aquatic organisms up to its water solubility limit.

7.3. Environmental Risk Assessment

A Risk Quotient (PEC/PNEC) has not been calculated as the notified chemical is not expected to be harmful up to its water solubility limit. Also, release to the aquatic environment will be limited based on its reported use pattern. Therefore, based on its low hazard and reported use (as a component of automotive engine oil), the notified chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Pour Point -22 °C

Method ASTM D5950 Pour Point of Petroleum Products (Automatic Tilt Method)
Remarks According to the study author ASTM D5950 was used as it offers better repeatability and reproducibility relative to ASTM D97 (recommended by OECD TG 102). The test substance is heated to approx. 45 °C and inserted into the automatic pour point apparatus. The sample is then cooled and examined at 1 °C intervals. The lowest temperature at which movement of the test substance is detected (using the automatic equipment) is displayed as the pour point.
Test Facility Chevron (2017a)

Boiling Point 325-490 °C at 101.3 kPa

Method Petroleum and Materials Characterization Test Code 10309 Thermogravimetric Analysis
Remarks Measured using thermogravimetric analysis. Onset of evaporation occurred at approx. 190 °C. However, the majority of test substance weight loss occurred between approx. 325 °C and 490 °C. By 490 °C, the test substance had completely evaporated.
Test Facility Chevron (2017b)

Density 942.0 kg/m^3 at 20 °C

Method EC Council Regulation No 440/2008 A.3 Relative Density (2008)
Remarks Pycnometer method
Test Facility Harlan (2012a)

Vapour Pressure 4×10^{-6} kPa at 25 °C

Method OECD TG 104 Vapour Pressure (2006)
EC Council Regulation No 440/2008 A.4 Vapour Pressure (2008)
Remarks Vapour pressure balance method
Test Facility Harlan (2012b)

Water Solubility 29×10^{-6} g/L at 25 °C

Method OECD TG 105 Water Solubility
Remarks Flask Method. The main component of the test substance was used to determine the water solubility.
Test Facility Chevron (2017c)

Hydrolysis as a Function of pH

Method OECD TG 111 Hydrolysis as a Function of pH

pH	*T (°C)*	*$t_{½}$ <year >*
4	50	> 1
7	50	> 1
9	50	> 1

Remarks The test substance was analysed by HPLC-MS. Based on the obtained data, the notified UVCB is considered hydrolytically stable at pH 4, 7 and 9.
Test Facility Chevron (2018)

Partition Coefficient (n-octanol/water) log Pow = 8.77

Method OECD TG 117 Partition Coefficient (n-octanol/water).
Remarks HPLC Method. Experiment temperature not listed. The log Kow for the three major components of the test substance ranged from 8.0 – 9.22. The weighted average for all

components of the test substance was 8.77.
Test Facility Chevron (2012a)

Adsorption/Desorption log K_{oc} = 6.8 – 8.1 at 25 °C

Method OECD TG 121 Estimation of the Adsorption Coefficient (K_{oc}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)
Remarks None
Test Facility Chevron (2012b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method (2001)
Species/Strain	Rat/Albino (Crl:CD(SD))
Vehicle	None
Remarks - Method	No noted deviations from the study protocol.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	6F	2000	0/6

LD50	> 2000 mg/kg bw
Signs of Toxicity	None
Effects in Organs	None
Remarks - Results	All animals showed expected body weight gains during the study.
CONCLUSION	The notified chemical is of low acute toxicity via the oral route.
TEST FACILITY	WIL (2014a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test (1987)
Species/Strain	Rat/Albino (Crl:CD(SD))
Vehicle	None
Type of dressing	Semi-occlusive
Remarks - Method	No deviations from the study protocol were noted.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Very slight erythema was present in 3/5 males up to Day 3. These same males presented with desquamation from Day 3 to Day 10 of the study. Very slight erythema occurred in 2/5 females, and persisted till Day 6 of the study. Desquamation was present in all female mice from Day 3 to Day 10.
Signs of Toxicity - Systemic	None
Effects in Organs	None
Remarks - Results	All animals showed expected body weight gains during the study, except for one female (slight weight loss during the first week).
CONCLUSION	The notified chemical is of low acute toxicity via the dermal route.
TEST FACILITY	WIL (2014b)

B.3. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion (2002)

Species/Strain	Rabbit/New Zealand White Albino
Number of Animals	3
Vehicle	None
Observation Period	14 days
Type of Dressing	Semi-occlusive
Remarks - Method	No significant 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			
Erythema/Eschar	1	0	1	1	< 14 days	0
Oedema	0	0	0	0	-	0

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

Remarks - Results	Very slight erythema was noted in 2/3 animals up to 4 days after treatment. One animal continued to present this symptom on Day 7 of the study. All signs of irritation were resolved at the 14 day observation.

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY WIL (2014c)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion (2012)

Species/Strain	Rabbit/New Zealand White albino
Number of Animals	3
Observation Period	14 days
Remarks - Method	No significant 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	0.33	0.67	0.67	1	< 72 hours	0
Conjunctiva: chemosis	0.67	0.33	0.33	1	< 72 hours	0
Conjunctiva: discharge	0	0	0	-	-	0
Corneal opacity	1	0	0	1	< 7 days	0
Iridial inflammation	0	0	0	-	-	0

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

Remarks - Results	Slight conjunctival irritation was observed in all treated eyes up to the 48 hour observation. All signs of conjunctival irritation were resolved at the 72 hour observation. One animal displayed minor corneal opacity up to Day 4. At the 24 hour observation, opacity was present in up to half the cornea area; by the 48 hour observation, this had regressed to up to quarter of the cornea area.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY WIL (2014d)

B.5. Skin sensitisation – Guinea pig maximisation test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 406 Skin Sensitisation – guinea pig maximisation test (1992)
Species/Strain	Guinea pig/Crl:HA
PRELIMINARY STUDY	Maximum Non-irritating Concentration: topical: 100%
MAIN STUDY	
Number of Animals	Test Group: 10M/10F Positive Control Group: 5M/5F Naïve Control Group: 5M/5F
Vehicle	Mineral oil
Positive control	α–hexylcinnamaldehyde (HCA)
INDUCTION PHASE	Induction Concentration: topical: 100%
Signs of Irritation	Induction 1: Only negligible signs of irritation present in 2/10 males at 24 hours after dosing and in 3/10 males at the 48 hours after dosing. Negligible signs of irritation present in 3/10 females at 24 hours after dosing and in 1/10 females 48 hours after dosing. Induction 2: Slight irritation present in 2/10 males and 5/10 females at 24 hours after dosing; this completely regressed or was reduced to a negligible severity by the 48 hour timepoint. Negligible signs of irritation present in 6/10 males at 24 hours after dosing; this had completely regressed or remained at negligible severity by the 48 hour timepoint. Negligible signs of irritation present in 4/10 females at 24 hours after dosing; these signs had completely regressed in these females at 48 hours after dosing. Induction 3: Slight irritation present in 3/10 males and 1/10 females at 24 hours after dosing; this completely regressed or was reduced to a negligible severity by the 48 hour timepoint. Negligible signs of irritation present in 8/10 males and 4/10 females at 24 hours after dosing; this had completely regressed or remained at negligible severity by the 48 hour timepoint.
CHALLENGE PHASE	
1st challenge	topical: 75%
Remarks - Method	The induction phase consisted of three induction doses of the test substance spaced one week apart for a total duration of three weeks. Each exposure occurred for approx. 6 hours.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*	
		24 h	*48 h*
Test Group	75%	1/20	0/20
Naïve Control Group	75%	2/10	1/10
Positive Group	10%	4/10	0/10
	20%	7/10	4/10

Remarks - Results	The skin reactions presented by animals in the test group were of similar severity and frequency as those from animals in the naïve control group. As such, the test group skin reactions were not considered as positive skin sensitisation responses. The positive control performed as expected, confirming the validity of the test system. Body weight gains were comparable to those seen in the naïve control group animals.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.

TEST FACILITY WIL (2014e)

B.6. Repeat dose toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (1996)

Species/Strain Rat/ Sprague-Dawley (Crl:CD BR)

Route of Administration Oral – gavage

Exposure Information Total exposure days:

- 28 - 29 days for males selected for mating (2 weeks prior to mating, during mating and until scheduled necropsy)
- 39 - 43 days for females selected for mating (2 weeks prior to mating, during mating and until lactation day 3).
- 39 days for females with no evidence of mating (2 weeks prior to mating and during period designated for mating).
- 52 days for females who failed to deliver offspring (2 weeks prior to mating, during mating and until post-cohabitation day 25).
- 29 days for males not selected for pairing
- 39 days for females not selected for pairing

Dose regimen: 7 days per week

Post-exposure observation period: 14 days (only for animals not selected for mating)

Vehicle Corn oil

Remarks - Method No statistical analysis was conducted on histopathological findings. This and all other noted protocol deviations in the study report were not considered to have affected the integrity of the study.

Dose levels were selected based on the results of a previous 14-day repeat dose toxicity study (study no. WIL-187161).

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
vehicle control	12M/12F	0	1/24
low dose	12M/12F	250	0/24
mid dose	12M/12F	500	0/24
high dose	12M/12F	1000	0/24
vehicle control recovery	5M/5F	0	0/10
low dose recovery	5M/5F	250	0/10
high dose recovery	5M/5F	1000	0/10

Mortality and Time to Death

One female in the vehicle control group died during gestation. The cause of death was due to a gavage error.

Clinical Observations

Females treated at 500 and 1,000 mg/kg bw/day presented statistically significant increases in food consumption compared to controls after the first week of treatment (an increase of 15% for both groups). This increase in food consumption continued in the 1,000 mg/kg bw/day group after the second week and fifth weeks of treatment, where statistically significant increases of 23% and 30% were observed, respectively. During gestation days 0, 4 and 7, females in the 1,000 mg/kg bw/day group had statistically significantly higher mean food consumption compared to controls (increases of 15%, 13% and 13% respectively). Mean food consumption over the entire gestation period and the entire treatment period was also statistically significantly higher in females treated at 1,000 mg/kg bw/day compared to controls (increases of 13% and 14%, respectively). All noted increases in food consumption were not accompanied by noticeable increases in body weight and/or body weight changes. As such, these differences in food consumption were considered by the study authors to be test-substance related, but not adverse.

During the treatment period, males treated at 1,000 mg/kg showed a statistically significant increase in the mean

hindlimb footsplay value compared to the control group. The authors of this study did not consider this effect to be attributed to the test substance, as there were no corresponding effects on gait, mobility or motor activity and no similar effect was observed in females.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
No test substance related effects were noted.

Effects in Organs
No test substance related effects were noted.

Reproductive/developmental findings
The number of implantation sites, pups born, unaccounted sites, corpora lutea, partially cannibalised pups, and missing pups were not affected. Mating index, fertility index, copulation index, gestation length, live litter size, mean postnatal survival rate, mean pup body weights, mean pup body weight changes and general physical condition of the pups were also not affected.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) for parental and reproductive and developmental toxicity was established as 1,000 mg/kg bw/day in this study, based on an absence of toxicity at the maximum dose tested.

TEST FACILITY	WIL (2014f)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test EC Directive No 440/2008 B.13/B14 Mutagenicity – Reverse Mutation Test using Bacteria (2008) Plate incorporation procedure
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100 *Escherichia coli*: WP2uvrA
Metabolic Activation System	S9 fraction from phenobarbitone/β-napthoflavone induced rat liver
Concentration Range in Main Test	<u>Test 1</u> a) With metabolic activation: 1.5 – 5000 µg/plate b) Without metabolic activation: 1.5 – 5000 µg/plate <u>Test 2</u> a) With metabolic activation: 15 – 5000 µg/plate b) Without metabolic activation: 15 – 5000 µg/plate
Vehicle	Acetone
Remarks - Method	No noted deviations from the study plan. The dose range used for Test 2 was determined by the results of Test 1. Positive controls: *Without metabolic activation* N-ethyl-N'-nitro-N-nitrosoguanidine (TA100, TA1535, WP2uvrA) 9-Aminoacridine (TA1537) 4-Nitroquinoline-1-oxide (TA98) *With metabolic activation* 2-Aminoanthracene (TA100, TA1535, TA1537, WP2uvrA) Benzo(a)pyrene (TA98)

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	*Cytotoxicity in Preliminary Test**	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	-	> 5000	≥ 500	Negative
Test 2	-	> 5000	≥ 500	Negative
Present				
Test 1	-	> 5000	≥ 500	Negative
Test 2	-	> 5000	≥ 500	Negative

*Not conducted

Remarks - Results	No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with the test substance at any dose level, in the presence or absence of metabolic activation. Vehicle and positive controls performed as expected, confirming the validity of the test system.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Harlan (2013a)

B.8. Genotoxicity – *in vitro* chromosome aberration test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test EC Directive 440/2008 B.10 Mutagenicity - *In vitro* Mammalian Chromosome Aberration Test
Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 fraction from phenobarbitone/β-napthoflavone induced rat liver
Vehicle	Acetone
Remarks - Method	No noted deviations from the study plan. A preliminary experiment was performed to determine the dose range for the main test. The doses in this experiment were: 19.5, 39.1, 78.1, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/mL. Test 1 and 2 samples designated for metabolic activation were exposed to S9-mix at 2% and 1% concentration, respectively. Positive controls: *Without metabolic activation* Mitomycin C *With metabolic activation* Cyclophosphamide

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	10, 20*, 40*, 80*, 160, 320	4h	24h
Test 2	5, 10*, 20*, 40*, 80, 160	24h	24h
Present			
Test 1	10*, 20*, 40*, 80*, 160, 320	4h	24h
Test 2	10, 20*, 40*, 80, 160, 320	4h	24h

*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	> 5000	> 320	≥ 80	Negative
Test 2	> 5000	> 160	≥ 40	Negative
Present				
Test 1	> 5000	> 320	≥ 80	Negative
Test 2	-	> 320	≥ 40	Negative

*Preliminary toxicity test performed using the exposure conditions for Test 1 and Test 2
Indicated by > 50% reduction in mitotic index

Remarks - Results

In Test 1 in the presence of metabolic activation a modest reduction in mitotic index was observed at 20, 40 and 80 μg/mL (decreases of 38%, 43% and 42%, respectively). No reduction in mitotic index was observed in Test 1 in the absence of metabolic activation or in Test 2, either in the absence or presence of metabolic activation.

In Test 1 and 2, the test substance did not induce any statistically significant increases in the frequency of cells with chromosome aberrations, either in the absence or presence of metabolic activation.

In Test 2, a small but statistically significant increase in the frequency of cells with chromosome aberrations was observed at 40 μg/mL (p= 0.03) in the 24-hour continuous group. As this increase was within the historical range of vehicle control values and there was no marked increase of mitotic index at the next dose level tested, the study authors considered the response was not biologically relevant. To confirm this assumption an additional 100 cells were scored from the duplicate slides of both 0 μg/mL and 40 μg/mL. The additional analysis of metaphases diluted the small response to the extent that the statistical significance disappeared.

In Test 1 and 2, there were no biologically relevant increases in polypoid cells after treatment with the test substance, either in the absence or presence of metabolic activation.

The solvent and positive controls performed as expected, confirming the validity of the test system.

CONCLUSION

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

TEST FACILITY

Harlan (2014a)

B.9. Genotoxicity – *in vivo* micronucleus test

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 474 Mammalian Erythrocyte Micronucleus Test (1997)
EC Directive 440/2008 B.12 Mutagenicity - Mammalian Erythrocyte Micronucleus Test

Species/Strain: Mouse/Hsd:ICR (CD-1®) Albino
Route of Administration: Intraperitoneal injection
Vehicle: Arachis Oil
Physical Form: Emulsion
Remarks - Method: No noted deviation from the study plan. A range-finding toxicity test was performed using 6 male and 4 female mice with the test substance at 1000

and 2000 mg/kg bw. No deaths or clinical signs of toxicity were noted for up to 2 days after dosing. On the bases of this study, 2000 mg/kg bw was determined to be a suitable limit dose for the main study.

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Sacrifice Time (hours)*
I (vehicle control) 1	7M	0	24
I (vehicle control) 2	7M	0	48
II (low dose)	7M	500	24
III (mid dose)	7M	1000	24
IV (high dose) 1	7M	2000	24
IV (high dose) 2	7M	2000	48
V (positive control, CP)	5M	5	24

CP=cyclophosphamide.

RESULTS

Doses Producing Toxicity	No clinical signs of toxicity were noted.
Genotoxic Effects	The test substance induced no biologically relevant increases in micronucleated, polychromatic erythrocytes (PCEs) at any of the doses or sacrifice times.
Remarks - Results	The PCE/NCE ratio was slightly decreased (but not statically significant) after 24 and 48-hour treatment with the test item at 2000 mg/kg bw in comparison with the concurrent vehicle control groups. These decreases were taken to indicate that the test substance had reached the target tissue. There was a statistically significant increase in the mean number of PCEs in the 24-hour 500 mg/kg bw group in comparison to the concurrent vehicle control groups. However, there was no dose-related effect and the group mean was within the historical range for the concurrent vehicle control. As such, the noted increase was not considered toxicologically relevant. The vehicle and positive controls performed as expected, confirming the validity of the test system.

CONCLUSION The notified chemical was not clastogenic under the conditions of this *in vivo* mouse micronucleus test.

TEST FACILITY Harlan (2013b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO2 Evolution Test (1992) EC Council Regulation No 440/2008 C.4-C Ready Biodegradability: CO2 Evolution Test
Inoculum	Activated sludge from a domestic sewage treatment plant (STP)
Exposure Period	29 days
Auxiliary Solvent	Chloroform
Analytical Monitoring	CO_2 by Tekmar-Dohrmann Apollo 900 TOC analyser
Remarks - Method	No major deviations from the test guidelines were reported. A stock solution was prepared by dissolving the test substance (786 mg) in chloroform (10 mL). An aliquot (500 µL) of this stock solution was dispensed onto a filter paper and the solvent allowed to evaporate to dryness for approximately 15 minutes. The filter paper was dispersed in approximately 400 mL of mineral medium with the aid of high shear mixing for 5 minutes prior to addition to inoculated mineral medium. The volume was then adjusted to 3 litres to give a final concentration equivalent to 10 mg carbon/L before testing. A toxicity control was run.

RESULTS

Test Substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
2	1	2	50
14	8	14	70
21	6	21	80
29	5	29	74

Remarks - Results	All validity criteria for the test were satisfied. The toxicity control exceeded 25% biodegradation after 14 days showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the test substance after 29 days was 5%.
CONCLUSION	The test substance is not readily biodegradable
TEST FACILITY	Harlan (2015a)

C.1.2. Bioaccumulation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 305 Bioaccumulation in fish: aqueous and dietary exposure (2012)
Species	Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period	Exposure: 28 days
Auxiliary Solvent	Dimethylformamide (DMF)
Concentration Range	Nominal: 0.001 mg/L Actual: 0.000659 mg/L
Analytical Monitoring	High Performance Liquid Chromatography with Mass Spectrometry (HPLC-MS)
Remarks - Method	No major deviations from the test guidelines were reported. A concentrated stock solution of 30 mg/L nominal concentration was

prepared in DMF before adding to the test tank. Samples of water and fish were taken on Days 0, 1, 4, 7, 14, 21 and 28 for chemical analysis of the test substance.

RESULTS

Bioconcentration Factor: The measured concentrations of the test substance in fish were below the limit of quantification (0.0208 µg/g) throughout the 28 days of exposure indicating the test substance did not significantly bioaccumulate in fish.

Remarks - Results: All validity criteria for the test were satisfied. As the test substance was not detected in the test fish, the depuration stage was not considered necessary. Dissolved oxygen in the test water was ≥ 73% during the test. The measured test substance of all samples was within ± 20% of the mean measured concentration during the test.

CONCLUSION: The test substance does not significantly bioaccumulate in fish.

TEST FACILITY: Envigo (2016)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 203 Fish, Acute Toxicity Test (1992)
EC Council Regulation No 440/2008 C.1 Acute Toxicity for Fish

Species: Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period: 96 hours
Auxiliary Solvent: None
Water Hardness: 140 mg $CaCO_3$/L
Analytical Monitoring: HPLC-MS
Remarks – Method: A range test with the test species was not conducted. Instead, data from studies previously conducted in algae and *Daphnia magna* (Harlan study numbers 41301980 and 41309877) were used to determine the experimental exposure concentration of the test substance; this led to the experiment being conducted as a limit test. No major deviations from the test guidelines were noted.

A nominal concentration of 100 mg/L of the test substance was stirred for 23 hours and stood for 1 hour. The aqueous phase or Water Accommodated Fraction (WAF) was removed by mid-depth siphoning to give the 100 mgWAF/L used for the test. The test water was renewed daily. Due to analytical equipment failures and a technical error, chemical analysis of the freshly prepared test water was only done at 48 hours. Chemical analyses of the used test water were done at 24, 48 and 72 hours.

RESULTS

Concentration (mg WAF/L)		*Number of Fish*	*Mortality*
Nominal	*Actual*		*96 h*
Control	< LOQ*	7	0
100	0.000418	7	0

*LOQ: limit of quantitation (determined to be 0.00011 mg/L)

LC50: > 100 mg/L at 96 hours
Remarks – Results: The validity criteria for the test were satisfied. During the test, dissolved oxygen in the test water was ≥ 9.4 mg/L at 15 °C (≥ 93% saturation; USGS 2011). The fresh test preparation at 48 hours had a measured test concentration of 0.000428 mg/L. At 72 hours, the measured concentration

in used test media had declined to below the LOQ. Used test media at 24 and 48 hours contained the test substance at 0.000418 mg/L and < LOQ, respectively. Toxicity results were based on nominal loading rates only.

CONCLUSION The test substance is not harmful to fish up to its water solubility limit.

TEST FACILITY Harlan (2015b)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test – Static (2004)
EC Council Regulation No 440/2008 C.2 Acute Toxicity for Daphnia - Static

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	Gas Chromatography (GC)
Remarks - Method	The loading rate to be used in the limit test was determined by a preliminary range-finding test. The limit test was run with no major deviations from the test guidelines. A nominal concentration of 100 mg/L of the test substance was stirred for 23 hours and stood for 1 hour. The aqueous phase or Water Accommodated Fraction (WAF) was removed by mid-depth siphoning to give the 100 mgWAF/L used for the test. Samples of the test water were taken at 0 and 48 hours for analysis of the test substance.

RESULTS

Concentration (mg WAF/L)		*Number of D. magna*	*Number Immobilised*
Nominal	*Actual*		*48h*
Control	Control	20	0
100	< LOQ*	20	0

*LOQ: limit of quantitation (determined to be 0.035 mg/L)

EL50	> 100 mg/L at 48 hours
Remarks - Results	All the validity criteria for the test were satisfied. During the test, dissolved oxygen in the test water was ≥ 8.9 mg/L at 20°C (≥ 98% saturation; USGS 2011). The toxicity results were based on nominal loading rates only.

CONCLUSION The test substance is not harmful to aquatic invertebrates up to its water solubility limit.

TEST FACILITY Harlan (2013c)

C.2.3. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 211 *Daphnia magna* Reproduction test (2008)
EC Council Regulation No 440/2008 C.20 *Daphnia magna* Reproduction Test

Species	*Daphnia magna*
Exposure Period	21 days
Auxiliary Solvent	None
Water Hardness	230-244 $CaCO_3$ mg/L
Analytical Monitoring	Gas chromatography

Remarks - Method	No major deviation from the test guidelines was reported. Each nominal concentration of the test substance was stirred for 23 hours and stood for 1 hour. The aqueous phase or Water Accommodated Fraction (WAF) was removed by mid-depth siphoning to give the WAFs for testing. The test preparations were renewed 3 times per week on Days 0, 2, 5, 7, 9, 12, 14, 16 and 20. Samples of the fresh test water were taken at Days 0, 5, 9, 14, and 20. Samples of used media were taken at Days 2, 7, 12, 16, 21 for analysis of the test substance.

RESULTS

	Test Concentration (nominal; mg WAF/L)					
	Control	1.0	3.2	10	32	100
Survival (% parental generation)	90	100	80	90	90	90
No. offspring released by surviving *Daphnia*	132	119	137	126	111	125

21 day EL50	> 100 mg/L
21 day NOEL	100 mg/L
Remarks - Results	All the validity criteria for the test were satisfied. During the test, dissolved oxygen in the test water was ≥ 8.0 mg/L at 19-20 °C (≥ 86% saturation; USGS 2011). Chemical analysis of the 100 mg WAF/L test water showed measured test concentration to be less than the limit of quantitation (0.034 mg/L). The toxicity results were based on nominal loading rates only.

CONCLUSION	The test substance does not affect the reproduction of aquatic invertebrates up to its water solubility limit.
TEST FACILITY	Harlan (2014a)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006) EC Council Regulation No 761/2009 C.3 Algal Inhibition Test
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 100 mg/L Actual: Less than Limit of Quantitation (LOQ) of 0.013 mg/L
Auxiliary Solvent	None
Water Hardness	15 mg $CaCO_3$/L
Analytical Monitoring	Gas chromatography
Remarks - Method	The loading rate to be used in the limit test was determined by a preliminary range-finding test. The limit test was run with no major deviation from the test guidelines. A nominal concentration of 100 mg/L of the test substance was stirred for 23 hours and stood for 1 hour. The aqueous phase or Water Accommodated Fraction (WAF) was removed by mid-depth siphoning to give 100 mg WAF/L for testing. Samples of the test water were taken at 0 and 72 hours for analysis of the test substance.

RESULTS

Biomass		*Growth*	
EL50 (mg WAF/L at 72 h)	*NOEL (mg WAF/L)*	*EL50 (mg WAF/L at 72 h)*	*NOEL (mg WAF/L)*
> 100	100	> 100	100

Remarks - Results	All the validity criteria for the test were satisfied. The mean cell density in

	the control increased by a factor of 229 after 72 hours. At 0 and 72 hours test concentration in test samples was < LOQ. The toxicity results were based on nominal loading rates only.
CONCLUSION	The test substance is not harmful to alga up to its water solubility limit.
TEST FACILITY	Harlan (2013d)

BIBLIOGRAPHY

Aither (2013) Third Independent Review of the Product Stewardship (Oil) Act 2000. Canberra, Australia.

Chevron (2012a) [Notified Chemical]: Partition Coefficient (No study no. provided, July, 2012). California, USA, Chevron Energy Technology Co (Unpublished report submitted by the notifier).

Chevron (2012b) [Notified Chemical]: Adsorption Coefficient (Koc) by OECD 121 (No study no. provided, July, 2012). California, USA, Chevron Energy Technology Co (Unpublished report submitted by the notifier).

Chevron (2017a) Melting Point Determination of [Notified Chemical] (No study no. provided, July, 2017). California, USA, Chevron Energy Technology Co (Unpublished report submitted by the notifier).

Chevron (2017b) Boiling Point Determination of [Notified Chemical] (No study no. provided, July, 2017). California, USA, Chevron Energy Technology Co (Unpublished report submitted by the notifier).

Chevron (2017c) [Notified Chemical] Determination of Water Solubility (No study no. provided, August, 2017). California, USA, Chevron Energy Technology Co (Unpublished report submitted by the notifier).

Chevron (2018) [Notified Chemical] Hydrolysis as a Function of pH (No study no. provided, March, 2018). California, USA, Chevron Energy Technology Co (Unpublished report submitted by the notifier).

Envigo (2016) [Notified Chemical]: Bioaccumulation in Fish: Aqueous Exposure (Study No. 41400792, December, 2016). Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2012a) [Notified Chemical] Determination of Vapor Pressure (Study No. 41204072, December, 2012). Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2012b) [Notified Chemical] Determination of Infrared Spectrum and Density (Study No.41203840, October, 2012). Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2013a) [Notified Chemical]: *Salmonella typhimurium* and *Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay (Study No. 41301982, November, 2013). Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2013b) [Notified Chemical]: Micronucleus Test in the Mouse (Study No. 41301983, November, 2013). Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2013c) [Notified Chemical]: Daphnia sp. 48-Hour Acute Immobilization Test (Study No. 41302877, November, 2013). Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2013d) [Notified Chemical]: Algal Growth Inhibition Test (Study No. 41301980, November, 2013). Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2014a) [Notified Chemical]: Chromosome Aberration Test in Human Lymphocytes *in vitro* (Study No.41301984, January, 2014). Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2014b) [Notified Chemical]: *Daphnia magna* Reproduction Test (Study No.41303009, January, 2014). Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2015a) [Notified Chemical]: Assessment of Ready Biodegradability; CO_2 Evolution Test (Study No.41403789, March, 2015). Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2015b) [Notified Chemical]: Acute Toxicity to Rainbow Trout (Study No.41403788, April, 2015). Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

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 >

USGS (2011) U.S. Geological Survey, Change to solubility equations for oxygen in water: Office of Water Quality Technical Memorandum 2011.03, accessed https://water.usgs.gov/software/DOTABLES/ v 3.5, 8 January 2018

WIL (2014a) Acute Oral Toxicity Study of [Notified Chemical] in Albino Rats (Acute Toxic Class Method) (Study No. WIL-187160, January, 2014). Ohio, USA, WIL Research Laboratories, LLC (Unpublished report submitted by the notifier).

WIL (2014b) Acute Dermal Toxicity Study of [Notified Chemical] in Albino Rats (Study No. WIL-187156, January, 2014). Ohio, USA, WIL Research Laboratories, LLC (Unpublished report submitted by the notifier).

WIL (2014c) Acute Dermal Irritation Study of [Notified Chemical] in Albino Rabbits (Study No. WIL-187157, January, 2014). Ohio, USA, WIL Research Laboratories, LLC (Unpublished report submitted by the notifier).

WIL (2014d) Acute Eye Irritation Study of [Notified Chemical] in Albino Rabbits (Study No. WIL-187158, January, 2014). Ohio, USA, WIL Research Laboratories, LLC (Unpublished report submitted by the notifier).

WIL (2014e) Skin Sensitization Study of [Notified Chemical] in Albino Guinea Pigs (Modified Buehler Method) (Study No. WIL-187159, January, 2014). Ohio, USA, WIL Research Laboratories, LLC (Unpublished report submitted by the notifier).

WIL (2014f) A Combined 28-Day Repeated Dose Oral (Gavage) Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of [Notified Chemical] in Rats, with Recovery (Study No. WIL-187162, April, 2014). Ohio, USA, WIL Research Laboratories, LLC (Unpublished report submitted by the notifier).