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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Chemical in HiTEC 11170, HiTEC 11180 and HiTEC 11145

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment and Energy.

This Public Report is available for viewing and downloading from the NICNAS website or available on request, free of charge, by contacting NICNAS. For requests and enquiries please contact the NICNAS Administration Coordinator at:

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Director
NICNAS

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SUMMARY

The following details will be published in the NICNAS *Chemical Gazette:*

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1665	Afton Chemical Asia Pacific LLC	Chemical in HiTEC 11170, HiTEC 11180 and HiTEC 11145	ND*	< 100 tonnes per annum	Component in automotive lubricants

*ND = not determined

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
As only limited toxicity data were provided, based on analogue data the notified chemical cannot be classified 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

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure to the notified chemical during reformulation operations:
  - Automated and enclosed processes

- 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 handling:
  - Gloves
  - Safety goggles
  - Coveralls

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

- A copy of the 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 component in automotive lubricants, 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 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)
Afton Chemical Asia Pacific LLC (ABN: 99 109 644 288)
Level 12, 20 Berry Street
NORTH SYDNEY NSW 2060

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, residual impurities, additives/adjuvants, use details, import volume, site of reformulation and identity of recipients.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: water solubility, hydrolysis as a function of pH, partition coefficient, adsorption/desorption, dissociation constant, explosive properties, oxidising properties, acute oral, dermal and inhalation toxicity, skin and eye irritation, skin sensitisation, repeated dose toxicity, bioaccumulation, acute toxicity to fish, acute immobilisation/reproduction of daphnia sp. and growth inhibition of alga.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Canada (2018)
United States of America (1993)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
HiTEC® 11170
HiTEC® 11180
HiTEC® 11145

MOLECULAR WEIGHT
Value for chemicals > 500 g/mol

ANALYTICAL DATA
Reference NMR, IR, LCMS, UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 95%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Tar like solid

Property	Value	Data Source/Justification
Melting Point	Not observed	Measured. Decomposed without melting above ~ 333 °C
Boiling Point	Not observed	Measured. Chemical did not boil.
Density	1471 ± 56 kg/m^3 at 19.7 °C	Measured
Vapour Pressure	0.002 kPa at 20 °C	Measured

Property	Value	Data Source/Justification
Water Solubility	Insoluble	Observed from a preliminary solubility evaluation
Hydrolysis as a Function of pH	Could not be determined*	Contains no hydrolysable functionalities
Partition Coefficient (n-octanol/water)	Could not be determined	Expected to partition from water to n-octanol based on insolubility in water and partial solubility in n-octanol
Adsorption/Desorption	Could not be determined*	Expected to have low mobility in soil based on insolubility in water
Dissociation Constant	Not determined	Expected to be ionised in the environmentally relevant range (pH 4 - 9).
Particle size	Inhalable fraction (< 100 μm): 8.38% Respirable fraction (< 10 μm): 0.28%	Measured
Flash Point	Not determined	Expected to be high based on flammability. Product which contains the chemical at < 5% concentration has a flash point of 135 °C.
Flammability	Not highly flammable	Measured
Autoignition Temperature	> 400 °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 oxidative properties.

* Measurement was not possible due to lack of sensitivity of analytical methods.

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 classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)*, as adopted for industrial chemicals in Australia.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. The notified chemical will be imported into Australia at a concentration of < 5% as a component of an additive pack for use in automotive engine oils.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 10	< 50	< 60	< 70	< 100

PORT OF ENTRY
Melbourne and Sydney

TRANSPORTATION AND PACKAGING
The imported lubricant additive containing the notified chemical (at < 5% concentration) will be packed in 205 L or 250 L drums or 24,000 L ISO tanks for distribution by road or rail. Finished engine oil lubricants will be packed in plastic containers suitable for retail sale (such 1 L, 4 L or 20 L) or in 205 L steel drums.

USE
The notified chemical is a detergent compound for use in automotive (specifically passenger cars) engine oils by industry and the public. The concentration of the notified chemical in final consumer products will not exceed 1% concentration.

OPERATION DESCRIPTION

Reformulation
The imported lubricant additive containing the notified chemical (at < 5% 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.

End-use
The finished engine oil products containing the notified chemical at < 1% concentration will be available to commercial and public consumers. End-use products are expected to be added to the engine through closed systems (original equipment manufacturers and commercial operations) or manually (public).

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)*
QC/QA after importation	0.5	30
Unloading of drums and containers	1 – 2	30
Blending of lubricant product	2 – 4	100
Sampling of finished lubricant after blending	0.5	100
Filling/packaging containers with lubricant	2 – 4	100
Maintenance workers (blending facility)	2 – 4	12
Commercial lubricant installation	8	220

EXPOSURE DETAILS

Transport and storage
Transport and storage workers may come into contact with the notified chemical as a component of lubricant additives (at < 5% concentration) or as a component of end-use products (at concentrations < 1%) only in the event of accidental rupture of containers. The notifier states that such exposures will be minimised through the use of personal protective equipment (PPE) including protective coveralls, chemical resistant gloves, safety glasses and respiratory protection where appropriate.

Formulation of end products
During reformulation, dermal, ocular and inhalation exposure of workers to the notified chemical (at < 5% concentration) may occur during weighing and transfer stages, blending, quality control analysis, packaging of materials and cleaning and maintenance of equipment. The notifier states that exposure is expected to be minimised through the use of PPE such as protective coveralls, chemical resistant gloves, safety glasses and respiratory protection where appropriate.

End users of engine oil
Personnel from engine manufacturers and service technicians may be exposed to the notified chemical (at < 1% concentration) during addition of engine oils. Dermal, ocular and inhalation exposure of workers to the notified chemical may occur during transfer stages and cleaning and maintenance of equipment. The notifier states that

exposure is expected to be minimised through the use of PPE such as protective coveralls, chemical resistant gloves, safety glasses and respiratory protection where appropriate.

6.1.2. Public Exposure

The public may be exposed to the notified chemical when performing do-it-yourself (DIY) oil changes. Such exposure is expected to be limited to 2 – 3 occasions per year. Dermal, ocular and inhalation exposure to the notified chemical may occur during transfer stages and cleaning and maintenance of equipment. Such users may use PPE to minimise exposure, but PPE use is not always expected.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical and two analogue chemcials are summarised in the following table. Analogue 1, analogue 2 and the notified chemical are considered to be similar in chemical composition and the analogues are likely to reflect the toxicity of the notified chemical. For full details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity (analogue 2)	LD50 > 5,000 mg/kg bw; low toxicity
Rat, acute oral toxicity (analogue 1)	LD50 > 25,100 mg/kg bw; low toxicity
Rat, acute dermal toxicity (analogue 1)	LD50 > 10,000 mg/kg bw; low toxicity
Rat, acute inhalation toxicity (analogue 1)	> 0.23 mg/L/6 hours
Rabbit, skin irritation (analogue 1)	irritating
Rabbit, eye irritation (analogue 1) (Pharmakon 1986)	slightly irritating
Rabbit, eye irritation (analogue 1) (Younger 1966)	irritating
Human, skin sensitisation – RIPT (100%) (analogue 1) (Hill Top 1991a)	no evidence of sensitisation
Human, skin sensitisation – RIPT (25, 50 and 100%) (analogue 2)	inconclusive due to severe irritation
Human, skin sensitisation – RIPT (100%) (analogue 1) (Hill Top 1991b)	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days (analogue 2).	NOAEL = 150 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* Mammalian Chromosome Aberration Test (Chinese Hamster Ovary Cells)	potential to inhibit cell cycle progress
Genotoxicity – *in vivo* Mammalian Erythrocyte Micronucleus Test (mouse)	non genotoxic

Toxicokinetics, metabolism and distribution

No toxicokinetic data on the notified chemical were submitted. For dermal and gastrointestinal absorption, molecular weights below 100 g/mol are favourable for absorption and molecular weights above 500 g/mol do not favour absorption (ECHA, 2017). Dermal uptake is likely to be low to moderate if the water solubility is between 1-100 mg/L and may be limited if the partition coefficient (log Pow) values are greater than 4 (ECHA, 2017). Gastrointestinal absorption is also likely to be high if the partition coefficient (log Pow) values are greater than 4. Absorption of the notified chemical through the skin and gastrointestinal tract is expected to be low based on the insolubility in water (< 0.1 mg/L) and molecular weight (> 500 g/mol).

Acute toxicity

Analogue 1 is of low acute oral and dermal toxicity based on studies conducted in rats. Analogue 2 is of low acute oral toxicity based on a study conducted in rats. Therefore, the notified chemical is expected to be of low acute oral and dermal toxicity.

Due to the insufficient test conditions of the acute inhalation toxicity study provided on analogue 1 (see Appendix B.4), the acute inhalation toxicity of the notified chemical remains uncertain.

Irritation

Analogue 1 was irritating to eyes and skin of rabbits. In the skin irritation study, very slight to severe erythema was observed at the 24 hour observation, with one animal exhibiting slight oedema. Recovery was observed in one animal at the 3 day observation, and in all animals at the 5 day observation.

In an eye irritation study of analogue 1, slight to moderate conjunctival redness was observed in all animals (24 hours) persisting in one animal for at least 48 hours. All animals had recovered with 72 hours. In the second

study on analogue 1, no test details were provided. Oedema and corneal opacity increased over the first 24 hours after exposure. Slight iridial inflammation was observed following exposure (after 1 hour), but decreased following washing of the eye. Full recovery from iridial effects was observed by the end of the study period (7 days).

Based on the available information, the notified chemical is likely to be irritating to the eyes and skin.

Sensitisation
Analogue 1 showed no evidence of skin sensitisation in two associated Human Repeated Insult Patch tests (HRIPTs) at 100% concentration. Under the conditions of the second test, some test subjects (15/45) individually exhibited skin irritation effects (weak erythema including skin glazing, cracking or peeling) at varying times following the induction procedure. All skin irritation effects were resolved by the start of the next induction (within 24 – 48 hours). No skin irritation effects were observed during the challenge period.

In the HRIPT, individuals exposed to analogue 2 at 25%, 50% and 100% exhibited moderate to severe erythema, oedema, closed fissures and papules following exposure. One individual was withdrawn from the study after one induction procedure based on the severity of the skin irritation effects (including itching beyond the test site) observed. Based on the severity of skin irritation following exposure, the remaining test subjects were not exposed to further doses of analogue 2, or to a challenge phase. Test subjects continued to be observed over the remaining induction period, and all showed recovery from the skin irritation effects at the commencement of the challenge phase (approximately 5 weeks after initial exposure). The test results were inconclusive for skin sensitisation.

Repeated dose toxicity
Information on repeated dose toxicity following oral exposure for 28 days is available for analogue 2.

Oral exposure to the test substance did not produce mortality or significant clinical, neurological or clinical pathology abnormalities at up to 1,000 mg/kg bw/day. Males exposed to 500 or 1,000 mg/kg bw/day showed a lower overall weight gain in week 3 of the study (9% and 6% reduction respectively), with males exposed to 500 mg/kg bw/day also exhibiting statistically lower food consumption during this week. Males in the high-dose recovery group also showed an overall reduction in weight gain at the end of the study period. No statistically significant changes were observed in the body weight gain of females, although mean body weight gain of females exposed to 500 or 1,000 mg/kg bw/day was 3% lower than the body weight gains in the control animals.

Adverse effects including irritation to the non-glandular stomach and oedema in the submucosa were observed in the stomach of males exposed to 500 or 1,000 mg/kg bw/day and females exposed to 150, 500 or 1,000 mg/kg bw/day). However, based on the absence of these effects in animals that had a two week recovery after exposure to 1,000 mg/kg bw/day, the study authors considered the effects to be transient. One female exposed to 500 mg/kg bw/day exhibited an ulcer with inflammation, hyperplasia, haemorrhage and oedema in the stomach.

In males, a statistically significant increase in platelet counts were observed at 500 or 1,000 mg/kg bw/day on day 28. The study authors did not consider this as toxicologically significant. Recovery group males also had increased platelet count (not statistically significant). Increased platelets can result in abnormal blood clotting

Based on effects reported in the study, the No Observed Adverse Effect Level (NOAEL) for analogue 2 was considered to be 150 mg/kg bw/day.

Mutagenicity/Genotoxicity
The notified chemical was non-genotoxic in a bacterial reverse mutation assay and an *in vivo* mammalian erythrocyte micronucleus test. In an *in vitro* mammalian chromosome aberration test in cultured Chinese Hamster ovary cells, the notified chemical showed the potential to inhibit cell cycle progress under the conditions of the test.

Health hazard classification
Based on limited available information, the notified chemical cannot be classified 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 from the analogues, the notified chemical is expected to have potential for skin and eye irritation. Absorption of the notified chemical through the skin and gastrointestinal tract is expected to be low based on the insolubility in water and high molecular weight.

Exposure of workers to the notified chemical at < 5% concentration may occur during transfer, application, and cleaning operations. Although the notified chemical is considered to be irritating, at the reduced concentration of < 5%, irritant effects from the notified chemical are not expected. Ocular and dermal exposure may occur during blending operations, or when adding automotive lubricants containing the notified chemical to engine cases. Control measures including the use of automated processes and use of PPE (such as impervious gloves, goggles, and coveralls) will minimise worker exposure.

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 public may come into contact with automotive lubricants containing the notified chemical at < 5% concentration when changing engine oil on vehicles. Given the low end use concentration irritation is not expected from exposures. The high molecular weight of the notified chemical limits the systemic absorption.

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 into Australia as a component of additive packs for local reformulation into end-use automotive engine oil. In general, the reformulation processes are expected to involve automatic blending operations in closed systems, followed by automated filling of the reformulated products into end-use containers. 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 commercial and public consumers. End-use products are expected to be added to the engine through closed systems (original equipment manufacturers and commercial operations) or manually (public).

At original equipment manufacturers and commercial operations, used oil will be collected by approved waste management contractors for recycling, re-refining or disposal of in accordance with local government regulations. As a result, no release to aquatic environment is expected from these activities.

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

RELEASE OF CHEMICAL FROM DISPOSAL

Empty containers containing residues of the notified chemical are expected to be reused or disposal of in accordance with local government regulations. The used oil containing the notified chemical are expected to be

collected at the end of their useful lives and recycled, re-refined or disposed of in accordance with local government regulations.

7.1.2. Environmental Fate

The biodegradability study conducted on the notified chemical shows that it is not readily biodegradable (16% biodegradation in 35 days). For details of the biodegradability study, refer to Appendix C.

Any used or waste 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. The notified chemical in the environment is expected to eventually degrade into water, oxides of carbon and sulphur, and magnesium 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% (1 tonne per annum) may be incorrectly disposed of. However, this will be in a dispersed manner and not all of the released notified chemical 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 acceptable analogue chemicals are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity of analogue 1 and analogue 2	96 h LC50 > 10,000 mg WAF*/L	Not harmful to fish up to its water solubility limit
Daphnia Toxicity of analogue 2 and analogue 3	48 h EC50 > 1,000 mg WAF*/L	Not harmful to aquatic invertebrates up to its water solubility limit
Algal Toxicity of analogue 2 and analogue 3	96 h EC50 > 1,000 mg WAF*/L	Not harmful to alga up to its water solubility limit

*WAF: Water Accommodated Fraction (nominal concentration)

The above ecotoxicological endpoints of acceptable analogue chemicals indicate that the notified chemical is not harmful to aquatic life up to its water solubility limit. 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

A predicted no-effect concentration (PNEC) for the aquatic compartment has not been calculated as the notified chemical is not considered 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, and release to the aquatic environment will be limited, based on its reported use pattern. Therefore, based on the low hazard and the 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

Melting Point Not observed

Method	OECD TG 102 Melting Point/Melting Range
Remarks	Notified chemical did not melt. Decomposition was observed at ~ 333 °C. Colour change to dark brown was observed at ~ 344 °C with no further physical changes.
Test Facility	Maxxam (2018)

Boiling Point Not observed

Method	OECD TG 103 Boiling Point
Remarks	Ebulliometer method. No definite stages of boiling were observed up to 350 °C. The notified chemical changed colour and showed a drop in temperature indicating degradation of the notified chemical.
Test Facility	Maxxam (2018)

Density 1471 ± 56 kg/m^3 at 19.7 °C

Method	OECD TG 109 Density of Liquids and Solids
Remarks	Pycnometer method.
Test Facility	Maxxam (2018)

Vapour Pressure 0.002 kPa at 20 °C

Method	OECD TG 104 Vapour Pressure
Remarks	Test conducted by Chilworth Technology Ltd (Southampton, Hampshire, United Kingdom).
Test Facility	Maxxam (2018)

Particle Size 6 – 2,000 μm

Method OECD TG 110 Particle Size Distribution/Fibre Length and Diameter Distributions

Range (μm)	*Mass (%)*
0.02 - 10	0.28
10 – 100.237	8.38
100.237 - 200	9.16
200 – 2,000	82.46

Volume weighted mean	630.148 μm
Median	565.432 μm
Mode	758.847 μm
10% of the notified chemical is <	117.782 μm
50% of the notified chemical is <	565.432 μm
90% of the notified chemical is <	1241.385 μm

Remarks	Test conducted by Chilworth Technology Ltd (Southampton, Hampshire, United Kingdom).
Test Facility	Maxxam (2018)

Flammability Not highly flammable

Method	EC Council Regulation No 440/2008 A.10 Flammability (Solids)
Remarks	Sample ignited briefly, but extinguished when the ignition source was removed. Test conducted by Chilworth Technology Ltd (Southampton, Hampshire, United Kingdom).
Test Facility	Maxxam (2018)

Autoignition Temperature > 400 °C

Method	EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids
Remarks	A multi-exothermic event was observed at ~ 162 °C, with a second event observed at 213 °C. The sample did not reach the required temperature by self-heating. Decomposition of the sample was observed at end of the test. Test conducted by Chilworth Technology Ltd (Southampton, Hampshire, United Kingdom).
Test Facility	Maxxam (2018)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral (analogue 2)

TEST SUBSTANCE	Analogue 2
METHOD	Similar to OECD TG 401 Acute Oral Toxicity. Animals were exposed to the test substance by oral gavage. Total observation period was 14 days, with observations made at 0, 2 and 4 hours post-exposure and then at least once in the morning and afternoon over the observation period.
Species/Strain	Rat/Sprague-Dawley
Vehicle	None
Remarks - Method	GLP Compliant. Individual animal weights and doses were provided in the study report. However, individual observations post-exposure were not provided.

RESULTS

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

LD50	> 5,000 mg/kg bw
Signs of Toxicity	Following exposure, the study authors reported slight diarrhoea in 2/5 males (at 2 hour observation), slight diarrhoea and yellow anal stains in most rats (day 1 observation), and dried bloody nasal discharge in 1/5 males and 1/5 females (day 2 and day 3 observations). All animals were reported as appearing normal on day 4 and over the remainder of the observation period.
Effects in Organs	None observed.
Remarks - Results	All males and 4/5 females made the expected body weight gains. One female exhibited a non-significant loss in body weight.

CONCLUSION	The test substance is of low acute toxicity via the oral route.
TEST FACILITY	Bioresearch (1981)

B.2. Acute toxicity – oral (analogue 1)

TEST SUBSTANCE	Analogue 1
METHOD	Summary only provided. Animals were exposed to the test substance by oral gavage in increasing doses.
Species/Strain	Rat/Sprague-Dawley
Vehicle	None
Remarks - Method	Doses were increased at increments of 0.3 and 0.2 fractional log intervals.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	1 F	201	0/1
2	1 M	1,000	0/1
3	1 F	1,580	0/1
4	1 M	2,510	0/1
5	1 F	3,980	0/1
6	1 M	6,310	0/1
7	1 F	10,000	0/1
8	1 M , 1 F	15,800	0/2
9	2 M, 1 F	25,100	0/3

LD50	> 25,100 mg/kg bw

Signs of Toxicity	Severe diarrhoea and loss of appetite were reported. No details were provided regarding at which dose these effects occured. No observations of nervousness or paralysis.
Effects in Organs	No description provided.
Remarks - Results	None.

CONCLUSION The test substance is of low acute toxicity via the oral route.

TEST FACILITY Younger (1966)

B.3. Acute toxicity – dermal (analogue 1)

TEST SUBSTANCE Analogue 1

METHOD Summary only provided. Animals were exposed to the test substance in increasing doses. Test substance was applied to closely clipped, intact skin. Following exposure, animals were placed in stocks for up to 24 hours before being assigned to individual cages. Animals were re-weighed 5 days after exposure.

Species/Strain	Rabbit/New Zealand White
Vehicle	None
Type of dressing	Occlusive (plastic strips)
Remarks - Method	Doses were increased at increments of 0.2 fractional log intervals.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	1 F	1,000	0/1
2	1 M	1,580	0/1
3	1 F	2,510	0/1
4	1 M	3,980	0/1
5	1 F	6,310	0/1
6	1 M	10,000	0/1

LD50	> 10,000 mg/kg bw
Signs of Toxicity - Local	None described
Signs of Toxicity - Systemic	Moderate weakness and reduced activity was observed in the animals exposed to the two highest doses (6,310 mg/kg bw and 10,000 mg/kg bw) for three to four days. No muscular impairment was noted. No significant changes in body weight recorded.
Effects in Organs	No animals died so no necropsies were performed.
Remarks - Results	None

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

TEST FACILITY Younger (1966)

B.4. Acute toxicity – inhalation (analogue 1)

TEST SUBSTANCE Analogue 1

METHOD Summary only provided. Animals were exposed to the test substance for 6 hours in a glass desiccator. Vapours were produced by passing a stream of air through 75 mL of the test substance (contained within a 250 mL Erlenmeyer flask), followed by passing through a 1 L bottle to remove droplets prior to entering the test chamber. Air flow was measured at 4 L/min. There was sufficient oxygen present to meet animal requirements and no supplementary air was introduced. Animals were observed for 10 days following exposure.

Species/Strain	Rat

Vehicle	None
Method of Exposure	Whole-body exposure.
Exposure Period	6 hours
Physical Form	Vapour
Remarks - Method	None

RESULTS

Group	*Number and Sex of Animals*	*Test Substance (mL/6 hours)*			*Mortality*
		Starting	*Residual*	*Vaporised*	
1	4 M	75.0	74.7	0.3*	0/4

* Approximately equivalent to 0.23 mg/L (vapour)

LC50	> 0.23 mg/L/6 hour
Signs of Toxicity	Mild lethargy.
Effects in Organs	No description provided
Remarks - Results	No adverse effects on animal behaviour were recorded. Animals resumed normal activity and respiration thirty minutes after exposure with mild lethargy observed after this point. No description provided if animals recovered from this effect. No respiratory complications observed.

CONCLUSION The test substance is of low acute toxicity via inhalation under the conditions of the test.

TEST FACILITY Younger (1966)

B.5. Irritation – skin (analogue 1)

TEST SUBSTANCE Analogue 1

METHOD Summary only provided. Test substance was applied to closely clipped, intact skin and removed after 24 hours.

Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	None
Observation Period	24 days
Type of Dressing	Occlusive (plastic strips)
Remarks - Method	Data were scored according to Draize method (1944).

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 and Oedema	1.6	0.6	2	3	5 days	0

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

Remarks - Results	No adverse reactions were observed one hour after exposure. Very slight to moderate to severe erythema was observed at the 24 hour observation, with one animal exhibiting slight oedema. After removal of the test substance (24 hours following exposure), recovery was observed in one animal at the 3 day observation, and in all animals at the 5 day observation.

CONCLUSION The test substance is irritating to the skin.

TEST FACILITY Younger (1966)

B.6. Irritation – eye (analogue 1)

TEST SUBSTANCE	Analogue 1
METHOD	Similar to OECD TG 405 Acute Eye Irritation/Corrosion
Species/Strain	Rabbit/Albino New Zealand White
Number of Animals	6 (3 males, 3 females)
Observation Period	3 days
Remarks - Method	GLP compliant. Eye irritation graded according to Draize method (1944, 1959a, 1965).

RESULTS

Lesion	*Mean Score**						*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3	4	5	6			
Conjunctiva: redness	0.6	0.6	0	0.6	0.3	0	2	24 hour	0
Conjunctiva: chemosis	0	0	0	0	0	0	0	-	0
Conjunctiva: discharge	0	0	0	0	0	0	0	-	0
Corneal opacity	0	0	0	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	0	0	0	-	0

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

Remarks - Results	All animals exhibited moderate to severe conjunctival redness, and 4/6 animals (2/3 males, 2/3 females) exhibited slight conjunctival swelling one hour after exposure to the test substance. Slight (2/3 females) to moderate (2/3 males) conjunctival redness was observed at the 24 hour observation with the effect persisting in one female at the 48 hour observation. Recovery was observed in all animals at the 72 hour observation.
CONCLUSION	The test substance is slightly irritating to the eye.
TEST FACILITY	Pharmakon (1986)

B.7. Irritation – eye (analogue 1)

TEST SUBSTANCE	Analogue 1
METHOD	Summary only provided. 0.1 mL of test substance (undiluted) was applied to the conjunctival sac of the right eye of each animal. The eyes were washed with warm isotonic saline solution 24 hours after exposure.
Species/Strain	Rabbit/New Zealand White
Number of Animals	2 M, 1 F
Observation Period	7 days
Remarks - Method	Irritation effects were scored according to the Draize method (1944).

RESULTS

Remarks - Results	Individual results for severity of conjunctival irritation (redness, chemosis, discharge), corneal opacity or iridial inflammation were not provided. Moderate discharge, mild swelling and redness, and slight iridial inflammation were observed one hour following exposure. Oedema and corneal opacity increased over the first 24 hours after exposure. Following washing of the eye, iridial inflammation decreased with full recovery by day 7. Recovery was indicated in all animals over the course of the study

(lessening in severity of effects recorded at the 48, 72 and 120 hour observations) and all animals had recovered fully at the end of the observation period (7 days).

CONCLUSION The test substance is irritating to the eye.

TEST FACILITY Younger (1966)

B.8. Skin sensitisation – human volunteers (analogues 1 and 2)

TEST SUBSTANCE Analogue 1 and Analogue 2

METHOD Repeated insult patch test with challenge

Study Design Induction Procedure: patches were applied to lateral surface of the right and left upper arms. Nine induction applications were made for analogue chemical 1 and 1 – 3 induction applications were made for analogue chemical 2 (see Remarks-results below for details) A naïve alternate site and the original induction site were used for the challenge applications (Draize 1959b).
Rest Period: 10 - 15 days
Challenge Procedure: sample of test substance (concentration not provided) applied to naïve site for 24 hours with observations made at 48 and 96 hours.

Study Group 19 F, 1 M; age range 18 - > 60 years
The age of oldest participant was not provided.
21 test subjects started the study. However one subject experienced an adverse reaction and was withdrawn from the study. No details on the age or sex of this subject were presented.

Vehicle 100 N Process Oil (supplied by test sponsor to testing facility)

Remarks - Method Occluded. The test substance (0.2 mL) was applied using the 25 mm Hill Top Chamber.

Under the protocol, application patches were to be worn for 24 hours. A number of test subjects lost their sample within 6 to 12 hours following application of the test substance during the first (1 test subject) or second (3 test subjects) induction phases, with 1 test subject losing vehicle control patch 12 hours after application during the challenge phase. These deviations did not affect the outcome of the study.

RESULTS

Test Sample	*Test Concentration (%)*	*Status*	*Irritation*	*Sensitisation*
Analogue 1	100	Completed	Negative	Negative
Analogue 2	100, 50 and 25	Discontinued	Severe	Inconclusive

Remarks - Results Of the 21 test subjects that started the study, one test subject was withdrawn based on an adverse reaction to the test substances (test report did not indicated if this was analogue 1, analogue 2 or both, or at what concentration). The subject exhibited moderate erythema and papules with itching spreading beyond the test site. Four days after exposure, eczematous plaques away from the test site were observed.

Of the 20 test subjects that completed the study, no evidence of clinical sensitisation or irritation was observed for analogue 1 at 100% concentration.

In the test subjects that completed the study, moderate to severe reactions consisting of erythema, oedema, fissures and papules were observed for analogue 2 at 100% concentration. Application of this chemical at 100% concentration was discontinued after the third application and was not

applied during the challenge phase. Skin reactions to analogue 2 continued to be recorded over the induction period, with the severity of clinical reactions reducing over time. Mild erythematous residual reactions were observed at the end of the induction period.

Analogue 2 was applied to test subjects at 25% and 50% concentration (induction number 4). Moderate to severe erythema, oedema, closed fissures and papules were observed and further applications were discontinued. Skin reactions continued to be recorded over the induction period with full recovery from effects at the end of the study period.

The intensity and clinical patterns for analogue 2 at 25%, 50% and 100% concentration were suggestive of moderate to severe clinical irritation.

CONCLUSION Analogue 1 was non-sensitising under the conditions of the test.
Analogue 2 was severely irritating with no conclusion for sensitisation.

TEST FACILITY Hill Top (1991a)

B.9. Skin sensitisation – human volunteers (analogue 1)

TEST SUBSTANCE Analogue 1

METHOD Repeated insult patch test with challenge

Study Design This study was a continuation of above HRIPT (Appendix B.8)

Induction Procedure: patches were applied to lateral surface of the right and left upper arms. Nine induction applications were made. A naïve alternate site and the original induction site were used for the challenge applications
Rest Period: 10 - 15 days
Challenge Procedure: sample of test substance applied to naïve site for 24 hours with observations made at 48 and 96 hours.

Study Group 35 F, 10 M; age range 18 - > 60 years
The age of oldest participant was not provided.
46 test subjects started the study. However one subject withdrew for reasons unrelated to the study. No details on the age or sex of this subject were presented.

Vehicle 100 N Process Oil (supplied by test sponsor to testing facility)

Remarks - Method Occluded. The test substance (0.2 ml) was applied using the 25 mm Hill Top Chamber.

Under the protocol, application patches were to be worn for 24 hours. Three test subjects wore the test sample for < 24 hours [2 test subjects wore the test substance patch for 1 hour only (inductions 1 and 6), and one test subject wore the control patch for 6 hours only (induction 5)]. One subject missed a skin assessment. These deviations did not affect the outcome of the study.

RESULTS

Remarks - Results Of the 45 test subjects that completed the study, weak erythema (including weak superficial skin reactions such as glazing, cracking or peeling) were observed following the first (1/45 test subjects), second (1/45 test subjects), third (2/45 test subjects), fourth (4/45 test subjects), fifth (3/45 test subjects), sixth (2/45 test subjects), seventh (1/45 test subjects) and eighth (1/45 test subjects) inductions.

No evidence of any skin reactions were observed in the challenge phase.

CONCLUSION There was no evidence of skin sensitisation to the test substance under the

conditions of the test.

TEST FACILITY Hill Top (1991b)

B.10. Repeat dose toxicity (analogue 2)

TEST SUBSTANCE Analogue 2

METHOD OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents
Species/Strain Rat/ Sprague Dawley Crl:CD® (SD)IGS BR
Route of Administration Oral – gavage
Exposure Information Total exposure days: 28 days
Dose regimen: 7 days per week
Post-exposure observation period: 14 days
Vehicle Corn oil
Remarks - Method GLP compliant.
No significant deviations from the protocol.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
control	5 M, 5 F	-	0
low dose	5 M, 5 F	50	0
mid dose 1	5 M, 5 F	150	0
mid dose 2	5 M, 5 F	500	0
high dose	5 M, 5 F	1,000	0
control recovery	5 M, 5 F	-	0
high dose recovery	5 M, 5 F	1,000	0

Mortality and Time to Death
No mortality, significant clinical abnormalities or toxicologically relevant neurological changes were observed during the main or recovery phases of the study.

Clinical Observations
Males in mid-dose 2 and high dose groups showed decreased weight gain during week 3 of the study and an overall lower weight gain (9% and 6% reduction respectively) compared with the control group over the course of the study. Males in mid-dose group 2 showed statistically significant lower food consumption during week 3. Males in the high-dose recovery group also exhibited an overall 6% lower weight gain than the control recovery group.

While no statistically significant changes in female body weight were observed, mean body weight was 3% lower than controls in mid-dose 2 and high-dose females. Females in the high-dose group showed statistically significant lower food consumption in the second week of the study. Females in the high-dose recovery group showed the expected body weight gains.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
No toxicologically relevant changes in the haematology, coagulation, clinical chemistry or urinalysis parameters were observed during the main and recovery phases of this study.

Males in the high-dose and mid-dose-2 groups exhibited a statistically significant increase in platelet counts. The study authors did not consider this as toxicologically relevant stating that as abnormalities in platelet counts are expected to be reflected in a decreased count rather than an increased count. The males in the recovery group showed decreased (not statistically significant) platelet counts. Statistically significant increases were observed in eosinophil and basophil count (mid-dose group 1 males), mean corpuscular haemoglobin concentration (high-dose recovery males), and a statistically significant decrease was observed in prothrombin time (mid-dose group 2 females). The study authors did not consider these effects as toxicologically relevant.

Males in all dosed groups showed a statistically significant increase in gamma glutamyl transferase (GGT). The study authors considered that the result may have been artificially high as the control group showed an abnormally low level of GGT. This interpretation is supported by the expected levels of GGT in control animals

in the recovery phase, and the absence of any correlative microscopic changes in the liver or kidney of exposed animals.

Statistically significant changes in serum alanine aminotransferase (increased in high-dose males), alanine aminotransferase (increased in mid-dose 2 and high-dose females), serum phosphorous (increased in high-dose males), bilirubin (increased in mid-dose group 2 males), sodium and chloride levels (both decreased in mid-dose group 2 females) were not considered toxicologically relevant, or attributable to exposure to the test substance by the study authors as no correlative microscopic changes or other biochemical indicators, or dose-response relationships were observed.

Urine pH was lower in high-dose recovery males. However, no correlative changes in kidney pathology were observed.

Effects in Organs

No toxicologically significant differences were observed in the organ weights of animals exposed to the test substance.

Minimal to mild pulmonary irritation was observed in high-dose (1/5 males, 2/5 females) and high-dose-recovery (3/5 males, 1/5 females) animals. The study authors considered that the irritation was associated with a foreign body response to incidentally aspirated test substance suspension rather than a result of oral exposure to the test substance.

Remarks – Results

Oral exposure to the test substance did not produce mortality or significant clinical or neurological abnormalities at up to 1,000 mg/kg/day. Minimal irritation in the nonglandular stomach was observed in females exposed to 150 and 1,000 mg/kg/day and these effects were resolved by the end of the recovery phase. An ulcer with inflammation, hyperplasia, haemorrhage and oedema were present in the stomach of one females exposed to 500 mg/kg/day.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 150 mg/kg bw/day in this study based on adverse irritation effects on the stomach observed.

TEST FACILITY	SLI (1991)

B.11. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test Plate incorporation procedure
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100 *Escherichia coli*: WP2uvrA
Metabolic Activation System	S9 fraction from Aroclor 1254-induced rat liver.
Concentration Range in Test 1 (Preliminary Test)	a) With metabolic activation: 25 - 5,000 µg/plate b) Without metabolic activation: 25 - 5,000 µg/plate
Concentration Range in Test 2 (Main Test)	a) With metabolic activation: 1 – 1,000 µg/plate b) Without metabolic activation: 100 – 5,000 µg/plate
Vehicle	Tetrahydrofuran
Remarks - Method	GLP compliant. No significant deviations from the protocol. Positive controls: without metabolic activation – ICR-191 acridine (TA1537), 2-nitrofluorene (TA98), sodium azide (TA100, TA1535), 4-nitroquinoline-N-oxide (WP2uvrA); with metabolic activation – 2-aminoanthracene (all strains).

RESULTS

Metabolic	*Test Substance Concentration (µg/plate) Resulting in:*

Activation	*Cytotoxicity in Preliminary Test*	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5,000	> 5,000	≥ 1,000	negative
Test 2		> 5,000	≥ 2,500	negative
Present				
Test 1	> 5,000	> 5,000	≥ 25	negative
Test 2		> 5,000	≥ 1,000	negative

Remarks - Results

Precipitates were observed at ≥ 25 µg/plate (test 1) and ≥ 1,000 µg/plate (test 2) in all strains in the presence of metabolic activation and at ≥ 1,000 µg/plate (test 1) and ≥ 2,500 µg/plate (test 2) in all strains in the absence of metabolic activation.

In test 2, in the presence of metabolic activation, tester strain TA98 was not added to plates dosed with 10 and 25 µg of the test substance. In the absence of bacteria, these plates were omitted from the results. However, based on the results observed for TA98 for concentrations > 25 µg/plate, the study authors concluded that this deviation did not negatively impact on the overall results of the study.

Cytotoxicity was not observed in any tester stain in the presence or absence of metabolic activation under the conditions of test 1 and test 2.

Positive and negative controls performed as expected.

CONCLUSION

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

TEST FACILITY

CRL (2018a)

B.12. Genotoxicity – *in vitro* Mammalian Chromosome Aberration Test

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test

Species/Strain: Chinese Hamster

Cell Type/Cell Line: CHO-WBL-cell line

Metabolic Activation System: S9 fraction from Aroclor 1254-induced rat liver.

Vehicle: Tetrahydrofuran

Remarks - Method: GLP compliant.
No significant deviations from the protocol.

A range finding assay was performed using a test substance concentration range of 3.91 – 2,000 µg/mL. Precipitates were observed at ≥ 250 µg/mL in the presence and absence of metabolic activation following a 3 hr exposure period, and at ≥ 500 µg/mL in the absence of metabolic activation following a 24 hr exposure period. Changes in cell morphology were observed in the absence of metabolic activation at ≥ 400 µg/mL following an exposure period of 3 hours and at ≥ 150 µg/mL following 24 hour exposure.

Positive controls: without metabolic activation – mitomycin C; with metabolic activation – cyclophosphamide monohydrate.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1A	11.9, 16.9, 24.2, 34.6, 49.4, 70.6, 101, 144*, 206*, 294*, 420, 600	3	24
Test 1B	25, 50, 100, 200*, 220*, 240*, 260, 280, 300, 400, 450, 600	3	24

Test 2A	11.9, 16.9, 24.2, 34.6, 49.4*, 70.6*, 101*, 144*, 206, 294, 420, 600	24	24
Test 2B	12.5, 25, 50, 70, 80, 90, 100, 110*, 125*, 150*, 200*, 300	24	24
Present			
Test 1A	11.9, 16.9, 24.2, 34.6, 49.4, 70.6, 101, 144*, 206*, 294*, 420, 600	3	24

*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*	
				Structural	*Numerical**
Absent					
Test 1A	≥ 250	≥ 600	≥ 294	equivocal	positive
Test 1B		≥ 400	≥ 240	negative	positive
Test 2A		≥ 101	≥ 294	positive[#]	negative
Test 2B		≥ 200	≥ 200	negative	negative
Present					
Test 1A	≥ 250	≥ 600	≥ 294	negative	negative

* Endoreduplication # with excessive cytotoxicity

Remarks - Results

In the absence of metabolic activation a statistically significant increase was observed in the number of cells with structural aberrations at concentrations ≥ 294 µg/mL (test 1A) following a 3 hour exposure period and at ≥ 49.4 µg/mL (test 2A) following a 24 hour exposure period. No statistically significant increase in structural aberrations was observed following a 3 hour exposure period in the presence of metabolic activation (test 1A).

Based on inconsistency in the cytotoxicity observed in the absence of metabolic activation following 3 and 24 hour exposures, as well as excessive cytotoxicity and/or aberration increases observed (Excessive mitotic index cytotoxicity was observed following 24 hour exposure in the absence of metabolic activation at a concentration of 144 µg/mL and was not included in the statistical analysis), these assays were repeated (test 1B and 2B).

Under the conditions of the repeat assays, no statistically significant increases in the percent of cells with structural aberrations were observed in the absence of metabolic activation following 3 and 24 hour exposure periods. A statistically significant increase in endoreduplication was observed at 240 µg/mL in the absence of metabolic activation following exposure to the test substance for 3 hours.

Positive and negative controls performed as expected.

CONCLUSION

The notified chemical showed the potential to inhibit cell cycle progress to Chinese Hamster Ovary cell cultures treated *in vitro* under the conditions of the test.

TEST FACILITY

CRL (2018b)

B.13. Genotoxicity – *in vivo* Mammalian Erythrocyte Micronucleus Test

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 474 Mammalian Erythrocyte Micronucleus Test
EC Guideline No. 440/2008 B.12 Mutagenicity – *In vivo* Mammalian Erythrocyte Micronucleus Test

Species/Strain: Mouse/NMRI (SPF)
Route of Administration: Oral – gavage

Vehicle	Mineral oil
Remarks - Method	GLP compliant.

No significant deviations from the protocol. Test substance formulations for groups II and IV were found to be homogenous and stable. The test substance formulation for group III was not homogenous or stable. However, as the highest dose tested met the criteria for homogeneity and stability, the study authors did not consider this deviation to have significantly affected the overall integrity or outcome of the study.

A dose-range finding study was performed. Animals (3 males, 3 females) were exposed to 2,000 mg/kg bw twice by oral gavage (24 h between doses). No adverse effects were observed and no sex-related differences were observed.

Within groups I and IV, an additional 3 animals (males) per blood sampling time (1, 2, 4, 6 and 8 hours after second exposure) were included as satellite groups for the purposes of blood sampling and plasma preparations.

Animals were sacrificed 48 hours after the first exposure.

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Sacrifice Time (hours)*
I (vehicle control)	20 M*	0	48
II (low dose)	5 M	500	48
III (mid dose)	5 M	1,000	48
IV (high dose)	20 M*	2,000	48
V (positive control, CP)	5 M	40	48

* 5 M in main test and 15 M in satellite group CP = cyclophosphamide.

RESULTS

Doses Producing Toxicity	> 2,000 mg/kg bw
Genotoxic Effects	No biological relevant increase in the mean frequency of micronucleated polychromatic erythrocytes was observed in the bone marrow of animals exposed to the test substance. No decrease in the ratio of polychromatic to normochromatic erythrocytes was observed in animals exposed to the vehicle control or test substance.
Remarks - Results	No unscheduled deaths were recorded. No treatment related effects were observed in control animals, or animals exposed to the test substance. The test substance lacked cytotoxic effects on erythropoiesis and it was hence impossible to determine whether the test substance reached the bone marrow of the test animals. Positive and negative controls performed as expected.

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

TEST FACILITY CRL (2018c)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test
Inoculum	Secondary effluent from a domestic STP
Exposure Period	35 days (extended due to a prolonged acclimation lag phase)
Auxiliary Solvent	None
Analytical Monitoring	Oxygen consumption by specific electrode
Remarks - Method	No significant deviations from the test guidelines were reported. A nominal 3,000 mg/L stock solution was stirred for 23 hours and allowed to settle for 1 hour. The first 100 mL were discarded from the bottom of the bottle and the next 500 mL were used as the test stock. A test solution of nominal Chemical Oxygen Demand (COD) concentration of 4 mg/L was prepared by diluting the test stock. A toxicity control was run.

RESULTS

Test substance		*Potassium hydrogen phthalate*	
Day	*% Degradation*	*Day*	*% Degradation*
		14	85
35	16.2	35	95

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 35 days was 16.2%.
CONCLUSION	The test substance is not readily biodegradable.
TEST FACILITY	AquaTox (2017)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Analogue 1
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-Static
Species	Sheepshead minnow (*Cyprinodon variegatus*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	Not determined
Analytical Monitoring	Total Organic Carbon (TOC) by Horiba PIR 2000 analyser
Remarks – Method	A limit test was run with no significant deviations from the test guidelines. 15 L of a nominal test solution of 10,000 mg/L was stirred overnight and settled for 2 hours. To separate from floating and settled materials, 5 L of water accommodated fraction (WAF) was gently pumped from a point approximately midway between the bottom, surface and sides of the jar. The WAF was renewed daily during the test.

RESULTS

Concentration mg WAF/L		*Number of Fish*	*Mortality*
Nominal	*Actual*		*96 h*
Control	Control	10	0
10,000	Not determined	10	0

LC50 > 10,000 mg WAF/L (nominal) at 96 hours
Remarks – Results All validity criteria for the test were satisfied. The dissolved oxygen concentration in the test solution during the test was ≥ 5.5 mg/L at 22 °C and salinity of 32 ‰ (≥ 76%, USGS, 2011).

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

TEST FACILITY Springborn (1986a)

C.2.2. Acute toxicity to fish

TEST SUBSTANCE Analogue 2

METHOD OECD TG 203 Fish, Acute Toxicity Test – Semi-Static
Species Sheepshead minnow (Cyprinodon variegatus)
Exposure Period 96 hours
Auxiliary Solvent None
Water Hardness Not determined
Analytical Monitoring TOC by Horiba PIR 2000 analyser
Remarks – Method A limit test was run with no significant deviations from the test guidelines. 15 L of a nominal test solution of 10,000 mg/L was stirred overnight and settled for 2 hours. To separate from floating and settled materials, 5 L of WAF was gently pumped from a point approximately midway between the bottom, surface and sides of the jar. The WAF was renewed daily during the test.

RESULTS

Concentration mg WAF/L		*Number of Fish*	*Mortality*
Nominal	*Actual*		*96 h*
Control	Control	10	0
10,000	Not determined	10	0

LC50 > 10,000 mg WAF/L (nominal) at 96 hours
Remarks – Results All validity criteria for the test were satisfied. The dissolved oxygen concentration in the test solution during the test was ≥ 6.0 mg/L at 22 °C and salinity of 32 ‰ (≥ 83%, USGS, 2011).

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

TEST FACILITY Springborn (1986b)

C.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Analogue 2

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - Static
Species *Daphnia magna*
Exposure Period 48 hours
Auxiliary Solvent None
Water Hardness 190 mg $CaCO_3$/L
Analytical Monitoring TOC

Remarks - Method No significant deviations from the test guidelines were reported. The test solutions were prepared, stirred for 24 hours and settled for 1 hour. Then the WAFs were siphoned and used for the test.

RESULTS

Concentration (mg WAF/L)		*Number of D. magna*	*Number Immobilised*
Nominal	*Actual*		*48 h*
Control	Control	20	0
100	Not determined	20	0
300	Not determined	20	0
1,000	Not determined	20	0

LC50 > 1,000 mg WAF/L (nominal) at 48 hours

Remarks - Results All validity criteria for the test were satisfied. The dissolved oxygen concentration in the test solution during the test was ≥ 7.9 mg/L at 20 °C (≥ 87%, USGS, 2011).

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

TEST FACILITY EnviroSystems (1993a)

C.2.4. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Analogue 3

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - Static

Species *Daphnia magna*
Exposure Period 48 hours
Auxiliary Solvent None
Water Hardness 180 mg $CaCO_3$/L
Analytical Monitoring TOC
Remarks - Method No significant deviations from the test guidelines were reported. The test solutions were prepared, stirred for 24 hours and settled for 1 hour. Then the WAFs were siphoned and used for the test.

RESULTS

Concentration (mg WAF/L)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	Control	20	0	0
100	Not determined	20	1	2
300	Not determined	20	0	0
1,000	Not determined	20	0	0

LC50 > 1,000 mg WAF/L (nominal) at 48 hours

Remarks - Results All validity criteria for the test were satisfied. The dissolved oxygen concentration in the test solution during the test was ≥ 7.8 mg/L at 20 °C (≥ 86%, USGS, 2011). Some immobilisation occurred in the 100 mg/L WAF, test solution, but there was no dose response.

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

TEST FACILITY EnviroSystems (1993b)

C.2.5. Algal growth inhibition test

TEST SUBSTANCE	Analogue 2
METHOD	OECD TG 201 Alga, Growth Inhibition Test
Species	*Selenastrum capricornutum*
Exposure Period	96 hours
Concentration Range	Nominal: 0; 100; 300; 1,000 mg WAF/L
Auxiliary Solvent	None
Water Hardness	Not determined
Analytical Monitoring	TOC
Remarks - Method	No significant deviations from the test guidelines were reported. The test solutions were prepared, stirred for 24 hours and settled for 1 hour. Then the WAFs were siphoned and used for the test.

RESULTS

Biomass		*Growth*	
EC50	*NOEC*	*EC50*	*NOEC*
mg WAF/L at 96 h	*mg WAF/L*	*mg WAF/L at 96 h*	*mg WAF/L*
> 1,000	1,000	> 1,000	1,000

Remarks - Results	All validity criteria for the test were satisfied. The mean cell density in the control increased by 171 times.
CONCLUSION	The test substance is not harmful to alga up to its water solubility limit.
TEST FACILITY	Wilbury (1994a)

C.2.6. Algal growth inhibition test

TEST SUBSTANCE	Analogue 3
METHOD	OECD TG 201 Alga, Growth Inhibition Test
Species	*Selenastrum capricornutum*
Exposure Period	96 hours
Concentration Range	Nominal: 0; 100; 300; 1,000 mg WAF/L
Auxiliary Solvent	None
Water Hardness	Not determined
Analytical Monitoring	TOC
Remarks - Method	No significant deviations from the test guidelines were reported. The test solutions were prepared, stirred for 24 hours and settled for 1 hour. Then the WAFs were siphoned and used for the test.

RESULTS

Biomass		*Growth*	
EC50	*NOEC*	*EC50*	*NOEC*
mg WAF/L at 96 h	*mg WAF/L*	*mg WAF/L at 96 h*	*mg WAF/L*
> 1,000	1,000	> 1,000	1,000

Remarks - Results	All validity criteria for the test were satisfied. The mean cell density in the control increased by 128 times.
CONCLUSION	The test substance is not harmful to alga up to its water solubility limit.
TEST FACILITY	Wilbury (1994b)

BIBLIOGRAPHY

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

AquaTox (2017) [notified chemical]: Ready Biodegradability Closed Bottle Test (OECD 301D) (Project no. 162775749-301, November, 2017). Puslinch, Canada, AquaTox Testing and Consulting Inc.

Bioresearch (1981) Acute Oral Toxicity Study [Analogue 2] (Study No. 1770-D, November, 1981). Louisiana, United States of America, Elars Bioresearch Laboratories Inc. (Unpublished report submitted by the notifier).

CRL (2018a) [notified chemical] *Salmonella-E. Coli*/ Mammalian Microsome Reverse Mutation Assay (Study No. 00349518, January, 2018). Illinois, United States of America, Charles River Laboratories (Unpublished report submitted by the notifier)

CRL (2018b) [notified chemical] *In vitro* Chromosome Aberration Test in Chinese Hamster Ovary (CHO-WBL) Cells (Study No. 00349519, April, 2018). Illinois, United States of America, Charles River Laboratories (Unpublished report submitted by the notifier)

CRL (2018c) Micronucleus test in Bone Marrow Cells of the Mouse with [notified chemical] (Study No. 519763, April, 2018). DD's-Hertogenbosch, The Netherlands, Charles River Laboratories Den Bosch BV (Unpublished report submitted by the notifier)

Draize J., Woodard G., Calvery H.. (1944) Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharm Exp Ther 82:377 - 390.

Draize J.H.. (1959a) Appraisal of Chemicals in Food, Drugs and Cosmetics. Association of Food and Drug Officials of the U.S., Austin, Texas, pp 36 – 45.

Draize J.H.. (1959b) "Dermal Toxicity" in Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics. Association of Food and Drug Officials of the U.S., Austin, Texas, pp 52.

Draize J.H.. (1965) Appraisal of Chemicals in Food, Drugs and Cosmetics – Dermal Toxicity. Association of Food and Drug Officials of the U.S., Topeka, Kansas, pp 49 – 52.

EnviroSystems (1993a) Acute Toxicity of the Water Accommodated Fractions (WAF) of [Analogue 2] to the Daphnid, *Daphnia magna* (Study No. 9178-CMA/ESI-604, May, 1993). New Hampshire, United States of America, EnviroSystems Division of Resource Analysts Inc. (Unpublished report submitted by the notifier).

EnviroSystems (1993b) Acute Toxicity of the Water Accommodated Fractions (WAF) of [Analogue 3] to the Daphnid, *Daphnia magna* (Study No. 9178-CMA/ESI-605, May, 1993). New Hampshire, United States of America, EnviroSystems Division of Resource Analysts Inc. (Unpublished report submitted by the notifier).

Hill Top (1991a) Repeated Insult Patch Test for Ethyl Corporation (Study No. 91-1005-70, May, 1991). Ohio, United States of America, Hill Top Research Inc. (Unpublished report submitted by the notifier).

Hill Top (1991b) Revised Report I. Repeated Insult Patch Test (Modified Draize Procedure) for Ethyl Corporation (Study No. 91-1112-70, October, 1991). Ohio, United States of America, Hill Top Research Inc. (Unpublished report submitted by the notifier).

Maxxam (2018) Product Chemistry Testing of X-20109 (Study No. AFTO921-170053, February, 2018). Burnaby, British Colombia, Canada, Maxxam Analytics (Unpublished report submitted by the notifier).

NTC (2017) Australian Code for the Transport of Dangerous Goods by Road and Rail (ADG code), Edition 7.5, National Transport Commission, Commonwealth of Australia.

Pharmakon (1986) Primary Eye Irritation [Analogue 1] (Study No. PH 421-ET-019-85, March, 1986). Pennsylvania, United States of America, Pharmakon Research International Inc. (Unpublished report submitted by the notifier).

SLI (1991) A 28-Day Oral Toxicity Study in Rats with a 14-Day Recovery Phase Using [Analogue 2] (Study No. PH 421-ET-019-85, March, 1986). Ohio, United States of America, Springborn Laboratories Inc. (Unpublished report submitted by the notifier).

Springborn (1986a) Acute Toxicity of [Analogue 1] to Sheepshead Minnow: Tier I Testing (Study No. 10823-0186-6100-500-514, April, 1986). Massachusetts, United States of America, Springborn Bionomics Inc. (Unpublished report submitted by the notifier).

Springborn (1986b) 96-Hour Static Renewal Toxicity Test: Tier I [Analogue 2] (Study No. 10823-0186-6100-500-515, April, 1986). Massachusetts, United States of America, Springborn Bionomics Inc. (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

Wilbury (1994a) Acute Toxicity of Water Accommodated Fractions (WAFs) of [Analogue 2] to the Fresh Water Alga, *Selenastrum capricornutum* (Study No. 73-CM-604, September, 1994). Massachusetts, United States of America, T.R. Wilbury Laboratories Inc. (Unpublished report submitted by the notifier).

Wilbury (1994b) Acute Toxicity of Water Accommodated Fractions (WAFs) of [Analogue 3] to the Fresh Water Alga, *Selenastrum capricornutum* (Study No. 73-CM-605, September, 1994). Massachusetts, United States of America, T.R. Wilbury Laboratories Inc. (Unpublished report submitted by the notifier).

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

Younger (1966) Toxicological Investigation of [Analogue 1] (Study No. Y-66-2, February, 1966). Missouri, United States of America, Younger Laboratories (Unpublished report submitted by the notifier).