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September 2014

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

EXP1200078

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.

For the purposes of subsection 78(1) of the Act, this Public Report may be inspected at our NICNAS office by appointment only at Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

This Public Report is also 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/1520	IMCD Australia Limited	EXP1200078	Yes	≤ 1,000 tonnes per annum	Industrial lubricating oils

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard classification	*Hazard statement*
Skin irritation (Category 2)	H315: Causes skin irritation
Skin sensitisation (Category 1)	H317: May cause an allergic skin reaction

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:

R38: Irritating to skin
R43: May cause sensitisation by skin contact

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 assessed use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Skin Irritation (Category 2): H315 – Causes skin irritation
  - Skin Sensitisation (Category 1): H317 – May cause an allergic skin reaction

The above should be used for products/mixtures containing the notified chemical, if applicable, based on the concentration of the notified chemical present and the intended use/exposure scenario.

Health Surveillance

- As the notified chemical is a skin sensitiser, employers should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of skin sensitisation.

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical:
  - Enclosed, automated processes, where possible

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

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical:
  - coveralls
  - impervious gloves
  - eye protection

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

- A copy of the (M)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 for the 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 unavailable or impracticable, dispose of the 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 industrial lubricating oils, or is likely to change significantly;
  - the amount of chemical being introduced has increased, or is likely to increase, significantly;
  - the chemical has begun to be manufactured in Australia;

- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

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

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
IMCD Australia Limited (ABN: 44 000 005 578)
1st Floor, 372 Wellington Rd
MULGRAVE, VIC 3170

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 use details.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: hydrolysis as a function of pH, dissociation constant, explosive and oxidising properties, and acute inhalation toxicity.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None.

NOTIFICATION IN OTHER COUNTRIES
Korea (2014)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
EXP1200078

MOLECULAR WEIGHT
UVCB substance

ANALYTICAL DATA
Reference NMR, IR, HPLC, LC-MS, ICP, UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
UVCB substance

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
The notified chemical (UVCB substance) is noted to contain a residual component at < 1% concentration, which is considered to be a category 3 reproductive toxicant (R62: Possible risk of impaired fertility; classification provided by the notifier).

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: very dark brown viscous liquid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	-16.8 ± 0.4 °C	Measured
Boiling Point	No boiling point or range could be determined	Measured (the test substance thermally degraded as the instrument block temperature approached 350 °C)
Density	1017.4 kg/m^3 at 20 ± 0.1 °C	Measured
Vapour Pressure	8.30 x 10^{-7} ± (5.41 x 10^{-8}) kPa at 20 ± 1 °C	Measured
Water Solubility	2.1 x 10^{-3} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Does not contain hydrolysable functionalities
Partition Coefficient (n-octanol/water)	≤-1.02 (4.41%) to ≥ 6.5 (66.3%)	Measured
Surface Tension	67.3 ± 0.5 mN/m at 20 ± 0.5 °C	Measured
Adsorption/Desorption	≤0.85 (2.83%) to ≥ 5.63 (22.7%)	Measured
Dissociation Constant	Not determined	The notified chemical is a salt, and therefore it is expected to be ionised in the environment
Flammability	Not hazardous in contact with water.	Measured
Autoignition Temperature	375 °C at 98.32 – 100.51 kPa	Measured
Explosive Properties	Not determined	Contains no functional groups that imply explosive properties
Oxidising Properties	Not determined	Contains no functional groups that imply oxidative 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 for the 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 be imported into Australia as part of finished lubricant oil products at < 20% concentration. The notified chemical may also be imported into Australia as part of additive products at < 80% concentration for reformulation into lubricant oils.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 500	< 1,000	< 1,000	< 1,000	< 1,000

IDENTITY OF MANUFACTURER/RECIPIENTS
IMCD Australia Limited

TRANSPORTATION AND PACKAGING
The additive products (containing the notified chemical at < 80% concentration) will be imported in either bulk quantity iso-containers or 205 L steel drums and will be shipped to distributor warehouses or directly to reformulation sites. Finished lubricant oil products (containing the notified chemical at < 20% concentration)

will be imported in various types of containers. Both the additive products and finished products containing the notified chemical will be transported by road within Australia.

USE
The notified chemical will be used at < 20% concentration in marine diesel cylinder lubricant engine oils.

OPERATION DESCRIPTION
Formulation of lubricant oils
At the reformulation sites, the imported additive products containing the notified chemical (at < 80% concentration) will be transferred into storage tanks via a drum transfer system using flexible hoses. The additive products could also be used directly from the drums. From these tanks (or drums, as applicable) the additive products will be transferred into blending tanks and will be blended with mineral oil and other components in a fully enclosed and automated system. Samples may be taken by laboratory staff prior to and after reformulation for quality control testing. The finished engine oils (containing < 20% notified chemical) will then be transferred into drums or appropriate containers via automated filling lines, then transferred to trucks for distribution to professional end-use sites.

End-use
At end-use sites, the finished marine lubricant engine oils containing the notified chemical at < 20% concentration will be transferred (by automated means) to vessel storage tanks and then to engines (as required), that function in a closed system.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

EXPOSURE DETAILS
Transport and storage
Transport and storage workers may come into contact with the notified chemical (at < 80% concentration) only in the unlikely event of accidental rupture of containers.

Reformulation of products
Dermal and ocular exposure to the notified chemical at < 80% concentration may occur during reformulation processes, particularly when connecting and disconnecting transfer hoses and when taking samples from blending vessels for quality assurance testing. Exposure is expected to be minimised through the use of enclosed, automated systems and through the use of personal protective equipment (PPE), such as impervious gloves, coveralls and safety glasses. Inhalation exposure to the notified chemical during reformulation processes is not expected due to the low vapour pressure of the notified chemical.

End use
There is potential for dermal and ocular exposure to the notified chemical at < 20% concentration by end user operators during transfer processes of the finished lubricant for use in marine engines. However, exposure is expected to be limited as the notifier has indicated that the engine oils will be transferred using automated systems. Any potential exposure may be further minimised by the use of PPE, such as gloves, safety glasses and protective clothing, as well as good hygiene practices. Again inhalation exposure is not expected due to the low vapour pressure of the notified chemical.

6.1.2. Public Exposure
The products containing the notified chemical are expected to be used in industrial settings only. No 'do-it-yourself' (DIY) applications of the finished marine engine oil products containing the notified chemical are intended. Therefore, given the proposed use pattern, public exposure is not expected except in the event of accidental release.

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 > 2,000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Skin irritation (in vitro)	irritating
Eye irritation (in vitro)	non-irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL = 1,000 mg/kg bw
Rat, repeat dose oral toxicity – 90 days.	NOAEL = 1,000 mg/kg bw
Mutagenicity – bacterial reverse mutation	equivocal
Genotoxicity – in vitro chromosome aberration	non genotoxic
Genotoxicity – in vitro gene mutation test	non genotoxic
Genotoxicity – in vivo micronucleus assay	non genotoxic
Rat, reproductive and developmental toxicity	NOAEL = 1,000 mg/kg bw

Toxicokinetics, metabolism and distribution.
The notified chemical is a UVCB substance and dermal absorption and absorption of components of the notified chemical across the GI tract may occur. However, absorption may be limited by the water solubility (2.1×10^{-3} g/L at 20 °C) and partition coefficient (66.3% log Pow > 6.5).

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

No data was available for acute toxicity via the inhalation route.

Irritation.
The notified chemical was found to be irritating in an *in vitro* skin irritation study using the Reconstructed Human Epidermis Test Method (EpiSkin), yielding a relative mean viability of 33%.

The eye irritation potential of the notified chemical was examined in an acute eye irritation and corrosion study in rabbits and an *in vitro* Bovine Corneal Opacity and Permeability Test. The notified chemical was not irritating under the condition of the *in vitro* test and was slightly irritating in the rabbit study. Conjunctival irritation was seen in all animals, with the effects subsiding by the end of the observation period (day 10). The irritation scores did not warrant classification of the chemical as an eye irritant.

Sensitisation.
The notified chemical was found to be a sensitiser in a Local Lymph Node Assay (LLNA) study in mice. Concentrations of 25, 50 and 100% of the notified chemical were tested, producing stimulation indices of 2.2, 8.3 and 8.4, respectively. An EC3 of 28.3% was derived.

Repeated dose toxicity.
In 28-day and 90-day repeated dose oral toxicity studies, the notified chemical was tested in rats at doses of 100, 300 and 1,000 mg/kg bw/day, with the NO(A)EL established by the study authors as 1,000 mg/kg bw/day.

In the 28-day study, the main clinical sign observed was clear material around the mouth. Various haematological effects were seen in animals of both sexes, e.g. decreased mean absolute neutrophil counts (low and mid-dose group males) and elevated mean white blood cell and mean absolute lymphocyte counts (high dose males and females). However, in general, the study authors deemed these observations as not having a dose-response relationship or not being statistically significant (and attributed them to individual animal biological variation).

At necropsy, higher mean absolute and relative liver weights were noted in animals of both sexes in the high dose group (statistically significant in males). However, these changes were considered non-adverse adaptive responses by the study authors, as there were no correlating clinical pathology or histologic changes noted and all individual animal absolute liver weight values fell within the study centre's historical database range of

values (with the exception of one high dose group female animal). A lower mean relative seminal vesicle weight (statistically significant) was noted in high dose males, but the statistical significance was not reached for the absolute weight or weight relative to the brain weight and as all individual animal absolute vesicle weight values fell within the study centre's historical database range of values in conjunction with a lack of correlated histologic changes, the study authors considered the decrease to be of no toxicological importance.

In the 90-day study, one female animal from the highest dose group died on day 50 (the animal did not show any clinical signs prior to death). The cause of death was considered to be acute pulmonary haemorrhage, with the study authors considering that this may have been due to inhalation of the test substance.

The main clinical sign of significance was the observation of clear and/or red material on various body surfaces (mouth, nose and/or ventral neck) in several mid and high dose test animals (most prevalent in the latter part of the study).There were several effects seen on blood chemistry parameters, e.g. high dose group females had statistically significantly higher mean corpuscular volume and mean corpuscular haemoglobin values and mean activated partial thromboplastin time was shorter (statistically significant) in the low and high dose group males compared to control animals. Various serum effects were also seen in animals of the high dose group, e.g. decreased alanine aminotransferase (females) and aminotransfersase (males and females; statistically significant in females) values relative to the control group results. Overall, any alterations were considered by the study authors to be not test substance related or of no toxicological importance.

At necropsy, higher mean absolute and relative (to final body weight and brain weight) liver weights were noted in animals of both sexes in the high dose group. Statistical significance was only reached for the male animals in mean liver weight relative to final body weight, and the study authors again noted that all values fell within the study centre's historical group mean control range. Microscopic examination revealed hepatocyte hypertrophy in the portal areas of the hepatic lobules at grade 1 (minimal) severity in all ten males and eight females of the high dose group. The female that died on day 50 showed grade 2 hepatocyte hypertrophy (mild) at necropsy. Although no control group animals presented with this effect, it was not considered by the study authors to be an adverse finding.

Mutagenicity/Genotoxicity.
In a bacterial reverse mutation assay, a 2.7 fold increase in revertant counts was noted with the tester strain TA1537 in the presence of metabolic activation (initial assay). However, the study authors did not consider this observation as indicative of mutagenic activity as the response showed no dose-dependent relationship, was not reproduced in the confirmatory assay and the plate count fell within the study centre's historical vehicle control range.

Equivocal results were obtained in an *in vitro* chromosome aberration assay, with statistically significant increases in the number of cells with structural aberrations noted in both the presence and absence of metabolic activation. However, no dose-response relationship was evident.

Negative results were obtained in an *in vitro* cell gene mutation test and an *in vivo* micronucleus assay.

Toxicity for reproduction.
The notified chemical (UVCB substance) is noted to contain a residual component at < 1% concentration, which is considered to be a category 3 reproductive toxicant (R62: Possible risk of impaired fertility; classification provided by the notifier). The notified chemical was tested in a reproduction/developmental toxicity screening study in rats at test concentrations 100, 300 and 1,000 mg/kg bw/day. The notifier indicated that the level of this hazardous residual component in the substance tested in this study was < 1%. The NO(A)EL for the notified chemical was established as 1,000 mg/kg bw/day for reproductive toxicity and maternal and neonatal systemic toxicity in this study.

Similar test substance related clinical signs were noted in treated animals (e.g. clear material around the mouth) and were deemed by the study authors to be non-adverse. No treatment related effects on reproductive performance were reported. At necropsy, higher mean liver weights (for absolute values and relative to both final body weight and brain weights) were noted in the high dose group adult animals of both sexes. These changes were dismissed by the study authors as being incidental and toxicologically unimportant (microscopic examination not performed), as they considered the increases to be of minimal magnitude and lacking correlating adverse clinical pathology or histologic changes (based on the abovementioned repeat dose toxicity studies on the notified chemical).

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

Hazard classification	*Hazard statement*
Skin irritation (Category 2)	H315: Causes skin irritation
Skin sensitisation (Category 1)	H317: May cause an allergic skin reaction

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase(s):
R38: Irritating to skin
R43: May cause sensitisation by skin contact

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Exposure of workers to the notified chemical during reformulation processes (at < 80% concentration) and to a much more limited extent, during end-use as a component of marine lubricant engine oils (< 20% notified chemical) may occur via the dermal and ocular routes. Based on the available studies, the notified chemical is considered to be a skin irritant and skin sensitiser. Slight irritation was also noted in an eye irritation study. While the toxicity of the notified chemical by the inhalation route is not known, inhalation exposure is not expected under the proposed use. Systemic toxicity effects from repeated exposure to the notified chemical are also not expected based on the proposed use. Overall, based on the identified hazards, skin and eye contact should be avoided during reformulation and end-use processes.

Therefore, provided that measures are in place to minimise worker exposure, including the use of enclosed and automated processes (where possible), and use of appropriate PPE (such as protective clothing, impervious gloves and eye protection), the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

6.3.2. Public Health
The products containing the notified chemical are intended to be used in industrial settings only, hence public exposure is not expected. Therefore, 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 manufactured overseas and will either be imported in finished lubricants or as a component of additive products that will be reformulated locally into lubricant oils (engine oils). Local blending or repackaging will be performed in an enclosed system, therefore spills from this activity are expected to be minimal. It is expected that any spillage will be contained with earth or sand and be disposed of according to State/Territory regulations. The notified chemical is not expected to be released to water drains or site sewer systems. The drumming/re-packing of the finished lubricant products containing the notified chemical into drums/iso-containers will be via an automated process. Any spill/leak is expected to be contained and be sent to an approved industrial facility for appropriate disposal. Empty containers, transfer hoses, pipelines, and pumps are expected to be air/nitrogen blown dry, and/or cleaned by flushing through with mineral base oil. Waste oils generated after cleaning are expected to be disposed of according to State/Territory regulations.

RELEASE OF CHEMICAL FROM USE

The marine lubricants, engine oils, containing the notified chemical will be poured into marine engines, which are closed systems, in industrial settings. The notifier indicated that there will be no do-it-yourself (DIY) application for the lubricant oils containing the notified chemical. Release of the notified chemical from professional activities is expected to be limited by the requirement for appropriate disposal of waste oil according to State/Territory regulations.

RELEASE OF CHEMICAL FROM DISPOSAL

Products containing the notified chemical are expected to be disposed of in accordance with State/Territory regulations. Consequently, the notified chemical is expected to be recycled, re-refined or used as low grade burner fuel. It is likely that the notified chemical will be degraded into simpler compounds during re-refining with any residue partitioning to the heavy fractions such as lubricating oils or asphalt. Similarly, during metal recycling of automotive components with chemical residues, the notified chemical is expected to be completely combusted.

7.1.2. Environmental Fate

A study submitted by the notifier indicates that the notified chemical is not considered to be readily biodegradable. For the details of the environmental fate study please refer to Appendix C. The notified chemical is not expected to be bioaccumulative or bioavailable to aquatic organisms due to its low water solubility and anticipated limited release to the aquatic environment. Most of the notified chemical will be either thermally decomposed during use, recycling or refinement. Notified chemical disposed of to landfill is expected to sorb strongly to soil and sediment. The notified chemical is expected to be degraded into water and oxides of carbon by thermal decomposition in industrial facilities.

7.1.3. Predicted Environmental Concentration (PEC)

The notifier stated that 100% of the notified chemical will be used in marine diesel lubricant (engine oil). The notifier also indicated that the diesel lubricant will not be used by DIY users. Therefore, the notified chemical is not anticipated to be released to the aquatic environment through improper disposal by DIY users. Release of the notified chemical to the marine environment through leakage is not expected under normal operating conditions. The predicted environmental concentration (PEC) has not been calculated since no significant release of the notified chemical to the aquatic environment is expected from the reported use pattern.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity (96 h)	LL50 > 100 mg/L* (filtered WAF)	Not harmful to fish up to its water solubility limit
Daphnia Toxicity (48 h)	EL50 > 100 mg/L* (filtered WAF)	Not harmful to aquatic invertebrates up to its water solubility limit

Algal Toxicity (72 h)	E_rL50 > 100 mg/L* (filtered WAF)	Not harmful to algae up to its water solubility limit
Inhibition of Bacterial Respiration	EC50 (3 h) > 1000 mg/L	Not inhibitory to microbial respiration up to its water solubility limit

* Water accommodated fraction

Based on the above reported endpoints for the notified chemical, it is not considered to be harmful to fish, daphnia and algae. Therefore, the notified chemical is not harmful to aquatic organisms. Consequently, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009), the notified chemical has not been formally classified for acute and chronic toxicities.

7.2.1. Predicted No-Effect Concentration

A predicted no-effect concentration (PNEC) for the aquatic compartment has not been calculated since the notified chemical is not considered to be harmful up to the limit of its solubility in water and no significant aquatic exposure is expected based on the reported use pattern.

7.3. Environmental Risk Assessment

The risk quotient, Q (= PEC/PNEC), of the notified chemical has not been determined due to its low potential for release to the aquatic compartment. The majority of the notified chemical will be thermally decomposed during its use as an additive in oils. Engine oil containing the notified chemical, after its useful life, is expected to be disposed of according to State/Territory regulations. Exposure of the notified chemical to the aquatic compartment is unlikely based on the reported use pattern. On the basis of its limited aquatic exposure and assessed use pattern, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point -16.8 ± 0.4 °C

Method	OECD TG 102 Melting Point/Melting Range.
Remarks	Determined using a dry ice/acetone bath
Test Facility	Wildlife International (2013a)

Boiling Point No boiling point or range could be determined

Method	OECD TG 103 Boiling Point.
Remarks	Modified Siwoloboff procedure In the approximation test, a measurable boiling point of 310 °C and a boiling range of 200 – 310 °C were determined. In the definitive test, bubbles were initially observed at 178.3-184.1 °C. However, on continued heating significant colour changes in the test substance were noted, with the test substance thermally degraded as the instrument block temperature approached 350 °C.
Test Facility	Wildlife International (2013b)

Density 1017.4 kg/m^3 at 20 ± 0.1 °C

Method	OECD TG 109 Density of Liquids and Solids.
Remarks	Determined using a pycnometer. The mean specific gravity was determined as 1.0192.
Test Facility	Wildlife International (2013c)

Vapour Pressure $8.30 \times 10^{-7} \pm (5.41 \times 10^{-8})$ kPa at 20 ± 1°C

Method	OECD TG 104 Vapour Pressure.
Remarks	Spinning rotor gauge method A molecular weight of 600 g/mol was used in the calculations. Measured vapour pressure ranged from 7.92×10^{-7} to 8.92×10^{-7} kPa.
Test Facility	Wildlife International (2013d)

Water Solubility 2.1×10^{-3} g/L at 20 °C

Method	OECD TG 105 Water Solubility.
Remarks	Column Elution Method. The mean measured water solubility of the test substance at 20 °C was 1.91 ± 0.08 mg/L (CV = 4.2%, N = 5) at a flow rate of approximately 1.0 mL/min and 2.28 ± 0.18 mg/L (CV = 7.9%, N = 5) at a flow rate of approximately 0.5 mL/min. The mean concentration of the test substance differed by 17.5%, between the two different flow rates, which was well within the ± 30% test criterion. The overall mean measured water solubility at 20 °C was 2.1 ± 0.2 mg/L (CV = 9.5%, N = 10).
Test Facility	Wildlife International (2013e)

Partition Coefficient (n-octanol/water) log Pow = < -1.02 (4.41%) to > 6.5 (66.3%)

Method	OECD TG 117: Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method
Remarks	HPLC Method. The column retention times for some of the components of the notified chemical are shorter or longer than that for the standards (chemicals) with the shortest and longest retention time.
Test Facility	Wildlife International (2013f)

Surface Tension 67.3 ± 0.5 mN/m at 20 ± 0.5 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions
Remarks	Determined using a surface tensiometer at 2.2 mg/L (~90% of the water solubility). The study authors noted that the test substance may have produced micelles in the aqueous

medium.
Test Facility Wildlife International (2013g)

Adsorption/Desorption – screening test log K_{oc} = < 0.85 (2.83%) to > 5.63 (22.7%)

Method OECD TG 121: Estimation of the Adsorption Coefficient (K_o) on soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)
Remarks HPLC Method. The column retention times for some of the components of the notified chemical are shorter or longer than that for the standards (chemicals) with the shortest and longest retention time.
Test Facility Wildlife International (2013h)

Flash Point 172.2 °C at 102.8 kPa

Method EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks Determined using Pensky-Martens closed cup apparatus
Test Facility Wildlife International (2013i)

Flammability Not hazardous in contact with water

METHOD EC Council Regulation No 440/2008 A.12 Flammability (Contact with Water).
Remarks No reaction or gas evolution observed on contact with water
Test Facility Wildlife International (2013j)

Autoignition Temperature 375 °C at 98.32 – 100.51 kPa

Method EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks Test conducted in triplicate using a commercially available apparatus.
Test Facility WIL Research (2014a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Albino Rat/ Crl:CD(SD)
Vehicle	Mineral oil
Remarks - Method	No significant protocol deviations. GLP Certificate.

RESULTS

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

LD50	> 2,000 mg/kg bw
Signs of Toxicity	No clinical observations were noted during the study.
Effects in Organs	There were no macroscopic findings observed at necropsy.
Remarks - Results	There were no mortalities or remarkable body weight changes noted during the study.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	WIL Research (2013a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity.
Species/Strain	Albino Rat/Crl:CD(SD)
Vehicle	Used as supplied
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations. GLP Certificate.

RESULTS

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

LD50	> 2,000 mg/kg bw
Signs of Toxicity - Local	Dermal findings noted during the study consisted of very slight (grade 1) to slight (grade 2) erythema and/or desquamation (3/10 animals). All dermal symptoms had resolved by day 8 and for the remainder of the observation period.
Signs of Toxicity - Systemic	There were no systemic clinical findings observed during the study.
Effects in Organs	There were no macroscopic findings observed at necropsy.
Remarks - Results	There were no mortalities or remarkable body weight changes noted during the study. Most female test animals (4/5) had very slight weight losses (1 g) from day 0 to day 7. However, these animals gained weight the following week.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.

TEST FACILITY WIL Research (2013b)

B.3. Irritation – skin (in vitro)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 439 In vitro Skin Irritation: Reconstructed Human *Epidermis* Test Method
EC Council Regulation No 440/2008 B.46. In vitro Skin Irritation – Reconstructed Human Epidermis Model Test

Vehicle None

Remarks - Method GLP Certificate.
The test substance (25 µL) was applied to the tissues in triplicate. Following 15 minute exposure periods, the tissues were rinsed and then incubated at 37 °C for approximately 42 hours.

Following treatment with MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 0.3 mg/mL], the tissues were incubated at 37 °C for 3 hours.

Positive (sodium dodecyl sulphate; 5%) and negative (phosphate buffered saline) controls were run in parallel with the test substance. As the test substance was shown to directly reduce MTT, additional controls were included to detect and correct for the test substance interference

The optical densities were determined at 570 nm.

RESULTS

Test material	*Mean OD_{570} of triplicate tissues (SD)*	*Relative mean Viability (%)*
Negative control	1.069 (0.113)	100*
Test substance	0.353 (0.151)	33
Positive control	0.207 (0.130)	19

OD = optical density; SD = standard deviation
*The mean viability of the negative control tissues is set as 100%.

Remarks - Results The non-specific reduction of MTT (NSMTT) by the test substance was 49% of the negative control tissues. The study authors noted that the NSMTT fell outside the acceptability criteria of the assay and hence was not taken into account for the determination of the viability of the test substance treated skin tissues.

The relative mean viability of the test substance treated tissues was 33% after a 15-minute exposure period (without correction for the NSMTT).

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

CONCLUSION The notified chemical was irritating to the skin under the conditions of the test.

TEST FACILITY WIL Research (2013c)

B.4. Irritation – eye (in vitro)

TEST SUBSTANCE Notified chemical

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

Vehicle None

Remarks - Method
No significant protocol deviations.
GLP Certificate.
Physiological saline was used as a negative control and Benzalkonium Chloride (10% w/v) was used as a positive control in the study.

RESULTS

Test material	*Mean opacities of triplicate tissues*	*Mean permeabilities of triplicate tissues*	*IVIS*
Vehicle control	-1	0.000	-1.0
*Test substance**	0	0.015	0.2
*Positive control**	83	2.780	125

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

Remarks - Results
The mean in vitro irritancy score was determined as 0.2 after 10 minutes of treatment.

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

CONCLUSION
The notified chemical was not irritating under the conditions of the test.

TEST FACILITY
WIL Research (2013d)

B.5. Irritation – eye

TEST SUBSTANCE
Notified chemical

METHOD
OECD TG 405 Acute Eye Irritation/Corrosion.
EPA OPPTS Guideline 870.2400 (1998)
Species/Strain
Rabbit/New Zealand White [Hra: (NZW)SPF]
Number of Animals
3M
Observation Period
10 days
Remarks - Method
No significant protocol deviations.
GLP Certificate.

Prior to the test substance administration (~1 hour), buprenorphine (0.01 mg/kg was administered by subcutaneous injection. In addition, ~5 minutes prior to administration, proparacaine hydrochloride (0.5%; 1 drop) was applied to each eye. Following test substance administration (~8 hours) and at subsequent 12- and/or 24-hour intervals buprenorphine and meloxicam were administered subcutaneously, until all signs of irritation and/or animal distress had resolved.

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	1.33	1	1	2	< 10 days	0
Conjunctiva: chemosis	0	0	0.33	1	< 48 hours	0
Conjunctiva: discharge	0	0	0	1	< 24 hours	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

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

Remarks - Results
Conjunctival irritation was noted in all test animals. Positive (grade 2)

irritation was seen in one animal 24 hours post dosing. The other two animals were noted with grade 1 irritation. All ocular irritation had subsided by day 10.

A light brown staining of the hair surrounding the eye was seen in one animal from 24 hours post instillation up to day 4. In addition, dried light brown material (residual test substance) around the eye was seen in two animals from 24 hours post instillation up to day 4.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY WIL Research (2014b)

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

TEST SUBSTANCE Notified chemical

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)
EPA OPPTS 870.2600 Skin Sensitisation (2003)

Species/Strain Mouse/CBA/J
Vehicle Methyl ethyl ketone
Remarks - Method No significant protocol deviations.
GLP Certificate.

A pre-screen test was conducted where no irritation or signs of systemic toxicity were observed in any of the test animals.

No concurrent positive control test was run, however, it had been previously conducted in the test laboratory using α-Hexylcinnamaldehyde.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/animal)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	514	-
25	1,119	2.2
50	4,266	8.3
100	4,292	8.4

Remarks - Results No irritation of the ears (erythema) was observed in any of the test animals. No test substance related mortalities occurred and no clinical signs of toxicity were observed (1 animal in the low dose group died prior to radiolabel injection). The majority of auricular lymph nodes examined were noted by the study authors to be enlarged, especially in animals of the 50% and 100% concentration test groups. An EC3 value of 28.3% was calculated.

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

TEST FACILITY WIL Research (2013e)

B.7. Repeat dose toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.

	EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/Crl:CD(SD)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week
Vehicle	Mineral oil
Remarks - Method	No significant protocol deviations. GLP Certificate.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 per sex	0	1/10
low dose	5 per sex	100	0/10
mid dose	5 per sex	300	0/10
high dose	5 per sex	1,000	0/10

Mortality and Time to Death

A single control group male died during blood collection procedures for clinical pathology on day 28 of the study. There were no other unscheduled deaths in this study.

Clinical Observations

Clear material around the mouth was noted in animals of both sexes in the high dose group. This was seen 1 to 2 hours post dosing beginning on day 11 in males and at the time of dosing and 1 to 2 hours post-dosing beginning on day 8 in females. All other clinical findings in treated group animals were not considered by the study authors to be toxicologically significant as they were not statistically significant, were noted in similar incidence in the control group, were limited to single animals, the incidence showed no dose relationship and/or the signs were common to the strain and age of test animal.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Lower (statistically significant) mean absolute neutrophil counts were seen in the low and mid dose group males. However, the study authors did not correlate a clear dose-related response in these findings. Mean white blood cell and mean absolute lymphocyte counts were slightly elevated for males and females in the high dose group, although these observations were not statistically significant and the study authors attributed them to individual animal biological variation. Statistically significant findings for percentage leukocyte differential counts were discounted by the study authors as not toxicologically important as the absolute cell counts were considered more relevant for interpretative purposes.

Effects in Organs

Higher mean absolute and relative liver weights were noted in animals of both sexes in the high dose group (statistically significant in males). Liver weight changes were considered by the study authors as non-adverse adaptive responses, as there were no correlating clinical pathology or histologic changes noted and all individual animal absolute liver weight values fell within the study centre's historical database range of values, with the exception of one high dose group female animal.

Mean relative seminal vesicle weight was statistically significantly decreased in males of the high dose group, but the statistical significance was not reached for the absolute weight, or weight relative to the brain weight. In addition, as all individual animal absolute vesicle weight values fell within the study centre's historical database range of values in conjunction with a lack of correlated histologic changes, the study authors considered the decrease to be of no toxicological importance.

No test substance-related histologic changes were reported.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established by the study authors as 1,000 mg/kg bw/day in this study.

TEST FACILITY WIL Research (2013f)

B.8. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents. EC Directive 88/302/EEC B.26 Sub-Chronic Oral Toxicity Test: 90-Day Repeated Oral Dose Study using Rodent Species.
Species/Strain	Rat/ Crl: CD(SD)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 90 - 91 days Dose regimen: 7 days per week
Vehicle	Mineral oil
Remarks - Method	No significant protocol deviations. GLP Certificate.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10 per sex	0	0/20
low dose	10 per sex	100	0/20
mid dose	10 per sex	300	0/20
high dose	10 per sex	1,000	1/20

Mortality and Time to Death

A single high dose group female died on day 50 of the study. The animal was examined microscopically with the cause of death determined as acute pulmonary haemorrhage. The study authors considered that this was potentially associated with inhalation of the test substance. The animal did not show any significant clinical observations prior to death. There were no other unscheduled deaths in this study

Clinical Observations

Clear and/or red material was noted on various body surfaces (mouth, nose and/or ventral neck) in several test animals. Mid dose group males exhibited these signs initially on day 39 of the study period, but primarily in the latter part of the study, developing 1 to 2 hours post dosing. High dose group animals of both genders also exhibited these signs, first noted on day 4, but again noted to be most prevalent in the latter part of the study.

All other clinical findings in test group animals were dismissed by the study authors for various reasons, including not being of statistical significance, signs occurring with similar incidence as the control group animals, they were limited to single animals and/or were common findings for the age and strain of animal.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Lower (statistically significant) mean values for red blood cell (RBC) counts were noted in the low and mid dose group males, compared to the control animals. High dose group females had statistically significantly higher mean corpuscular volume and mean corpuscular haemoglobin values. Mean activated partial thromboplastin time was shorter (statistically significant) in the low and high dose group males compared to control animals. While these various changes were considered statistically significant, the study authors stated that all values fell within the study centre's historical group mean control ranges. Overall, the study authors did not deem any alterations in haematology and coagulation parameters to be test substance related.

Various serum effects were seen in females of the high dose group, including decreased alanine aminotransferase and aminotransfersase (AST; statistically significant) values relative to the control group results. AST decreases were also seen in males of that group, but it did not reach statistical significance. Low dose group males also exhibited statistically significantly increased mean potassium levels relative to the control group. As all relevant values for animals of both sexes fell within the study centre's historical group mean control range, the findings were considered to be of no toxicological significance.

There were no statistically significant changes in urinalysis parameters in test animals of any dose group noted by the study authors.

Effects in Organs

No gross necropsy observations were considered by the study authors to be direct effects of the test substance.

The liver was the main organ showing variance in weight at examination. Higher mean absolute and relative (to final body weight and brain weight) liver weights were noted in animals of both sexes in the high dose group. Statistical significance was only reached for the male animals in mean liver weight relative to final body weight, and the study authors again noted that all values fell within the study centre's historical group mean control range.

Microscopic examination revealed hepatocyte hypertrophy in the portal areas of the hepatic lobules at grade 1 (minimal) severity in all ten males and eight females of the high dose group. The high dose group female animal that died on day 50 of the study showed grade 2 hepatocyte hypertrophy (mild) at necropsy. The remaining high dose group female was considered within the normal limits. No control group animals presented with this effect. Despite this, the study authors did not deem hepatocyte hypertrophy to be an adverse finding.

Histologic examination was, in general, not conducted for the mid and low dose group animals (only gross lesions were examined microscopically).

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established by the study authors as 1,000 mg/kg bw/day in this study.

TEST FACILITY WIL Research (2013g)

B.9. Genotoxicity – bacteria

TEST SUBSTANCE

METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 fraction from Aroclor 1254-induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate b) Without metabolic activation: 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate
Vehicle	Tetrahydrofuran (THF)
Remarks - Method	No significant protocol deviations. GLP Certificate. Test 1 (initial toxicity-mutation assay) was treated as a dose-finding test. Tests 1 and 2 were conducted on separate days. The concentration range was amended in Test 2 (50-5000 µg/plate) Vehicle and positive controls were used in parallel with the test material. Positive controls: i) without S9: 2-nitrofluorene (TA98), sodium azide (TA100, TA1535), 9-aminoacridine (TA1537) and methyl methanesulfonate (WP2uvrA); ii) with S9: 2-aminoanthracene (TA98, TA100, TA1535, TA1537, WP2uvrA$^-$). All positive controls were prepared in dimethyl sulphoxide (DMSO), except sodium azide which was diluted in water.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*		
	Cytotoxicity in Main Test	*Precipitation*	*Genotoxic Effect*

Absent			
Test 1	> 5,000	≥ 1,500	negative
Test 2	> 5,000	≥ 1,500	negative
Present			
Test 1	> 5,000	≥ 1,500	negative
Test 2	> 5,000	≥ 1,500	negative

Remarks - Results

In the initial toxicity-mutation assay, no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. There was a 2.7 fold increase in revertant counts with the tester strain TA1537 in the presence of S9 activation. However, the study authors did not consider this observation as indicative of mutagenic activity as the response showed no dose-dependent relationship, was not reproduced in test 2 and the plate count fell within the study centre's historical vehicle control range.

In the confirmatory mutagenicity assay, no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation.

CONCLUSION

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

TEST FACILITY

BioReliance (2013a)

B.10. Genotoxicity – in vitro

TEST SUBSTANCE

Notified chemical

METHOD

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

Species/Strain: Human
Cell Type/Cell Line: Peripheral blood lymphocytes (HPBL)
Metabolic Activation System: S9 fraction from Aroclor 1254-induced rat liver
Vehicle: Tetrahydrofuran (THF)
Remarks - Method: No significant protocol deviations.
GLP Certificate.
A preliminary toxicity study was performed (4 hr exposure, with and without activation and 20 hr exposure without activation) at concentrations 0.25-2,500 µg/mL. Based on this, dose levels of 2.5, 5, 10, 25, 50, 75 and 100 µg/mL were selected for the main tests. Precipitate was seen in the preliminary test ≥ 75 µg/mL.

Vehicle and positive controls (mitomycin C without metabolic activation and cyclophosphamide with metabolic activation) were used in parallel with the test material.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	2.5, 5, 10*, 25*, 50*, 75, 100	4 h	20 h
Test 2	2.5, 5, 10*, 25*, 50*, 75, 100	20 h	20 h
Present			
Test 1	2.5, 5, 10*, 25*, 50*, 75, 100	4 h	20 h

*Cultures selected for metaphase analysis.

RESULTS

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

Activation	*Cytotoxicity in Preliminary Test*	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 2,500	> 50	≥ 50	Equivocal
Test 2	≥ 2,500	> 50	≥ 50	Equivocal
Present				
Test 1	≥ 2,500	> 50	≥ 50	Equivocal

Remarks - Results

Statistically significant increases in the number of cells with structural aberrations were noted in all three treatment groups (at 10 µg/mL in Test 1, with and without activation, and at 50 µg/mL in Test 2), however, the study authors did not deem the increases to exhibit dose-response relationships.

In order to increase the statistical power of the data analysis, additional metaphases were scored, with all data pulled together for analysis. Statistically significant increases in the number of cells with structural aberrations were noted at 10 µg/mL in Test 1 (with activation) and at 50 µg/mL in Test 2. However, no dose-response relationship was evident.

The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.

CONCLUSION

The results for the notified chemical were equivocal for clastogenicity to human lymphocytes treated in vitro under the conditions of the test.

TEST FACILITY

BioReliance (2013b)

B.11. Genotoxicity – in vitro

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 476 In vitro Mammalian Cell Gene Mutation Test..

Species/Strain: Mouse/ Sprague-Dawley

Cell Type/Cell Line: Lymphoma/ L5178Y/TK$^{+/-}$

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

Vehicle: Tetrahydrofuran (THF)

Remarks - Method:

No significant protocol deviations.

GLP Certificate.

A preliminary toxicity study was performed (4 hr exposure, with and without activation and 24 hr exposure without activation) at concentrations 0.5 - 2,000 µg/mL. Based on this, dose levels of 2.5 - 200 µg/mL were selected for the main tests. Precipitate was seen in the preliminary test ≥ 150 µg/mL.

Vehicle and positive controls (Methyl methanesulfonate (MMS) without metabolic activation and 7,12-dimethylbenz(a)anthracene (DMBA) with metabolic activation) were used in parallel with the test material.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	2.5, 5*, 10*, 25*, 50*, 75*, 100, 125, 150, 175, 200	4 h	2 days	10-14 days
Test 2	2.5, 5*, 10*, 25*, 50*, 75*, 100, 125, 150, 175, 200	24 h	2 days	10-14 days
Present				
Test 1	2.5, 5*, 10*, 25*, 50*, 75*, 100, 125, 150, 175, 200	4 h	2 days	10-14 days

*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	≥ 500	> 75	≥ 75	negative
Test 2	≥ 150	> 75	≥ 75	negative
Present				
Test 1	≥ 50	> 75	≥ 75	negative
Test 2	-	-	-	-

Remarks - Results: There was no concentration-related increase in mutant frequency in cultures with or without metabolic activation.

The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.

CONCLUSION: The notified chemical was not clastogenic to mouse lymphoma L5178Y cells treated in vitro under the conditions of the test.

TEST FACILITY: BioReliance (2013c)

B.12. Genotoxicity – in vivo

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
EC Directive 2000/32/EC B.12 Mutagenicity - Mammalian Erythrocyte Micronucleus Test.

Species/Strain: Rat/Sprague-Dawley (Hsd:SD)
Route of Administration: Oral – gavage
Vehicle: Mineral oil
Remarks - Method: No significant protocol deviations.
GLP Certificate.

The study was performed using male rats only.

Vehicle and positive controls (cyclophosphamide monohydrate) were used in parallel with the test material.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	10 M	0	24 and 48
II (test material)	10 M	2,000	24 and 48
III (positive control: CP)	5 M	40	24

CP=cyclophosphamide monohydrate

RESULTS

Doses Producing Toxicity: > 2,000 mg/kg bw
Genotoxic Effects: No significant increases in the frequency of micronucleated polychromatic erythrocyte cells were recorded.
Remarks - Results: No mortalities occurred during the study and all animals appeared normal throughout the observation period.
The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.

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

TEST FACILITY BioReliance (2014)

B.13. Developmental toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 421 Reproduction/Developmental Toxicity Screening Test.
Species/Strain Rats/ Crl:CD(SD)
Route of Administration Oral – gavage
Exposure Information Exposure days: up to 28 days for males and up to 66 days for females
Post-exposure observation period: None
Vehicle Mineral oil
Remarks - Method No significant protocol deviations.
GLP Certificate.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	12 per sex	0	0/24
Low	12 per sex	100	0/24
Mid	12 per sex	300	0/24
high	12 per sex	1,000	0/24

Mortality and Time to Death
There were no unscheduled deaths of adult test animals during the study.

Pups from each group were found dead in the post natal period before the scheduled euthanasia. Deceased animals included three animals from the control group, compared to six animals, two animals and four animals from the low, mid and high dose groups respectively. Two animals from the low dose group and five from the high dose group were missing by day 4 post natal. These animals were presumed to have been cannibalized.

Clinical Observations
Some test substance related clinical signs were noted for animals of both sexes in the mid and high dose groups. Clear material around the mouth was noted in eleven males and all twelve females in the high dose group and five males and six females of the mid dose group. This effect was generally noticed one to two hours following daily dosing and persisted over the treatment period. Incidences of red material around the mouth were also noted. These clinical findings were deemed by the study authors to be non-adverse.

Other clinical observations, including red material around the eye and hair loss on various body surfaces, occurred infrequently, lacked a dose response relationship or occurred at similar frequencies in the control group animals.

There were no changes or effects deemed to be adverse by the study authors with respect to adult mean body weights or weight gains and food consumption detected in male or female test animals throughout the respective pre-mating, gestation or lactation treatment periods.

With respect to the offspring, general physical condition, mean offspring body weights and body weight gains were deemed unaffected by parental test substance administration in all groups.

Effects on Reproductive Performance
The study authors did not find any treatment related effects on reproductive performance of the test animals, with dose group animals showing similar results to control group animals in the mean number of days between pairing and coitus, female conception and male copulation rates. The gestation lengths of the test group animals were comparable to the control animals. No signs of dystocia were noted in these groups.

Mean number of pups born and live litter size in the test group animals were comparable to the control group values. Both post natal survival and sex ratio were unaffected in all dose groups, with values comparable to

controls.

Effects on Organs

For the offspring, the study authors did not note any macroscopic findings that they considered to indicate adverse effect of maternal treatment, with the only finding of note being the absence or presence of milk in the stomach.

With respect to the adults, no macroscopic findings or effects on implantation sites and corpora lutea were deemed test substance related as the differences between test group and control animals were slight and not statistically significant.

Higher mean liver weights (for absolute values and relative to both final body weight and brain weights) were noted in the high dose group animals of both sexes (statistically significant in females). These changes were dismissed by the study authors as being incidental and toxicologically unimportant findings (microscopic examination not performed), as they considered the increases to be of minimal magnitude and lacking correlating adverse clinical pathology or histologic changes (based on the abovementioned repeat dose toxicity studies on the notified chemical).

Histopathological inspection of the organs did not reveal any microscopic observations in test animals of any dosage level that were considered by the study authors to be statistically significant or treatment related.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1,000 mg/kg bw/day for reproductive toxicity and maternal and neonatal systemic toxicity in this study.

TEST FACILITY WIL Research (2013h)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B: Ready Biodegradability: CO_2 Evolution Test.
Inoculum	Activated Sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	TOC-5050A Total Organic Carbon (TOC) Analyser
Remarks - Method	No significant protocol deviations. GLP Certificate.

RESULTS

Test substance		*Sodium benzoate*	
% Degradation ($ThCO_2$)	*Day*	*% Degradation ($ThCO_2$)*	*Day*
28	19.1	14	98.5

Remarks - Results	All validity criteria for the test were satisfied. The reference compound, sodium benzoate, reached the 60% pass level by day 3 indicating the suitability of the inoculum. It was not mentioned in the study that a toxicity control was included. The degree of degradation of the notified chemical after the cultivation period was 19.1%. Therefore, the test substance is classified as not readily biodegradable according to the OECD (301 B) guideline.
CONCLUSION	The notified chemical is not readily biodegradable
TEST FACILITY	Wildlife International (2013k)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test –Static Test
Species	Fathead minnow (*Pimephales promelas*)
Exposure Period	96 hours
Auxiliary Solvent	Not reported
Water Hardness	128 mg $CaCo_3$/L
Analytical Monitoring	Analysis of the test substance was not performed
Remarks – Method	No significant protocol deviations. GLP Certificate. The fish ecotoxicity test was conducted in a water accommodated fraction (WAF) of the notified chemical as it is a complex mixture and has low water solubility. A WAF of the nominal loading rate of 100 mg/L was prepared by sonicating, and then stirring the test substance in dilution water for 46 hours. Following the mixing period, the WAF was allowed to settle for approximately 1 hour. WAF treatments were filtered using a 20-25 µm filter paper.

RESULTS

Concentration	*Number of Fish*	*Cumulative mortality (%)*				
Nominal (filtered WAF;mg/L)		*2 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	30	0	0	0	0	0
100	30	0	0	0	0	0

LL50	> 100 mg/L at 96 hours (filtered WAF)
NOEL	100 mg/L at 96 hours (filtered WAF)
Remarks – Results	All validity criteria for the test were satisfied. The toxicity test was conducted as a limit test. Treatment solutions used in the toxicity test appeared clear and colourless, with no evidence of precipitation observed. The end points were determined based on the nominal loading rates as the toxicity cannot be attributed to a single component or mixture of components but to the test substance as a whole. The end points were determined by visual observations.
CONCLUSION	The notified chemical is not harmful to fish
TEST FACILITY	Wildlife International (2013l)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Static Test
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Not reported
Water Hardness	136 mg $CaCo_3$/L
Analytical Monitoring	Total Organic Carbon (TOC) was measured using a Shimadzu TOC-VCSH analyser
Remarks - Method	No significant protocol deviations. GLP Certificate. The daphnia ecotoxicity test was conducted in WAF of the notified chemical as it is a complex mixture and has low water solubility. WAFs of five nominal loading rates were prepared individually by sonicating, and then stirring the test substance in dilution water for 48 hours. Following the mixing period, the WAFs were allowed to settle for approximately 1 hour. WAF treatments were filtered using a 20-25 µm filter paper.

RESULTS

Concentration	*Number of D. magna*	*Cumulative % Immobilised*	
Nominal (filtered WAF;mg/L)		*24 h*	*48 h*
Control	20	0	0
6.3	20	0	0
13	20	0	0
25	20	0	0
50	20	0	0
100	20	0	0

EL50	> 100 mg/L at 48 hours (filtered WAF)
NOEL	100 mg/L at 48 hours (filtered WAF)
Remarks - Results	All validity criteria for the test were satisfied. Treatment solutions used in

the toxicity test appeared clear and colourless, with no evidence of precipitation observed. Analysis of the test substance was not performed. The measured TOC value on day zero was less than 1 mg/L. The end points were determined based on the nominal loading rates as the toxicity cannot be attributed to a single component or mixture of components but to the test substance as a whole. The end points were determined by visual observations.

CONCLUSION The notified chemical is not harmful to aquatic invertebrates

TEST FACILITY Wildlife International (2013m)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test
Species *Pseudokirchneriella subcapitata*
Exposure Period 96 hours
Concentration Range Nominal: 6.3, 13, 25, 50, and 100 mg/L
Auxiliary Solvent Not reported
Water Hardness Not reported
Analytical Monitoring Analysis of the test substance was not performed
Remarks - Method No significant protocol deviations.
GLP Certificate..

The algae ecotoxicity test was conducted in water accommodated fractions (WAF) of the notified chemical as it is a complex mixture and has low water solubility. WAFs of fix nominal loading rates were prepared individually by stirring the test substance in dilution water for 47 hours. Following the mixing period, the WAFs were allowed to settle for approximately 1 hour. WAF treatments were filtered using a vacuum filter through a 0.45 µm alpha cellulose disc.

RESULTS

Biomass (72 h)		*Growth (72 h)*	
E_yL50 (filtered WAF) (mg/L)	*NOE_yL (mg/L)*	*E_rL50 (filtered WAF) (mg/L)*	*NOE_rL (mg/L)*
> 100	13	> 100	13

Remarks - Results All validity criteria for the test were satisfied. Treatment solutions used in the toxicity test appeared clear and colourless, with no evidence of precipitation observed. The end points were determined based on the nominal loading rates as the toxicity cannot be attributed to a single component or mixture of components but to the test substance as a whole. The test was conducted for 96 hours; however, the endpoints at 72 hours were reported as standard.

The E_rL50 and E_yL50 were calculated using the statistical software, SAS System for Windows, Version 8.2.

CONCLUSION The notified chemical is not harmful to algae

TEST FACILITY Wildlife International (2013n)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE Notified chemical

METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 10, 100 and 1000 mg/L
Remarks – Method	No significant protocol deviations. GLP Certificate.
RESULTS	
EC20	Not reported
EC50	> 1000 mg/L
Remarks – Results	All validity criteria for the test were satisfied. The EC50 was out of the tested concentration range (> 1000 mg/L).
CONCLUSION	The notified chemical is not expected to inhibit microbial respiration.
TEST FACILITY	Wildlife International (2013o)

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