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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

138492

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/1628	Cintox Australia Pty Ltd	138492	Yes	< 20 tonnes per annum	Lubricant oil additive

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 of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

Hazard classification	*Hazard statement*
Skin corrosion/irritation (Category 2)	H315 – Causes skin irritation
Serious eye damage/eye irritation (Category 1)	H318 – Causes serious eye damage
Skin sensitisation (Category 1B)	H317 – May cause an allergic skin reaction

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

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

Environmental risk assessment
On the basis of the maximum annual importation volume and the reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Skin corrosion/irritation (Category 2): H315 – Causes skin irritation
  - Skin sensitisation (Category 1B): H317 – May cause an allergic skin reaction
  - Serious eye damage/eye irritation (Category 1): H318 – Causes serious eye damage

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

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 during the handling of products containing the notified chemical:
  - Enclosed and automated systems

- 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 products containing the notified chemical:
  - Avoid direct skin and eye contact

- 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:
  - Gloves
  - Face shield, chemical glasses or goggles
  - Protective clothing

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

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

- If products and mixtures containing the 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.

Public Health

- Due to the risk of skin sensitisation the notified chemical in products should only be used by the public at ≤ 1% concentration.

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(1) of the Act; if
- Further information becomes available on the skin sensitisation potential of the notified chemical.
- The notified chemical in products will be used by the public at > 1% concentration;

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

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

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Cintox Australia Pty Ltd (ABN: 63 122 874 613)
Suite 1, Level 2, 38-40 George Street
PARRAMATTA NSW 2150

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

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

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, Absorption/Desorption, Dissociation Constant, Flammability Limits, Explosive Properties, Oxidising Properties, and Reactivity.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
US EPA 2010
Canada 2016

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
138492 (product containing the notified chemical)

MOLECULAR WEIGHT
< 500 Da (UVCB substance)

ANALYTICAL DATA
Reference elemental analysis, NMR, IR, HPLC, GC-MS, and UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Clear colourless liquid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	1 °C	Measured
Boiling Point	> 271 °C at 101.3 kPa	Measured
Density	818.7 kg/m^3 at 20 °C	Measured
Vapour Pressure	3×10^{-8} kPa at 20 °C	Calculated
Water Solubility	0.62 µg/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Not expected to hydrolyse in environmental pH of 4-9.
Partition Coefficient (n-octanol/water)	log Pow = 6.1 at 20 °C	Measured

Property	Value	Data Source/Justification
Adsorption/Desorption	Not determined	Expected to sorb to soil and sediment based on its low water solubility and surface activity.
Dissociation Constant	Not determined	Contains potential cationic functionalities. However, it is not expected to ionise in the environmental pH range (4 to 9).
Flash Point	164 °C at 101.4 kPa	Measured
Flammability	Not determined	Expected to be of low flammability based on the relatively high flash point.
Autoignition Temperature	341 °C	Measured
Explosive Properties	Not determined	Not expected to have explosive properties based on the chemical structure
Oxidising Properties	Not determined	Not expected to have oxidising properties based on the chemical structure

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

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. It will be imported as a component of a formulation containing up to 10% notified chemical.

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

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

PORT OF ENTRY
Throughout Australia

TRANSPORTATION AND PACKAGING
The imported formulations containing the notified chemical at ≤ 10% concentration will be transported by road or rail to the formulation sites in 205 L drums. The finished products containing the notified chemical at ≤ 1% concentration will be transported by road to end-users warehouses in 205 L drums or small plastic bottles (1 – 5 L).

USE
The notified chemical will be used as a component of engine oil.

OPERATION DESCRIPTION

Reformulation
After importation, additive packages containing the notified chemical (at ≤ 10%) will be transferred into storage tanks through hoses with air back flush systems to prevent spillage, before being transferred into the blending facilities and formulated into engine oil products by mixing with other components. Transfer from the storage tanks to the blending facilities and the blending process itself is expected to involve automated, well ventilated and enclosed systems. The resulting engine oil products containing the notified chemical (≤ 1%) will be filled into drums and smaller containers which will be distributed to end-users. Samples may be taken during the blending process for quality control testing.

End use
Engine oil products containing ≤ 1% of the notified chemical will primarily be used by commercial automotive and industrial engine service outlets, and to a lesser extent, by the public. Use by the public will involve the engine oils being manually decanted into automobile engines, while at industrial sites the engine oils will predominantly be pumped from the drums into the engine oil reservoir.

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)*
Forklift/truck driver	1	30
Lab technician	0.3	220
Loading oil into tank trucks operators	0.5	220
Service stations and workshop workers	0.5	220

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

Reformulation
During reformulation dermal and ocular exposure of workers to the notified chemical at ≤ 10% concentration may occur when connecting and disconnecting hoses and during sample testing. Dermal exposure will also be possible when cleaning up spills or leaks and during maintenance of the blending equipment.

Exposure of workers to the notified chemical will be reduced through the use of automated and in an enclosed systems with ventilation in place, and personal protective equipment (PPE) including protective clothing, safety glasses, and impervious gloves.

Transfer of the finished lubricant containing the notified chemical at ≤ 1% concentration to packaging will mainly be performed by automated processes; hence, exposure to workers is expected to be minimal.

End-use
Dermal and ocular exposure of workers at automotive service centres may occur when handling the finished oil lubricants containing the notified chemical at ≤ 1% concentration. It is expected that at automotive service centres, processes will be mostly enclosed or supplied with engineering controls and good general ventilation to reduce exposure from splashes, mists and vapours (if generated). Exposure may be minimised through the use of PPE such as goggles or face shield, gloves, and protective clothing.

6.1.2. Public Exposure
Products containing the notified chemical at ≤ 1% will be sold to the public who change oil in their own automotives. Dermal and ocular exposure of "do-it-yourself" (DIY) users may occur when handling the finished oil lubricants containing the notified chemical in small containers at ≤ 1% concentration. Gloves and goggles are recommended by the notifier when handling DIY containers to minimise exposure to skin and eyes.

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation	irritating
Rabbit, eye irritation	irritating
Mouse, skin sensitisation – Local lymph node	evidence of sensitisation

Endpoint	*Result and Assessment Conclusion*
assay	
Rat, repeat dose oral (gavage) toxicity – 90 days.	NOAEL = 40 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* Chromosome Aberration Test in Human Lymphocytes	non genotoxic
Genotoxicity – *in vitro* L5178Y TK+/- mouse lymphoma cells	non genotoxic
Rat, repeat dose oral (gavage) toxicity – 28 days, with the Reproduction/Developmental Toxicity Screening Test	NOAEL = 200 mg/kg bw/day (systemic toxicity) NOAEL > 200 mg/kg bw/day (reproductive/developmental)

Toxicokinetics, metabolism and distribution
No toxicokinetics data for the notified chemical were provided. Based on the low molecular weights (< 500 Da), the notified chemical has a potential to be dermally absorbed after exposure. However, the potential for absorption may be reduced to some extent given the low water solubility (0.48-0.62 $\times 10^{-6}$ g/L).

Acute toxicity
The notified chemical was of low acute oral and dermal toxicity in rats (LD50 > 2000 mg/kg bw). The notified chemical is expected to be of low acute inhalation toxicity due to its very low vapour pressure (3×10^{-8} kPa).

Irritation
Based on studies conducted in rabbits, the notified chemical was found to be irritating to the skin and to the eye. Information on irritation to the respiratory tract is not available.

Sensitisation
The notified chemical was a skin sensitiser in a mouse local lymph node assay (LLNA). No EC3 value was calculated as the SI was well above 3 for all of the concentrations tested and hence extrapolation would be inaccurate. The skin sensitisation with autoxidation prediction using OASIS Times (version 2.28.1.4) for the notified chemical and 19 metabolites (all of which were within domain) was that all of them were non sensitisers.

An analogue of the notified chemical was assessed in a guinea pig maximisation test with challenge concentrations of 3% or 0.5% w/v (LSR, 1991). Slight erythema was observed in 1/20 and 3/20 animals at concentrations of 3% and 0.5% respectively with effects primarily confined to the 24 hour observation. A further seven analogues were also not sensitising in a range of animal studies (SIDS, OECD).

In conclusion the notified chemical is expected to be a skin sensitiser based on the LLNA study. However, sufficient additional information on analogues suggests that it may not be a strong sensitiser.

Repeated dose toxicity
A 90-day repeat dose study was conducted in rats, with the notified chemical administered through oral gavage doses of 0, 10, 40, 200 and 1000 mg/kg bw/day. The No Observed Adverse Effect Level (NOAEL) was established as 40 mg/kg bw/day.

Mutagenicity/Genotoxicity
The notified chemical was not mutagenic in a bacterial reverse mutation test, and non clastogenic in an *in vitro* mammalian chromosome aberration test using cultured human lymphocytes and in an *in vitro* mammalian cell gene mutation test using mouse lymphoma L5178Y cells.

Reproductive and developmental toxicity
A 28-day repeat dose study combined with a reproduction/developmental toxicity screening test was conducted in rats, with the notified chemical administered via oral gavage doses of 0, 10, 40, 200 and 1000 mg/kg bw/day.

The NOAEL for F0 systemic toxicity was considered to be 200 mg/kg bw/day due to deaths and adverse clinical findings, severe body weight deficits during gestation, and adverse microscopic findings (hyperplasia, ulceration, necrosis, and neutrophil inflammation in the glandular and non-glandular stomach and decreased lymphoid cellularity in the thymus and spleen) at 1000 mg/kg bw/day dose.

The NOAEL for male and female reproductive toxicity and neonatal toxicity of the test substance was considered to be > 200 mg/kg bw/day.

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

Hazard classification	*Hazard statement*
Skin corrosion/irritation (Category 2)	H315 – Causes skin irritation
Serious eye damage/eye irritation, eye (Category 1)	H318 – Causes serious eye damage
Skin sensitisation (Category 1B)	H317 – May cause an allergic skin reaction

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the information available the notified chemical is irritating to the eye and skin, and is a skin sensitiser.

Workers handling the notified chemical at ≤ 10% concentrations during transport, reformulation and repackaging processes will be at risk of irritation and sensitising effects. The use of PPE such as coveralls, goggles, impervious gloves (and respiratory protection if required) in addition to the use of enclosed and automated systems with local or general ventilation should minimise the potential for exposure and risk. End-users of the finished product (containing the notified chemical at ≤ 1% concentrations), may be exposed to the notified chemical when handling the product into industrial vehicles or service stations. Appropriate PPE (coveralls, impervious gloves, eye protection) and engineering controls will be used to limit workers' exposure.

Therefore, provided engineering controls are instituted, workers wear appropriate PPE, and safe work practices are maintained to reduce exposure, the risk to workers is not considered to be unreasonable.

6.3.2. Public Health
The public may have dermal or ocular exposure to products containing the notified chemical at ≤ 1% concentrations. The use of PPE is less likely than for professional workers, but use of products containing the notified chemical by the public is also expected to be less frequent. Therefore, products containing the notified chemical at ≤ 1% are not considered to pose an unreasonable risk to the public.

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 for reformulation into engine oil products. Accidental spills of the notified chemical during import, transport, reformulation or storage are expected to be physically contained within suitable materials, and collected for treatment or disposal of in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE
Engine oil products containing ≤ 1% of the notified chemical will primarily be used by commercial automotive and industrial engine service outlets, and a small amount, estimated by the notifier to account for 0-5% of the import volume of the notified chemical, will be used by do-it-yourself (DIY) consumers.

At commercial automotive and industrial engine service outlets, used oil will be recovered from the engine and collected by a licensed contractor for recycling or disposal in accordance with local government regulations. As a result, no release to aquatic environment is expected from this activity.

According to a survey tracing the fate of used lubricating oil in Australia (Snow, 1997), approximately 20% of oil used by DIY consumers is collected for recycling, 25% is buried or disposed of to landfill, 5% is disposed of

into stormwater drains, and the remaining 50% is used in treating fence posts, killing grass and weeds or disposed of in other ways. In the worst case scenario involving the 5% of oil used by DIY consumers, up to 0.25% (5% × 5% stormwater disposal) of the total import volume of the notified chemical (or 50 kg) may enter the aquatic environment via disposal to stormwater drains. Since the use of the engine oils will occur throughout Australia, releases resulting from use or disposal of used oil are expected to be diffuse.

Release during use may arise from drips while adding the finished oil to the engine or from the engine itself, and is expected to be very low.

RELEASE OF CHEMICAL FROM DISPOSAL

Empty drums containing residues of the notified chemical will be steam cleaned, and the residual waste, estimated by the notifier to account for up to 1% of the import volume of the notified chemical, will be treated at on-site wastewater treatment facilities. The treatment facilities include a pond where the notified chemical is treated by induced air flotation and biological processes. The notified chemical is expected to be effectively removed through the treatment processes as reported by the notifier. The waste biological sludge from the biological treatment will be disposed of safely in accordance with local government regulations.

Any used or waste product containing the notified chemical is expected to be recycled, re-refined or used as low grade burner fuel, or disposed of by approved waste management facilities. It is likely that the notified chemical will be degraded into simpler compounds during refining, with any residue partitioning to the heavy fractions such as asphalt.

7.1.2. Environmental Fate

The biodegradability study conducted on the notified chemical shows that it is readily biodegradable (65% degradation in 28 days). For details of the environmental fate study, please refer to Appendix C.

The majority of the notified chemical will be thermally decomposed during use, or recycling and re-refining. Up to 0.25% of annual import volume of the notified chemical (or 50 kg) may be released by DIY consumers into stormwater drains from incorrect disposal of wastes and used engine oils. As estimated by the notifier, up to 1% of the import volume of the notified chemical from cleaning of empty drum may go to on-site wastewater treatment facilities where the notified chemical is expected to be effectively removed through biodegradation and adsorption to sludge based on its readily biodegradability and high partition coefficient (log Pow = 6.1). The notified chemical is not expected to bioaccumulate due to its readily biodegradability. The waste biological sludge from wastewater treatment process is expected to be disposed of to landfill. The notified chemical is expected to eventually degrade into water and oxides of carbon and nitrogen by thermal decomposition during its use, and via biotic and abiotic pathways in landfill and surface water.

7.1.3. Predicted Environmental Concentration (PEC)

For the worst case scenario, the percentage of the imported quantity of notified chemical inappropriately disposed to stormwater drains is estimated to be 0.25%. That is, 5% (fraction collected by DIY users) × 5% (fraction disposed to stormwater). The release of the notified chemical may be up to 50 kg/year (= 20 tonnes/year × 0.25%). In this worst case scenario, it is assumed that the release goes into stormwater drains in a single metropolitan area with a geographical footprint of 500 km^2 and an average annual rainfall of 500 mm, all of which drains to stormwater. With a maximum annual release into this localised stormwater system of 50 kg and the annual volume of water drained from this region estimated to be 250×10^6 m^3, the calculated PEC will be up to 0.2 µg/L. This result reflects the worst-case scenario upper limit, as in reality releases of the notified chemical will be distributed over multiple regions and it will be further diluted if it reaches surface waters.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 > 100 mg/L (WAF*)	Not harmful to fish
Daphnia Toxicity	48 h EC50 > 100 mg/L (WAF*)	Not harmful to aquatic invertebrates
Algal Toxicity	72 h EC50 = 20 mg/L (WAF*) NOEC = 0.64 mg/L	Not harmful to algae up to its water solubility limit
Inhibition of Bacterial Respiration	3 h IC50 > 1000 mg/L (WAF*)	Not inhibitory to microbial respiration up to its water solubility limit

*Water accommodated fraction

Based on the above ecotoxicological endpoints, the notified chemical is not harmful to aquatic organisms 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, since the notified chemical is not harmful to aquatic life up to the limit of its solubility in water. Based on the calculated PEC, the notified chemical is not expected to reach ecotoxicologically significant concentrations in surface waters.

7.3. Environmental Risk Assessment

The Risk Quotient (Q = PEC/PNEC) has not been calculated, since the PNEC is not available. The notified chemical is considered readily biodegradable, and is not expected to be harmful to aquatic life. On the basis of the maximum annual importation volume, low expected aquatic exposure and assessed use pattern in engine oils, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 1 °C

Method	ASTM D5950
Remarks	The sample was heated then inserted into the automatic pour point apparatus. The sample was cooled according to the cooling profile listed in Table 1 of ASTM D5950 and examined at 1°C intervals. The lowest temperature at which movement of the sample was detected by the automatic equipment was displayed as the pour point.
Test Facility	(EI report)

Boiling Point > 271 °C at 101.3 kPa

Method	In House Testing Method - A vacuum thermogravimetric analysis was used to determine rate of sample weight change as a function of temperature.
Remarks	Weight loss due to evaporation begins at 271°C.
Test Facility	(EI report)

Density 818.7 kg/m3 at 20 °C

Method	ASTM D4052 using an oscillating densitometer.
Remarks	The sample of the test substance was introduced into a U-shaped cell which is housed in a thermostatically-controlled chamber and electromagnetically excited to oscillate at its natural frequency. When the sample was introduced into the cell, the mass of the sample changed the frequency of the cell's oscillation and the density was calculated from this change.
Test Facility	(EI report)

Vapour Pressure 3×10^{-8} kPa at 20 °C

Method	In House Method – Developed Maxwell-Bonnell Method.
Remarks	Using the Maxwell-Bonnell calculation in conjunction with a correlation from distillation data (software by Simulation Sciences ProVision, v.10) represents an approach to compute vapour pressures for broad boiling mixtures expected to have very low vapour pressures.
Test Facility	(EI report)

Water Solubility 0.62 µg/L at 20 °C

Method	OECD TG 105 Water Solubility
Remarks	Column Elution Method
Test Facility	(EI report)

Partition Coefficient (n-octanol/water) log Pow = 6.1 at 20 °C

Method	OECD TG 117 Partition Coefficient (n-octanol/water)
Remarks	HPLC Method
Test Facility	(EI report)

Flash Point 164 °C at 101.4 kPa

Method	ASTM D93
Remarks	Pensky-Martens Closed Cup Tester, was used to determine the flash point
Test Facility	(EI report)

Autoignition Temperature 341°C

Method	In house Testing Method - ASTM E-659 Auto-ignition/Hot Flame Autoignition
Remarks	The sample of the test substance did not produce a hot flame autoignition.
Test Facility	(EI report)

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: Rat/Crl:CD(SD)
- Vehicle: None
- Remarks - Method: No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	3F	2000	0/3
2	3F	2000	0/3

- LD50: > 2000 mg/kg bw
- Signs of Toxicity: One female was observed to have opacity in the right eye on study days 11, 12 and 14. This effect was not considered to be test substance-related. No significant body weight changes were noted during the study.
- Effects in Organs: No effects were detected.
- Remarks - Results: No deaths occurred during the study and no macroscopic findings were observed

CONCLUSION: The notified chemical is of low acute toxicity via the oral route.

TEST FACILITY: Charles River (2016a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 402 Acute Dermal Toxicity.
- Species/Strain: Rat/Crl:CD(SD)
- Vehicle: None
- Type of dressing: Semi-occlusive
- Remarks - Method: No protocol deviations

RESULTS

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

- LD50: > 2000 mg/kg bw
- Signs of Toxicity - Local: Very slight or slight erythema, scabbing and desquamations were observed in males and females. This correlated with scabbing at the application site, which was noted in one male and one female at necropsy.
- Signs of Toxicity - Systemic: None
- Effects in Organs: None
- Remarks - Results: No deaths occurred. No significant body weight changes or test substance related clinical observations and no internal gross findings were observed.

CONCLUSION: The notified chemical is of low acute toxicity via the dermal route.

TEST FACILITY: Charles River (2016b)

B.3. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Council Regulation No 440/2008 B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	None
Observation Period	14 Days
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of 7 day Observation Period*
	1	2	3			
Erythema/Eschar	1.3	1.3	1.3	3	> 14 days	1
Oedema	0	0	0	1	< 14 days	0

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

Remarks - Results	Very slight erythema was observed in all three animals at up to and including the 48 hour observation after patch removal. The erythema increased to slight in two animals on day 4 with the remaining animal having moderate to severe erythema. At the 7 day observation all three animals had slight erythema, desquamation and very slight oedema. At the 14 day observation no oedema was present but all animals still showed very slight erythema and desquamation with one animal also having scab formation.
CONCLUSION	The notified chemical is irritating to the skin.
TEST FACILITY	Charles River (2016c)

B.4. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Council Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Observation Period	21 Days
Remarks - Method	No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect(days)*	*Maximum Value at End of 7 day Observation Period*
	1	2	3			
Conjunctiva: redness	0.7	0.7	1	1	> 21	1
Conjunctiva: chemosis	1	1	1	1	< 14	0
Conjunctiva: discharge	0	0	0	2	< 1	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

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

Remarks - Results	Slight conjunctival redness was observed in all three animals. This was resolved after 17 days for two animals and remained present in the third

animal at the end of the study.
Slight chemosis was noted in all animals from 24 hours post-instillation and resolved after ten days in one animal and after 14 days in the remaining two animals.
Conjunctival discharge was observed in all animals at the one-hour post-instillation observation only.
Other clinical observations noted for all animals included reddened right eyelid, partial closure of the right eye, and/or brown or yellow staining of the anogenital area.

CONCLUSION The notified chemical is severely irritating to the eye.

TEST FACILITY Charles River (2016d)

B.5. 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)

Species/Strain	Mouse/CBA/J female
Vehicle	Propylene glycol
Preliminary study	Yes
Positive control	1-Chloro-2,4,-dinitrobenzene (DNCB) 0.15% and α-hexylcinnamaldehyde (HCA) 85%. Conducted in parallel with the test substance
Remarks - Method	A preliminary dermal irritation screening was conducted on three groups (n=2/group) of female CBA/J mice. Three concentrations of the test substance were tested: 10 %, 25 % and 50 % (v/v). None of the test concentrations caused significant dermal irritation And hence were used for the main LLNA study.

RESULTS

Concentration (% v/v)	*Number and sex of animals*	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	5F	15557	1.0
25	5F	515530	33.1
50	5F	1051783	67.6
100	5F	885025	45.9
Positive Control			
0.15 % DNCB	5F	685972	44.1
25 % HCA	5F	342709	22.0

Remarks - Results No systemic toxicity or significant body weight changes were observed. No signs of irritation were observed.

The stimulation index (SI) in the groups treated with the test substance was > 3 at all tested concentrations. A satisfactory response was observed in both the positive controls.

The EC3 value was not extrapolated as the SI values were not close to 3 and hence any extrapolation is likely to be inaccurate.

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

TEST FACILITY MB Research Labs (2016)

B.6. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents.
Species/Strain	Sprague Dawley [Crl:CD(SD)] rats
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 90 days Dose regimen: 7 days per week Post-exposure observation period: 1-day
Vehicle	Corn oil
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
control	30 (15 M/15 F)	0	0/30
low dose	20 (10 M/10 F)	10	1/20
mid dose I	20 (10 M/10 F)	40	1/20
mid dose II	20 (10 M/10 F)	200	2/20
high dose	30 (15 M/15 F)	1000	3/30

Mortality and Time to Death

One male animal in the 10 mg/kg bw/day dose group died on day 87, due to an undetermined cause. One female animal in the 40 mg/kg bw/day dose group died on day 17, due to estimated procedural cause. Two females in the 200 mg/kg bw/day died or were euthanised on day 26 and day 28, due to test-substance related erosion/ulcerations of the non-glandular stomach. One male in the 1000 mg/kg bw/day was euthanised in extremis on day 29, due to test-substance related degeneration/regeneration in the jejunum. In the 1000 mg/kg bw/day group, one female was euthanised in extremis on day 33, due to undetermined cause and one female was found dead on day 33 due to estimated procedural cause.

Clinical Observations

The mean body weight in the 1000 mg/kg bw/day group males was 9.8% lower than the mean control group body weight at the end of the dosing period. During the recovery period, cumulative body weights in the 1000 mg/kg bw/day group males were comparable to the control group. Significant amount of body weight losses (at least 20%) were noted in one female dosed at 200 mg/kg bw/day and another female at 1000 mg/kg bw/day groups). One female at 1000 mg/kg bw/day group also lost a substantial amount of weight from study weeks 2 to 4 and was found dead during study week 4.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There were no test substance-related effects on urinalysis parameters, or functional observational battery and motor activity assessments.

In the haematology findings of males in the 200 and 1000 mg/kg bw/day dose groups and females in all dose groups, a dose-dependent increase in mean absolute reticulocyte counts was observed, although it was only statistically significant for both sexes at 1000 mg/kg bw/day. Male animals dosed at 1000 mg/kg bw/day also showed a statistically significant decrease in haemoglobin, increase in neutrophils, red cell distribution width and haemoglobin distribution width. Red cell distribution width and haemoglobin distribution width showed increases that were statistically significant in male animals dosed at 200 mg/kg bw/day. White blood cells in females in the 1000 mg/kg bw/day dose group showed statistically significant increases (49 %) as did neutrophils (53 %). Females in the 1000 mg/kg bw/day were also observed with elevated lymphocytes (48 %), monocytes (59 %) and minimally increased platelets (13 %).

Statistically significant reductions in the total protein, albumin, globulin, cholesterol, and calcium levels were observed at 1000 mg/kg bw/day.

Effects in Organs

Test substance-related changes in organ weights included higher spleen, adrenal gland, kidney, and liver weights in the 1000 mg/kg bw/day group females, and spleen and kidney weights in males in the 1000 mg/kg bw/day group.

Males in the 1000 mg/kg bw/day group and females in the 200 and 1000 mg/kg bw/day groups had cytoplasmic vacuolation of cells in the jejunum. Cytoplasmic vacuolation of cells was also present in the adrenal cortex at 1000 mg/kg bw/day in both sexes, and mesenteric lymph nodes in all treatment groups in both sexes. Other effects caused by administration of the notified chemical were erosion/ulceration of the non glandular stomach in the males and females in the 200 and 1000 mg/kg bw/day groups.

Following a minimum 28-day recovery period, organ weight changes in the 1000 mg/kg bw/day groups (the only dose assessed) were restored or recovering. Microscopic changes in the non glandular stomach seen at the primary necropsy were not seen at the recovery necropsy and were considered resolved; however, the test substance-related microscopic changes in the jejunum, mesenteric lymph node, lungs, and adrenal cortex persisted in the 1000 mg/kg bw/day recovery necropsy groups.

Remarks – Results

Adverse granulomatous/pyogranulomatous inflammation of the lungs was observed in males and females in various treatment groups. This was considered to be secondary to administration of the notified chemical for all treatment groups, since this was likely to be due to reflux of the notified chemical into the lungs during gavage.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 40 mg/kg bw/day in this study, based on clinical and histopathological findings and treatment related deaths in both sexes at ≥ 200 mg/kg bw/day.

TEST FACILITY Charles River (2017a)

B.7. Repeat dose toxicity

TEST SUBSTANCE Notified chemical

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

Species/Strain	Sprague Dawley [Crl:CD (SD)] rats
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: Males 28-29 days Females, from 14 days before mating to day 13 of lactation Dose regimen: 7 days per week Post-exposure observation period: 1 day
Vehicle	Corn oil
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	20 (10 M/10 F)	0	0/20
low dose	20 (10 M/10 F)	10	0/20
mid dose I	20 (10 M/10 F)	40	0/20
mid dose II	20 (10 M/10 F)	200	0/20
high dose	20 (10 M/10 F)	1000	3/20

Mortality and Time to Death

Three females at 1000 mg/kg bw/day group were euthanised in extremis (on study day 21 or during gestation days 10 and 21), following adverse clinical signs (thin, pale and/or cool body) and/or severe body weight losses. One female in the same group was euthanised soon after the parturition due to total loss of the litter on post-natal day (PND) 0 and the remaining 6 females in this group failed to deliver and were necropsied on post-mating day 25.

Clinical Observations

No change in the mean body weights, body weight gains and food consumption for F0 females were observed during the pre-mating period (study days 0-13). Lower mean body weight gains were recorded at 200 and 1000 mg/kg bw/day during gestation days 7-20. Mean body weights at 200 and 1000 mg/kg bw/day were 6.9 % and 17.3 % lower, respectively, than the control group at gestation day 20. Mean food consumption in the 1000 mg/kg bw/day group was slightly lower than control group during gestation days 14-20. Reduced food consumption was noted in the 200 mg/kg bw/day group at the beginning of lactation. No significant treatment-related effects on mean body weights, body weight gains and food consumption for F0 males at any dose tested or in the F0 females at 10 and 40 mg/kg bw/day were reported.

Histopathological findings in the three females in the 1000 mg/kg bw/day euthanised in extremis at day 21 included squamous cell hyperplasia in the non-glandular stomach; degeneration of the olfactory epithelium and neutrophil inflammation on the nasal cavity; decreased lymphoid cellularity in the thymus, decreased cellularity in the bone marrow and vacuolisation and hypertrophy of the zona fasciculata in the adrenal gland cortex. Squamous cell hyperplasia and hyperkeratosis in the non-glandular stomach were noted in the F0 males at 200 and 1000 mg/kg bw/day and in F0 females at 1000 mg/kg bw/day. Epithelial degeneration with neutrophil inflammation was also noted in the non-glandular stomach for F0 males at 200 mg/kg bw/day and mixed inflammation with type II pneumocyte hyperplasia in the lungs and epithelial degeneration with neutrophil inflammation in the nasal cavity were noted in males and females at 1000 mg/kg bw/day. Macrophage hyperplasia in the medulla of the mesenteric lymph node was also noted in the 1000 mg/kg bw/day group F0 males and females.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

In the clinical pathological findings of the F0 males at 1000 mg/kg bw/day statistically significant higher mean absolute neutrophil counts were observed at necropsy. This finding correlated with histopathological findings in the lungs and nasal cavity. Statistically significant higher mean absolute neutrophil counts were also noted in the 10, 40 and 200 mg/kg bw/day group F0 females.

Statistically significant lower mean serum albumin values and serum protein values were noted in the 200 and 1000 mg/kg bw/day group F0 males. Males in the 40, 200 and 1000 mg/kg bw/day groups had statistically significant lower mean T4 values and females in these groups had higher mean neutrophil counts.

Effects in Organs

In the 200 mg/kg bw/day, the F0 females had statistically significant lower mean heart weights with no histopathological correlation. No other treatment-related alteration in organ weights for F0 male and females were observed at any dose tested.

Histopathology findings in male and female animals in the 1000 mg/kg bw/day group included: hyperplasia, hyperkeratosis, ulceration, neutrophil inflammation, epithelial degeneration and necrosis of the stomach; inflammation and hyperplasia in the lungs; epithelium degeneration and neutrophil inflammation in the nasal passage; decreased lymphoid cellularity in the thymus, spleen and mesenteric lymph node; hyperplasia of the mesenteric lymph node; decreased cellularity of the bone marrow; and vacuolation and hypertrophy of the adrenal cortex. The only effect at lower doses was squamous cell hyperplasia in the non-glandular stomach of males in the 200 mg/kg bw/day.

Reproductive Toxicity

Effects on Parental (F0) animals:

Lower mean fertility (44.4 %), male copulation (44.4 %), and female conception indices (44.4 %) were noted in the 1000 mg/kg bw/day group, due to 5/9 mated females being non gravid at necropsy. Five males at 1000 mg/kg bw/day didn't sire a litter. No other signs of dystocia were noted at any dosage level. F0 estrous cycle length and reproductive performance and gestation lengths were unaffected at 10, 40 and 200 mg/kg bw/day.

Effects on neonatal pups (F1):

No viable pups were reported in the 1000 mg/kg bw/day. The mean number of F1 pups in the 200 mg/kg bw/day showed a statistically significant decrease when compared to the concurrent control group. No significant differences in the mean numbers of implantation sites, unaccounted sites, or live litter size were reported at 200 mg/kg bw/day. There were no treatment-related effects on the number of F1 pups born and live litter size at 10 and 40 mg/kg bw/day.

Remarks – Results

Due to the loss of all the F0 females at 1000 mg/kg bw/day due to various test-substance related effects, the systemic toxicity NOAEL was considered to be 200 mg/kg bw/day.

The NOAEL for male and female reproductive toxicity and neonatal toxicity was established at > 200 mg/kg bw/day and in the absence of viable pups and due to early termination of all F0 females.

CONCLUSION

The NOAEL for F0 systemic toxicity was considered to be 200 mg/kg bw/day.

The NOAEL for reproductive neonatal toxicity was established as > 200 mg/kg bw/day based on the absence of viable F1 offspring at 1000 mg/kg bw/day.

TEST FACILITY Charles River (2017b)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98 and TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 fraction from phenobarbital/beta-naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 1.5 to 5000 µg/plate b) Without metabolic activation: 1.5 to 5000 µg/plate
Vehicle	Tetrahydrofuran
Remarks - Method	No deviations from the study plan. Plate incorporation method

RESULTS

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

Remarks - Results

In the preliminary dose range finding study with dose levels of up to 5000 µg/plate no toxicity was observed.

No substantial increase in revertant colony numbers of any of the tester strains were observed following treatment with the notified chemical at any dose level, with or without metabolic activation, in either mutation test.

The concurrent positive control compounds demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

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

TEST FACILITY ENVIGO (2016)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Species/Strain *Human*
Cell Type/Cell Line Human Lymphocytes
Metabolic Activation System S9 fraction from phenobarbital/5,6-benzoflavone-induced rat liver.
Vehicle Dimethyl sulphoxide (DMSO)
Remarks - Method No deviations from the study protocol.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 10, 20, 40*, 80*, 160*, 240*, 320	4 hr	24 hr
Test 2	0*, 10, 20, 40*, 80*, 160*, 240*, 320	24 hr	24 hr
Present			
Test 1	0*, 10, 20, 40*, 80*, 160*, 240*, 320	4 hr	24 hr

*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	≥ 312.5			
Test 1		> 240	≥ 240	Negative
Test 2		> 240	≥ 240	Negative
Present	> 1250			
Test 1		> 240	≥ 240	Negative

Remarks - Results The notified chemical did not induce chromosomal aberrations with or without metabolic activation at any dose level studied for both short and continuous treatments (including 1250 µg/mL), the treatment limit concentration).

The concurrent positive control compounds (Mitomycin C (MMC) used without S9 Mix and Cyclophosphamide (CP) used with S9 Mix) demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

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

TEST FACILITY ENVIGO (2017a)

B.10. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 490 *In vitro* Mammalian Cell Gene Mutation Test.
EC Directive 2000/32/EC B.17 Mutagenicity - *In vitro* Mammalian Cell Gene Mutation Test
Species/Strain Mouse
Cell Type/Cell Line Lymphoma L5178Y cells
Metabolic Activation System S9 fraction from phenobarbital/beta-naphthoflavone induced rat liver
Vehicle DMSO
Remarks - Method No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0, 19.53, 39.06, 78.13, 156.25, 312.5, 625	4 hr	24 hr
Test 2	0, 4.88, 9.77, 19.53, 39.06, 78.13, 156.25	24 hr	48 hr

Present			
Test 1	0, 19.53,39.06, 78.13, 156.25, 312.5, 625	4 hr	24 hr

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*		
	Cytotoxicity in Main Test	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	> 625	> 625	Negative
Test 2	> 156.25	> 156.25	Negative
Present			
Test 1	> 625	> 625	Negative

Remarks - Results

In both the absence and presence of S9-mix, the notified chemical was not mutagenic. The notified chemical was not cytotoxic in the main study but was in the preliminary study in the absence of metabolic activation from 156.25 µg/mL.

The positive control substances (Ethylmethanesulphonate (EMS) used without S9-Mix, and Cyclophosphamide (CP) used with S9-Mix) induced marked increases in the mutant frequency, sufficient to indicate the satisfactory performance of the test and of the activity of the metabolizing system.

CONCLUSION

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

TEST FACILITY

ENVIGO (2017b).

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO2 Evolution Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Theoretical Carbon Dioxide ($ThCO_2$)
Remarks - Method	The test was conducted in accordance with the test guideline above, with no significant deviation in protocol reported.

RESULTS

Notified chemical		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
1	0.5	1	11
3	1.7	3	32
6	2	6	52
8	8	8	64
11	10	11	79
14	22	14	88
16	38	16	88
18	51	18	91
21	62	21	91
24	64	24	92
28	64	28	92
29*	65	29*	93

* Corrected for the last gas wash

Remarks - Results	All validity criteria for the test were satisfied. The percentage degradation of the reference compound, sodium benzoate, surpassed the threshold level of 60% by 14 days (88%), therefore, the tests indicate the suitability of the inoculum. The percentage degradation of the toxicity control reached the threshold level of 25% by 8 days (25%; 71% in 28 days), showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The mean biodegradation of notified chemical was 62% during the 10-d window and 65% during the 28 days window. The test substance is, therefore, considered to be readily biodegradable
CONCLUSION	The notified chemical is readily biodegradable.
TEST FACILITY	Ecotoxicological Testing Laboratory of Shanghai Environmental Protection Co. Ltd., 2016

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Static renewal
Species	*Onchorhynchus mykiss*
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	144-168 mg $CaCO_3$/L

Analytical Monitoring	HPLC-MS/MS
Remarks – Method	No significant deviations to the test protocol were reported. The test substance was prepared as a Water Accommodated Fraction (WAF) due to its low water solubility. Nominal WAF loading rates were 6.3, 13, 25, 50, and 100 mg/L.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*2 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	Control	7	0	0	0	0	0
6.3	0.51	7	0	0	0	0	0
13	0.17	7	0	0	0	0	0
25	0.17	7	0	0	0	0	0
50	0.65	7	0	0	0	0	0
100	0.23	7	0	0	0	0	0

LL50	>100 mg/L at 96 hours.
NOEL (or LOEC)	100 mg/L at 96 hours
Remarks – Results	All validity criteria for the test were satisfied. The test solutions were renewed every 48 hours during the 96 hour test period. Since the test substance is poorly soluble in water, the individual test solutions, prepared as WAF for each loading rate, were all above the solubility limit of the test substance. The results of the study were based on the nominal WAF loading rates. The LL_{50} was >100 mg/L based on the WAF nominal test concentration.

CONCLUSION The notified chemical is not considered to be harmful to fish.

TEST FACILITY EAG Laboratories, 2017a

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

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	140-176 mg $CaCO_3$/L
Analytical Monitoring	HPLC-MS/MS
Remarks - Method	The test substance was prepared as a Water Accommodated Fraction (WAF) due to its low water solubility. Nominal loading rate test solutions were prepared by stirring for 23 hours and settling for 1 hour to allow phase separation, prior to removal of aqueous phase. Aqueous phase was filtered through glass wool to ensure removal of undissolved oil droplets.

RESULTS

Concentration mg/L		*Number of D. magna*	*Cumulative Immobilised %*	
Nominal WAF	*Actual*		*24 h*	*48 h*
Control	Control	20	0	0
1.9	0.01	20	0	0
4.3	0.02	20	0	0
9.4	0.03	20	0	0
21	0.02	20	0	0
45	0.16	20	5	5
100	0.07	20	0	0

EL50	>100 mg/L at 48 hours (WAF)
NOEL	1.9 mg/L at 48 hours (WAF)
Remarks - Results	All validity criteria for the test were satisfied. The test solutions were not renewed during the 48 h test period. Test concentrations were measured at the beginning and end of the test. The 48 h EL_{50} was >100 mg/L. The no-observed effect loading rate (NOEL) was 1.9 mg/L (WAF), based on nominal loading concentrations. Percent immobility in the 45 mg/L treatment group was 5%, and was considered incidental to treatment.
CONCLUSION	The notified chemical is not considered to be harmful to aquatic invertebrates up to its water solubility limit.
TEST FACILITY	EAG Laboratories, 2017b

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Freshwater Alga and Cyanobacteria, Growth Inhibition Test.
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 0.26, 0.64, 1.6, 4.0, 10 & 25 mg/L Actual: below the limit of analytical quantification
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	HPLC-MS/MS
Remarks - Method	The test substance was prepared as a Water Accommodated Fraction (WAF) due to its low water solubility. Water accommodated fractions were stirred for 23 hours, allowed to settle for 1 hour then decanted from mid-depth via tubing, prior to use. Samples of test medium were collected from each treatment and control group at the start and end of the test for analysis, all were below the 0.1 mg/L limit of quantification of the analysis method.

RESULTS

Biomass		*Growth*	
EyL50 *mg/L at 72 h*	*NOEL* *mg/L*	*ErL50* *mg/L at 72 h*	*NOEL* *mg/L*
2.6 (95% CI: 1.7-4.2)	0.64	20 (95% CI: 16-25)	0.64

Remarks - Results	All validity criteria of the test guideline were satisfied. The ErL_{50} and EyL_{50} values and their corresponding were calculated, on the basis of nominal WAF loading rates. The Growth rate EL_{50} was 20 (95% CI: 16-25) and the NOEL 0.64 mg/L.
CONCLUSION	The notified chemical is not considered to be harmful to algae up to the limit of its water solubility.
TEST FACILITY	EAG Laboratories, 2017c

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 & 1000 mg/L Actual: not determined
Remarks – Method	The test was conducted in accordance with the test guideline above with no significant deviation from the protocol reported. Based on the poor solubility of the test substance it was dosed into the inoculum mix by direct weight addition. The inhibitory effect of the test substance to microbial activity was determined as the inhibition of respiration by the notified chemical compared to a reference dosed at 3, 15 and 50 mg/L with 3,5-dichlorophenol. An abiotic control was dosed with the test substance at a concentration of 1000 mg/L to examine the potential for abiotic reactions of the test substance to consume or release oxygen under the tested conditions.

RESULTS

EC50	>1000 mg/L
NOEC	Not calculated
Remarks – Results	All validity criteria for the test were satisfied. The EC_{50} for the reference substance was within the 2 to 25 mg/L range considered acceptable for the test. The abiotic treatment mixture dosed with 1000 mg/L of the notified chemical had a respiration rate of 0.0 mg O_2/L/hr showing there was no significant uptake or release of oxygen resulting from abiotic reactions of the test substance. The EC_{50} value for the notified chemical was greater than 1000 mg/L, the highest concentration tested.

CONCLUSION	The notified chemical does not inhibit microbial activity up to the limit of its water solubility.
TEST FACILITY	EAG Laboratories, 2016

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