File No: STD/1630

January 2018

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Amides, tallow, *N,N*-bis(2-hydroxypropyl)

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/1630	Lanxess Solutions Australia Pty Ltd	Amides, tallow, *N,N*-bis(2-hydroxypropyl)	ND*	≤ 100 tonnes per annum	Component of motor oil

* ND = not determined

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical cannot be recommended for classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS), as adopted for industrial chemicals in Australia.

The environmental hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS) is presented below. Environmental classification under the GHS is not mandated in Australia and carries no legal status but is presented for information purposes.

Hazard classification	*Hazard statement*
Hazardous to the aquatic environment, short-term (Acute Category 1)	H400 – Very toxic to aquatic life

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 PEC/PNEC ratio and the reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical or products containing the notified chemical:
  - Avoid 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:
  - Protective clothing
  - Gloves
  - Safety glasses or goggles

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

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

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

Disposal

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

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by containment, physical 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 on the skin sensitisation potential of the notified chemical has become available;

or

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

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

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT
Lanxess Solutions Australia Pty Ltd (ABN: 79 600 792 569)
5 Comserv Close
WEST GOSFORD NSW 2250

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: other names, 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 for: acute inhalation toxicity, genotoxicity (*in vivo*), and bioaccumulation.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Canada (2013)
EU REACH (2017, pending)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
MLA-3202

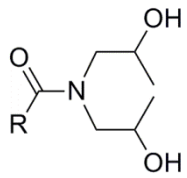
CAS NUMBER
1454803-04-3

CHEMICAL NAME
Amides, tallow, *N,N*-bis(2-hydroxypropyl)

MOLECULAR FORMULA
Unspecified

The notified chemical is a substance of Unknown, of Variable Composition, or of Biological Origin (UVCB).

STRUCTURAL FORMULA



R = predominantly C_{14-18} and $C_{16-18(unsatd.)}$

(Structural formula provided by the notifier)

MOLECULAR WEIGHT
343.6 – 399.7 g/mol

ANALYTICAL DATA
Reference ^{1}H NMR, ^{13}C NMR, FTIR, GC-MS, and UV-VIS spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Clear amber-red liquid

Property	Value	Data Source/Justification
Melting Point	-50 to 10 °C	Measured
Boiling Point	Decomposes at > 200 °C; no boiling observed	Measured
Density	941 kg/m^3 at 20 °C	Measured
Viscosity	1,116 mm2/s at 20 °C	Measured
Vapour Pressure	2.1×10^{-9} kPa at 20 °C 4.6×10^{-9} kPa or 25 °C	Measured
Water Solubility	5.4×10^{-4} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Stable	Measured; contains functionalities susceptible to hydrolysis, but was shown to be stable in the environmental pH range (4-9)
Partition Coefficient (n-octanol/water)	log Pow ≥ 5.3 at 35 °C	Measured
Surface Tension	57.2 mN/m at 20 °C	Measured, regarded as surface active
Adsorption/Desorption	log Koc = 5.4 to > 6.3 at 35 °C	Measured
Dissociation Constant	Not determined	No dissociable functionality
Flash Point	Not observed	Measured
Flammability	Not highly flammable in contact with water	Expert statement provided by the notifier
Autoignition Temperature	360 °C	Measured
Explosive Properties	No explosive properties	Expert statement provided by the notifier
Oxidising Properties	No oxidising properties	Expert statement provided by the notifier

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. Based on measured surface tension, the notified chemical is considered to be surface active.

Physical hazard classification
Based on the submitted physical-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 into Australia both in neat form and as a component of motor oil products (at concentrations ≤ 3%).

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	1 – 10	10 – 50	50 – 100	50 – 100	50 – 100

PORT OF ENTRY
Sydney, Melbourne, Brisbane and Perth

TRANSPORTATION AND PACKAGING
The neat form of the notified chemical will be imported in 200 L drums, stored in warehouses and transported by road for distribution to reformulation sites.

The finished motor oil products will be packaged in 1 L and 5 L high density polyethylene (HDPE) containers or 205 L and 209 L steel drums, and distributed by road to automotive service centres, car dealerships and independent repair shops for commercial use (approximately 80% of the total import volume). Some finished oil products will also be distributed to retailers for do-it-yourself (DIY) use (approximately 20% of the import volume).

USE
The notified chemical will be used as an additive in motor oils for passenger vehicles at ≤ 3% concentration.

OPERATION DESCRIPTION

Reformulation
Reformulation of the notified chemical will be carried out in a controlled, automated system. The neat form of the notified chemical in 200 L drums will be transferred by forklift to the blending area, weighed, and added into a blending vessel with other ingredients. After the motor oil containing the notified chemical at a maximum concentration of 3% is blended, it will be transferred from the blending vessel to automated filling machines that will pack the motor oil into 1 L and 5 L HDPE containers or 205 L and 209 L steel drums.

End Use
Workers at original equipment manufacturers (OEM) will pump the oil products containing the notified chemical into lubricating oil reservoirs in assembled vehicles during process.

Some commercial servicing facilities, such as vehicle service centres, will receive the finished motor oil products in smaller containers. In these facilities, the oil products may be transferred from the containers into vehicles using automated valves, or decanted manually into the oil reservoirs of vehicles by trained workers wearing appropriate personal protective equipment (PPE).

Any waste or spilled oil during the commercial end use will be collected for disposal by approved waste management facilities.

Certain amount of the motor oil products will be available to DIY consumers. These consumers are expected to follow the safe use instructions provided with the products to handle the motor oils containing the notified chemical.

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)**
Importer/dock worker	2 – 4	260
Blending operator	2	260
Filling operator	8	260
Analytical/Quality Control (QC) worker	1 – 2	260
Decant operator	1 – 2	260
Warehouse operator	4 – 6	260
Pack driver	4 – 6	260
Drum/product disposal worker	1 – 2	260
End user (commercial)	2 – 3	260

* As the market for the notified chemical has not yet been established, the exposure frequency is assumed to be all working days in an average year as a worst case scenario.

EXPOSURE DETAILS

Transport workers
During transport, storage and delivery, exposure of workers to the notified chemical is not expected, except in an accident where the packaging is breached. Potential for exposure can be minimised by the use of appropriate PPE.

Reformulation operators
The blending and packaging processes will be automated and within closed systems, and operators will not be in direct contact with the notified chemical or products containing the chemical. Incidental dermal or ocular exposure may occur during blending and packaging processes, and during cleaning of the equipment or QC analysis. The potential of exposure may be reduced by safe work practices and the use of PPE such as impervious gloves, goggles and respirators if ventilation is inadequate.

End users
Professional workers using the motor oil products are potentially to be exposed via dermal and ocular routes to the notified chemical at concentration ≤ 3% during filling vehicles with the oil products. PPE such as gloves, goggles, and overalls will minimise potential for exposure. Inhalation exposure to the notified chemical is not expected unless aerosols are formed. Once the motor oil is filled into the vehicles, it will be contained within the engine compartments.

6.1.2. Public Exposure
The neat form of the notified chemical will not be available to the public.

DIY users may use motor oil products containing the notified chemical in low quantity and frequency, and be potentially exposed to the notified chemical at ≤ 3%. Main route of exposure is expected to be dermal with ocular exposure also possible. In the event of skin contact, the DIY users are expected to use routine personal hygiene practices, such as washing of hands and collection of spills, to avoid prolonged exposure. DIY users are also expected to follow the safe use instructions to handle the products containing the notified chemical to minimise potential for exposure.

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 > 5,000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 5,000 mg/kg bw; low toxicity
Rabbit, skin irritation	slightly irritating
Skin corrosion (*in vitro* reconstructed human epidermis test)	non-corrosive
Skin irritation (*in vitro* reconstructed human epidermis test)	non-irritating
Rabbit, eye irritation	slightly irritating
Eye irritation (*in vitro* BCOP Test)	non-irritating
Guinea pig, skin sensitisation – non-adjuvant test.	no evidence of sensitisation
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation (EC3 = 30%)
Rat, repeat dose oral toxicity – 28 days.	NOAEL > 1,000 mg/kg bw/day*
Mutagenicity – bacterial reverse mutation	non mutagenic
Mutagenicity – *in vitro* mammalian cell gene mutation	non mutagenic
Genotoxicity – *in vitro* mammalian chromosome aberration	non genotoxic
Rat, reproductive and developmental toxicity	NOAEL (parental, reproduction, developmental) > 1,000 mg/kg bw/day*

*Established by the study authors

Toxicokinetics, metabolism and distribution
No toxicokinetics, metabolism and distribution study data were submitted for the notified chemical.

Based on the molecular weight (< 500 g/mol) of the notified chemical, there is potential for the chemical to cross biological membranes. However, absorption is expected to be limited based on the low water solubility (0.54 mg/L at 20 °C) and partition coefficient (log Pow > 5.3) of the notified chemical.

Acute toxicity
The notified chemical was found to have low acute oral and dermal toxicity in rats.

No information was submitted on acute inhalation toxicity; however, the notified chemical has a low vapour pressure and is not expected to form aerosols during normal use. Therefore, it is unlikely to pose an acute inhalation risk under normal use conditions.

Irritation
Based on studies conducted in rabbits, the notified chemical was considered to be slightly irritating to the skin and eyes. In addition, the notified chemical was not considered as an eye irritant in an *in vitro* bovine corneal opacity and permeability test (BCOP Test). It was also shown to be non-irritating and non-corrosive to the skin in two separate *in vitro* human skin model tests.

Sensitisation
The notified chemical showed no evidence of sensitisation in a guinea pig skin sensitisation test, but showed evidence of sensitisation in a local lymph node assay (LLNA) in mice with an EC3 of 30%. However, based on the surface tension measured, the notified chemical is surface active. Surface active substances, especially compounds containing unsaturated carbon chains, are known to give false positive results in LLNA studies (Kreiling, 2008). The study authors of the LLNA concluded that although the notified chemical may be a skin sensitiser, a false positive outcome cannot be ruled out. The notified chemical cannot therefore be considered for classification as a skin sensitiser due to uncertainty of the LLNA results.

Repeated dose toxicity
A 28 day repeated dose oral toxicity study was conducted on the notified chemical. Does levels tested were 100, 300 and 1,000 mg/kg bw/day taking water as the vehicle control. Microscopic examination of the animals treated at 1,000 mg/kg bw/day revealed absence of haematopoiesis in the spleen, and minimal hepatocellular hypertrophy of the liver, which corresponded to reduced spleen weights and increased liver weights. No treatment-related effects or toxicologically relevant changes were observed in the 100 and 300 mg/kg bw/day dose levels tested. A No Observed Adverse Effect Level (NOAEL) was established by the study authors for the notified chemical as > 1,000 mg/kg bw/day in rats, based on the highest dose level tested.

Mutagenicity/Genotoxicity
The notified chemical was not mutagenic in a bacterial reverse mutation test and an *in vitro* mammalian cell gene mutation test. The notified chemical was not clastogenic in an *in vitro* mammalian chromosome aberration test.

Toxicity for reproduction
A reproduction/developmental toxicity screening test was conducted on the notified chemical at the dose levels of 100, 300 and 1,000 mg/kg bw/day. No parental, reproduction or developmental toxicity effects was observed in any of the dose levels tested. Therefore, a NOAEL was established for the notified chemical in the study as > 1,000 mg/kg bw/day in rats.

Carcinogenicity potential
Based on a published report of toxicology and carcinogenesis of a structurally similar analogue (lauric acid diethanolamine condensate, CAS number 120-40-1) (NTP, 1999), the notified chemical is not expected to be carcinogenic. Two year studies with the analogue chemical showed no evidence of carcinogenic activity in rats when administered at 50 or 100 mg/kg bw/day or in male mice when administered at 100 or 200 mg/kg bw/day. There was some evidence of carcinogenic activity in female mice based on increased incidences of hepatocellular neoplasms. These increases were associated with free diethanolamine, which was present as a contaminant in the analogue test substance.

Dermal administration of the analogue to rats and mice for 2 years resulted in increased incidences of epidermal and sebaceous gland hyperplasia, hyperkeratosis, chronic inflammation, and parakeratosis at the site of application. The administration of the analogue also resulted in increased incidences of thyroid gland follicular cell hyperplasia in dosed male mice.

Health hazard classification
Based on the available information, the notified chemical cannot be recommended for classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Significant systemic toxicity potentially caused by exposure to the notified chemical is not expected based on the submitted toxicology data and predicted limited absorption across biological membranes. However, the potential for the chemical to cause skin sensitisation cannot be ruled out as the LLNA study report showed evidence of weak skin sensitisation, although false positive outcome may be possible.

There is potential for dermal and ocular exposure of workers to the notified chemical at 100% concentration during reformulation processes. Exposure is expected to be minimised through the use of enclosed, automated processes, sufficient ventilation and appropriate PPE.

There is also potential for dermal and ocular exposure of workers to the notified chemical at ≤ 3% concentration during end use when transferring motor oils containing the notified chemical between containers and the engine's oil reservoirs. The risk to workers is expected to be mitigated through the use of automated processes and use of PPE.

Overall, provided engineering controls are instituted during reformulation, workers wear appropriate PPE, and safe work practices are maintained to reduce exposure, the risk to workers from use of the notified chemical is not considered to be unreasonable.

6.3.2. Public Health

The potential for dermal and ocular exposure of DIY users to the notified chemical at up to 3% is expected during transferring motor oils containing the notified chemical between containers and oil reservoirs. However, by following the safe use instructions the risk to the DIY users from use of the notified chemical is not considered to be unreasonable, given the low end-use concentrations and expected low use frequency.

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 end use products or neat chemical for further reformulation. The notifier has indicated that the formulated passenger car engine oil will be used in the industrial and commercial sector (approximately 80% total volume) at automotive service centres, dealerships and independent repair shops. The balance (approximately 20% of total volume), will be sold to consumers for personal automotive maintenance.

The application in Australia will be as base fluids or additives for lubricant oils used for industrial application. Significant release of the notified chemical to the environment is not expected during transport and storage except in the unlikely event of accidental spills or leaks.

Any notified chemical spilled during reformulation is expected to be contained with bunds and either reclaimed or sent to on-site waste treatment facilities. At the on-site waste treatment facilities, residues of the notified chemical will be separated from the aqueous waste stream by the American Petroleum Industry (API) process. As a result of this treatment, greater than 90% of the notified chemical is estimated to be removed. The aqueous waste undergoes further treatment involving pond aeration and biological treatment before being released to the sewage system. The remaining non-aqueous waste is expected to be disposed of according to local regulations, which is most likely to landfill. Therefore, the accidental release from reformulation of the notified chemical and finished oils is unlikely to be significant.

RELEASE OF CHEMICAL FROM USE

The finished products containing the notified chemical will be used as a component of lubricants. Release during its use may come from spills when pouring lubricants into the machinery or leaks from the machinery, which is expected to be negligible.

RELEASE OF CHEMICAL FROM DISPOSAL

After reformulation, empty import drums containing residues of the notified chemical (0.1% of the total import volume) are expected to be steam cleaned, with the residual waste sent to on-site wastewater treatment facilities. Assuming 0.1% of the notified chemical remains in the empty drums after use, 100 kg/yr (100 tonnes/yr × 0.1%) of the notified substance will be sent to the on-site waste treatment. It is estimated that greater than 90% of the notified chemical may be removed during waste treatment processes. Therefore, the amount of the notified chemical released to sewer from the cleaning of empty drums is estimated to be 10 kg/yr. The wastewater will be further treated at the sewage treatment plants. Therefore, the release of the notified chemical to surface waters is expected to be limited from the cleaning of empty drums.

The majority of the formulated products containing the notified chemical will be used as lubricant products. At the end of life, the fluids will be drained from the machinery for disposal. The main method of disposal will be by recycling or thermal decomposition.

The notified chemical may be released to the environment during disposal of waste or used oils. Oil products containing the notified chemical will be poured into engines by automotive manufacturers, service centres or by do-it-yourself (DIY) consumers. A survey by the Australian Institute of Petroleum (AIP, 1995) indicates that of the annual sales of engine oils in Australia, 60% of oils are potentially recoverable (i.e. not burnt in the engines during use). This report also indicates that around 86% of oil changes take place in specialised automotive service centres, where old oil drained from crankcases is disposed of responsibly (e.g. oil recycling or incineration). Assuming this is the case, negligible release of the notified chemical should result from these professional activities. The remaining 14% of oil is handled by DIY consumers. This is broadly consistent with the notifier's estimate of the proportion of the volume likely to be sold to the commercial and consumer markets.

In the case of DIY consumers, some of the used oil would be either incinerated, left at transfer stations where it is again likely to be recycled, or deposited of into landfill. It was estimated that DIY activities account for approximately 7 – 10% of the unaccounted used oil (Meinhardt, 2002).

According to a survey tracing the fate of used lubricating oil in Australia (Snow, 1997), only approximately 20% of used oil removed by DIY consumers is collected for recycling, approximately 25% is buried or disposed of in 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 a worst case scenario involving the 14% of used oil removed by DIY consumers, up to 0.7% (= 14% × 5%) of the total import volume of the notified chemical may enter the aquatic environment via disposal to stormwater drains. Therefore, the amount of the notified chemical released to the aquatic environment from disposal of used oil due to DIY consumers is expected to be 700 kg/yr. In addition to this, considering the unknown fate of some of the oil used by DIY consumers, a small proportion may also be disposed of to the sewer. Since the use of the lubricating oils will occur throughout Australia, all releases resulting from use or disposal of used oil will be very diffuse, and release of the notified chemical in neat concentrations is unlikely except as a result of transport accidents.

7.1.2. Environmental Fate

The notified chemical was shown to be biodegradable. However, the notified chemical cannot be formally classified as readily biodegradable according to the strict definition of the test guidelines. A study report indicates that it is inherently biodegradable. Bioaccumulation of the notified chemical is not expected due to its biodegradability and limited potential for exposure to the aquatic compartment. The limited amount of notified chemical anticipated to be disposed of to landfill (e.g. residues in drums) is expected to bind to soil due to the presence of cationic functionality, and undergo slow degradation processes via biotic and abiotic pathways. In landfill, the notified chemical is expected to decompose into water and oxides of carbon and nitrogen. For details of the environmental fate study refer to Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)

It is not necessary to calculate the Predicted No-Effect Concentration (PNEC) since no significant release of the notified chemical is expected from the proposed use pattern.

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 = 0.91 mg/L	Not toxic to fish up to the limit of water solubility

Fish Toxicity (96 h)	LC50 = 0.5 mg/L	Very toxic to fish
Daphnia Toxicity (48 h)	EC50 = 0.14 mg/L	Very Toxic to aquatic invertebrates
Algal Toxicity (96 h)	E_rC50 = 9.8 mg/L	Not toxic to algae up to the limit of water solubility

Under the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS; United Nations, 2009) the notified chemical is acutely very toxic to fish and aquatic invertebrates. The notified chemical is therefore formally classified 'Acute Category 1; Very toxic to aquatic life'. As the notified chemical is expected to be inherently biodegradable with a NOEC of >1, it is not classified under the GHS for chronic toxicity.

7.2.1. Predicted No-Effect Concentration

The PNEC has not been calculated as no significant aquatic exposure is expected based on the reported use pattern.

7.3. Environmental Risk Assessment

The Risk Quotient, Q (= PEC/PNEC), has not been determined due to the notified chemical's low potential for release to the aquatic compartment. The majority of the formulated products containing the notified chemical will be used as lubricant products. At the end of life, the fluids will be drained from the machinery for disposal. The main method of disposal will be by recycling or thermal decomposition. If notified chemical is spilt to soil or disposed of to landfill, it is expected to degrade biotically and abiotically to water and oxides of carbon and nitrogen. Based on its reported use pattern, the notified chemical is not expected to pose an unacceptable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point -50 to 10 °C

Method	OECD TG 102 Melting Point/Melting Range EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature
Remarks	Differential scanning calorimetry (DSC)
Test Facility	Charles River (2016a)

Boiling Point Decomposes at > 200 °C; No boiling observed

Method	OECD TG 103 Boiling Point EC Council Regulation No 440/2008 A.2 Boiling Temperature
Remarks	Differential scanning calorimetry (DSC)
Test Facility	Charles River (2016a)

Density 941 kg/m^3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids EC Council Regulation No 440/2008 A.3 Relative Density
Remarks	Pycnometer, D^{20}_4 = 0.941
Test Facility	Charles River (2016a)

Viscosity 1,116 mm^2/s at 20 °C

Method	OECD TG 114 Viscosity of Liquids
Remarks	Glass capillary viscometer
Test Facility	Charles River (2016a)

Vapour Pressure 4.6×10^{-9} kPa at 25 °C
2.1×10^{-9} kPa or 20 °C

Method	OECD TG 104 Vapour Pressure EC Council Regulation No 440/2008 A.4 Vapour Pressure
Remarks	Isothermal thermogravimetric method
Test Facility	Charles River (2016a)

Water Solubility 5.4×10^{-4} g/L at 20 °C

Method	OECD TG 105 Water Solubility EC Council Regulation No 440/2008 A.6 Water Solubility
Remarks	Flask Method
Test Facility	Charles River (2017a)

Hydrolysis as a Function of pH Stable

Method	OECD TG 111 Hydrolysis as a Function of pH EC Council Regulation No 440/2008 C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH
Remarks	The preliminary test (Tier 1) and main study (Tier 2) were performed for the determination of the rate of hydrolysis of the test substance. Based on the relatively low recovery at start of the tests and the steep decrease in concentration during the first hours of the tests at 50 and 60°C, it is expected that the decrease in concentration observed in the various tests at pH 4, pH 7 and pH 9 is most probably due to adsorption and/or limited solubility in the buffer solutions and not due to hydrolysis. The amide bond in the various compounds present in the test substance is expected to be stable at pH 4, 7 and 9.
Test Facility	Charles River (2017b)

Partition Coefficient (n-octanol/water) log Pow ≥ 5.3 at 35 °C

Method	OECD TG 117 Partition Coefficient (n-octanol/water). EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	HPLC Method
Test Facility	Charles River (2017a)

Surface Tension 57.2 mN/m at 20 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions EC Council Regulation No 440/2008 A.5 Surface Tension
Remarks	Concentration: 90% of saturation concentration. The notified chemical is regarded as surface active.
Test Facility	Charles River (2016a)

Adsorption/Desorption log Koc = 5.4 to > 6.3 at 35 °C

Method	OECD TG 121 –Estimation of Adsorption Coefficient on Soil and Sewage Sludge using High Performance Liquid Chromatography (HPLC) Method
Remarks	The HPLC method using soil-adsorption-reference data was applied for the determination of the adsorption coefficient (Koc) of the test substance.
Test Facility	Charles River (2017b)

Flash Point Not observed

Method	EC Council Regulation No 440/2008 A.9 Flash Point
Remarks	Closed cup method
Test Facility	Charles River (2016a)

Flammability Not highly flammable in contact with water

Method	EC Council Regulation No 440/2008 A.12 Flammability (Contact with Water)
Remarks	Based on chemical structure and water solubility; statement provided by the study authors
Test Facility	Charles River (2016a)

Autoignition Temperature 360 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases)
Remarks	Commercial apparatus was used and the notified chemical was auto-ignitable.
Test Facility	Charles River (2016a)

Explosive Properties No explosive properties

Method	EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks	Based on chemical structure; statement provided by the study authors
Test Facility	Charles River (2016a)

Oxidizing Properties No oxidising properties

Method	EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids)
Remarks	Based on chemical structure; statement provided by the study authors
Test Facility	Charles River (2016a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method EC Council Regulation No 440/2008 B.1 tris Acute Oral Toxicity – Acute Toxic Class Method
Species/Strain	Rat/Wistar Crl:WI (Han)
Vehicle	None. The notified chemical was dosed undiluted.
Remarks - Method	GLP Certificate No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	3 F	2,000	0/3
2	3 F	2,000	0/3
3	1 F	5,000	0/1
4	2 F	5,000	0/2

LD50	> 5,000 mg/kg bw
Signs of Toxicity	Hunched posture was seen for all animals on Day 1, and for one animal at 2,000 mg/kg bw on Days 2 – 3. Piloerection was observed on most animals on Day 1. Two animals in the 5,000 mg/kg bw dose group showed abnormal licking.
Effects in Organs	Isolated red foci on the thymus was observed on one animal dosed at 2,000 mg/kg bw. No abnormalities were observed in the other animals.
Remarks - Results	The animals showed expected body weight gain over the observation period.
CONCLUSION	The notified chemical is of low acute toxicity via the oral route.
TEST FACILITY	Charles River (2016b)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal)
Species/Strain	Rat/Wistar Crl:WI (Han)
Vehicle	None. The notified chemical was directly applied.
Type of dressing	Occlusive
Remarks - Method	GLP Certificate No significant protocol deviations

RESULTS

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

LD50	> 5,000 mg/kg bw
Signs of Toxicity - Local	Focal erythema was observed on the nose and treated skin-area on animals in the 2,000 mg/kg bw dose group. Chromodacryorrhoea was observed on Day 1 in 3 males and 2 females in the 5,000 mg/kg bw dose group. Erythema and scales were seen in the

	treated-skin area on all animals in the 5,000 mg/kg bw dose group.
Signs of Toxicity - Systemic	No abnormalities were observed.
Effects in Organs	Isolated red foci on the thymus was observed on one female dosed at 5,000 mg/kg bw. No abnormalities were observed in the other animals.
Remarks - Results	The animals showed expected body weight gain over the observation period.
CONCLUSION	The notified chemical is of low acute toxicity via the dermal route.
TEST FACILITY	Charles River (2016c)

B.3. Corrosion – skin (*in vitro* reconstructed human epidermis test)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 431 *In vitro* Skin Corrosion – Reconstructed Human Epidermis Test EC Council Regulation No 440/2008 B.40. *In vitro* Skin Corrosion – Reconstructed Human Epidermis Test
Vehicle	None. The notified chemical was directly applied.
Remarks - Method	GLP Certificate No significant protocol deviations EpiDerm Skin Model Negative control (milli-Q water) and positive control (potassium hydroxide) were run concurrently with the notified chemical.

RESULTS

3 Minute Exposure

Test material	*Mean OD_{570} of triplicate tissues*	*Relative mean Viability (%)*	*SD of mean viability*
Negative control	2.192	100	0.067
Test substance	1.467	67	0.040
Positive control	0.149	7	0.004

1 Hour Exposure

Test material	*Mean OD_{570} of triplicate tissues*	*Relative mean Viability (%)*	*SD of mean viability*
Negative control	2.057	100	0.034
Test substance	1.760	86	0.006
Positive control	0.179	9	0.055

OD = optical density; SD = standard deviation

Remarks - Results	Because the relative mean tissue viability was > 50% after the 3 minute treatment and > 15% after the 1 hour treatment with the notified chemical, it is categorised as non-corrosive according to the test guidelines. The test substance did not show colour interference and direct MTT reduction under the conditions of the tests. The mean OD570 from the negative control and positive control were within the historical control values. Therefore, it is concluded by the study authors that the test conditions of this study were adequate and functioned properly.
CONCLUSION	The notified chemical was non-corrosive to the skin under the conditions of the test.
TEST FACILITY	Charles River (2016d)

B.4. Irritation – skin (*in vitro* reconstructed human epidermis test)

TEST SUBSTANCE	Notified chemical

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

Vehicle: None. The notified chemical was directly applied.

Remarks - Method: GLP Certificate
No significant protocol deviations
EpiSkin Small Model.

Negative control (phosphate buffered saline) and positive control (5% sodium dodecyl sulfate) were run concurrently with the notified chemical.

RESULTS

Test material	*Mean OD_{570} of triplicate tissues*	*Relative mean Viability (%)*	*SD of mean viability*
Negative control	0.882	100	0.112
Test substance	0.827	94	0.128
Positive control	0.107	12	0.035

OD = optical density; SD = standard deviation

Remarks - Results: Because the relative mean tissue viability was > 50% with treatment of the notified chemical, it is categorised as non-irritating accordingly to the test guidelines.

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

TEST FACILITY: Charles River (2016e)

B.5. Irritation – skin

TEST SUBSTANCE

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. The notified chemical was directly applied.
Observation Period: 14 days
Type of Dressing: Semi-occlusive
Remarks - Method: GLP Certificate
No significant protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	1.3	0	0	2	< 7 days	0
Oedema	0.3	0.3	0.3	1	< 48 h	0

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

Remarks - Results: Slight oedema was observed in all animals within four hours of applying the notified chemical, and was reversible within 48 hours. Slight to well-defined erythema was observed in all animals and was reversible within 24 hours for two animals, but was persistent in one animal. This animal showed scaliness after 7 days but recovered within 14 days.

CONCLUSION: The notified chemical is slightly irritating to the skin.

TEST FACILITY Charles River (2016f)

B.6. Irritation – eye (*in vitro* bovine corneal opacity and permeability test)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage

Vehicle None. The notified chemical was directly applied.

Remarks - Method GLP Certificate
No significant protocol deviations were noted. One of the negative control eyes was excluded from the study since it was slightly translucent resulting in an opacity value outside of normal range. The study authors stated that since the other two eyes met the criteria, the test results were not influenced by this exclusion.

RESULTS

Test material	*Mean opacities of triplicate tissues*	*Mean permeabilities of triplicate tissues*	*IVIS*
Vehicle control	-1.0	0.010	-0.8
*Test substance**	2.2	0.006	2.3
*Positive control**	22.3	2.636	61.8

IVIS = *in vitro* irritancy score
*Corrected for background values

Remarks - Results The notified chemical did not induce ocular irritation. As its IVIS is ≤ 3, no classification is required for eye irritation or severe eye damage.

The IVIS from the vehicle control and positive control were within the historical control means. Therefore, it is concluded by the study authors that the test conditions of this study were adequate and functioned properly.

CONCLUSION The notified chemical was not corrosive or severely irritating to the eyes under the conditions of the test.

TEST FACILITY Charles River (2016g)

B.7. 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 3 days

Remarks - Method GLP Certificate
No significant protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0	0	0.3	1	< 48 h	0
Conjunctiva: chemosis	0	0	0	1	< 24 h	0
Conjunctiva: discharge	0	0	0	1	< 24 h	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 No corneal or iridial effects were noted during the study. Slight conjunctival irritation was noted in all treated eyes 1 hour after treatment. All effects on treated eyes were fully reversible within 48 hours.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY Charles River (2016h)

B.8. Skin sensitisation

TEST SUBSTANCE Notified chemical

METHOD OECD TG 406 Skin Sensitisation – Guinea-Pig Maximization Test
US OCSPP 870.2600

Species/Strain Guinea pig/Hartley-Albino

PRELIMINARY STUDY Maximum Non-irritating Concentration:
topical: 25%, 50%, 75%, and 100%

MAIN STUDY

Number of Animals Test Group: 20 (10M, 10F) Negative control Group: 10 (5M, 5F)

Vehicle

Positive control Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using α-hexylcinnamaldehyde.

INDUCTION PHASE Induction Concentration:
topical: 100%

Signs of Irritation None observed

CHALLENGE PHASE

1st challenge topical: 100%

Remarks - Method GLP Certificate
No significant protocol deviations

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after Challenge*	
		24 h	*48 h*
Test Group	100%	0/20	0/20
Negative Control Group	100%	0/10	0/10

Remarks - Results

CONCLUSION There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.

TEST FACILITY StillMeadow (2016)

B.9. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
EC Directive No 440/2008 B.42 Skin Sensitisation (Local Lymph Node Assay)

Species/Strain	Mouse/CBA/J
Vehicle	Acetone/olive oil (4:1)
Preliminary study	Yes – notified chemical at 50% and 100% concentration
Positive control	Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using α-hexylcinnamaldehyde.
Remarks - Method	GLP Certificate. No significant protocol deviations.

RESULTS

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	5 F	531	1.0
10	5 F	822	1.5
25	5 F	1,219	2.3
50	5 F	3,222	6.1
Positive Control (historical)			
0 (vehicle control)	5 F	1,044	1.0
5	5 F	1,062	1.0
10	5 F	1,536	1.5
25	5 F	4,551	4.4

EC3	30%
Remarks - Results	No deaths or signs of systemic toxicity observed in the main study. Scaliness was noted on the ears of animals dosed at 25% and 50% concentrations on Day 6, which was not considered by the study authors to have a toxicologically significant effect on the activity of the draining lymph nodes. Enlarged auricular lymph nodes were found in animals dosed at 50% concentration. All other animals had normal-sized nodes. No macroscopic abnormalities of the surrounding area were noted for any animal. The study showed positive results with dose-dependent response, indicating potential for skin sensitisation for the notified chemical. However, the notified chemical is surface active. Surface active substances, especially compounds containing unsaturated carbon chains, are known to cause false positive outcomes in LLNA studies (Kreiling, 2008) . Therefore, it cannot be excluded that a non-specific stimulation of the lymph nodes had occurred.

CONCLUSION The study authors conclude that the notified chemical may be regarded as a skin sensitiser, but a false positive outcome cannot be ruled out.

TEST FACILITY Charles River (2016i)

B.10. Repeat dose toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents
EC Directive 440/2008 B.7 Repeated Dose (28 Days) Toxicity (Oral)

Species/Strain	Rat/Wistar Crl:WI (Han)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days

	Dose regimen: 7 days per week
	Post-exposure observation period: None
Vehicle	Water
Remarks - Method	GLP Certificate
	No significant protocol deviations
	Dose levels selected based a 5 day range finding study

RESULTS

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

Mortality and Time to Death
There were no unscheduled deaths of animals in all dose groups.

Clinical Observations
No clinical signs of toxicity were observed in treated animals. Body weight gain was statistically significantly reduced in males of the high dose group, but increased in females of the mid and high dose groups compared with controls. The study authors did not consider these changes to be adverse.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Alanine aminotransferase (ALAT) and cholesterol levels were statistically significantly increased in all animals in the high dose group. Calcium levels were significantly increased in males in the high dose group. No other significant treatment-related changes in haematology and biochemistry were observed.

Effects in Organs
Reduced spleen weights in males and increased liver weights in females of the high dose group were reported. Microscopic examination revealed an absence of haematopoiesis in the spleen, and minimal hepatocellular hypertrophy of the liver, which corresponded to the changed spleen and liver weights.

No treatment-related effects on the organs were observed in low and mid dose group animals.

Remarks – Results
The study authors stated that there were no toxicologically relevant adverse effects observed in animals treated up to 1,000 mg/kg bw/day.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as > 1,000 mg/kg bw/day by the study authors.

TEST FACILITY Charles River (2017c)

B.11. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD	OECD TG 471 Bacterial Reverse Mutation Test
	EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100
	Escherichia coli: WP2uvrA
Metabolic Activation System	S9 mix from Aroclor 1254 induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 5.4 – 5,000 µg/plate
	b) Without metabolic activation: 5.4 – 5,000 µg/plate
Vehicle	DMSO
Remarks - Method	GLP certificate
	No significant protocol deviations

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 512	≥ 1,600	≥ 1,600	Negative
Test 2		≥ 1,568	≥ 2,800	Negative
Test 3		≥ 1,600	≥ 1,600	Negative
Present				
Test 1	≥ 1,600	≥ 5,000	≥ 1,600	Negative
Test 2		≥ 878	≥ 2,800	Negative
Test 3		≥ 1,600	≥ 1,600	Negative

Remarks - Results The notified chemical did not increase the number of revertant colonies more than 2-fold in the presence or absence of metabolic activation in all strains tested.

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

TEST FACILITY WIL (2016)

B.12. Genotoxicity – *in vitro* mammalian chromosome aberration test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test
Species/Strain Human blood cells
Cell Type/Cell Line Lymphocyte
Metabolic Activation System S9-Mix from phenobarbital (PB)/β-naphthoflavone (NF) induced rat liver
Vehicle DMSO
Remarks - Method GLP certificate.

A dose range-finding study was carried out at 5.4 – 512 µg/mL. The dose selection for the main experiments was based on toxicity observed in the range-finding study and solubility test.

Vehicle and two positive controls (mitomycin C and cyclophosphamide) were run concurrently with the notified chemical.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	17*, 52*, 164*	3 h	24 h
Test 2	10*, 25, 40, 50*, 60, 70*	24 h	24 h
Test 3	10*, 25*, 40, 50*, 60, 70	48 h	48 h
Present			
Test 1	17*, 52*, 164*	3 h	24 h

*Cultures selected for metaphase analysis

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1		≥ 164	≥ 164	Negative
Test 2	≥ 52	≥ 70	≥ 70	Negative
Test 3	≥ 52	≥ 50	≥ 60	Negative
Present				
Test 1		> 164	≥ 164	Negative

Remarks - Results	The notified chemical did not cause any increase in the number of structurally aberrant metaphases in either the absence or presence of metabolic activation. There was a statistically significant increase of cells with chromosomal aberrations at 70 µg/mL concentration with 24 hours exposure time, but these increases were within control limits of the historical data range. No increases in the frequency of cells containing numerical chromosome aberrations were noted in other samples. The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.
CONCLUSION	The notified chemical was not genotoxic to human lymphocytes treated *in vitro* under the conditions of the test.
TEST FACILITY	Charles River (2016j)

B.13. Mutagenicity – *in vitro* mammalian cell gene mutation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 490 *In vitro* Mammalian Cell Gene Mutation Test Using the Thymidine Kinase Gene
Species/Strain	Mouse
Cell Type/Cell Line	L5178Y Mouse lymphoma cells ($TK^{+/-}$-3.7.2C)
Metabolic Activation System	S9-Mix from phenobarbital (PB)/β-naphthoflavone (NF) induced rat liver
Vehicle	DMSO
Remarks - Method	GLP certificate. A dose range-finding study was carried out at 9.8 – 156 µg/mL. The dose selection for the main experiments was based on toxicity observed in the range-finding study and the solubility test. Vehicle and two positive controls (methyl methanesulfonate and cyclophosphamide) were run concurrently with the notified chemical.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*
Absent			
Test 1	0.63, 1.25*, 2.5*, 5*, 10*, 20*, 25*, 30*, 35*, 40, 45, 50	3 h	2 days
Test 2	2.5*, 5*, 10*, 15*, 20*, 22.5*, 25, 27.5, 30, 35	24 h	2 days
Present			
Test 1	5*, 10, 20*, 30*, 40, 50*, 60*, 70*, 80*, 90*, 100	3 h	2 days

* Cultures selected for mutation frequency (MF) 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	≥ 78	≥ 30	≥ 156*	Negative
Test 2	-	≥ 20	-	Negative
Present				
Test 1	≥ 156	≥ 90	≥ 156*	Negative

* Observed in preliminary test.

Remarks - Results	The notified chemical did not lead to a statistically significant increase in the number of mutation frequencies at the TK-locus, either in the presence

	or absence of metabolic activation. The number of small and large colonies in treated cultures was within the range of the concurrent vehicle control and the historical negative control data. The increase in the frequencies of mutant colonies induced by the positive control demonstrated the sensitivity of the test method and the metabolic activity of the S9 mix
CONCLUSION	The notified chemical was not mutagenic to mouse lymphoma cells treated *in vitro* under the conditions of the test.
TEST FACILITY	Charles River (2017d)

B.14. Toxicity to reproduction/development – screening test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 421 Reproduction/Developmental Toxicity Screening Test OPPTS 870.3550, Reproduction/Developmental Toxicity Screening Test
Species/Strain	Rat/Wistar Crl:WI (Han)
Route of Administration	Oral – gavage
Exposure Information	Exposure days: Males: 29 days (including 14 days pre-mating, during mating and up to termination) Females: 42 – 46 days (most females) or 56 days (2 females) (including 14 days pre-mating, during mating, during pregnancy and 5 – 6 days of lactation)
Vehicle	Water
Remarks – Method	GLP certificate No significant protocol variation

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
1 (vehicle control)	20 (10 M/10 F)	0	0/20
2	20 (10 M/10 F)	100	0/20
3	20 (10 M/10 F)	300	0/20
4	20 (10 M/10 F)	1,000	0/20

Mortality and Time to Death

No mortality was reported during the test.

Effects on Parental (P) animals:

Systemic Toxicity

No treatment-related adverse effects were observed in all animals up to the highest dose tested. Salivation occurred after dosing in all animals treated at 300 or 1,000 mg/kg bw/day and in a few males at 100 mg/kg bw/day. This observation was considered by the study authors to be a physiological response.

Reproductive Toxicity

No significant changes in the reproductive organs of males and females were observed.

The mean numbers of corpora lutea and implantation sites were lower in all groups treated with the test item compared with the vehicle control. However, this difference was not statistically significant, showed no dose-related trend, and remained within the historical control range.

Effects on 1st Filial Generation (F1)

No treatment-related adverse effects were observed in any pups.

Remarks - Results

At the highest dose level (1,000 mg/kg bw/day), body weight gain was reduced in all animals in the first week

of the pre-mating period. Food consumption was also decreased, and mean body weights were 5% lower than the control from Day 8 onwards. Some females also showed decreased food consumption in the second week of the pre-mating period and the post-coitum period. However, these findings were not considered by the study authors to be of toxicological relevance.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) for reproduction and development toxicity was established as > 1,000 mg/kg bw/day by the study authors.

TEST FACILITY Charles River (2017e)

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	Total Organic Carbon (TOC)
Remarks - Method	The test was conducted in accordance with the test guideline above with no significant deviation from the protocol reported.

RESULTS

Test substance		*Sodium acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
5	10	5	29
9	25	7	48
14	35	9	60
23	44	14	73
29	48		

Remarks - Results	All validity criteria were met. The positive control, sodium acetate, reached 73% biodegradation after 14 days, thus confirming suitability of inoculum and test conditions. The test substance did not reach the pass level of 60% for ready biodegradability in the test within the 10-d window and, therefore, cannot be termed as readily biodegradable.
CONCLUSION	The notified chemical is not readily biodegradable
TEST FACILITY	Charles River (2016k)

C.1.2. Inherent biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 302 C Inherent Biodegradability: Modified MITI Test (II)
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biological Oxygen Demand (BOD)
Remarks – Method	The test was conducted in accordance with the test guideline above with no significant deviation from the protocol reported.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
5	42.1	5	73
7	47.2	7	78.6
9	53.7	9	82.9
14	69.7	14	84.9

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
23	75.2	23	89
28	78	28	88.8

Remarks – Results

All validity criteria were met.

The positive control, sodium benzoate, reached 84.9% biodegradation after 14 days, thus confirming suitability of inoculum and test conditions.

The test substance is considered to be inherently biodegradable since the inherent degradation rate was greater than 20% during the 28 day test period.

CONCLUSION

The test substance is inherently biodegradable.

TEST FACILITY

Nanjing Institute of Environmental Sciences (2016)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 203 Fish, Acute Toxicity Test – Semi-Static
EC Council Regulation No 440/2008 C.1 Acute Toxicity for Fish

Species	*Cyprinus carpio*
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	180 mg $CaCO_3$/L
Analytical Monitoring	Ultra-Performance Liquid Chromatography (UPLC)
Remarks – Method	After a range finding test, a definitive test was conducted in accordance with the guidelines above and in compliance with GLP standards and principles. Test solutions were changed daily. No significant deviations to the test protocol were reported.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*
Nominal	*Actual*		*96 h*
Control		7	0
0.22	0.16	7	0
0.46	0.33	7	0
1.0	0.85	7	3
2.2	1.83	7	7
4.6	4.0	7	7

n.d.- Not determined, no surviving fish were present in this concentration.

LC50

0.91 mg/L at 96 hours. (ToxRat, Method not specified)

Remarks – Results

The validity criteria for the test were met.

The actual concentrations measured in the freshly prepared solutions were in agreement with nominal (89-100%). Concentrations measured in the spent solutions at the end of the first refreshment period were between 44 and 90% of initial. The stability of the concentrations was increasing with the nominal dose. The actual concentrations in the spent solutions measured at the end of the last renewal period were at the level of 72-84% of initial. Therefore, the results are based on actual average exposure concentrations (95% confidence interval between 0.69 and 1.2 mg/L).

CONCLUSION	The notified chemical is not toxic to fish up to the limit of its water solubility.
TEST FACILITY	Charles River (2017f)

C.2.2. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Static EC Council Regulation No 440/2008 C.1 Acute Toxicity for Fish
Species	*Gobiocyprus rarus*
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	164-175 mg $CaCO_3$/L
Analytical Monitoring	Gas Chromatography (GC)
Remarks – Method	After a range finding test, a definitive test was conducted in accordance with the guidelines above and in compliance with GLP standards and principles. No significant deviations to the test protocol were reported. In the definitive test, the test medium was prepared as a slow stir stock solution by adding 1.0035 g of the test substance in 10 L dilution water. The aqueous test substance mixture was stirred for 1 hrs on a magnetic stirrer. The stock solution was kept for 2 hour at room temperature prior to the removal of any undissolved test item by filtration through 0.45 µm membrane to produce the stock solution. Test solutions were prepared by using 1 %, 2%, 4%, 6%, 8% and 10% concentrations of the stock.

RESULTS

Concentration (mg/L) Measured	*Number of Fish*	*Mortality 96 h*
Control	10	0
0.12	10	0
0.24	10	0
0.5	10	4
0.69	10	8
0.85	10	10
1.27	10	10

LC50	0.5 mg/L at 96 hours (Trimmed Spearman-Karber Method).
Remarks – Results	The validity criteria for the test were met. The results are based on actual concentrations.
CONCLUSION	The notified chemical is very toxic to fish.
TEST FACILITY	Nanjing Institute of Environmental Sciences (2017a)

C.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - Static> EC Council Regulation No 440/2008 C.2 Acute Toxicity for Daphnia - Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None

Water Hardness	180 mg $CaCO_3$/L
Analytical Monitoring	Ultra-Performance Liquid Chromatography (UPLC)
Remarks - Method	After a range finding test, a definitive test was conducted in accordance with the guidelines above and in compliance with GLP standards and principles. No significant deviations to the test protocol were reported.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual**		*24 h*	*48 h*
Control		20	0	0
0.1	0.029	20	0	0
0.18	0.10	20	0	4
0.32	0.22	20	7	17
0.56	0.44	20	18	20
1.0	0.81	20	20	20

*Based on the geometric mean of the concentration at t = 0 and t = 48

LC50	0.14 mg/L at 48 hours geometric mean of the measured concentration.
Remarks - Results	The validity criteria for the test were met. Measured concentrations were at the level of nominal concentrations at the start of the test (85-91%) and decreased to 10-84% of initial at the end of the exposure period. Therefore, the results are based on actual concentrations.
CONCLUSION	The notified chemical is very toxic to aquatic invertebrates
TEST FACILITY	Charles River (2017g)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test EC Council Regulation No 440/2008 C.3 Algal Inhibition Test
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 0.1, 0.35, 1.2, 4.3 and 15 mg/L Actual: 0.035, 0.19, 0.68, 3.3 and 14 mg/L
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	Ultra-Performance Liquid Chromatography (UPLC)
Remarks - Method	After a range finding test, a definitive test was conducted in accordance with the guidelines above and in compliance with GLP standards and principles. No significant deviations to the test protocol were reported

RESULTS

Biomass		*Growth*	
EC50 *mg/L at 72 h*	*NOEC* *mg/L*	*EC50* *mg/L at 72 h*	*NOEC* *mg/L*
0.43	0.19	9.8	0.19

Remarks - Results	The validity criteria for the test were met.
CONCLUSION	The notified chemical is not toxic to algae up to the limit of water solubility.
TEST FACILITY	Charles River (2017h)

C.2.5. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 1,000 mg/L
Remarks – Method	The test was conducted in accordance with the test guideline without significant deviations. Good Laboratory Practice (GLP) was followed.
RESULTS	
IL50	> 1,000 mg/L
NOEL	1,000 mg/L
Remarks – Results	All validity criteria for the test were satisfied.
CONCLUSION	The notified chemical is not expected to inhibit microbial respiration
TEST FACILITY	Charles River (2016l)

C.2.6. Earthworm Acute toxicity test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 207 Earthworms, Acute toxicity test
Remarks - Method	The test was conducted in accordance with the test guideline without significant deviations. Good Laboratory Practice (GLP) was followed. The 7 day and 14 day LC50 of the test substance was both determined to be > 1,000 mg/kg dry soil. 2-Chloracetamide was used as a reference substance and the EC50 was within the expected range.
RESULTS	14 d LC50 > 1,000 mg/kg dry soil.
Remarks - Results	All validity criteria for the test were satisfied.
CONCLUSION	The notified chemical is not toxic to earthworm.
TEST FACILITY	Nanjing Institute of Environmental Sciences (2017b)

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