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September 2014

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

EXP0700332

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

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

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

Street Address:	Level 7, 260 Elizabeth Street, SURRY HILLS NSW 2010, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX:	+ 61 2 8577 8888
Website:	www.nicnas.gov.au

Director
NICNAS

TABLE OF CONTENTS

SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1519	IMCD Australia Ltd	EXP0700332	No	< 10 tonnes per annum	Component of lubricants

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals* (GHS), as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

Human health risk assessment
Under the conditions of the occupational settings described, the notified chemical is considered to pose/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 reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

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

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

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

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

Disposal

- The notified chemical should be disposed of in accordance with local regulations for recycling, re-use or recovery.

Emergency procedures

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

Regulatory Obligations

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

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

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

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

No additional secondary notification conditions are stipulated.

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

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

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

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

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Europe (2013), Japan (2012), US (2012), Philippines (2013), China (2014), Korea (2014)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
EXP0700332

MOLECULAR WEIGHT
> 500 Da

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

3. COMPOSITION

DEGREE OF PURITY
> 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: viscous amber/brown liquid

Property	Value	Data Source/Justification
Pour Point	-3 °C	Measured
Boiling Point	> 400 °C at 102.31 kPa	Measured
Density	979 kg/m^3 at 20 °C	Measured
Vapour Pressure	4.8×10^{-13} kPa at 25 °C 3.2×10^{-6} kPa at 25 °C	Measured
Water Solubility	$\leq 6.8 \times 10^{-6}$ g/L at 20 ± 5 °C	Measured
Hydrolysis as a Function of pH	Not determined	The notified chemical contains hydrolysable functionalities and is expected to hydrolyse slowly under normal environmental conditions (pH 4-9).
Partition Coefficient (n-octanol/water)	log Pow > 6.5	Measured

Property	Value	Data Source/Justification
Adsorption/Desorption	log K_{oc} > 5.63	Measured
Dissociation Constant	Not determined	Does not contain any dissociable functional groups.
Particle Size	Not determined	Liquid
Flash Point	211 °C at 101.3 kPa	Measured
Flammability (contact with water)	Not highly flammable	Measured
Autoignition Temperature	> 400 °C	Measured
Explosive Properties	Predicted negative	Estimated based on chemical structure
Oxidising Properties	Predicted negative	Estimated based on 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 for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported into Australia as a component of automatic transmission fluid (ATF) additive packages at < 50% concentration or within finished ATF fluids (lubricant) at < 10% concentration.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 10	< 10	< 10	< 10	< 10

PORT OF ENTRY
Unknown

IDENTITY OF RECIPIENTS
IMCD Australia Limited

TRANSPORTATION AND PACKAGING
ATF additive packages containing the notified chemical at < 50% concentration will be imported in 205 L drums or bulk vessels such as iso-containers. Finished ATF lubricants containing the notified chemical at < 10% concentration will be imported within drums or iso-containers. Both ATF additive packages and finished ATF lubricants will be transported by rail or road within Australia.

USE
The notified chemical will be used as a component of finished ATF fluids (lubricant) at < 10% concentration.

OPERATION DESCRIPTION
Reformulation
The ATF additive packages (containing the notified chemical at < 50% concentration) will be reformulated locally using a typical liquid blending process.

During reformulation, the ATF additive packages will be transferred from the original containers into blending vessels using automated pumping/dosing equipment. The containers will be opened by workers and connected to the blending vessels with pipes/hoses using connect fittings. The blending vessels will be sealed. Quality assurance (QA) staff will take samples from sampling ports for analysis. When blending processes are completed, the finished products will be fed via an automated filling line where the products will be filled into various types of end use containers.

End use
Finished ATF lubricant containing the notified chemical at < 10% concentration will be used at automotive manufacturing facilities and mechanic workshops. In automotive manufacturing sites, workers will open the drums or road containers and connect pumping equipment and hoses to dispense fixed volumes of lubricant into motor vehicles on the assembly lines.

Finished ATF lubricant containing the notified chemical at < 10% concentration in smaller containers will be used by aftermarket service mechanics who will manually pour the lubricant into the vehicle reservoirs. Mechanics will also be involved in draining spent lubricant from vehicles during servicing.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and warehousing	4	30
Blending plant operators	5	12
Blending QA staff	2	12
End-use plant operators	4-12	200
End-use maintenance workers	4-12	200
Vehicle manufacturing and mechanics	8	200

EXPOSURE DETAILS
Transport and warehousing
Transport and storage workers will only handle sealed containers and exposure is only expected to occur in the event of container breach.

Blending plant operators and QA
At the reformulation sites, workers may be exposed to the notified chemical at < 50% concentration when connecting or disconnecting transfer hoses, when taking samples for QA testing and during maintenance activities. Exposure at other times is not expected given the enclosed automated processes. Dermal and ocular is expected to be the main routes of exposure. All workers are expected to wear as a minimum coveralls, eye protection and impervious gloves.

Vehicle manufacturing and mechanics
There is potential for dermal and possibly ocular exposure to the notified chemical at < 10% concentration by workers at automotive manufacturing facilities and mechanic workshops when charging or replacing lubricant from engines. At automotive manufacturing facilities, PPE including coveralls, eye protection and impervious gloves is expected to be worn. At mechanic workshops the level of PPE worn by workers may vary.

6.1.2. Public Exposure
Products containing the notified chemical will not be sold to the public. The notified chemical is intended for use in automobile manufacturing plants or mechanic workshops. Hence, the public is not expected to come into contact with the notified chemical.

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
Skin irritation (*in vitro*)	non-irritating
Eye irritation (*in vitro*)	non-irritating

Rabbit, eye irritation	non-irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days	NOEL = 1,000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* mammalian chromosome aberration test	non genotoxic
Genotoxicity – *in vivo* mammalian erythrocyte micronucleus test	non genotoxic
Rat, reproductive and developmental toxicity	NOEL = 1,000 mg/kg bw/day

Toxicokinetics, metabolism and distribution
No toxicokinetic data on the notified chemical were submitted. Absorption across biological membranes is expected to be limited by the relatively high molecular weight (> 500 Da) and high partition coefficient (log Pow > 6).

Solubility of the notified chemical in both fasted simulated intestinal fluid (FaSSIF) and pH 7.4 phosphate buffer containing 0.05% Polysorbate 80 was evaluated and appeared to be in the low to moderate µM range. Using the Polysorbate 80 vehicle, bidirectional permeability of the notified chemical cross Caco-2 cells, as a model of the human intestinal mucosa, was evaluated as a predictor of oral absorption potential. Low but detectable permeability of the notified chemical across Caco-2 cells, about 2% of the applied dose over two hours, was found in the absorptive permeability (apical to basolateral) direction. Lower values were found in the opposite secretory direction. The results showed that the notified chemical may be absorbed orally but absorption would likely to be very low (CeeTox, 2013).

Acute toxicity
In studies conducted in rats the notified chemical was found to have low acute oral and dermal toxicity.

An inhalation toxicity study was not carried out due to the very low vapour pressure of the notified chemical.

Irritation
The notified chemical was non-irritating in an *in vitro* skin irritation study using the Reconstructed Human *Epidermis* test method.

The notified chemical was not irritating to the eye in a study conducted in rabbits. The notified chemical was also found to be non-irritating in an *in vitro* eye irritation study using the SkinEthic Reconstituted Human Corneal Epithelium model.

Sensitisation
The notified chemical in a mouse Local Lymph Node Assay showed no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation.

Repeated dose toxicity
A 28 day repeat dose study by oral gavage was conducted in rats with the notified chemical at dose levels of 30, 300 and 1000 mg/kg bw/day. The No Observed adverse Effect Level (NOAEL) was established as 1000 mg/kg bw/day, based on no changes observed at the highest dose tested.

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

Toxicity for reproduction
The No Observed Effect Level (NOEL) was established as 1,000 mg/kg bw/day in a reproduction/development toxicity screening test based on the absence of treatment-related effects at the highest dose tested.

Health hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the available information, the notified chemical is of low hazard. Therefore, the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

6.3.2. Public Health
Based on the available information, the notified chemical is of low hazard. Furthermore, the notified chemical is only intended for use in industrial settings and hence exposure to the public is not expected. Therefore, when used in the proposed manner, the risk to public health from the notified chemical is not considered to be unreasonable.

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 for repackaging and reformulation into automatic transmission fluid (ATF). 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.

The blending operation occurs in closed pipes and vessels, where the notified chemical will be blended with mineral oil and other additives and automatically pumped out for distribution to customers in Australia via road tanker/drums. If incidental spillage of the additive package occurs during normal blending procedures, it will be contained and soaked up with earth or sand before being transported off-site to an approved industrial facility for appropriate disposal.

RELEASE OF CHEMICAL FROM USE
The finished products containing the notified chemical will be used as a component of ATF. Release during its use may come from spills when pouring the fluid into engines or leaks from the engines, which is expected to be negligible.

RELEASE OF CHEMICAL FROM DISPOSAL
After reformulation, empty import drums containing residues of the notified chemical (1% of the total import volume) are expected to be steam cleaned, with the residual waste sent to on-site wastewater treatment facilities. Assuming 1% of the notified chemical remains in the empty drums after use, 100 kg/yr (10 tonnes/yr × 1%) of the notified chemical 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 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 formulated ATF containing the notified chemical will be used in an enclosed system. The systems will require initial loading and occasional top-up. At the end of life, the fluids will be drained from the machinery for disposal. The main method of disposal will be recycling or thermal decomposition.

7.1.2. Environmental Fate
A ready biodegradability study for the notified chemical showed a 28 day biodegradation of 42%. Therefore, the notified chemical is not considered readily biodegradable. However, it is expected to be biodegradable in the environment (> 60% biodegraded after 42 days). For the details of the environmental fate studies please refer to Appendix C. Most of the notified chemical will be either thermally decomposed during use, recycled or re-refined. Bioaccumulation and bioavailability of the notified chemical is not expected due to its biodegradability and limited potential for exposure to the aquatic compartment.

The notified chemical has very low water solubility at $\geq 6.8 \times 10^{-6}$ g/L. With a high adsorption/desorption coefficient (log K_{OC} > 5.63), and high partition coefficient (log P_{OW} > 6.5), the notified chemical is expected to partition to organic matter and to sediments and soils in the environment, and is therefore considered highly immobile within a landfill environment. Notified chemical released to surface water is expected to partition to

sediment based on its limited water solubility and high log Koc value. The notified chemical has a tendency to partition to organic phases as indicated by its n-octanol/water partition coefficient (log Pow > 6.5), which indicates a potential to bioaccumulate. However, given the notified chemical is expected to be biodegradable, not significantly bioavailable and is not expected to be released to the aquatic environment, the notified chemical has limited potential for bioaccumulation.

Despite the presence of a hydrolysable functionality, the potential for hydrolysis is low due to poor water solubility of the notified chemical. However, a reasonable level of biotic degradation (> 60% in 42 days) has been detected. Therefore, the notified chemical is expected to quickly break down in either a landfill or aquatic environment to form water and oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) is not calculated since no significant release to the aquatic environment is expected based on 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*
Acute toxicity		
Fish Toxicity	96 h LL50 > 102 mg/L (WAF)	Not harmful to fish
Fish Toxicity	96 h LL50 > 100 mg/L (WAF)	Not harmful to fish
Daphnia Toxicity	48 h EL50 > 100 mg/L (WAF)	Not harmful to aquatic invertebrates
Algal Toxicity	72 h EL50 > 100 mg/L (WAF)	Not harmful to algae
Inhibition of Bacterial Respiration	3 h EC50 > 1000 mg/L	Not harmful to bacteria
Earthworm Toxicity	14 d LC50 > 1000 mg/kg dry soil	Not harmful to earthworm
Chronic toxicity		
Fish Toxicity	14 d NOEL > 102 mg/L (WAF)	Not harmful to fish
Daphnia Toxicity	21 d NOEL = 100 mg/L	Not harmful to daphnia

The toxicity data to fish, daphnia and alga in the table above suggest that the notified chemical is not harmful to aquatic organisms up to the limit of water solubility. The chronic toxicity data also suggest that the notified chemical is not harmful to aquatic organisms up to the limit of water solubility. The notified chemical is expected to be biodegradable in the environment (> 60% biodegraded after 42 days). Therefore, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009), the notified chemical is not expected to be harmful to fish, invertebrates and algae on an acute or long term basis and is not formally classified under the GHS.

7.2.1. Predicted No-Effect Concentration

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.

7.3. Environmental Risk Assessment

The risk quotient (RQ = PEC/PNEC) has not been calculated. The notified chemical is not harmful to the aquatic environment. The notified chemical is not expected to persist in the environment due to its biodegradability. Therefore, based on the assessed use pattern and low potential for aquatic exposure, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Pour Point -3 ± 3 °C

Method OECD TG 102 Melting Point/Melting Range.
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Test Facility Harlan Laboratories Ltd (2009a)

Boiling Point > 400 ± 0.5 °C at 102.31 kPa

Method OECD TG 103 Boiling Point.
EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks Differential scanning calorimetry was used.
Test Facility Harlan Laboratories Ltd (2009a)

Density 979 kg/m^3 at 20.0 ± 0.5 °C

Method OECD TG 109 Density of Liquids and Solids.
EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks The pycnometer method was used.
Test Facility Harlan Laboratories Ltd (2009a)

Vapour Pressure 4.8×10^{-13} kPa at 25 °C

Method OECD TG 104 Vapour Pressure.
EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks The vapour pressure balance method was used.
Test Facility Harlan Laboratories Ltd (2009b)

Vapour Pressure 3.2×10^{-6} kPa at 25 °C

Method OECD TG 104 Vapour Pressure.
EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks The vapour pressure balance method was used.
Test Facility Harlan Laboratories Ltd (2013a)

Water Solubility ≤ 6.8 x 10^{-6} 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 Harlan Laboratories Ltd (2009a)

Partition Coefficient (n-octanol/water) log Pow > 6.5 at 40 °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 Harlan Laboratories Ltd (2009a)

Adsorption/Desorption – screening test log K_{oc} > 5.63

Method OECD TG 121 Adsorption Coefficient using HPLC Method.
Remarks During the test it was observed that the test substance did not elute from the HPLC column during the 45 minute run time. Therefore, The adsorption coefficient is expressed as being greater than that of the last eluting reference standard (4,4'-DDT) of 5.63.
Test Facility Charles River (2013a)

Flash Point 211 ± 2 °C at 101.3 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks	A closed cup equilibrium method was used.
Test Facility	Harlan Laboratories Ltd (2009b)

Flammability Not highly flammable

Method	EC Council Regulation No 440/2008 A.12 Flammability (Contact with Water).
Remarks	No gases evolved when in contact with water.
Test Facility	Harlan Laboratories Ltd (2013b)

Autoignition Temperature > 400 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks	It was determined by heating aliquots od the test substance in a flask and observing any ignition.
Test Facility	Harlan Laboratories Ltd (2009b)

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) Sprague-Dawley
Vehicle	None
Remarks - Method	Minor protocol deviations occurring during the study were not considered to have compromised the validity or integrity of the study.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 F	300	0
2	3 F	2000	0
3	3 F	2000	0

LD50	> 2000 mg/kg bw
Signs of Toxicity	No signs of systemic toxicity were observed.
Effects in Organs	Macroscopic findings at necroscopy were restricted to dark discolouring to all lobes of the lungs in two animals which were dose at 2000 mg/kg bw in group 3. No other macroscopic abnormalities were observed. As this finding is occasionally observed in rats of this strain at these laboratories, it was regarded by study authors as a background finding rather than an effect related to treatment.
Remarks - Results	All animals showed expected body weight gains during the study.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Charles River Laboratories (2014a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity.
Species/Strain	Rat/Crl:CD(SD) Sprague-Dawley
Vehicle	None
Type of dressing	Occlusive
Remarks - Method	Minor protocol deviations occurring during the study were not considered to have compromised the validity or integrity of the study.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 per sex	2000	0

LD50	> 2000 mg/kg bw
Signs of Toxicity - Systemic	No signs of systemic toxicity were observed.
Effects in Organs	Macroscopic findings were restricted to dark foci to all lobes of the lungs at necroscopy in one female animal. No other macroscopic abnormalities were observed. As this finding is commonly observed in rats of this strain at these laboratories, it was regarded by study authors not to be an effect related to treatment.
Remarks - Results	All animals showed expected body weight gains during the study.

CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	Charles River Laboratories (2013b)

B.3. Irritation – skin (*in vitro*)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method
Vehicle	None
Remarks - Method	Prior to the conduct of the irritation assay, a preliminary test was conducted to assess intrinsic ability of the test substance to reduce methylthiazoldiphenyl-tetrazolium bromide (MTT) to formazan by visual assessment of the formation of purple-coloured formazan. The test substance did not reduce MTT to formazan while the positive control (eugenol) reduced the MTT solution to purple-coloured formazan. Based on this, non-viable controls were not necessary, and the ≤ 30% non-specific MTT reduction acceptance criterion was assumed to be met.

RESULTS

Test material	*Mean OD_{570} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	0.799	100.00	1.17
Test substance	0.798	99.90	9.42
Positive control	0.085	10.62	3.54

OD = optical density; SD = standard deviation

Remarks - Results	The results for negative controls, positive controls and test substance were within the acceptance criteria defined in the ECVAM validation SOP.
CONCLUSION	The notified chemical was non-irritating to the skin under the conditions of the test.
TEST FACILITY	Charles River Laboratories (2013c)

B.4. Irritation – eye (*in vitro)*

TEST SUBSTANCE	Notified chemical
METHOD	Determination of Ocular Irritation Potential Using the SkinEthic Reconstituted Human Corneal Epithelium Model
Vehicle	None
Remarks - Method	Prior to the conduct of the irritation assay, a preliminary test was conducted to assess intrinsic ability of the test substance to reduce methylthiazoldiphenyl-tetrazolium bromide (MTT) to formazan, which is a measure of cell viability in the assay. The test substance did not reduce MTT to formazan. Minor protocol deviations occurring during the study were not considered to have compromised the validity or integrity of the study.

RESULTS

Test material	*Mean OD_{570} of duplicate tissues*	*Relative mean viability (%)*
Negative control	0.929	100
Test substance	0.936	100.81
Positive control	0.002	0.23

OD = optical density

Remarks - Results	Exposure to the test substance resulted in a tissue viability of 100.81%. Exposure to the positive control resulted in a tissue viability of 0.23%. Cell viability values below 50% of the negative control viability indicate that the test substance is not an irritant.
CONCLUSION	The notified chemical was considered to be non-irritating to the eye under the conditions of the test.
TEST FACILITY	Charles River Laboratories (2013d)

B.5. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	2 M
Observation Period	72 hours
Remarks - Method	Minor protocol deviations occurring during the study were not considered to have compromised the validity or integrity of the study.

RESULTS

Lesion	*Mean Score* Animal No.*		*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2			
Conjunctiva: redness	0	0	1	< 24 h	0
Conjunctiva: chemosis	0	0	0	-	0
Conjunctiva: discharge	0	0	1	< 24 h	0
Corneal opacity	0	0	0	-	0
Iridial inflammation	0	0	0	-	0

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

Remarks - Results	Slight conjunctival irritation was observed in both animals at 1 and 4 hours after instillation. No other ocular signs were observed in either rabbit at any other observation point.
CONCLUSION	The notified chemical is non-irritating to the eye.
TEST FACILITY	Charles River Laboratories (2014b)

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

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)
Species/Strain	Mouse/CBA/Ca
Vehicle	Acetone: olive oil (4:1)
Remarks - Method	Minor protocol deviations occurring during the study were not considered to have compromised the validity or integrity of the study. The positive control was not tested concurrently.

RESULTS

Concentration (% w/w)	*Mean Scintillation Count (DPM)*	*Stimulation Index (Test/Control Ratio)*

Test Substance		
0 (vehicle control)	230 ± 135	1
25	240 ± 103	1.0
50	382 ± 137	1.7
100	403 ± 48	1.8
Positive Control (hexylcinnamicaldehyde)		
25%	not reported	8.9

Remarks - Results

There were no signs of systemic toxicity or local irritation and there was no effect on body weight in any animal.

Dixon's Q-test for the detection of a single outliner was performed on the disintegrations per minute (DPM) data. Applying 95% confidence limits for a population of 4, the DPM value recorded by one animal that received a 25% concentration of test substance, was found to be an outliner and it was excluded from the group mean and SI calculations.

CONCLUSION

There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY

Charles River Laboratories (2013e)

B.7. Repeat dose toxicity

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).

Species/Strain: Rat/Wistar (RccHan:WIST)
Route of Administration: Oral – gavage
Exposure Information: Total exposure days: 28 days
Dose regimen: 7 days per week
Recovery period: 14 days
Vehicle: Arachis oil BP
Remarks - Method: No protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 per sex	0	0
low dose	5 per sex	30	0
mid dose	5 per sex	300	0
high dose	5 per sex	1,000	0
control recovery	5 per sex	0	0
high dose recovery	5 per sex	1,000	0

Mortality and Time to Death

There were no unscheduled deaths.

Clinical Observations

No clinical signs were observed. No treatment-related effects were detected for behaviour assessment, in functional performance parameters or in sensory reactivity parameters. No adverse effects on body weight change, dietary intake, food conversion efficiency or water consumption were detected.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There were no treatment-related adverse changes detected for haematological parameters, blood chemical parameters, urinalytical parameters or the stage of oestrus cycle.

Effects in Organs

There were no toxicologically significant changes in organ weight measurements, no treatment-related necropsy findings or no treatment-related histopathological changes observed at terminal kill.

CONCLUSION

The No Observed Effect Level (NOEL) was established as 1,000 mg/kg bw/day in this study, based on the absence of treatment-related effects at the highest dose tested.

TEST FACILITY Harlan Laboratories Ltd (2009c)

B.8. 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. Plate incorporation procedure (test 1)/Pre incubation procedure (test 2)
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 0, 50, 150, 500, 1500, 5000 µg/plate b) Without metabolic activation: 0, 50, 150, 500, 1500, 5000 µg/plate
Vehicle	Acetone
Remarks - Method	No protocol deviations.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	> 5,000			
Test 1		> 5,000	≥ 5,000	negative
Test 2		> 5,000	≥ 5,000	negative
Present	> 5,000			
Test 1		> 5,000	≥ 5,000	negative
Test 2		> 5,000	≥ 5,000	negative

Remarks - Results

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test substance, either with or without metabolic activation or with or without pre-incubation.

The positive controls produced satisfactory responses, thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

The test substance caused no visible reduction in the growth of the bacterial background lawn at any dose level and was therefore tested up to the maximum recommended dose level of 5,000 µg/plate. A globular precipitate was observed at 5,000 µg/plate, this observation did not prevent the scoring of revertant colonies.

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

TEST FACILITY Harlan Laboratories Ltd (2009d)

B.9. Genotoxicity – *in vitro*

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test. EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.
Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 fraction from phenobarbital/β-naphthoflavone induced rat liver
Vehicle	Acetone
Remarks - Method	No protocol deviations.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 39.06, 78.13*, 156.25*, 312.5*, 625*, 1,250*	4	24
Test 2	0*, 39.06*, 78.13*, 156.25*, 312.5*, 625, 1,250	24	24
Present			
Test 1	0*, 39.06, 78.13*, 156.25*, 312.5*, 625*, 1,250	4	24

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5,000	≥ 625	≥ 1,250	negative
Test 2	> 5,000	= 156.25	≥ 625	negative
Present				
Test 1	> 5,000	> 1,250	≥ 625	negative

Remarks - Results

The top dose for the main test was chosen on the precipitating dose level before the onset of a greasy/oily precipitate where it was considered that the cells were not fully exposed to the test substance. In the preliminary toxicity test the test substance induced some mild evidence of toxicity in all exposure groups.

In the main test, the test substance did not induce any statistically significant increases in the numbers of polyploid cells at any dose level either in the absence or presence of metabolic activation.

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

The results of the mitotic indices from the cultures after their respective treatments showed a 35% growth inhibition achieved in the absence of S9 (test 1). However no toxicity was seen in the presence of S9 (test 1). In the continuous exposure no toxicity was seen at the top dose, however, 14% toxicity was seen at 156.25 μg/mL (test 2).

The test substance induced small by statistically significant increases in the frequency of cells with aberrations in the presence of metabolic activation (test 1). However the significant increases observed were within or close to the upper limit of the historical vehicle control range and well below the positive control in the exposure group. They were also predominantly due to chromatid break type aberrations and were compared to a very low vehicle control value. It was therefore considered by the study authors that these small increases had no biological relevance

and were of no toxicological significance. No significant increases in the frequency of chromosome aberrations were observed in either of the exposure groups without metabolic activation (tests 1 and 2).

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

TEST FACILITY Harlan Laboratories Ltd (2009e)

B.10. Genotoxicity – *in vivo*

TEST SUBSTANCE Notified chemical

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

Species/Strain	Rat/Sprague Dawley (CD)
Route of Administration	Oral – gavage
Vehicle	Peanut oil
Remarks - Method	No protocol deviations. Rats only received a maximum dose level of 2000 mg/kg bw in the dose range finding and main micronucleus tests. In the main micronucleus test, the rats were dosed orally at 0 h and 24 h.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	5 per sex	0	48 h
II (high dose)	7 per sex	2000	48 h
III (positive control, CP)	3 M	50	48 h
IV (untreated control)	3 per sex	0	48 h

CP=cyclophosphamide

RESULTS

Doses Producing Toxicity	No mortality was observed in the treatment group. All rats in the vehicle control group appeared normal during the study.
Genotoxic Effects	There was no indication that the test substance induced bone marrow micronuclei in the treated rats.
Remarks - Results	The MN-PCE frequency of the vehicle and untreated groups conformed to the established in-house control range for vehicle treated rats of the Sprague Dawley strain. Exposure of rats to the positive control induced large increases in bone marrow micronuclei. Based upon this, the test was considered valid. There is no definitive evidence indicating that the test substance reached the bone marrow of the rats.

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

TEST FACILITY Charles River Laboratories (2013f)

B.11. Developmental toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 421 Reproduction/Development Toxicity Screening Test.

Species/Strain	Rat/Wistar (RccHan:WIST)
Route of Administration	Oral – gavage
Exposure Information	Exposure days: Males 28 days from 2 weeks prior to mating. Females 6-8 weeks from 2 weeks prior to mating until day 4 of lactation. Post-exposure observation period: none

Vehicle	Arachis Oil BP
Remarks - Method	Minor protocol deviations occurring during the study were not considered to have compromised the validity or integrity of the study.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
1	10 per sex	0	0
2	10 per sex	30	0
3	10 per sex	300	0
4	10 per sex	1000	0

Mortality and Time to Death

There were no unscheduled deaths.

Effects on Dams

Litter survival, litter weights and mean pup weights were similar between litters derived from control females and litters derived from females treated with the test substance.

All observations recorded for dams and their pups were common findings for this species and strain, and the distributions of observations across the dose groups indicated no relationship to the test substance.

Effects on Foetus

No test substance-related organ weight changes, gross findings and microscopic findings were observed.

Mating performance, fertility indices, corpora lutea and implantation counts, duration of gestation, and the mean number of liver pups born per litter were unaffected by the test substance.

At 1,000 mg/kg/day, 3/10 females passed 1 or more oestruses without mating, versus 0/10 in the 0, 30 and 300 mg/kg/day dose groups. One of these animals passed 3 oestruses in total over 14 days mating period, and only displayed one equivocal mating sign during this time; this animal did not become pregnant. The other 2 animals mated successfully at the next available oestrus, and as there were no other problems noted with the overall reproductive performance of these animals, or indeed in any of the other animals receiving 1,000 mg/kg/day, the distribution of these findings at 1,000 mg/kg/day was considered by study authors to be incidental.

The fertility index at 1,000 mg/kg/day for both males and females was slightly lower than control (80% versus 90%, respectively). However given the minor magnitude of difference from control, this finding was considered by study authors to be incidental.

Remarks - Results

No clinical signs related to the test substance were observed. Body weight gains and food consumption values were similar between control animals and animals treated with the test substance.

CONCLUSION

The No Observed Effect Level (NOEL) was established as 1,000 mg/kg bw/day in this study, based on the absence of treatment-related effects at the highest dose tested.

TEST FACILITY Charles River Laboratories (2013g)

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	42 days
Auxiliary Solvent	None
Analytical Monitoring	Dissolved Organic Carbon (DOC)
Remarks - Method	The test was conducted according to the above mentioned OECD test guidelines. No significant deviations from the test guidelines were reported.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
6	6	6	79
8	16	8	91
14	37	14	90
28	51	28	84
42	61	42	82

Remarks - Results: All test validation criteria were satisfied.

The test substance attained 61% degradation after 42 days. However, despite attaining in excess of 50% biodegradation, the test substance failed to satisfy the 10 day window validation criterion by which 60% degradation must be attained within 10 days of the degradation exceeding 10%. Therefore, the test substance is not considered to be readily biodegradable under the strict terms of the test.

The toxicity control exceeded 37% biodegradation within 14 days showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the notified chemical after 42 days was 67%.

CONCLUSION: The test substances, and hence the notified chemical, is not readily biodegradable.

TEST FACILITY: Harlan Laboratories Ltd (2010)

C.1.2. Bioaccumulation

TEST SUBSTANCE: Notified chemical

METHOD: New Chemical Substances Bioconcentration test of chemical substances in fish and shellfish (Yakushokuhatsu No.1121002, Heisei 15.11.13 Seikyoku No.2, Kanpokihatsu No.031121002, November 21, 2003): Continuous flow-through dilution system.

Species	Carp *(Cyprinus carpio)*
Exposure Period	Exposure: 62 days
Auxiliary Solvent	Tetrahydrofuran (50 ppm)
Concentration Range	High: 0.2 mg/L Low: 0.02 mg/L
Analytical Monitoring	Liquid Chromatography- Mass Spectrometry (LCMS)
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the

test guidelines were reported.

During the exposure period, the concentration of the test substance in water and fish was measured periodically. There was no depuration period. The bioconcentration factor (BCF) was determined by comparing the concentration of the test substance in the fish to the mean concentration of test substance in the test water. Test conditions were: 24 ± 2 °C, pH 6.0-8.5 and ≥ 5 mg O_2/L.

RESULTS

The bioconcentration factors (BCF) during exposure period are shown in the table below.

Component	BCF at high Concentration level	BCF at low concentration level
A	56*	≤ 90*
B	≤ 87**	< 667***
C	≤ 39**	< 64*
D	≤ 39**	< 33*

* The variation of mean BCF at the last three consecutive measurements (over 48 h) were confirmed to fall within ± 20%, the concentration factor was considered to reach the steady-state during 62 days of exposure period.

** The variation of mean BCF at the three consecutive measurements were not confirmed to fall within ± 20%. However, all of the BCF values during the exposure period were less than 100, thus BCF were considered to reach the steady-state.

*** The variation of mean BCF at the three consecutive measurements (over 48 h) of component B was not confirmed to fall within ± 20%, and did not reach the steady-state. Therefore, the BCF value was not calculated.

Bioconcentration Factor: High concentration = 39-87
Low concentration = 33-90

CT50: Not determined

Remarks - Results: The validity criteria for the test were met.

As the test substance is a mixture, many peaks were detected in LC/MS chromatograms. Four quantifiable components (peak A-D) were measured in the determination of BCF.

The lipid content of the fish ranged from 4.6% (n = 3, 4.0-5.8%) at the beginning of the test to 5.1 % (n = 3, 4.2-6.4%) at the end of the test.

During the exposure period of 62 days, the mortalities in both control and treated fish were less than 10% at the end of the test. There were no abnormality in shape of the body or in swimming and eating behaviour during the test period. The test is considered reliable.

CONCLUSION: Under the conditions of this test, the notified chemical is not considered to be bioaccumulative to fish.

TEST FACILITY: MCM (2010)

C.1.3. Inherent biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 302C 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 according to the above mentioned OECD test guidelines. No significant deviations from the test guidelines were reported.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	0	7	90.1
14	0	14	90.6
21	0	21	-
28	0	28	-

Remarks – Results	The test substance attained 0% degradation after 28 days. Therefore, the test substance cannot be considered to be readily biodegradable under the terms of the test. The toxicity control attained 60.1% biodegradation within 14 days showing that toxicity was not a factor inhibiting the biodegradability of the test substance.
CONCLUSION	The test substance is not readily biodegradable under the conditions of the Modified MITI Test.
TEST FACILITY	GDC (2014a)

C.1.4. Ready Biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	New Chemical Substances Biodegradation test of chemical substances by Microorganisms (Yakushokuhatsu No.1121002, Heisei 15.11.13 Seikyoku No.2, Kanpokihatsu No.031121002, November 21, 2003):
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biological Oxygen Demand (BOD)
Remarks – Method	The test was conducted according to the above mentioned OECD test guidelines. No significant deviations from the test guidelines were reported.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	1.47	7	59
14	1.43	14	67
21	1.89	21	75
28	1.5	28	75

Remarks – Results	All test validation criteria were satisfied. The test substance attained a maximum of 2% degradation after 28 days.

Therefore, the test substance cannot be considered to be readily biodegradable under the terms of the test.

CONCLUSION The test substance is not readily biodegradable under the conditions of the test.

TEST FACILITY MSC (2008)

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
Species Zebra fish (*Brachydanio rerio*)
Exposure Period 96 hours
Auxiliary Solvent None
Water Hardness 100 mg $CaCO_3$/L
Analytical Monitoring HPLC
Remarks – Method The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported.

Following a range finding test, a definitive test was conducted at nominal concentration of 102 mg/L. Due to the limited water solubility of the notified chemical, water accommodated fraction (WAF) was used in the test. The test solution was prepared by direct addition of the notified chemical (0.51g) into 5000 L of test water This mixture was stirred for 24 hours and was allowed to stand for 1 hour. The solution was removed by mid-depth siphoning to give the 102 mg/L WAF.

RESULTS

Concentration mg/L	*Number of Fish*	*Mortality*			
Nominal		*24 h*	*48 h*	*72 h*	*96 h*
Control	10	0	0	0	0
102	10	0	0	0	0

LL50 > 102 mg/L at 96 hours (WAF)
NOEL = 102 mg/L at 96 hours (WAF)

Remarks – Results All validity criteria for the test were satisfied. Since a WAF method was used to prepare the treatment solutions, the endpoints were based on the nominal loading rates used to prepare the WAF solutions.

CONCLUSION The notified chemical is not harmful to fish.

TEST FACILITY GDC (2014b)

C.2.2. Acute toxicity to fish

TEST SUBSTANCE Notified chemical

METHOD OECD TG 203 Fish, Acute Toxicity Test – Semi static
Species Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period 96 hours
Auxiliary Solvent None
Water Hardness 140 mg $CaCO_3$/L
Analytical Monitoring HPLC
Remarks – Method The test was conducted according to the guidelines above and good

laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported.

Following a range finding test, a definitive test was conducted at nominal concentration of 100 mg/L. Due to the limited water solubility of the notified chemical, water accommodated fraction (WAF) was used in the test. The test solution was prepared by direct addition of the notified chemical (2100 mg) into 21 L of test water. This mixture was stirred for 23 hours and allowed to stand for 1 hour. The solution was removed by mid-depth siphoning to give the 100 mg/L WAF.

RESULTS

Concentration mg/L *Nominal*	*Number of Fish*	*Mortality* *24 h*	*48 h*	*72 h*	*96 h*
Control	7	0	0	0	0
102	7	0	0	0	0

LL50 > 100 mg/L at 96 hours (WAF)
NOEL = 100 mg/L at 96 hours (WAF)

Remarks – Results All validity criteria for the test were satisfied. Since a WAF method was used to prepare the treatment solutions, the endpoints were based on the nominal loading rates used to prepare the WAF solutions.

CONCLUSION The notified chemical is not harmful to fish.

TEST FACILITY Harlan Laboratories Ltd (2009e)

C.2.3 Chronic toxicity to fish

TEST SUBSTANCE Notified chemical
METHOD OECD TG 204 Fish, Prolonged Toxicity Test: 14-Day Study Semi - Static test.
Species Zebra fish (*Brachydanio rerio*)
Exposure Period 14 day
Auxiliary Solvent None
Water Hardness 100 mg $CaCO_3$/L
Analytical Monitoring HPLC
Remarks – Method The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported.

Following a range finding test, a definitive test was conducted at nominal concentration of 102 mg/L. Due to the limited water solubility of the notified chemical, water accommodated fraction (WAF) was used in the test. The test solution was prepared by direct addition of the notified chemical (0.51g) into 5000 L of test water This mixture was stirred for 24 hours and allowed to stand for 1 hour. The solution was removed by mid-depth siphoning to give the 102 mg/L WAF.

RESULTS

Concentration mg/L *Nominal*	*Number of Fish*	*Mortality* *2 d*	*8 d*	*10 d*	*14d*
Control	10	0	0	0	0
102	10	0	0	0	0

14 d LL50 > 102 mg/L (WAF)
NOEL = 100 mg/L (WAF)
Remarks – Results The validity criteria for the test were met. The result is based on the nominal concentration.

In the test, the body weights of the test fish increased in all groups. The specific growth rate of all treatment groups was not significantly different from that of the control.

The analytical measurements showed that the measured concentrations of the test substance was lower than the limit of detection (LOD = 0.21 mg/L). No mortality occurred in treatments groups. The LC50 for *Brachydanio rerio* exposed for 14 d was higher than the saturated solution of the test substance.

CONCLUSION The notified chemical is not harmful to fish up to the limit of its solubility.

TEST FACILITY GDC (2014b)

C.2.4. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test - Static
Species *Daphnia magna*
Exposure Period 48 hours
Auxiliary Solvent None
Water Hardness 250 mg $CaCO_3$/L
Analytical Monitoring HPLC
Remarks - Method The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported.

Following a range finding test, a definitive test was conducted at a nominal concentration of 100 mg/L. Due to the limited water solubility of the notified chemical, water accommodated fraction (WAF) was used in the test. The test solution was prepared by direct addition of the notified chemical (400 mg) into 4 L of test water. This mixture was stirred for 23 hours and allowed to stand for 1 hour. The solution was removed by mid-depth siphoning to give the 100 mg/L WAF.

RESULTS

Concentration mg/L Nominal	*Number of D. magna*	*Number Immobilised*	
		24 h	*48 h*
Control	30	0	0
100	30	0	0

EL50 > 100 mg/L at 48 hours (WAF)
NOEC (or LOEC) = 100 mg/L at 48 hours (WAF)
Remarks - Results All validity criteria for the test were satisfied. Since a WAF method was used to prepare the treatment solutions, the endpoints were based on the nominal loading rates used to prepare the WAF solutions.

CONCLUSION The notified chemical is not toxic to Daphnia.

TEST FACILITY Harlan Laboratories Ltd (2009f)

C.2.5. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 211 *Daphnia magna* Reproduction test – Semi static.
Species *Daphnia magna*

Exposure Period	21 d
Auxiliary Solvent	None
Water Hardness	150-180 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported. Following a range finding test, a definitive test was conducted at a nominal concentration of 100 mg/L. Due to the limited water solubility of the notified chemical, water accommodated fraction (WAF) was used in the test. The test solution was prepared by direct addition of the notified chemical into test water. This mixture was stirred for, 48 hours and suction filtered through 4µ glass filter. The filtrate was stirred for 30 minutes to recover dissolved oxygen concentration decreased by the suction filtration.

Results

Concentration tested, cumulative mean number of offspring released, number of adult daphnidsias immobilised, and survival of parental daphnids.

	Concentration (mg/L)	
Test Day 21	Control	100
Total no. of offspring released by survived *Daphnia*	96	78
No. of adult daphnids Immobilised	0	0
% Survival	100	100

21 day NOEL = 100 mg/L

Remarks - Results	The validity criteria for the test were met. The survival of the test animals at the end of the test 100% in the controls. This was observed at the nominal test concentration of 100 mg/L. Thus, the survival of *Daphnia magna* was not affected by the test substance up to the highest test concentration. The first young offspring released from their parent animals were recorded in the control and at the test concentration between day 8 and 9. Thus, the time of first brood was not affected by the test substance up to and including the highest test concentration. The NOEL was determined to be 100 mg/L. All the endpoints were determined by the study author and are considered acceptable.
CONCLUSION	The notified chemical is not considered toxic to daphnids on a chronic basis
TEST FACILITY	CERI (2013)

C.2.6. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Desmodesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 100 mg/L
Auxiliary Solvent	None
Water Hardness	Not provided

Analytical Monitoring	HPLC
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported. Following a range finding test, a definitive test was conducted at nominal concentration of 100 mg/L. Due to the limited water solubility of the notified chemical, water accommodated fraction (WAF) was used in the test. The test solution was prepared by direct addition of the notified chemical (250 mg) into 2.5 L of test water. This mixture was stirred for 23 hours and allowed to stand for 1 hour. The solution was removed by mid-depth siphoning to give the 100 mg/L WAF.

RESULTS

Biomass		*Growth*	
EyL50 *mg/L at 72 h*	*NOEL* *mg/L*	*ErL50* *mg/L at 72 h*	*NOEL* *mg/L*
> 100	100	> 100	100

Remarks - Results	All validity criteria for the test were satisfied. Chemical analyses of the test substance in WAFs were conducted at 0 and 72 hours of the test. Statistically significant reduction of growth rate was not found at the highest treatment, which was at the loading rate of 100 mg/L.

CONCLUSION The notified chemical is not harmful to algae at the loading rate of 100 mg/L.

TEST FACILITY Harlan Laboratories Ltd (2009g)

C.2.7. 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: 100 mg/L
Remarks – Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported.

RESULTS

EC50	> 1,000 mg/L
NOEC	1,000 mg/L
Remarks – Results	All validity criteria for the test were satisfied. The EC50 was out of the tested concentration range (> 1,000 mg/L).

CONCLUSION The notified chemical is not expected to inhibit microbial respiration

TEST FACILITY Charles River Laboratories (2013h)

C.2.8. Acute study in earthworm

TEST SUBSTANCE Notified chemical

METHOD OECD TG 207 Earthworms, Acute toxicity test

Remarks - Method	Test substance (2.75 g) was dissolved in an appropriate amount of acetone to make a stock solution. The stock solution (10 mL) was mixed with 10 g quartz sand. The solvent was removed from this solution and was mixed with 740 g of artificial soil to give the test system of 1000 mg/kg dry

weight soil.

The study was performed on *Eisenia foetida*. 20 animals were subjected to single exposure to nominal concentrations and observed for the following 14 days. The animals were observed on day 7 and 14 and mortality and other visible behavioural or pathological signs were reported.

RESULTS

Concentration mg/kg	Number of earthworms	Mortality 7 days	Mortality 14 days
Control	20	0	0
Solvent	20	0	0
1	20	0	0
10	20	0	0
100	20	0	0
1,000	20	0	0

Remarks - Results

14 d LC50 > 1,000 mg/kg dry soil.

All validity criteria for the test were satisfied. The 14 d LC50 was out of the tested concentration range (> 1,000 mg/kg dry weight).

CONCLUSION

The notified chemical is not toxic to earthworm.

TEST FACILITY

GDC (2014c)

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