File No: EX/221 (STD/1555)

November 2018

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Z-149

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

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This assessment report is for an extension of original assessment certificate for Z-149. Based on the submission of new information by the extension notifier, some sections of the original assessment report have been modified. These modifications have been made under the heading *'Extension Application'* in the respective sections.

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
EX/221 (STD/1555)	Total Oil Australia Pty Ltd	Z-149	Yes	< 110 tonnes per annum	Additive in engine oil

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard classification	*Hazard statement*
Skin sensitisation (Category 1)	H317 – May cause an allergic skin reaction

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

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

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*
Acute (Category 2)	H401 - 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

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:

- Skin sensitisation (Category 1): H317 – May cause an allergic skin reaction

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

Health Surveillance

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

CONTROL MEASURES

Occupational Health and Safety

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

- 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 during reformulation:
  - 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 during reformulation:
  - Impervious gloves
  - Safety glasses
  - Protective clothing

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

- A copy of the (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 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.

Storage

- The handling and storage of the notified chemical should be in accordance with the Safe Work Australia Code of Practice for *Managing Risks of Hazardous Chemicals in the Workplace* (SWA, 2012) or relevant State or Territory Code of Practice.

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 an additive in engine 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.

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

Extension Application (EX/210):
Applicant for the extension application has provided SDS for products containing the notified polymer. The accuracy of the information on the SDS remains the responsibility of the extension applicant.

Extension Application (EX/221):
Applicant for the extension application has provided SDS for products containing the notified polymer. The accuracy of the information on the SDS remains the responsibility of the extension applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)

Holders of Original Assessment Certificates (STD/1555)
Lubrizol International, Inc. (ABN: 52 073 495 603)
28 River Street
Silverwater NSW 2128

Applicant for an Extension (EX/210) of the Original Assessment Certificate:
BP Australia Pty Ltd (ABN: 53 004 085 616)
Level 17, 717 Bourke Street
DOCKLANDS VIC 3008

Applicant for an Extension (EX/221) of the Original Assessment Certificate:
Total Oil Australia Pty Ltd (ABN: 15 149 501 922)
415 Riverside Road
HAWTHORN EAST VIC 3178

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

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

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: hydrolysis as a function of pH, partition coefficient, adsorption/desorption and dissociation constant.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
US TSCA P-14-0306 (2014)

2. IDENTITY OF CHEMICAL

MARKETING NAME
Z-149

MOLECULAR WEIGHT
> 500 Da

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

3. COMPOSITION

DEGREE OF PURITY
> 99%

4. PHYSICAL AND CHEMICAL PROPERTIES

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

Property	Value	Data Source/Justification
Melting Point/Freezing Point	< -20 °C	Measured

Boiling Point	Decomposed without boiling from 186 °C	Measured
Density	856 kg/m^3 at 20 °C	Measured
Vapour Pressure	$9.1x10^{-6}$ kPa at 25 °C	Measured
Water Solubility	3.56×10^{-2} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	The notified chemical is unstable in water.
Partition Coefficient (n-octanol/water)	Not determined	The notified chemical is unstable in water
Adsorption/Desorption	Not determined	The notified chemical is unstable in water
Dissociation Constant	Not determined	The notified chemical is unstable in water
Particle Size	Not determined	Liquid
Flash Point	178 °C (closed cup)	Measured
Flammability	Not determined	Not expected to be flammable
Autoignition Temperature	258 °C	Measured
Explosive Properties	Not determined	Not expected to be explosive based on structure.
Oxidising Properties	Not determined	Not expected to be oxidising based on 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. The substance is incompatible with water or moisture, strong acids and strong oxidising agents.

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as part of an engine oil additive package at ≤ 10% concentration.

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

Original Introduction Volume

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 55	< 75	< 85	< 95	< 110

Extension Application Introduction Volume (EX/210)

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	39	41	44	46	48

Extension Application Introduction Volume (EX/221)

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 55	< 75	< 85	< 95	< 110

PORT OF ENTRY
Western Australia, Queensland and Victoria

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as part of engine oil additive packages in 200 L drums, 1200 L drums or 20 tonne bulk ISO tanks.

USE
The notified chemical will be used as a component of engine oils at ≤ 1% concentration for automotive use.

OPERATION DESCRIPTION
The notified chemical will not be manufactured in Australia, but the additive package containing the notified chemical (at ≤ 10% concentration) will be reformulated after importation.

Reformulation
After importation, it is expected that the additive packages containing the notified chemical at ≤ 10% concentration will be transferred into blending tanks (containing mineral oil and other additives) using automated, well ventilated and enclosed processes. After blending, it is expected that the end-use product containing the notified chemical at ≤ 1% concentration will be packaged using automated processes. The resulting engine oil products containing the notified chemical at ≤ 1% concentration may be supplied in bulk for industrial users or smaller containers for use in commercial service applications or DIY users.

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

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

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

Reformulation
Dermal and ocular exposure of workers to the notified chemical at ≤ 10% concentration may occur during reformulation when connecting and disconnecting hoses and during sample testing. The blending process and packaging is expected to be automated and within a closed system.

Dermal and ocular exposure to workers should be mitigated through the use of personal protective equipment (PPE) including protective clothing, impervious gloves and goggles, as anticipated by the notifier in the application dossier. Inhalation exposure is not expected given the enclosed systems and low vapour pressure of the notified chemical.

End-use
At automotive service centres, professional users such as mechanics may experience dermal or ocular exposure to the engine oil products containing the notified chemical at ≤ 1% concentration when transferring engine oils to cars. The potential for dermal and ocular exposure may be mitigated through the use of PPE (e.g. gloves, protective clothing, goggles and face shield), as recommended by the notifier.

6.1.2. Public Exposure
Dermal and ocular exposure to the notified chemical may occur to members of the public when topping up or changing engine oil containing the notified chemical at ≤ 1% concentration. Given the low concentration (≤ 1%) of the notified chemical in the engine oil and the fact that engine oil is changed infrequently, potential for exposure to the notified chemical is expected to be low.

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation	irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation – non-adjuvant test (Buehler test)	evidence of sensitisation

Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
Rat, repeat dose oral gavage toxicity – 28 days	NOAEL = 500 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vivo mammalian erythrocyte micronucleus	non genotoxic

Toxicokinetics.
Based on the molecular weight (> 500 Da) and low water solubility (0.036 g/L at 20 °C) of the notified chemical, dermal absorption may be limited.

Acute toxicity.
The notified chemical was found to have low acute oral and dermal toxicity in rats. No acute inhalation toxicity studies were provided.

Irritation.
In studies conducted in rabbits, the notified chemical was found to be irritating to the skin and slightly irritating to the eyes.

Sensitisation.
In a non-adjuvant skin sensitisation study in guinea pigs (modified Buehler method), the notified chemical was found to be a sensitiser at a challenge concentration of 100% and a rechallenge concentration of 75%.

In a LLNA study in mice, the notified chemical also induced a sensitisation response. The test was initially performed using cottonseed oil as the vehicle and test concentrations of 0.1%, 1% and 10%. However, due to an inconclusive result and concerns over the reactivity of the test item with the moisture content of the cottonseed oil, an additional test was performed using dried cottonseed oil as the vehicle. The test concentrations of the notified chemical using dried cottonseed oil as the vehicle were 0.1%, 0.4% and 100% resulting in stimulation index (SI) values of 1.05 (0.1% notified chemical), 1.31 (0.4% notified chemical), and 3.66 (100% notified chemical). Given there is a clear dose response and a SI of greater than 3 at 100% concentration, the results of this study indicate the notified chemical to be a weak sensitiser.

Repeated dose toxicity.
In a 28-day repeated dose oral gavage toxicity study in rats with the notified chemical at exposure doses of 0, 250, 500 or 1000 mg/kg bw/day, the NO(A)EL was established as 500 mg/kg bw/day based on uncertainty regarding the cause of death of one female, and testicular tubular degeneration observed at the highest exposure dose.

Mutagenicity/Genotoxicity.
The notified chemical was negative in a bacterial reverse mutation assay and in an *in vivo* mammalian erythrocyte micronucleus test.

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

Hazard classification	***Hazard statement***
Skin sensitisation (Category 1)	H317 – May cause an allergic skin reaction

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

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

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The critical health effects of the notified chemical are as a skin irritant and a weak sensitiser.

During reformulation workers may be exposed to the notified chemical at ≤ 10% concentration. At these concentrations, the potential risk of irritating and sensitising effects is expected to be low. Furthermore, this risk is expected to be further minimised by the expected use of personal protective equipment including protective clothing, imperious gloves and goggles, and largely automated and enclosed processes limiting exposure.

During end-use workers may be exposed to the notified chemical at ≤ 1% concentration when changing or topping-up engine oil. At these low end-use concentrations, the potential risk of irritating and sensitising effects is not expected.

Given the lower end-use concentration and stated controls in place to minimise exposure during reformulation, the risk to the health of workers is not considered unreasonable.

6.3.2. Public Health

Given the public will only be exposed to the notified chemical at low concentrations (≤ 1%) and on an infrequent basis, the risk to public health from use of the notified chemical is not considered 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 engine lubricating oils. 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 concrete bunds and either reclaimed or sent to on-site waste treatment facilities.

RELEASE OF CHEMICAL FROM USE

Finished products containing the notified chemical will be used as a component of engine lubricants. Release during use may come from spills when pouring lubricants 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 are expected to be steam cleaned, with the residual waste sent to on-site wastewater treatment facilities. 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 to the cleaning of empty drums.

The major release of the notified chemical to the environment will come from inappropriate disposal of waste or used oils. Oil products containing the notified chemical will be poured into engines by automotive 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 removed by DIY consumers. In these cases, some of the used oil would be either incinerated, left at transfer stations where it is again likely to be recycled, or deposited into landfill. It was estimated that DIY activities account for 7 - 10% of the unaccounted used oil (Meinhardt, 2002).

According to a survey tracing the fate of used lubricating oil in Australia (Snow, 1997), 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 770 kg/yr (= 110 tonnes/year × 0.7%). 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 test substance attained 74% biodegradation after 28 days. Under the strict terms and conditions of OECD Guideline No. 301B the test item cannot be considered to be readily biodegradable as the test item failed to satisfy the 10-Day window validation criterion, whereby 60% biodegradation must be attained within 10 days of the biodegradation exceeding 10%. However, the test item has exhibited the potential for rapid biodegradation. For details of the environmental fate studies please refer to Appendix C. Most of the notified chemical will be 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 majority of the notified chemical will be thermally decomposed during usage in engine oils, or thermally decomposed to recover the calorific value, or disposed of to landfill. In landfill, the notified chemical is not expected to leach from soil due to its surfactant properties. The notified chemical is expected to degrade in landfill or be thermally decomposed to form water and oxides of carbon, boron and nitrogen. Based on its surface active properties, the notified chemical is likely to partition to phase boundaries, and therefore expected to partition to sludge during sewage treatment processes. The notified chemical released to surface waters is expected to partition to sediment. Additionally, the notified chemical is not expected to bioaccumulate based on its surfactant property. Any notified chemical remaining in treated sewage effluents is likely to be released to surface waters or applied to land when used for irrigation. Notified chemical in sewage sludge is anticipated to be disposed of to landfill or applied to land when sludge is used for soil remediation.

7.1.3. Predicted Environmental Concentration (PEC)

For the worst case scenario, the percentage of the imported quantity of notified chemical inappropriately disposed to stormwater drains is estimated to be 0.7%. That is, 14% (fraction collected by DIY users) × 5% (fraction disposed to stormwater). The release of the notified chemical may be up to 770 kg/year (= 110 tonnes/year × 0.7%). In this worst case scenario, it is assumed that the release goes into stormwater drains in a single metropolitan area with a geographical footprint of 500 km^2 and an average annual rainfall of 500 mm, all of which drains to stormwater. With a maximum annual release into this localised stormwater system of 770 kg and the annual volume of water drained from this region estimated to be 250 × 10^6 m^3, the calculated PEC will be up to 3.08 µg/L. This result reflects a worst-case scenario upper limit, as in reality releases of the notified chemical will be distributed over multiple regions and it will be further diluted if it reaches the ocean.

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	LC50 1.3 mg/L	Toxic to fish
Daphnia Toxicity	EC50 2.6 mg/L	Toxic to aquatic invertebrates
Algal Toxicity	EC50 9.0 mg/L	Toxic to algae
Inhibition of Bacterial Respiration	EC50 230 mg/L	Not toxic to bacterial respiration

Under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009) the notified chemical is considered to be toxic to fish, aquatic invertebrates and algae and is formally classified as 'Acute Category 2: Toxic to aquatic life'. On the basis of its rapid biodegradability, the notified chemical is not been classified for its chronic toxicity.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) for the notified chemical has been calculated and presented in the table below. An assessment factor of 100 has been used to derive the PNEC as ecotoxicity data for aquatic species at three trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment	
LC50 (Fish)	1.3 mg/L
Assessment Factor	100
PNEC:	13 µg/L

7.3. Environmental Risk Assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	3.08	13	0.236
Q - Ocean	0.308	13	0.023

The Risk Quotients (Q = PEC/PNEC) have been calculated to be < < 1 for both river and ocean compartments. The notified chemical is not expected to persist in the environment as it is unstable in water, and is not expected to bioaccumulate. Therefore, on the basis of the PEC/PNEC ratio, maximum annual import volume and assessed use pattern, the notified polymer is not expected to pose an unreasonable risk to the environment.

8. RISK ASSESSMENT FOR EXTENSION APPLICATION

EX/210

There are no changes under the proposed extension to the use, or the occupational and public exposure. The introduction volume will be increasing by approximately 50%, however, the environmental risk quotients will still remain < 1 for both the river and ocean compartments. Therefore, the circumstances in the extension are not expected to impact on the original human health and environment risk assessment and recommendations.

EX/221

There are no changes under the proposed extension to the use, or to the occupational, public and the environmental exposure. Therefore, the circumstances in the extension are not expected to impact on the original human health and environment risk assessment and recommendations.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point < -20 °C

Method	OECD TG 102 Melting Point/Melting Range. EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks	Dry ice/acetone bath
Test Facility	Harlan (2014a)

Boiling Point Decomposed without boiling from approximately 186 °C

Method	OECD TG 103 Boiling Point. EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks	Differential scanning calorimetry
Test Facility	Harlan (2014a)

Density 856 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
Test Facility	Harlan (2014a)

Vapour Pressure 9.1×10^{-6} kPa at 25 °C

Method	OECD TG 104 Vapour Pressure. EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks	Vapour pressure balance method. Vapour pressure determined to be 1.5×10^{-5} kPa at 70 °C
Test Facility	Harlan (2014b)

Water Solubility 3.56×10^{-2} g/L at 20 °C

Method	OECD TG 105 Water Solubility. EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks	Flask Method. The notified chemical is hydrolytically unstable. Therefore, a specific analytical method was not available. Therefore, it was agreed to analyse the samples for total organic carbon and boron by inductively coupled plasma. The boron analysis would represent how much boric acid, the degradation product, was dissolved in solution.
Test Facility	Harlan (2014a)

Partition Coefficient (n-octanol/water) Not Determined

Method	OECD TG 117 Partition Coefficient (n-octanol/water). EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	The partition coefficient of the notified chemical could not be determined using a procedure designed to be compatible with Method 117 of the OECD Guidelines for Testing of Chemicals. It was concluded that the method was not applicable due to the test item being hydrolytically unstable.
Test Facility	Harlan (2014a)

Adsorption/Desorption – screening test Not determined

Method	OECD TG 121 Adsorption - Desorption Using a Batch Equilibrium Method.
Remarks	The adsorption coefficient of the notified chemical could not be determined using a procedure designed to be compatible with Method 121 of the OECD Guidelines for Testing of Chemicals, 22 January 2001 and Method C19 Adsorption Coefficient of Commission Regulation (EC) No 440/2008 of 30 May 2008. It was concluded that the method was not applicable due to the test item being hydrolytically unstable.

Test Facility Harlan (2014d)

Flash Point 178 °C at 101.3 kPa

Method EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks Closed cup
Test Facility Harlan (2014c)

Autoignition Temperature 258 °C

Method EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks Carbolite flask heater
Test Facility Harlan (2014c)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure. EC Council Regulation No 440/2008 B.1 bis Acute toxicity (oral) fixed dose method.
Species/Strain	Rat/Wistar (RCCHan™:WIST)
Vehicle	None
Remarks - Method	None

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	1 F	2000	0
2	4 F	2000	0

LD50	> 2000 mg/kg bw
Signs of Toxicity	Animal in Group 1 (sighting test) exhibited hunched posture on the day of dosing.
Effects in Organs	No abnormalities noted at necropsy
Remarks - Results	No deaths were recorded. No signs of systemic toxicity noted in Group 2. All animals showed the expected gains in body weight.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Harlan (2014e)

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) – Limit Test.
Species/Strain	Rat/ Wistar (RCCHan™:WIST)
Vehicle	None
Type of dressing	Semi-occlusive.
Remarks - Method	None

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	No signs of dermal irritation in 5/5 M and 1/5 F. Crust formation and/or glossy skin recorded in 4/5 F on days 5, 6 and 7, (recovery by day 8). In addition, 1 of the 4 F also exhibited very slight erythema (days 2 to 7, recovery by day 8) and small superficial scattered scabs (days 3 to 8, recovery by day 9). No other effects observed for the remainder of the observation period.
Signs of Toxicity - Systemic	None
Effects in Organs	No abnormalities noted at necropsy
Remarks - Results	No mortalities were recorded. All animals showed expected gains in body

weight.

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

TEST FACILITY Harlan (2014f)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Council Regulation No 440/2008 B.4 Acute Toxicity (Skin Irritation).

Species/Strain	Rabbit/New Zealand White (Hsdlf:NZW)
Number of Animals	3 (2M, 1F)
Vehicle	None
Observation Period	14 days
Type of Dressing	Semi-occlusive
Remarks - Method	None

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	0.3	2	2	2	< 7 days	0
Oedema	0.3	2	0.7	2	< 7 days	0

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

Remarks - Results All animals showed expected body weight gains. No mortality or corrosive effects observed.

Very slight erythema and oedema recorded in 1/3 animals (M) immediately after patch removal. Recovery occurred with 24 – 48 hours. Well-defined erythema and slight oedema was recorded in 1/3 animals (M) immediately after patch removal with recovery occurring between 3 and 7 days. One animal (F) exhibited no skin reactions immediately after patch removal. However, erythema developed over time from very slight (1 hr) to well-defined (72 hr). Desquamation was observed on day 7. Very slight oedema was observed at 24 and 48 hrs, with recovery occurring within 48 and 72 hr. All effects were reversed at the 14 day observation period.

CONCLUSION The notified chemical is irritating to the skin.

TEST FACILITY Harlan (2014g)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Council Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation).

Species/Strain	Rabbit/New Zealand White (Hsdlf:NZW)
Number of Animals	3 (2M, 1F)
Observation Period	7 days
Remarks - Method	A rabbit enucleated eye test performed prior to the *in vivo* test indicated that the notified chemical was unlikely to cause severe ocular irritancy.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1	0.7	1.7	2	< 7 d	0
Conjunctiva: chemosis	0.3	0.3	1	1	< 7 d	0
Conjunctiva: discharge	0.3	0	0.7	1	< 72 h	0
Corneal opacity	0	0	0.7	1	< 72 h	0
Iridial inflammation	0	0	0.7	1	< 72 h	0

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

Remarks - Results

1/3 animals showed significant body weight loss (more than 5%) while the remaining 2 animals showed expected gains in body weight.

Diffuse corneal opacity and iridial inflammation was observed in 1/3 animals (24 and 48 hr) with effects reversed between 48 and 72 hr. Moderate conjunctival irritation was observed in 3/3 animals 1 hr after exposure. Signs of recovery varied between the 3 animals, with moderate conjunctival irritation being maintained in 1/3 animals for 48 hr (decreasing to minimal irritation at 72 hr), 24 hr (1/3 animals with a decrease in irritation to minimal at 48 hr) and 1/3 animals exhibiting signs of recovery at 24 hr (minimal conjunctival irritation).

All effects appeared reversed in 2/3 animals at 72 hr, and in the remaining animal at 7 d.

CONCLUSION

The notified chemical is slightly irritating to the eye.

TEST FACILITY

Harlan (2014h)

B.5. Skin sensitisation

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 406 Skin Sensitisation – non-adjuvant test

Species/Strain

Guinea pig/Hartley-derived, albino

PRELIMINARY STUDY

Maximum Non-irritating Concentration:
topical: 100%

MAIN STUDY

Number of Animals

Test Group: 20 (10 M, 10 F) Control Group: 10

INDUCTION PHASE

Induction Concentration:
topical: 100%

Signs of Irritation

No effects were observed after the first induction. At the second induction, slight to moderate erythema was observed in all animals with erythema increasing over time (from 1/20 animals at 24 hr to 6/20 animals at 48 hr) Desquamation also increased over time (6/20 animals at 24 hr and 17/20 animals at 48 hr). Slight to moderate patchy erythema was observed in 19/20 animals after the third induction with a decrease in erythema over time. Moderate erythema was observed in 1/20 animals at 24 and 48 hr. Desquamation increased over time (1/20 animals at 24 hr and 6/20 animals at 48 hr).

CHALLENGE PHASE

1st challenge

topical: 100%

2nd challenge

topical: 75%

Remarks - Method

Positive control: α-Hexylcinnamaldehyde (HCA)
Positive control groups consisted of 10 test and 10 control animals. Test animals were inducted with 5% w/v HCA in ethanol and all animals were challenged with 2.5% w/v HCA in acetone (1st challenge) and 1.0% w/v HCA in acetone (2nd challenge).

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st challenge		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	100%	3/20	6/20	-	-
	75%	-	-	7/20	6/20
Negative Control Group	100%	1/10	0/10	-	-
	75%	-	-	0/10	0/10
Positive Control Group					
HCA Test	2.5%	10/10	10/10	-	-
	1.0%	-	-	9/10	8/10
HCA Control	2.5%	0/10	0/10	-	-
	1.0%	-	-	0/10	0/10

Remarks - Results

No mortalities occurred during the study. All animals gained the expected amount of weight. Negative and positive controls behaved as expected.

Following the 1st challenge with 100% notified chemical, slight or moderately patchy erythema (Grade 1) was recorded in 3/20 test animals and 1/10 challenge control animals at 24 hr and in 6/20 test animals and 0/10 challenge control animals at 48 hr.

Following rechallenge with 75% notified chemical, slight or moderately patchy erythema (Grade 1) was recorded in 7/20 test animals and 0/10 challenge control animals at 24 hr and in 6/20 test animals and 0/10 challenge control animals at 48 hr.

CONCLUSION

There was evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.

TEST FACILITY

Charles River (2014)

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/CaOlaHsd

Vehicle: Cottonseed oil

Remarks - Method: Positive control: α-Hexylcinnamaldehyde (85%).

Test was performed initially with cottonseed oil. The test was repeated using dried cottonseed oil based on an inconclusive result in the initial test and concerns over the reactivity of the notified chemical with the moisture content of the cottonseed oil (original vehicle).

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance (cottonseed oil)		
0 (vehicle control)	5532.18	
0.1	17738.60	3.21
1	16257.20	2.94
10	8038.01	1.45
100	5452.32	0.99
Test Substance (dried cottonseed oil)		
0 (blank)	1417.80	

0 (vehicle control)	6771.10	
0.1	7076.79	1.05
0.4	8885.19	1.31
100	5186.80	3.66#
Positive Control (cottonseed oil)		
50	23486.58	4.25
Positive Control (dried cottonseed oil)		
50	30694.90	4.53

The stimulation index was derived using the mean radioactive incorporation of the blank (untreated) control group i.e. 1417.80 dpm.

Remarks - Results

Positive and negative controls performed as expected.

No signs of systemic toxicity were observed. No deaths occurred during the test.

Where cottonseed oil was the vehicle, very slight erythema was observed on days 1 - 4 in 15/15 animals treated with dilutions (0.1, 1, 10%) of the notified chemical and the positive control (at 50% concentration) (5/5 animals). Negative control animals (5/5) exhibited very slight erythema on Day 2, with effect maintained in 3/5 animals on Day 3 and 2/5 animals on Day 4. The effect was reversed in all animals in all groups by day 5. No signs of irritation were recorded in 5/5 animals exposed to undiluted notified chemical.

Sensitisation appears to increase as the concentration of notified chemical decreased.

Where dried cottonseed oil was the vehicle, very slight erythema was recorded on day 1 in 5/5 animals treated with 0.4% notified chemical (effect reversed by day 2) and 5/5 animals in the positive control group (at 50% concentration) with the effect reversed by day 4. No signs of irritation were observed in negative control animals or animals treated with 0.1% or undiluted concentrations of the notified chemical.

A clear dose-response relationship (where sensitisation increased with increasing concentration of notified chemical) was observed when the notified chemical was tested using dried cottonseed oil as a vehicle.

CONCLUSION

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

TEST FACILITY

Harlan (2014i)

B.7. Repeat dose toxicity

TEST SUBSTANCE

Notified chemical

METHOD

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

Species/Strain: Rat/ Crl:CD(SD)

Route of Administration: Oral – gavage

Exposure Information: Total exposure days: 28 days
Dose regimen: 7 days per week
Post-exposure observation period: 0 days

Vehicle: Peanut oil

Remarks - Method: None

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 M, 5 F	0	0/10
low dose	5 M, 5 F	250	0/10
mid dose	5 M, 5 F	500	0/10
high dose	5 M, 5 F	1000	1/10

Mortality and Time to Death

There was one unscheduled death (1 F from the high-dose group on day 18). Cause of death was undetermined with no clinical or macroscopic findings associated to the notified chemical.

All other animals survived to the end of the study period.

Clinical Observations

All animals showed expected body weight gains.

Clinical effects attributed to the notified chemical were noted in the mid- and high-dose males and females, including clear and/or yellow material around the mouth, yellow material on various body surfaces (urogenital and anogenital areas, ventral trunk and hind limbs), and red material around the nose and/or mouth. Clear discharge from the eyes was noted for females in the high-dose group. Effects were recorded primarily at time of dosing and/or 1-2 hours post-dosing as early as study day 3 and generally persisted throughout the study.

Clear material around the mouth was recorded in males and females in the low-dose group. This was attributed to the notified chemical and was observed at the time of dosing and/or 1-2 hours post-dosing between study days 12 and 26.

Increased incidences of slightly soiled fur (males and females in the mid- and high-dose groups) and slightly increased ambulatory activity (males in mid- and high-dose groups) were recorded in week 3 and attributed to the notified chemical.

Slightly higher food consumption was observed in mid- and high-dose males and mid-dose females in the last week of the study (days 21 to 27), and in high-dose females from week 2 (days 7 to 27). The increased food consumption was attributed to the notified chemical, but not considered adverse as there was no correlative body weight gain.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Animals in the high-dose group showed higher reticulocytes (males and females), lower platelets (females), lower urine specific gravity (males and females), and higher urine total volume (males and females). Lower urine specific gravity and higher urine total volume was also noted in mid-dose males. There were no correlating microscopic findings.

Lower triiodothyronine (T3) values were noted in the mid- and high-dose males. However, there were no corresponding alterations in thyroid stimulating hormone (TSH) therefore the study authors concluded that the toxicological relevance and association of this change with the notified chemical is uncertain.

Effects in Organs

Minimal to mild hepatocellular hypertrophy was observed in 5/5 M and 3/5 F of the high-dose group animals and 2/5 of the mid-dose group males, corresponding to higher liver weights noted for 1000 mg/kg/day group animals. There were no accompanying alterations in clinical pathology or test substance-related microscopic findings indicative of hepatotoxicity. Therefore the change was considered non-adverse by the study authors.

Minimal single cell necrosis in the liver was also recorded in the mid- (2/5 M, 1/5 F) and high- (3/5 M, 3/5 F) dose groups. A single male in the control group also exhibited minimal single cell necrosis.

Changes to the thyroid glands were observed. Minimal to mild follicular cell hypertrophy was observed in males and females in the mid- (5/5 M, 2/5 F) and high- (5/5 M, 3/5 F) dose groups and a single male in the low-dose group. Decreased colloid was observed in males and females in the mid- (4/5 M, 2/5 F) and high- (3/5 M, 3/5 F) dose groups and a single male in the low-dose group. The authors considered these changes to be secondary to enzyme induction rather than evidence of direct thyroid toxicity.

Minimal germ cell degeneration was observed in the testes of 4/5 M in both the mid- and high-dose groups and 1/5 M in the low-dose group. Tubular degeneration was observed in the testes of 1/5 M in the high-dose group. A higher incidence of ovarian cysts was observed in the high-dose group females (3/4) when compared with the control and low-dose group (1/5 in both groups), corresponding to the higher ovaries/oviducts weights for these females. The authors are uncertain as to the cause of the effects and state that the effects may be a result of direct toxicity of the notified chemical on the reproductive tract or secondary to the changes observed in the thyroid gland.

Necrosis of the nasal turbinates was observed in the high-dose males (3/5) and females (3/4) and the mid-dose males (2/5) particularly within the posterior nasal cavity sections (nasal levels III and IV). Minimal necrosis was observed in the nasal cavity of a single low-dose male. The authors stated that this nonspecific extensive necrosis and inflammation of the posterior nasal cavity was consistent with gavage-related reflux of the notified chemical rather than an adverse effect.

Lower thymus weights were recorded for females in the low- and mid-dose groups and in males and females in the high-dose groups. No clear association was made between the lower weights and the notified chemical as no corresponding lymphoid depletion was observed microscopically and most individual animal weights were within the range of the historical control data.

Remarks – Results

Exposure to ≥ 250 mg/kg bw/day of the notified chemical resulted in clinical signs including clear and/or yellow material around the mouth, yellow material on various body surfaces, red material around the nose and/or mouth and clear discharge from the eyes. Testicular tubular degeneration observed in high-dose males was considered to be potentially adverse by the study authors due to the presence of scattered seminiferous tubules exhibiting loss of germ cells and vacuolisation. Other reproductive changes noted were not considered adverse due to minimal nature of changes. One animal (female, high-dose group) died. However, the cause of death and association with the notified chemical was uncertain. Any other effects observed could not be attributed solely to the presence of the notified chemical.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 500 mg/kg bw/day in this study, based on the uncertainty regarding the cause of death of one female, and testicular tubular degeneration observed at 1000 mg/kg bw/day.

TEST FACILITY	WIL (2015)

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
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	Test 1: a) With metabolic activation: 1.5 - 5000 µg/plate b) Without metabolic activation: 1.5 - 5000 µg/plate Test 2: a) With metabolic activation: 0.5 - 500 µg/plate b) Without metabolic activation: 1.5 – 1500 µg/plate
Vehicle	Acetone
Remarks - Method	Vehicle, negative (untreated) and positive controls were tested in parallel with the notified chemical. Positive controls: i) without S9: N-ethyl-N'-nitro-N-nitrosoguanidine (WP2*uvrA*, TA100, TA1535), 9-Aminoacridine (TA1537), 4-Nitroquinoline-1-oxide (TA98); ii) with S9: 1-Aminoanthracene (TA100,

TA1535, TA1537, WP2*uvrA*), Benzo(a)pyrene (TA98).

The dose range for Test 2 was determined by the results of Test 1.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/plate) Resulting in:*			
	*Cytotoxicity in Preliminary Test**	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	-	≥ 150	> 5000	Negative
Test 2		≥ 150	> 500	Negative
Present				
Test 1	-	≥ 500	> 5000	Negative
Test 2		≥ 500	> 1500	Negative

* Not conducted

Remarks - Results

Positive and negative controls performed as expected confirming the validity of the test system.

No significant increases in the frequency of the revertant colonies were recorded following treatment with the notified chemical at any dose level, with or without metabolic activation.

CONCLUSION

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

TEST FACILITY

Harlan (2014j)

B.9. 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: Mouse/ Hsd: ICR (CD-1®)
Route of Administration: Oral – gavage
Vehicle: Arachis oil
Remarks - Method: Range finding test found no marked difference in toxic response between the sexes. It was therefore considered acceptable to use males only for the main test.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	7 M	0	24
II (low dose)	7 M	250	24
III (mid dose)	7 M	500	24
IV (high dose)	7 M	1000	24
V (high dose)	7 M	1000	48
VI (positive control, CP)	5 M	50	24

CP=cyclophosphamide.

RESULTS

Doses Producing Toxicity: No statistically significant decreases in ratio of polychromatic erythrocytes to normochromatic erythrocytes were observed in any group exposed to the notified chemical.

Genotoxic Effects: No statistically significant increases in the frequency of micronucleated polychromatic erythrocytes were observed in the high dose group exposed for 48 hr or the low-dose group exposed for 24 hr.

	Statistically significant increases were observed in animals exposed to the mid- and high-dosages for 24 hr when compared to the vehicle control. However, the authors state that the values for the notified chemical were within the historical control range and as such the responses can be considered to be artefactual. No clear dose-response relationship was exhibited between the dose administered and the number of polychromatic erythrocytes.
Remarks - Results	No premature deaths observed. Animals in both high dose groups exhibited hunched posture, ptosis, ataxia, lethargy and splayed gait which continued for the duration of the test.
CONCLUSION	The notified chemical was not clastogenic under the conditions of this in vivo mammalian erythrocyte micronucleus test.
TEST FACILITY	Harlan (2014k)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO2 Evolution Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Total organic content (TOC)
Remarks - Method	The test was conducted according to the above mentioned test guidelines. No significant deviations from the test guidelines were reported.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
8	40	8	68
14	52	14	69
21	50	21	64
28	66	28	70
29	74	29	77

Remarks - Results

All test validation criteria were satisfied.

The test substance attained 66% degradation after 28 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. However, the test item has exhibited the potential for rapid biodegradation.

CONCLUSION The notified chemical is not readily biodegradable.

TEST FACILITY Harlan (2014l)

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	Rainbow trout *(Oncorhynchus mykiss).*
Exposure Period	96 hour
Auxiliary Solvent	None
Water Hardness	140 mg $CaCO_3$/L
Analytical Monitoring	Total Organic Carbon (TOC)
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. In view of the difficulties associated with the evaluation of aquatic toxicity of poorly water soluble test substances, a modification of the standard method for the preparation of aqueous media was performed.

A saturated solution was prepared by stirring an excess (100 mg/L) of test substance in test water for a period of 23 hours followed by a 1 hour standing period prior to removing any undissolved test item present by filtration through a glass wool plug (first approximate 75-100 mL discarded) to give a saturated solution of the test substance. A series of dilutions was made from this saturated solution to give the required test concentrations of 10, 5.6, 3.2, 1.8 and 1.0% v/v saturated solution.

RESULTS

Concentration mg/L Nominal(Saturated solution)	*Number of Fish*	*Mortality*			
		24 h	*48 h*	*72 h*	*96 h*
1.0		0	0	0	0
1.8		0	0	0	0
3.2		0	0	0	0
5.6		0	0	0	0
10		7*	7	7	7

* Fish removed and humanely killed due to prolonged sub lethal effects

LL50	1.3 mg/L at 96 hours.
NOEL	1.1 mg/L at 96 hours.
Remarks – Results	All validity criteria for the test were satisfied.

The LL50 value and associated confidence limits at 48, 72 and 96 hours were calculated using the geometric mean method.

The results of the definitive test showed the highest test concentration resulting in 0% mortality to be 1.1 mg/L, the lowest test concentration resulting in 100% mortality to be 1.6 mg/L.

Total Organic Carbon (TOC) analysis of the 1.0, 1.8,3.2,5.6 and 10% v/v saturated solution test preparations at 0 and 72 hours (fresh media) and at 24 and 96 hours (old media) showed measured carbon concentrations to range from less than the limit of quantification (LOQ) of the analytical method, assessed as being 1.0 mg C/L to 3.01 mg C/L (equivalent to test substance concentrations of less than the LOQ to 4.0 mg/L based on a test item carbon content of 75.78%).

It was considered appropriate to calculate the results based on the equivalent test substance concentrations obtained from TOC analysis as these results give an estimate of the total dissolved test substance concentrations present.

CONCLUSION	The notified chemical is toxic to fish.
TEST FACILITY	Harlan (2014m)

C.2.2. 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	Total Organic Carbon (TOC)
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.

In view of the difficulties associated with the evaluation of aquatic toxicity of poorly water soluble test substances, a modification of the standard method for the preparation of aqueous media was performed.

A saturated solution was prepared by stirring an excess (100 mg/L) of test substance in test water for a period of 23 hours followed by a 1 hour standing period prior to removing any undissolved test item present by filtration through a glass wool plug (first approximate 75-100 mL discarded) to give a saturated solution of the test substance. A series of dilutions was made from this saturated solution to give the required test concentrations of 10, 5.6, 3.2, 1.8 and 1.0% v/v saturated solution.

RESULTS

Concentration mg/L Nominal (saturated solution)	*Number of D. magna*	*Number Immobilised*	
		24 h	*48 h*
Control	20	0	0
1.0	20	0	0
1.8	20	0	0
3.2	20	0	0
5.6	20	2	14
10	20	10	20

LL50	2.6 mg/L at 48 hours
NOEL	1.7 mg/L at 48 hours
Remarks - Results	All validity criteria for the test were satisfied. Total Organic Carbon (TOC) analysis of the 3.2, 5.6 and 10% v/v saturated solution test preparations at 0 and 48 hours showed measured carbon concentrations to range from 1.4 to 3.7 mg/L (equivalent to test substance concentrations of 1.9 to 4.9 mg/L based on a test item carbon content of 75.78%). It was considered appropriate to calculate the results based on the equivalent test substance concentrations obtained from TOC analysis as these results give an estimate of the total dissolved test item concentrations present.
CONCLUSION	The notified chemical is toxic to aquatic invertebrates
TEST FACILITY	Harlan (2014n)

C.2.3. 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: 1.0, 3.2, 10, 32 and 100% v/v saturated solution
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	Total Organic Carbon (TOC)
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.

In view of the difficulties associated with the evaluation of aquatic toxicity of poorly water soluble test substances, a modification of the standard method for the preparation of aqueous media was performed.

A saturated solution was prepared by stirring an excess (100 mg/L) of test substance in test water for a period of 23 hours followed by a 1 hour standing period prior to removing any undissolved test item present by filtration through a glass wool plug (first approximate 75-100 mL discarded) to give a saturated solution of the test substance. A series of dilutions was made from this saturated solution to give the required test concentrations of 10, 5.6, 3.2, 1.8 and 1.0% v/v saturated solution.

RESULTS

Biomass		*Growth*	
EyL50	*NOEL*	*ErL50*	*NOEL*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
5.5	1.9	9.0	1.9

Remarks - Results

All validity criteria for the test were satisfied.

The results showed no effect on growth rate at the loading rates of 0.010, 0.10 and 1.0 mg/L. However, growth was observed to be reduced at 10 and 100 mg/L.

Total Organic Carbon (TOC) analysis of test preparations at 0 and 72 hours showed measured carbon concentrations to range from less than the limit of quantification (LOQ), determined to be 1.0 mg C/L at 1.0% v/v saturated solution, through to 32 mg/L at 100% v/v saturated solution (equivalent to test substance concentrations of less than the LOQ to 42 mg/L based on a test item carbon content of 75.78%).

It was considered appropriate to calculate the results based on the equivalent test substance concentrations obtained from TOC analysis as these results give an estimate of the total dissolved test substance concentrations present.

CONCLUSION The notified chemical is toxic to algae
TEST FACILITY Harlan (2015)

C.2.4. 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: 100, 108, 320, 560 and 1000 mg/L

Remarks – Method The test was conducted according to the guidelines above using good laboratory practice (GLP). No significant deviations from the test guidelines were reported.

RESULTS
EL50 230 mg/L
NOEC > 230 mg/L

Remarks – Results	All validity criteria for the test were satisfied
CONCLUSION	The notified chemical is not inhibitory to microorganisms
TEST FACILITY	Harlan (2014o)

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