File No: LTD/1719

April 2014

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Priolube 3952

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

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

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/1719	Croda Singapore Pte Ltd (trading as Croda Australia)	Priolube 3952	ND*	100 tonnes per annum	A component of an additive for oils and lubricating fluids

*ND = not determined

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified polymer cannot be classified 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).

The environmental hazard classification according to the *Globally Harmonised System for the 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 3)	H402 - Harmful to aquatic life
Chronic (Category 3)	H412 - Harmful to aquatic life with long lasting effects

Human health risk assessment
Under the conditions of the occupational settings described, the notified polymer is not considered to pose an unreasonable risk to the health of workers.

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

Environmental risk assessment
On the basis of the PEC/PNEC ratio and the assessed use pattern, the notified polymer 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 polymer 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 polymer 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 polymer in oil products should be disposed of in accordance with local regulations for recycling, re-use or recovery. However, the notified polymer in water-based lubricating fluids should be disposed of to landfill.

Emergency procedures

- Spills or accidental release of the notified polymer 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 polymer is listed on the Australian Inventory of Chemical Substances (AICS).

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

(1) Under Section 64(1) of the Act; if
- the polymer has a number-average molecular weight of less than 1000 Da;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the polymer has changed from a component of an additive for oils and lubricating fluids, or is likely to change significantly;
- the amount of polymer being introduced has increased, or is likely to increase, significantly;
- the polymer has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the polymer 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.

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Croda Singapore Pte Ltd (Trading as Croda Australia) (ABN: 34 088 345 457)
Suite 102, 447 Victoria Street
WETHERILL PARK NSW 2164

NOTIFICATION CATEGORY
Limited: Synthetic polymer with Mn ≥ 1000 Da.

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, polymer constituents, residual monomers, impurities and additives/adjuvants

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: all physico-chemical endpoints

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
USA, Canada, Korea, Japan

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Priolube 3952

MOLECULAR WEIGHT
> 1,000 Da

ANALYTICAL DATA
Reference GPC spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 90%

DEGRADATION PRODUCTS
The notified polymer is not expected to degrade under normal conditions of use.

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: brown liquid

Property	Value	Data Source/Justification
Melting Point	-35 °C	SDS
Boiling Point	> 200 °C (pressure unknown)	SDS
Density	940 kg/m^3	SDS
Vapour Pressure	< 0.1 kPa at 20 °C	SDS
Water Solubility	Not determined	Expected to be dispersible based on the structure
Hydrolysis as a Function of pH	Not determined	The notified polymer contains functionality that may slowly hydrolyse under normal environmental conditions of pH 4 – 9

Partition Coefficient (n-octanol/water)	Not determined	Expected to partition to phase boundaries based on its emulsifying properties
Adsorption/Desorption	Not determined	Expected to partition to sediment/sludge based on its high molecular weight
Dissociation Constant	Not determined …	The notified polymer does not contain dissociable functionalities
Particle Size		Liquid
Flash Point	> 325 °C (pressure unknown)	SDS
Flammability	Not determined	Not expected to be flammable based on flash point
Autoignition Temperature	> 325 °C	Estimated based on flash point
Explosive Properties	Not determined	Not expected to be explosive based on the chemical structure.
Oxidising Properties	Not determined	Not expected to possess oxidising properties based on the chemical structure.

DISCUSSION OF PROPERTIES

Reactivity
The notified polymer 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 polymer 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 polymer will not be manufactured in Australia. It will be imported neat for reformulation and as a component of formulated lubricants at concentrations of up to 50%.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	100	100	100	100	100

PORT OF ENTRY
Melbourne, Sydney, Brisbane and Perth

IDENTITY OF RECIPIENTS
Croda Singapore Pte Ltd

TRANSPORTATION AND PACKAGING
The notified polymer or formulated lubricants containing > 90% of the notified polymer will be imported by sea or air in sealed 190 kg drums which will be transported by road or rail to the notifier's warehouse for storage before being distributed to reformulation or end use sites. The formulated lubricants will be packaged in 20 L or 200 L drums.

USE
A component of an additive for oils and lubricating fluids

OPERATION DESCRIPTION
Reformulation
When imported in drums, the notified polymer will be loaded into the blending vessels by the operators either manually or with the help of mechanical devices and mixed with oils and other additives to form finished lubricants at the customer's site. This will most likely take place in enclosed automated systems with adequate ventilation and appropriate engineering controls. The finished lubricants containing the notified polymer at up to

50% will be packaged into 20 L or 200 L drums with automated processes and then be stored and distributed nationwide via road and rail for end use.

Cleaning and maintenance of the blending vessels and filling lines and quality control tests will occur from time to time.

End use
The imported or reformulated finished lubricants containing the notified polymer at up to 50% concentration will be used as an automobile lubricant (at car manufacturing sites, car dealerships and automotive service centres), as a chain lubricant, or in metal working fluids. They may be applied manually or through automated lubrication delivery systems.

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 Warehouse	2-3	25
Process Operator (formulation)	2-3	25
Quality Control	1-2	4-5
End Users	2-4	250
Waste Management	1	40
Packaging	2-3	25

EXPOSURE DETAILS
Transport and Storage
Transport and storage workers are not expected to be exposed to the notified polymer except in the unlikely event of an accidental rupture of the containers.

Reformulation, Packaging and Testing
During reformulation, procedures involved in the handling of the notified polymer and products containing it will occur in industrial areas with adequate ventilation and appropriate engineering controls. The blending processes are expected to take place in enclosed automated systems, reducing the potential for workers to be exposed to the notified polymer. Dermal and ocular exposure to the notified polymer at up to 100% concentration may occur during the charging of blending vessels, equipment cleaning and maintenance. Dermal and ocular exposure may occur to the notified polymer at up to 50% concentration during packaging and quality control testing. Inhalation exposure is not expected given the low vapour pressure of the notified polymer, unless aerosols or mists are formed.

PPE including coveralls, impervious gloves, and safety goggles are expected to be worn by workers to minimise exposure during reformulation and packaging.

End Use
Dermal and ocular exposure to the notified polymer at up to 50% concentration may occur during charging, topping up and maintenance activities. Inhalation exposure is not expected given the low vapour pressure of the notified polymer. PPE including coveralls, impervious gloves, and safety goggles are expected to be worn by workers to minimise exposure.

6.1.2. Public Exposure
The finished lubricant products containing the notified polymer at up to 50% concentration will be available to the general public. DIY users may experience inadvertent dermal and ocular exposure to final products containing up to 50% of the notified polymer when adding and/or replacing lubricants in their automobiles, particularly as they are unlikely to wear PPE. However, once lubricants containing the notified polymer are added to the engine, the general public are not expected to be exposed to the notified polymer.

Overall, public exposure to the notified polymer is expected to be limited by the fact that the adding and/or replacing of lubricants containing the notified polymer occurs on an infrequent basis.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified polymer are summarised in the following table. For full details of the studies, refer to Appendix A.

Endpoint	*Result and Assessment Conclusion*
Rat, repeat dose oral toxicity – 28 days.	NOAEL > 1000 mg/kg/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* mammalian chromosome aberration test	non genotoxic

Toxicokinetics, metabolism and distribution
Absorption of the notified polymer across biological membranes (gastrointestinal tract and skin) is expected to be limited based on the high molecular weight (> 1000 Da). However, the notified polymer contains a low proportion of low molecular weight species (< 500 Da) which may be absorbed.

Repeated dose toxicity
In an oral repeat dose toxicity study, rats were administered the notified polymer by gavage at 0, 25, 250 or 1000 mg/kg bw/day. The NOAEL for systemic toxicity was established as 1000 mg/kg bw/day, based on the lack of adverse effects. Therefore, the notified polymer is of low repeated dose toxicity.

Mutagenicity/Genotoxicity
The notified polymer was not mutagenic in a bacterial reverse mutation assay. The notified polymer was not clastogenic in an *in vitro* mammalian chromosome aberration test. Overall, the available evidence indicates that the notified polymer is unlikely to be mutagenic or genotoxic.

Health hazard classification
Based on the available information, the notified polymer cannot be classified 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
The available toxicity information indicates that the notified polymer is likely to be of low toxicity. The workers expected to experience the highest exposure are those handling the notified polymer as imported (≤ 100%) at reformulation and/or repackaging sites. Workers involved in reformulation processes may experience dermal and ocular exposure to the notified polymer during transfer of the notified polymer to blending vessels, quality control analysis, packaging, cleaning and maintenance tasks and occasionally from leaks and spills. The blending process is expected to be largely enclosed and automated, and therefore significant exposure is not expected to occur. Exposure of workers is expected to be further minimised by the use of PPE such as protective clothing, gloves and goggles. Given the likely low toxicity, the use of automated and enclosed systems and the appropriate use of PPE during handling of the notified polymer as imported, the risk of adverse health effects is not considered unreasonable.

Lubricant products containing the notified polymer at < 50% concentration will be used by workers. Exposure to these workers may occur frequently during lubricant changes and use in metal working fluids. The potential for adverse health effects from use of the notified polymer is not considered to be unreasonable as workers may use automated delivery systems and PPE to minimise exposure.

Overall, the potential for adverse health effects in workers exposed to the notified polymer (≤ 100%) is not considered to be unreasonable, given the use of appropriate engineering control measures to minimise exposure during reformulation and/or repackaging processes and the use of PPE and automated delivery systems to minimise exposure to the notified polymer in the lubricant and metal working products (<50%).

6.3.2. Public Health

Lubricant products containing < 50% notified polymer will be available to the public for DIY use. During lubricant changes, some dermal and accidental ocular exposure is possible. However, DIY engine lubricant change/top-up activities are only expected to occur on an infrequent basis. Therefore, the risk to the public from use of the notified polymer at < 50% concentration in lubricant products 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 polymer will be imported into Australia as a component of finished lubricating products for the automotive, manufacturing and construction industries. The notified polymer may also be imported for reformulation into oils for the same markets. Significant release of the notified polymer to the environment is not expected during transport and storage except in the unlikely event of accidental spills or leaks. During reformulation, it is expected that less than 1% of the total import volume of the notified polymer in the introduced oil may be released to the environment. Any notified polymer spilled during reformulation is expected to be collected and disposed of in accordance with the local and state regulations.

RELEASE OF CHEMICAL FROM USE

The lubricating fluid containing the notified polymer will be used in sealed units. During use, the notified polymer will be contained and its release is expected to be very low. No significant release of the notified polymer is expected from removal of used lubricating fluid containing the notified polymer. The used lubricating fluids are expected to be collected and stored for subsequent disposal. The release of the notified polymer from professional activities and by the general public is expected to be minimal as the product containing the notified polymer should be collected and disposed of to landfill.

In the manufacturing industry, lubricating fluid containing the notified polymer is expected to be used for the cooling and lubrication of machine tools. Up to 25% of the total import volume of the notified polymer is expected to be used for this industrial purpose. It is expected that the lubricating fluids will be disposed of via licensed waste disposal contractors.

RELEASE OF CHEMICAL FROM DISPOSAL

The used lubrication fluid in oil-based products containing the notified polymer, which is disposed of in accordance with the State/Territory regulations, is expected to be recycled, re-refined or used as low grade burner fuel. The notifier estimated that up to 50% of the notified polymer used as lubricating fluid in the manufacturing industry is expected to be released either to the sewer, storm water drains or disposed of to landfill.

7.1.2. Environmental Fate

The notified polymer is not readily biodegradable based on the biodegradability studies provided (about 3% and 21% biodegraded after 28 days). For details of the environmental fate studies please refer to Appendix B.

Most of the notified polymer will be either thermally decomposed during use, recycling or re-refining processes. It is estimated by the notifier up to 12.5% (50% × 25%) of the notified polymer is expected to be released to sewer. In Sewage Treatment Plants (STPs), up to 90% of the notified polymer is expected to partition to sludge due to its high molecular weight (Boethling and Nabholz, 1997). Sludge from the wastewater treatment plants containing the notified polymer is expected to be disposed of to landfill or applied to agricultural soils. Notified polymer released to surface water is expected to partition to sediment based on its high molecular weight and expected low water solubility. The notified polymer is not expected to be bioaccumulative due to its high molecular weight. It is expected to ultimately be degraded into water and oxides of carbon by thermal decomposition or by natural processes in water, soil and landfill.

7.1.3. Predicted Environmental Concentration (PEC)

The calculation for the Predicted Environmental Concentration (PEC) is summarised in the table below. Based on the reported use as lubricating fluid for manufacturing, it is estimated, as a worst case release scenario, that 50% of the total import volume of the notified polymer may be released to the sewer. As the release of the notified polymer is from lubricating fluids from industrial use, it is anticipated that such releases will occur 5 days per week into a local metropolitan STP (329 ML capacity). It is expected that 90% of the notified polymer will be removed to sludge during sewerage treatment plant (STP) processes (Boethling and Nabholz, 1997).

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	100,000	kg/year
Proportion expected to be released to sewer	50%	
Annual quantity of chemical released to sewer	50,000	kg/year
Days per year where release occurs	260	days/year
Daily chemical release:	192.308	kg/day
Individual Sewage Treatment Plant Average Daily Flow:	329	ML/day
Removal within STP	90%	Mitigation
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	58.45	µg/L
PEC - Ocean:	5.85	µg/L

Partitioning to biosolids in STPs Australia-wide may result in an average biosolids concentration of 5260.7 mg/kg (dry wt). Biosolids are applied to agricultural soils, with an assumed average rate of 10 t/ha/year. Assuming a soil bulk density of 1500 kg/m^3 and a soil-mixing zone of 10 cm, the concentration of the notified polymer may approximate 35.1 mg/kg in applied soil. This assumes that degradation of the notified polymer occurs in the soil within 1 year from application. Assuming accumulation of the notified polymer in soil for 5 and 10 years under repeated biosolids application, the concentration of notified polymer in the applied soil in 5 and 10 years may approximate 175.4 mg/kg and 350.7 mg/kg, respectively.

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified polymer in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 58.5 µg/L may potentially result in a soil concentration of approximately 389.7 µg/kg. Assuming accumulation of the notified polymer in soil for 5 and 10 years under repeated irrigation, the concentration of notified polymer in the applied soil in 5 and 10 years may be approximately 1.9 mg/kg and 3.9 mg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity (96 h)	LC50 >100 mg/L	Not expected to be harmful to fish
Daphnia Toxicity (48 h)	EC50 > 100 mg/L	Not expected to be harmful to aquatic invertebrates
Algal Toxicity (72 h)	$E_rC50 \geq 13$ mg/L	Expected to be harmful to algae

The notified polymer is not expected to be harmful to fish or aquatic invertebrates, but is considered toxic to algae. Therefore, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009), the notified polymer is formally classified as Acute Category 3; Harmful to aquatic life. Based on the acute toxicity and lack of ready biodegradability of the notified polymer, it has been formally classified under the GHS as Chronic Category 3; Harmful to aquatic life with long lasting effects.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) for the notified polymer has been calculated and is presented in the table below. The PNEC is calculated based on the endpoint for the most sensitive species (algae, E_rC50) for the notified polymer. Three acute ecotoxicity endpoints for aquatic species from three trophic levels are available. Therefore, an assessment factor of 100 has been used.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Alga).	13	mg/L
Assessment Factor	100	
PNEC:	130	µg/L

7.3. Environmental Risk Assessment

Based on the above PEC and PNEC values, the following Risk Quotient (Q) has been calculated:

Risk Assessment	*PEC µg/L*	*PNEC µg/L*	*Q*
Q - River:	58.45	130	0.45
Q - Ocean:	5.85	130	0.045

The Risk Quotients (Q = PEC/PNEC) for a worst case scenario have been calculated to be less than 1 for both the riverine and marine compartments. Release of the notified polymer in treated effluent to the aquatic environment in ecotoxicologically significant quantities is not expected based on its reported use pattern. The notified polymer is not expected to either be significantly bioavailable or bioaccumulative. Therefore, based on the assessed use pattern, the notified polymer is not expected to pose an unreasonable risk to the environment.

APPENDIX A: TOXICOLOGICAL INVESTIGATIONS

A.1. Repeat dose toxicity

TEST SUBSTANCE	Notified polymer
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	Rats/Sprague-Dawley Crl:CD (SD) IGS BR
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Distilled water
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	25	0
mid dose	5 per sex	250	0
high dose	5 per sex	1000	0
control recovery	5 per sex	0	0
high dose recovery	5 per sex	1000	0

Clinical Observations

No treatment-related clinical signs, including functional observations, behaviour differences, functional performance parameters, sensory reactivity, body weight development, food consumption and water consumption, were observed in any animals throughout the study.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No treatment-related effects were detected for haematological, blood chemistry and urinalysis parameters.

Effects in Organs

No treatment-related changes in the organ weight, macroscopic abnormalities or histopathological changes were observed.

CONCLUSION

The NOAEL for systemic toxicity was established as 1000 mg/kg bw/day under the conditions of the study, based on the lack of adverse effects at all treatment levels.

TEST FACILITY	SafePharm Laboratories (2006)

A.2. Genotoxicity – bacteria

TEST SUBSTANCE	Notified polymer
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	A liver microsomal preparation (S9-mix) prepared from rats pre-treated with a compound (phenobarbitone/β-naphthoflavone 80/100 mg per kg per day) known to induce an elevated level of these enzymes.
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				
Test	> 5000	> 5000	> 5000	negative
Present				
Test	> 5000	> 5000	> 5000	negative

Remarks - Results	Neither precipitation nor appreciable toxicity was observed. No positive mutagenic responses were observed with any of test strains in either the presence or absence of S9 activation. An opaque film was observed at 5000 μg/plate and it did not prevent the scoring of revertant colonies. All criteria for a valid study were met as described in the protocol.
CONCLUSION	The notified polymer was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	SafePharm Laboratories (2005a)

A.3. Genotoxicity – in vitro

TEST SUBSTANCE	Notified polymer
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test. EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.
Cell Type/Cell Line	Chinese Hamster Lung cell line
Metabolic Activation System	A liver microsomal preparation (S9-mix) prepared from rats pre-treated with a compound (phenobarbitone/β-naphthoflavone 80/100 mg per kg per day) known to induce an elevated level of these enzymes.
Vehicle	Dimethyl sulphoxide (DMSO)
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*, 1250	6 hours	18 hours
Test 2	0*, 39.06, 78.13, 156.25*, 312.5*, 625*, 1250	24 hours	0 hours
Present			
Test 1	0*, 39.06, 78.13*, 156.25*, 312.5*, 625*, 1250	6 hours	18 hours
Test 2	0*, 39.06, 78.13*, 156.25*, 312.5*, 625*, 1250	6 hours	18 hours

*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	> 5000	> 5000	≥ 156.25*	negative
Test 2		> 5000	≥ 156.25*	negative
Present				
Test 1	> 5000	> 5000	≥ 156.25*	negative
Test 2		> 5000	≥ 156.25*	negative

*A cloudy precipitate.

Remarks - Results	The test substance did not induce any statistically significant increases in the frequency of cells with aberrations or in the number of polyploidy cells either in the presence or absence of metabolic activation at any dose level in any exposure group. All criteria for a valid study were met as described in the protocol.
CONCLUSION	The notified polymer was not clastogenic to CHL cells treated in vitro under the conditions of the test.
TEST FACILITY	SafePharm Laboratories (2005b)

APPENDIX B: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

B.1 Environmental Fate

B.1.1. Ready biodegradability

TEST SUBSTANCE	Notified polymer
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I)
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	Not reported
Analytical Monitoring	Measured biochemical oxygen demand (BOD). The test substance was analysed by 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.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	0	7	56
14	1	14	66
28	3.3	28	69

Remarks - Results	All validity criteria for the test were satisfied. The toxicity control exceeded 25% biodegradation (required by guideline) showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the test substance after the cultivation period was 3.3% by BOD. However, the degree of degradation was 21% when analysing for residual test substance. Therefore, the test substance cannot be classified as readily biodegradable according to the OECD (301 C) guideline.
CONCLUSION	The notified polymer is not readily biodegradable.
TEST FACILITY	Mitsubishi (2003)

B.1.2. Ready biodegradability

TEST SUBSTANCE	Notified polymer
METHOD	Modified Sturm Test (OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test)
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	Not reported
Analytical Monitoring	Measured biochemical oxygen demand (BOD). The test substance was analysed by HPLC.
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles.

RESULTS

Test substance		*Reference*	
Day	*% Degradation*	*Day*	*% Degradation*
7	5.5	7	67.3
14	11.2	14	77.3
28	21.0	28	87.1

Remarks - Results	The toxicity control exceeded 25% biodegradation (required by guideline) showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the test substance after the cultivation period was 21%. Therefore, the test substance cannot be classified as readily biodegradable according to the OECD (301 B) guideline.
CONCLUSION	The notified polymer is not readily biodegradable
TEST FACILITY	BFB (1994)

B.2. Ecotoxicological Investigations

B.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified polymer
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Static Test
Species	Rainbow Trout (*Oncorhyncus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	Not reported
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.

RESULTS

Nominal Concentration	*Number of Fish*	*Mortality (%)*				
		3 h	*24 h*	*48 h*	*72 h*	*96 h*
Control (no replication)	7	0	0	0	0	0
100 (2 replicates)	14	0	0	0	0	0

LC50	>100 mg/L at 96 hours
NOEC	≥100 mg/L at 96 hours
Remarks – Results	All validity criteria for the test were satisfied. TOC analysis of the samples taken at the beginning and at every 24 h intervals until the end of the test. A preliminary solubility study showed the test substance formed a stable homogenous dispersion at 100 mg/L under the test conditions over a period of 24 h. However, at higher concentrations such as 1000 mg/L the test substance did not form a completely homogenous dispersion. The 100 mg/L suspension was analysed for TOC content. Three different types of samples were analysed including untreated samples, filtered samples (0.2 µm) and centrifuged samples (samples were centrifuged at 40000 rpm for 30 minutes). The TOC concentrations were comparable for the untreated and centrifuged samples, whilst the results for the filtered samples were low. The mean measured concentrations were close to the nominal test concentrations. Therefore, the results were calculated based on the nominal concentrations only.
CONCLUSION	The notified polymer is not harmful to fish.
TEST FACILITY	SafePharm (2005a)

B.2.2. Acute toxicity to fish

TEST SUBSTANCE	Notified polymer

METHOD	OECD TG 203 Fish, Acute Toxicity Test – Static Test
Species	Carp (*Cyprinum carpio*)
Exposure Period	96 hours
Auxiliary Solvent	Not reported
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	DC-190 High Temperature Total Organic Carbon (TOC) Analyser
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

Nominal Concentration	*Number of Fish*	*Mortality (%)*				
		2 h	*24 h*	*48 h*	*72 h*	*96 h*
Control	7	0	0	0	0	0
100	7	0	0	0	0	0

LC50	>100 mg/L at 96 hours
NOEC	≥100 mg/L at 96 hours
Remarks - Results	All validity criteria for the test were satisfied. TOC analysis of the samples taken at the beginning of the test showed an actual recovery rate of the test substance is 79%. This may be due to the undissolved particles present in the solution after thawing the sample. Although the sample was vigorously stirred in an ultrasonic bath, the complete re-dissolving of the test substance was probably not achieved. The sample taken at 96 hours showed that the TOC content in the treatment solution by the end of the test did not decrease by more than 20%.

CONCLUSION	The notified polymer is not harmful to fish.
TEST FACILITY	Notox (1996a)

B.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified polymer
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Static Test
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Not reported
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	DC-190 High Temperature Total Organic Carbon (TOC) Analyser
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

Nominal Concentration (mg/L)	*Number of D. magna*	*Cummulative % Immobilised 48 h*
Control	20	0
100	20	0

EC50	> 100 mg/L at 48 hours
NOEC	≥ 100 mg/L at 48 hours
Remarks - Results	All validity criteria for the test were satisfied. TOC analysis of the samples taken at beginning of the test showed an actual recovery rate of the test substance is 79%. This may be due to the undissolved particles present in the solution after thawing the sample. Although the sample was

	vigorously stirred in an ultrasonic bath, the complete re-dissolving of the test substance was probably not achieved. The sample taken at 96 hours showed that the TOC content in the treatment solution by the end of the test did not decrease by more than 20%.
CONCLUSION	The notified polymer is not harmful to aquatic invertebrates.
TEST FACILITY	Notox (1996b)

B.2.4. Algal growth inhibition test

TEST SUBSTANCE	Notified polymer
METHOD	OECD TG 201 Alga, Growth Inhibition Test
Species	*Selenastrum capricornutum*
Exposure Period	72 hours
Concentration Range	Nominal: 0.15, 0.32, 0.7, 1.5, 3.2, 7.0, 15 and 32 mg/L
Auxiliary Solvent	Not reported
Analytical Monitoring	DC-190 High Temperature Total Organic Carbon (TOC) Analyser
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

Growth rate (72 h)		*Growth (72 h)*	
E_bL50 (mg/L)	*NOE_bL (mg/L)*	*E_rL50 (mg/L)*	*NOE_rL (mg/L)*
2.2	1.5	13.0	1.5

Remarks - Results	All validity criteria for the test were satisfied. TOC analysis of the samples taken at the beginning of the test showed actual recovery rates of the test substance ranging from 68 to 84%. The measurements at the lower concentrations of 0.15 and 1.5 mg/L were difficult to interpret. The results based on the highest treatment concentration of 32 mg/L, the sample taken at 72 hours showed that the TOC content in the treatment solution by the end of the test did not decrease by more than 20%.
CONCLUSION	The notified polymer is harmful to algae.
TEST FACILITY	Notox (1996c)

B.2.5. Algal growth inhibition test

TEST SUBSTANCE	Notified polymer
METHOD	OECD TG 201 Alga, Growth Inhibition Test
Species	*Scenedesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 0.1, 0.32, 1.0, 3.2, 10, 32 and 100 mg/L
Auxiliary Solvent	Not reported
Analytical Monitoring	DC-190 High Temperature Total Organic Carbon (TOC) Analyser
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

Growth rate (72 h)		*Growth (72 h)*	
E_bL50	*NOE_bL*	*E_rL50*	*NOE_rL*

(mg/L)	*(mg/L)*	*(mg/L)*	*(mg/L)*
6.5	0.36	150	0.36

Remarks - Results	All validity criteria for the test were satisfied. TOC analyses of the samples were carried out at the beginning and end of the test. The E_rL50 was extrapolated from the equation for the fitted line as 50% inhibition was not achieved from the experiment. The analysis results showed that the loss of the test substance at the end of the test was greater than 20% for some treatments. Therefore, geometric mean measured concentrations were used as worst case for the calculations.
CONCLUSION	The notified polymer is not harmful to algae.
TEST FACILITY	SafePharm (2005b)

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