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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Polymer in CP2490 (Neat)

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 Ageing, 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, Water, Heritage and the Arts.

For the purposes of subsection 78(1) of the Act, this Full Public Report may be inspected at our NICNAS office by appointment only at 334-336 Illawarra Road, Marrickville NSW 2204.

This Full 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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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Oronite Australia Pty Ltd (ABN 16 101 548 716)
Level 10, 45 William Street
MELBOURNE VIC 3000

NOTIFICATION CATEGORY
Standard: Synthetic Polymer with Mn > 1000 Da (more than 1 tonne per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication:
Chemical Name, CAS Number, Molecular Formula, Structural Formula, Molecular Weight, Spectral Data, Purity, Identity of Toxic Impurities, Non-hazardous impurities, Identity and Percentage of Additives, Import Volumes, Concentration of Notified Polymer in Imported Product and Formulated Gasoline, Identity of Recipients

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, Dissociation Constant, Flammability Limits and Autoignition Temperature

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
US (2009)
Canada (2009)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
CP2490 (Neat) (product containing the notified polymer at 60-75%)

OTHER NAME(S)
Polyolefin aryl amine

MOLECULAR WEIGHT
> 1000 Da

ANALYTICAL DATA
Reference IR and GPC spectra were provided.

3. COMPOSITION

DEGREE OF PURITY 60-75%

LOSS OF MONOMERS, OTHER REACTANTS, ADDITIVES, IMPURITIES
None expected.

DEGRADATION PRODUCTS
Stable under normal conditions.

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: amber liquid

Property	Value	Data Source/Justification
Pour Point	3°C	Measured
Boiling Point	294-735°C (pressure unknown)	Measured
Density	926.2 kg/m^3 at 20°C	Measured
Vapour Pressure	7.108×10^{-8} kPa at 20°C	Calculated
Water Solubility	$\leq 3.7 \times 10^{-5}$ g/L	Measured
Hydrolysis as a Function of pH	Not tested	The notified polymer is not expected to hydrolyse over the environmental pH range 4–9 at ambient temperature.
Partition Coefficient (n-octanol/water)	log Pow > 7.4	Measured
Adsorption/Desorption	log K_{oc} > 4.87	Estimated by QSAR equation
Dissociation Constant	Not tested	The notified polymer contains functional groups that are expected to be ionised over the environmental pH range 4–9.
Particle Size	Not determined	Liquid
Flash Point	> 247°C (pressure unknown)	MSDS
Flammability Limits	Not determined	Not expected to be highly flammable.
Autoignition Temperature	Not determined	The notified polymer is not expected to autoignite under normal conditions of use.
Explosive Properties	Not determined	Expected to be stable under normal conditions of use. The notified polymer contains no functional groups that would imply explosive properties.
Viscosity	28469 cSt at 40 °C	MSDS

DISCUSSION OF PROPERTIES

Test substance for Physical and Chemical Properties is CP2490 (Neat) containing the notified polymer at 60-75% (Chevron Energy Technology Company 2009). For full details of tests on physical and chemical properties, refer to Appendix A.

Reactivity

The notified polymer is considered stable under normal conditions. It is not reactive with water or air. It may react with strong oxidizing agents, such as chlorates, nitrates, and peroxides. Hazardous polymerization will not occur.

Dangerous Goods classification

Based on the submitted physical-chemical data in the above table the notified polymer is not classified according to the Australian Dangerous Goods Code (NTC, 2007). However the data above do not address all Dangerous Goods endpoints. Therefore consideration of all endpoints should be undertaken before a final decision on the Dangerous Goods classification is made by the introducer of the polymer.

5. INTRODUCTION AND USE INFORMATION

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

The notified polymer will be imported as an ingredient (at < 10%) of gasoline additive packages.

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

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

PORT OF ENTRY
Typical ports include: Sydney, Melbourne, Perth and Brisbane

IDENTITY OF RECIPIENTS
Major gasoline manufacturers in Australia

TRANSPORTATION AND PACKAGING
The gasoline additive containing the notified polymer at < 10% will be transported either by:
1) marine vessel in 1000-liter isotanks and offloaded to tank trucks or rail cars for distribution to a blending facility, or
2) drum (such as 250-liter steel) which will be shipped directly to the customer.

USE
Gasoline additive

OPERATION DESCRIPTION
The gasoline additive containing < 10% of the notified polymer will be transported directly to fuel manufacturer's refinery terminal, and pumped into storage tanks. Later the gasoline additive will be metered into the tank trucks to be mixed with gasoline to form the fully formulated gasoline (containing the notified polymer at < 500 ppm) before delivery to refuelling stations. At the refuelling stations, workers or the public will refill vehicles with the fully formulated gasoline.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Analysing additive package on arrival	1	10 min	30
Unloading isotanks and drums	1-2	0.5	30
Sampling and analysing finished gasoline	1-2	10 min	220
Loading gasoline into tank trucks	1-2	0.5	220
Distribution to service stations	1-2	0.5	220
End-use fuelling	> 10,000	10-30 min	220

EXPOSURE DETAILS
The potential routes of occupational exposure are dermal, ocular and inhalation. However inhalation exposure is not expected as the polymer has a low vapour pressure, and the generation of mists/aerosols is not expected.

Transport and storage of gasoline additive
Transport workers are not expected to be exposed to the imported gasoline additive containing the notified polymer at < 10%, as they will be handling closed containers. Dermal or ocular exposure is possible in the event of an accident where the packaging is breached or during transfer to storage tanks.

Blending
At the fuel manufacturer's refinery terminal, blending of the gasoline additive (containing notified polymer at < 10%) with gasoline will be carried out automatically or semi-automatically in a closed system, usually through metering into tank trucks. Exposure to the notified polymer at < 10% may occur from accidental spillage. Exposure is expected to be low and further reduced by workers wearing personal protective equipment when handling gasoline.

Exposure to the notified polymer (at < 500 ppm) may also occur during sampling and analysis of blended gasoline at the refinery or during maintenance of refinery plant or pipelines. The exposure would be limited by appropriate personal protective equipment worn by workers.
Transport and storage of gasoline
Dermal or ocular exposure to drips and spills of gasoline containing the notified polymer at < 500 ppm is

possible during the connection and disconnection of transfer hoses. Exposure is expected to be limited during transportation as the protocols of loading and unloading are done with minimal spills. The drivers also usually wear gloves and long sleeves shirts when unloading the gasoline.

End users of gasoline
Personnel from commercial trucking fleet, marine tugs or small ships, agriculture users, railroads, service stations, truck stops and construction companies may be exposed to the notified polymer at < 500 ppm during handling and fueling of the vehicles. As most of the notified polymer will be combusted with the fuel, exposure is expected to be minimal during end-use/combustion.

6.1.2. Public exposure
The public may have incidental skin or eye contact with gasoline containing the notified polymer at < 500 ppm through operations such as refilling vehicles.

6.2. Human health effects assessment

The results from toxicological investigations conducted on CP2490 (Neat) (containing the notified polymer at 60-75%) are summarised in the table below. Details of these studies can be found in 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
In vitro skin corrosion: human skin model test	no reliable result due to viscosity of the test substance
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation –non-adjuvant test.	evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL = 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosome aberration test	non genotoxic
Genotoxicity – in vivo mammalian erythrocyte micronucleus test	non genotoxic

Toxicokinetics, metabolism and distribution.
Dermal absorption of the notified polymer is not expected to be significant based on its high molecular weight (> 1000 Da), low water solubility (≤ 3.7×10^{-5} g/L) and high partition coefficient (log Pow > 7.4). However, the low molecular weight species present in the notified polymer may be absorbed more readily. This is also suggested by the sensitisation responses observed with the notified polymer.

Absorption of the notified polymer from the gastro-intestinal tract is expected to be limited by its water solubility and molecular weight. Any uptake is likely to occur via micellular solubilisation, given its highly lipophilic nature and low water solubility. The low molecular weight species may also undergo some absorption.

Acute toxicity
The notified polymer is expected to be of low acute toxicity *via* the oral and dermal routes.

Irritation and sensitisation
Based on the studies provided, the notified polymer is expected to be slightly irritating to skin and eyes.

The notified polymer is expected to have the potential to cause skin sensitisation based on a skin sensitisation test using the Buehler method.

Repeated dose toxicity
The oral administration of the test substance (60-75% notified polymer) to rats for a period of twenty-eight consecutive days at dose levels of 60, 250 and 1000 mg/kg bw/day did not result in any toxicological significant effects at any dose level. The No Observed Adverse Effect Level (NOAEL) was therefore established as 1000 mg/kg bw/day in this study.

Mutagenicity.
The notified polymer at 60-75% was not mutagenic in a bacterial reverse mutation study and not genotoxic in an

in vitro mammalian chromosome aberration test and an *in vivo* mammalian erythrocyte micronucleus test.

Health hazard classification
Based on the skin sensitisation test using the Buehler method the notified polymer is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:
Xi; R43 May cause sensitisation by skin contact

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
The notified polymer is a skin sensitiser and is slightly irritating to the skin and eyes.

Workers most at risk will be those workers handling the notified polymer as introduced where the concentration of the notified polymer is up to 10%. Given the exposure should be limited by the expected use of personal protective equipment by those workers and engineering controls in place (automated and closed system), the risk to workers from use of the notifier polymer is not considered unacceptable.

The risk to all other workers is considered low given the low concentration of the notified polymer (< 500 ppm) in the gasoline.

6.3.2. Public health
The risk to the public from exposure to the notified polymer in gasoline is expected to be low based on the low concentration of the notified polymer in the gasoline (< 500 ppm) and the expected low exposure to the gasoline.

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 as a component in a petrol additive for blending in Australia. In the unlikely event of spills during the transportation or blending, large spillages would be collected and reblended into product, or sent to on-site wastewater treatment plants. The treatment plant separates oils from the water for recycling, and the water is sent for flocculation and biological treatment. Sludge generated through the water treatment processes is expected to be sent to landfill. Small spills from blending would be immediately soaked up using absorbent material and disposed of to landfill. Empty transport drums containing residue of the notified polymer (< 0.1% of the annual import volume) are likely to recycled, whereby the residue is removed by steam cleaning and the wastewater is sent to a wastewater treatment plant. If the transport drums are consigned to metal reclamation, then the notified polymer is expected to be thermally decomposed to water and oxides of carbon and nitrogen.

RELEASE OF CHEMICAL FROM USE
At service stations, release of the notified polymer would mainly result from accidental spills when unloading the petrol from trucks into the reservoir, or from drips and spills when pumping petrol at the bowsers.

RELEASE OF CHEMICAL FROM DISPOSAL
The majority of the notified polymer is expected to be consumed by combustion in auto-engines.

7.1.2 Environmental fate

The notified polymer has limited water solubility and is not readily biodegradable. The notified polymer is expected to partition to, and remain in, soil and sediment in waste treatment plants and landfill, or when spilt during refuelling, where it is likely to be immobile and slowly degrade *in situ*. The notified polymer will decompose, either thermally in engines or abiotically in landfill, into mainly water and oxides of carbon and nitrogen. Bioaccumulation of the notified polymer is not expected due to its limited potential for exposure to the aquatic compartment. For the details of the environmental fate studies refer to Appendix C.

7.1.3 Predicted Environmental Concentration (PEC)

The PEC was not calculated as very limited aquatic exposure is expected based on the reported use pattern.

7.2. Environmental effects assessment

The results from ecotoxicological investigations conducted on water accommodated fractions (WAFs) of the petrol additive CP2490 (up to 60-75% notified polymer) are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	LL50 (96 h) > 1000 mg/L	Not harmful to fish
Daphnia Toxicity	EL50 (48 h) = 50 mg/L	Harmful to aquatic invertebrates
Algal Toxicity	E_rL50 (96 h) = 25 mg/L	Harmful to algae
Inhibition of Bacterial Respiration	IC50 (3 h) > 1000 mg/L	Not harmful to microbial respiration

Under the Globally Harmonised System of Classification and Labelling of Chemicals (United Nations, 2009) the notified polymer is harmful to aquatic invertebrates and algae, but not harmful to fish and bacteria. Interestingly, the biodegradation test indicated that the test substance had inhibitory effects on micro-organisms, however the microbial respiration test indicated that the test substance was not harmful to microbial respiration. The endpoints of the studies are based on nominal loading rates due to the low water solubility of the notified polymer. The actual concentration of the notified polymer in the studies ranged from less than the limit of quantification to 2.35 mg/L (determined by HPLC), and therefore these values should be treated with caution. Details of these studies can be found in Appendix C.

7.2.1 Predicted No-Effect Concentration

The PNEC has not been calculated as very limited aquatic exposure is expected based on the reported use pattern.

7.3. Environmental risk assessment

The Risk Quotient, Q (= PEC/PNEC), has not been determined due to the notified polymer's low potential for release to the aquatic compartment. Based on its reported use pattern, the notified polymer is not expected to pose a risk to the environment.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

Based on the available data the notified polymer is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)]. The following risk phrases apply to the notified polymer:

− Xi; R43 May cause sensitisation by skin contact

and

As a comparison only, the classification of the notified polymer using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations 2009) is presented below. This system is not mandated in Australia and carries no legal status but is presented for information purposes.

	Hazard category	***Hazard statement***
Skin sensitisation	Category 1	May cause an allergic skin reaction
Environment	Acute category 3	Harmful to aquatic life
	Chronic Category 3	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 unacceptable risk to the health of workers.

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

Environmental risk assessment
On the basis of the reported use pattern, the notified polymer is not expected to pose a risk to the environment.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- Safe Work Australia should consider the following health hazard classification for the notified polymer:
  - Xi; R43 May cause sensitisation by skin contact

- Use the following risk phrases for products/mixtures containing the notified polymer:
  - concentration ≥ 1%: R43

Health Surveillance

- As the notified polymer is a skin sensitiser, employers should carry out health surveillance for any worker (exposed to the imported gasoline additive containing the notified polymer at < 10%) who has been identified in the workplace risk assessment as having a significant risk of skin sensitisation.

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified polymer:
  - Automation of blending processes
  - Closed blending vessels

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified polymer as introduced:
  - Avoid contact with skin and eyes

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified polymer as introduced:
  - Coveralls
  - Gloves

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

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

- If products and mixtures containing the notified polymer are classified as hazardous to health in accordance with the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Disposal

- The notified polymer 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 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 polymer has changed from gasoline additive, or is likely to change significantly;
- the concentration of the notified chemical is > 500 ppm in gasoline;
- the amount of polymer being introduced has increased from 3 tonne per year, 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.

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

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Pour Point 3°C

Method	OECD TG 102 Melting Point/Melting Range. ASTM D5950
Remarks	Section 20 of OECD Guideline 102 indicates Pour Point is an appropriate method for oily substances with low melting points. Although ASTM D97 is referenced in the Appendix to Guideline 102, ASTM D5950 using an automatic apparatus offers better repeatability and reproducibility. Test method ASTM D5950 was used to generate the pour point value.
Test Facility	Chevron Energy Technology Company (2009)

Boiling Point 294-735°C (pressure unknown)

Method	OECD TG 103 Boiling Point.
Remarks	A similar method to ASTM D 6352 was used. Gas chromatography was used to determine the boiling point range. The boiling point distribution corresponds to 53.5% recovered mass. Hence there are also components of the notified polymer with boiling point > 735°C.
Test Facility	Chevron Energy Technology Company (2009)

Density 926.2 kg/m^3 at 20°C

Method	OECD TG 109 Density of Liquids and Solids. ASTM D 4052
Remarks	An oscillating densitometer was used.
Test Facility	Chevron Energy Technology Company (2009)

Vapour Pressure 7.108×10^{-8} kPa at 20°C

Method	OECD TG 104 Vapour Pressure.
Remarks	Calculated by the Maxwell-Bonnell/ProVision Method
Test Facility	Chevron Energy Technology Company (2009)

Water Solubility $\leq 3.7 \times 10^{-5}$ g/L

Method	OECD TG 105 Water Solubility.
Remarks	Column Elution Method. An HPLC column was charged with an inert Teflon support previously coated with CP2490 (Neat) dissolved in dichloromethane. CP2490 (Neat) contains up to 60-75% notified polymer in a complex mixture of related polymers. Reverse phase HPLC with a fluorescence detector was used to measure the amount of test substance eluted from the column. The water soluble fraction of CP2490 (Neat) was found to have a water solubility of 3.7×10^{-5} g/L. Column temperature and run conditions were not reported.
Test Facility	Chevron Energy Technology Company (2009)

Partition Coefficient (n-octanol/water) log Pow > 7.4

Method	OECD TG 117 Partition Coefficient (n-octanol/water).
Remarks	Linear gradient HPLC method (60:40 methanol/water 1 mL/min → 100:0 methanol/water at 7 min). The retention time of CP2490 (Neat) was compared to those of reference compounds with log Pow values in the range 3.6–7.4. CP2490 (Neat) contains up to 60-75% notified polymer in a complex mixture of related polymers. The first component of CP2490 (Neat) to elute was measured to have a log Pow value of 5.5, however the extrapolated weighted average log Pow for CP2490 (Neat) was 16.4. The bulk of CP2490 (Neat), eluted after the last known reference substance, hence the notified polymer is expected to have a log Pow value > 7.4. Column temperature was not reported.
Test Facility	Chevron Energy Technology Company (2009)

Adsorption/Desorption $\log K_{oc} > 4.87$

Method QSAR calculation (as per Technical Guidance Document (EC, 2003))

Remarks The adsorption coefficient was estimated by QSAR calculation using the nonhydrophobic class formula ($0.52 \times \log Pow + 1.02$; $n = 390$, $r^2 = 0.63$, standard error = 0.56) to give a value > 4.87. The nonhydrophobic class is defined to be all chemicals that do not contain halogen atoms. The QSAR estimates for amine-containing polyaromatic compounds are typically underestimated, and as such the value is reported as a minimum value, which is sufficient to indicate negligible mobility in soil. High affinity for soil particles is expected for the notified polymer due to its predominantly oleophilic structure with potential cationic nature.

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

The test substance CP2490 (Neat) refers to a product containing 60-75% of the notified polymer.

B.1. Acute toxicity – oral

TEST SUBSTANCE CP2490 (Neat)

METHOD OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain Rat/Sprague-Dawley
Vehicle Corn oil
Remarks - Method No significant protocol deviations.

RESULTS

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

LD50 > 2000 mg/kg bw
Signs of Toxicity There were no adverse signs of reaction to treatment recorded in any animal during the observation period.
Effects in Organs There were no macroscopic findings recorded in any animal at necropsy.
Remarks - Results Body weight gain was considered to be acceptable for rats of this age and strain.

CONCLUSION The test substance is of low toxicity via the oral route.

TEST FACILITY Charles River (2009a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE CP2490 (Neat)

METHOD OECD TG 402 Acute Dermal Toxicity – Limit Test.
Species/Strain Rat/Sprague-Dawley
Vehicle None
Type of dressing Occlusive
Remarks - Method No significant protocol deviations.

RESULTS

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

LD50 > 2000 mg/kg bw
Signs of Toxicity - Local There were local findings at the dosing site with scabs on the abdomen observed in one male from days 5-7, and red skin noted on the abdomen of 2 females from day 9 until day 13 and 15 respectively.
Signs of Toxicity - Systemic There were no systemic signs noted in any animal at any observation timepoint.
Effects in Organs No abnormal findings were noted at necropsy.
Remarks - Results Body weight gain was considered to be acceptable for rats of this age and strain.

CONCLUSION The test substance is of low toxicity via the dermal route.

TEST FACILITY Charles River (2009b)

B.3. Irritation – skin

TEST SUBSTANCE	CP2490 (Neat)
METHOD	Analogous to OECD TG 431: In Vitro Skin Corrosion: Human Skin Model Test A preliminary test was conducted to assessment the ability of the test substance alone to reduce MTT (2*H*-tetrazolium, 2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-, bromide (1:1)). Any direct MTT reduction by the test substance would interfere with the assay results and necessitate the inclusion of water-killed controls in the system. The test substance did not directly reduce MTT. The irritation potential of the test substance was assessed by applying the test substance undiluted onto the surface of six EpiSkin reconstructed human epidermis (RHE) units for *ca* 15 min. The test substance was then washed from the surface of the EpiSkin and the units returned to the incubator for a recovery period of *ca* 42 h. After the recovery period, the skin units were transferred to assay medium containing 0.3 mg/mL MTT and returned to the incubator for *ca* 3 h. Biopsies of the EpiSkin membranes were then removed and added to the acidic isopropanol. The formazan production was assessed by measuring absorption at 550 nm and the viability of each individual tissue calculated as a percentage of the mean negative control viability. The SinkEthic EpiSkin irritation assay has been validated by the European centre for the Validation of Alternative Methods (ECVAM) as an *in vitro* model to assess skin irritation.
Remarks - Method	Due to viscosity and stickiness of the test substance, two wash methods were employed to remove the test substance from the epidermis after the 15 min exposure period. Three of the EpiSkin units dosed with the test substance were washed with Dulbecco's phosphate buffered saline (PBS) and swabbed with a sterile cotton swab to attempt to remove any test substance that remained bound to the skin after rinsing. It was suspected that the pressure required to swab the bound test substance from the skin surface may have resulted in some physical damage to the epidermis, leading to false positive results. As such, a further three EpiSkin units were washed by rinsing with Dulbecco's PBS without swabbing. This removed little, if any, of the test substance but had the advantage of causing no obvious physical damage to the epidermis. Acceptance criteria The assay was deemed acceptable if the following occurred: The mean optical density (OD) value of the 3 negative control tissues was ≥ 0.6 and the standard deviation value (SD) was ≤ 18. The mean % viability of the 3 positive control tissues was ≤ 30% and the SD was ≤ 18. The mean % viability SD of the 3 treated tissues (for each wash method) was ≤ 18.
RESULTS	
Remarks - Results	*MTT Direct Reduction Test* The test was scored by visual assessment of the formation of the formazan (purple). The positive control (Eugenol) reduced the MTT solution to formazan almost immediately, generating a dark purple colour even before incubation. The negative control (sterile, ultra-pure water) did not reduce MTT to formazan after approximate 3 h incubation. The test substance did not reduce MTT to formazan after approximate 3 h incubation. Since the test substance had no inherent capability to reduce MTT, the assay was conducted without the requirement for water-killed controls. *Negative Control Groups* The negative control results were within the acceptance criteria defined in the ECVAM (European Centre for the Validation of Alternative Methods)

validation SOP (standard operating procedure).
Positive Control Groups
The positive control results were within the acceptance criteria defined in the ECVAM validation SOP.
The Test Substance
The results of the irritation assay were similar for both wash methods and are expressed as mean ± SD. EpiSkin exposure to the test substance followed by rinsing alone resulted in a cell viability value of 21.27 ± 9.76%. Rinsing and swabbing of the epidermis resulted in a cell viability of 20.84 ± 7.48%. The positive and negative controls were within the defined acceptance criteria and demonstrated the efficacy of the test system.
The results of the test should be considered with caution, due to a number of factors that may have affected the viability of the EpiSkin tissues and therefore led to a false positive result. These factors all relate to the difficulties encountered when applying and removing the extremely viscous test substance to and form the EpiSkin as follows:
It was not possible to apply the recommended dose (10 ± 2 mg) to each of the EpiSkin units. Dose applications ranged from approximate 10-18 mg and the dose applied was higher than 12 mg for 5 of the 6 EpiSkin units. During the dosing procedure the test substance stuck to the surface of the epidermis on contact and resisted any attempts to distribute evenly over the surface using a circular spreading motion. It is possible that the increased pressure required to spread the test item over the entire exposed skin surface may have resulted in some damage to the skin tissue. The test substance could not be fully removed from the skin surface by either wash method employed. The EpiSkin was therefore afforded no period of recovery from weakly irritant effects. Removal of the test substance by swabbing may have resulted in damage to the skin tissue.

CONCLUSION — The results of the test were inconclusive. The positive response obtained in this study may be an artefact caused by the viscosity of the test substance and the associated difficulties in its application and removal from the EpiSkin surface.

TEST FACILITY — Charles River (2009c)

B.4. Irritation – skin

TEST SUBSTANCE — CP2490 (Neat)

METHOD — OECD TG 404 Acute Dermal Irritation/Corrosion.

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 M
Vehicle	Corn oil
Observation Period	10 days
Type of Dressing	Semi-occlusive.
Remarks - Method	Three concentrations were tested, 25%, 50% and 100% at different sites on the same animal. No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
100%	1	2	3			
Erythema/Eschar	1	2	1	2	< 9 days	0
Oedema	0	0	0	1 (1 hour)	< 1 day	0

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

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
50%	1	2	3			
Erythema/Eschar	1	2	2	2	< 9 days	0
Oedema	0	0	0.3	2 (1 hour)	< 2 days	0

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

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
25%	1	2	3			
Erythema/Eschar	0.7	1.3	1	2	< 9 days	0
Oedema	0	0	0	0	-	0

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

Remarks - Results

Reactions in all 3 treated animals were broadly similar among the 3 concentrations and there was complete recovery by 9 days after patch removal.
Oedema was only noted in one animal 1 h and 24 h after patch removal at 50% and 1 h after patch removal at 100%.
Very slight erythema was noted in one animal and very slight to well defined erythema was noted in the other 2 animals.

CONCLUSION

The test substance is slightly irritating to the skin.

TEST FACILITY

Charles River (2009d)

B.5. Irritation – eye

TEST SUBSTANCE

CP2490 (Neat)

METHOD

OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain: Rabbit/New Zealand White
Number of Animals: 2 M, 1 F
Observation Period: Up to 94 h
Remarks - Method: No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0.7	0.7	0	1	< 72 h	0
Conjunctiva: chemosis	0	0	0	0	-	0
Conjunctiva: discharge	1	0.3	0	1	< 94 h	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

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

Remarks - Results

Test eye
Reactions were confined to slight conjunctival redness and slight discharge.
Slight conjunctival redness (some blood vessels definitely hyperaemic) was seen in all 3 rabbits. Onset was from ½ h to 4 h after test substance instillation and persisted until 48 h after instillation.
Slight discharge was seen in 2 rabbits from ½ h after instillation. In one rabbit this had resolved 48 h after instillation and in the second rabbit resolution was complete 94 h after instillation.
Control eye
No irritation was noted in the control eye of any rabbit at any observation timepoint.

CONCLUSION The test substance is slightly irritating to the eye.

TEST FACILITY Charles River (2009e)

B.6. Skin sensitisation

TEST SUBSTANCE CP2490 (Neat)

METHOD OECD TG 406 Skin Sensitisation - Buehler Test Method
Species/Strain Guinea pig/Harley-derived albino
PRELIMINARY STUDY Maximum Non-irritating Concentration: 2.5% w/v in mineral oil, USP
topical 1: 1, 2.5, 5, 10, 25, 50, 75 and 100%
topical 2: 100%
MAIN STUDY
Number of Animals Test Group: 10 per sex Control Group: 5 per sex for 1st challenge and 2nd challenge
INDUCTION PHASE Induction Concentration:
topical: 100%
Signs of Irritation Dermal scores of 0 (no reaction), ± (slight patchy erythema) (some had irritation outside of test site) and 1 (slight, but confluent or moderate patchy erythema) were observed in test animals.
CHALLENGE PHASE
1st challenge topical: 2.5% w/v in mineral oil, USP
2nd challenge topical: 2.5% w/v in mineral oil, USP
Remarks - Method This study consisted of 2 topical range-finding phases, a test group, a challenge control group, a rechallenge group, and α-Hexylcinnamaldehyde (HCA) (positive control) test and vehicle control groups.

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	2.5%	4/20	4/20	5/20	5/20
Vehicle Control Group	2.5%	0/10	0/10	0/10	0/10

Remarks - Results All animals survived to scheduled euthanasia. All test animals gained weight during the test period and generally appeared in good health.
Following 1st challenge, dermal scores of 1 were noted in 4/20 test animals at the 24-hour and 48-hour scoring intervals. The remaining test and control animals had scores of ±. Group mean dermal scores were slightly higher in the test animals as compared to the control animals.
Following 2nd challenge, dermal scores of 1 were noted in 5/20 test animals at the 24-hour and 48-hour scoring intervals. The remaining test and control animals had scores of 0 or ±. Group mean dermal scores were slightly higher in the test animals as compared to the control animals.
Three animals out of 20 test animals (15%) responded at both 1st challenge and 2nd challenge. These animals also had scores of 1 at both the 24- and 48-hour scoring intervals.
The results of the HCA positive control study demonstrated that a valid test was performed and indicated that the test design would detect potential contact sensitisers.

CONCLUSION There was evidence of reactions indicative of skin sensitisation to the test substance under the conditions of the test.

TEST FACILITY Charles River (2009f)

B.7. Repeat dose toxicity

TEST SUBSTANCE	CP2490 (Neat)
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	Corn oil
Remarks - Method	No deviations from the protocol.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 per sex	0	0
low dose	5 per sex	60	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

Mortality and Time to Death
There were no unscheduled deaths during the study.

Clinical Observations
No test substance related clinical signs of toxicity were observed throughout the treatment period or during the recovery period.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
No test substance related effects were detected.

Effects in Organs
No test substance related effects were observed.

Remarks – Results
The oral administration of the test substance to rats for a period of twenty-eight consecutive days at dose levels of 60, 250 and 1000 mg/kg bw/day did not result in any test substance related effects at any dose level.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 1000 mg/kg bw/day in this study, based on no test substance related effects at the highest dose tested.

TEST FACILITY	Harlan Laboratories Limited (2009a)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE	CP2490 (Neat)
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 and TA100 *E. coli*: WP2uvrA$^-$
Metabolic Activation System	S9 was prepared from the livers of male Sprague-Dawley rats that had

	each orally received three consecutive daily doses of phenobarbital/β-naphthoflavone (80/100 mg/kg bw/day) prior to S9 preparation on Day 4.
Concentration Range in Main Test	a) With metabolic activation: 0, 15, 50, 150, 500, 1500, 5000 µg/plate b) Without metabolic activation: 0, 15, 50, 150, 500, 1500, 5000 µg/plate
Vehicle	Tetrahydrofuran
Remarks - Method	No deviations from the protocol.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	> 5000		≥ 1500	
Main Test		> 5000	≥ 1500	negative
Present	> 5000		≥ 1500	
Main Test		> 5000	≥ 1500	negative

Remarks - Results	The test substance caused no visible reduction in the growth of the bacterial background lawn at any dose level and was, therefore, tested up to the maximum recommended dose level of 5000 µg/plate. A light, oily precipitate was observed at and above 1500 µg/plate. This observation did not prevent the scoring of revertant colonies. No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains at any dose level, with or without metabolic activation. All of the positive control chemicals (N-ethyl-N'-nitrosoguanidine, 9-aminoacridine and 4-nitroquinoline-1-oxide) used in the test induced marked increases in the frequency of revertant colonies and the activity of the S9 fraction was shown to be satisfactory.
CONCLUSION	The test substance was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Safepharm Laboratories Limited (2008)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE	CP2490 (Neat)
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Cell Type	Human peripheral blood lymphocytes
Metabolic Activation System	The in vitro metabolic activation system consisted of a rat liver post-mitochondrial fraction (S9) and an energy-producing system (NADP plus isocitric acid). Various hepatic P450 isoenzymes levels are increased by treatment of the rats with Aroclor™ 1254 (single concentration of 500 mL/kg) which were sacrificed 5 days later.
Vehicle	Tetrahydrofuran
Remarks - Method	The protocol deviation did not affect the integrity or interpretability of the results of the study.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Main Test	0, 9.40, 18.8, 25.0*, 37.5*, 50.0*, 75.0, 100, 125, 150, 180	22 h	22 h
Present			
Main Test	0, 25.0, 37.5, 50.0*, 75.0*, 100*, 125, 180, 240	3 h	22 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	> 500			
Main Test		> 180	≥ 75.0	negative
Present	> 500			
Main Test		> 240	≥ 100	negative

Remarks - Results

The vehicle control cultures were in the historical control range for cells with chromosomal aberrations and the positive control cultures had significant increase in cells with chromosomal aberrations as compared with the vehicle control cultures. The high doses selected for analysis in the assay had either a precipitate at the end of the treatment period or a ≥ 50% reduction in mitotic index.
Under conditions without metabolic activation, the sensitivity of the cell cultures for induction of chromosomal aberrations is shown by the increased frequency of aberrations in the cells exposed to mitomycin C, the positive control agent. The test substance was considered negative for inducing chromosomal aberrations, polyploidy, or endoreduplication under conditions without metabolic activation.
Under conditions with metabolic activation, the successful activation of the metabolic system is illustrated by the increased incidence of cells with chromosomal aberrations in the cultures induced with cyclophosphamide, the positive control agent. The test substance was considered negative for inducing chromosomal aberrations, polyploidy, or endoreduplication under conditions with metabolic activation.

CONCLUSION

The test substance was not clastogenic under the conditions of this in vitro chromosome aberration test.

TEST FACILITY

Covance Laboratories Inc. (2009a)

B.10. Genotoxicity – in vivo

TEST SUBSTANCE

CP2490 (Neat)

METHOD

OECD TG 474 Mammalian Erythrocyte Micronucleus Test.

Species/Strain: Mouse/CD-1
Route of Administration: Intraperitoneal injection
Vehicle: Corn oil
Remarks - Method: No significant protocol deviations.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	10 M	0	24 & 48
II (low dose)	5 M	500	24
III (mid dose)	5 M	1000	24
IV (high dose)	10 M	2000	24 & 48
V (positive control, CP)	5 M	80	24

CP=cyclophosphamide

RESULTS

Survival and Clinical Observations	The test substance did not induce mortality at any dose level. Clinical signs of toxicity were observed in all animals in the 2000 mg/kg bw (48-hour dose group) which included non-formed faeces and rough haircoat. All animals in the 500, 1000, and 2000 mg/kg bw (24-hour) dose groups appeared normal immediately after dosing and remained normal until the end of observation period. All animals in the vehicle and positive control groups appeared normal after dosing and remained normal until the appropriate harvest timepoint.
Remarks - Results	The test substance did not induce statistically significant increases in micronucleated PCEs at any dose tested. However, the test substance was cytotoxic to the bone marrow (i.e., a statistically significant decrease in the PCE:NCE ratio) in the 2000 mg/kg bw dose group 48 hours after dose administration. The vehicle control group had approximately ≤ 0.05% micronucleated PCEs and the group mean was within the historical control range. The positive control, cyclophosphamide, induced a statistically significant increase in micronucleated PCEs as compared to that of the vehicle control, with a mean and standard deviation of 1.64 ± 0.33%.
CONCLUSION	The test substance was not clastogenic under the conditions of this in vivo mammalian erythrocyte micronucleus test.
TEST FACILITY	Covance Laboratories Inc. (2009b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

The test substance CP2490 (Neat) refers to a product containing 60-75% of the notified polymer.

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	CP2490 (Neat)
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test.
Inoculum	Activated sewage sludge from a domestic sewage treatment plant
Exposure Period	29 days
Auxiliary Solvent	None
Analytical Monitoring	Dissolved organic carbon (DOC) and total organic carbon (TOC) using Shimadzu TOC–5050A and TOC–V_{CSH} TOC analysers.
Remarks - Method	In an initial experiment, a toxicity control indicated that the test substance exhibited inhibitory effects at 10 mg C/L. In triplicate, test substance (adsorbed to silica gel, due to the low solubility of the test substance) was dispersed in inoculated culture medium to give a concentration of 5 mg C/L. Duplicate positive controls (sodium benzoate) and a toxicity control were run in parallel, all containing silica gel for consistency. The test solutions were incubated at 21°C in darkness for 29 days. Degradation was determined by measuring the amount of CO_2 produced, corrected with the blank inoculum, and expressed as % of theoretical amount of CO_2 ($ThCO_2$).

RESULTS

Test substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
1	0	1	0
10	0	7	68
20	0	21	85
29	0	29	108

Remarks - Results	The test substance attained 0% degradation after 29 days. The toxicity control attained 11% degradation after 14 d, and 4% after 28 d, indicating that the test substance is inhibitory at 5 mg C/L. The reference substance degraded by more than 60% in a ten day window, thus validating the test. The reference substance attained 108% degradation by 29 d, however values in excess of 100 are reported to be due to sampling/analytical variation. Over the duration of the test, all test vessels were observed to contain cloudy dispersions with no test or reference substance visible.
CONCLUSION	CP2490 (Neat) and, by inference, the notified polymer are not readily biodegradable.
TEST FACILITY	Harlan Laboratories Limited (2009b)

C.1.2. Bioaccumulation

METHOD	Test not conducted
Remarks - Results	The notified polymer is not expected to bioaccumulate due to its low potential for exposure to the aquatic environment.

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: CP2490 (Neat)

METHOD
- Species: Juvenile rainbow trout (*Oncorhynchus mykiss*)
- Exposure Period: 96 hours
- Auxiliary Solvent: None
- Water Hardness: 120 mg $CaCO_3$/L to 132 mg $CaCO_3$/L
- Analytical Monitoring: HPLC was used for determination of the concentration of the test substance.
- Remarks – Method: After a range-finding test, two tests were conducted in triplicate with water accommodated fractions (WAFs; due to the low water solubility of the test substance) at nominal loading rates of 100 mg/L and 1000 mg/L. The test substance (2100 mg and 21.00 g) was added to the surface of dechlorinated tap water (21 L) to achieve the loading rates of 100 mg/L and 1000 mg/L respectively. The test media were stirred for 24 h and allowed to stand for 4 h. The WAFs were removed by mid-depth siphoning (discarding the first 75–100 mL). Microscopic inspection of the WAFs showed no micro-dispersions or undissolved test material to be present. The fish, introduced to the WAFs and maintained at 14.8°C to 16.0°C under semi-static conditions for 4 days (pH 7.3 to 7.9, 5.3 mg O_2/L to 10.0 mg O_2/L), were observed for mortality and sub-lethal effects.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Loading rates	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	0	20	0	0	0	0	0
100	0.12–0.16	30	0	0	0	0	0
1000	0.59–2.35	30	0	0	0	1	2

- LL50: > 1000 mg/L at 96 hours. Based on loading rates.
- NOEL: 100 mg/L at 96 hours. Based on loading rates.
- Remarks – Results: Mortality and sub-lethal effects, including moderate to extreme loss of equilibrium, increased pigmentation, surface swimming and moribund fish, were observed in the test vessel replicates at 1000 mg/L loading rate. There were no sub-lethal effects of exposure observed at the 100 mg/L loading rate. The actual concentrations of the test substance were found to range from 0.59 mg/L to 2.35 mg/L for the 1000 mg/L loading rate, and 0.12 mg/L to 0.16 mg/L for the 100 mg/L loading rate. The measured concentrations (greater than the water solubility level) were considered to possibly be due to micro-emulsions of the test substance present, despite microscopic inspections indicating otherwise, and therefore these results should be treated with caution. Given that the toxicity of the test substance cannot be attributed to a single component, the results are based on nominal loading rates only. Hence, the test substance and, by inference, the notified polymer are not harmful to fish.

CONCLUSION: The notified polymer is not harmful to fish.

TEST FACILITY: Harlan Laboratories Limited (2009c)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE: CP2490 (Neat)

METHOD OECD TG 202 *Daphnia* sp., Acute Immobilisation Test - static
Species *Daphnia magna*
Exposure Period 48 hours
Auxiliary Solvent None
Water Hardness 250 mg $CaCO_3$/L
Analytical Monitoring HPLC was used for determination of the concentration of the test substance.
Remarks - Method After a range-finding test, twenty daphnids (2 replicates of 10 animals) were exposed to WAFs (prepared as per C.2.1. Acute toxicity to fish) with nominal loading rates of 10, 18, 32, 56 and 100 mg/L. Microscopic inspection of the WAFs showed no micro-dispersions or undissolved test substance to be present. The daphnia were observed for immobilisation over two days (test conditions: artificial light dark cycle of 16 to 8 hours, 21–22°C, pH 7.8–8.1, 8.1–8.6 mg O_2/L). A control was maintained under identical conditions, but without the test substance. Daphnia unable to swim within 15 seconds of gentle agitation were considered to be immobile. Statistical analysis was conducted by maximum-likelihood probit method using ToxCalc software.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Loading rates	*Actual*		*24 h*	*48 h*
0	0	20	0	0
10	0.250–0.257	20	0	0
18	0.138–0.168	20	0	0
32	0.391–0.459	20	0	1
56	0.473–0.533	20	0	13
100	0.477–0.519	20	1	20

EL50 50 mg/L (95% CI: 44 mg/L to 58 mg/L) at 48 hours. Based on loading rates.
NOEL 18 mg/L at 48 hours. Based on loading rates.
Remarks - Results There were no immobilised daphnia in the control group and the dissolved oxygen in the control group and test vessels were ≥ 3 mg/L, thus validating the test.
The actual concentrations of the test substance were found to range from 0.168 mg/L to 0.533 mg/L at 0 h, and 0.138 mg/L to 0.477 mg/L at 48 h. The measured concentrations (greater than the water solubility level) were considered to possibly be due to micro-emulsions of the test substance present, despite microscopic inspections indicating otherwise, and therefore these results should be treated with caution. Given that the toxicity of the test substance cannot be attributed to a single component, the results are based on nominal loading rates only. Hence, the test substance and, by inference, the notified polymer are harmful to aquatic invertebrates.

CONCLUSION The notified polymer is harmful to aquatic invertebrates.

TEST FACILITY Harlan Laboratories Limited (2009d)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE CP2490 (Neat)

METHOD OECD TG 201 Alga, Growth Inhibition Test.
Species *Pseudokirchneriella subcapitata*
Exposure Period 96 hours

Concentration Range	Nominal:	10 mg/L to 160 mg/L
	Actual:	0.098 mg/L to 2.3 mg/L
Auxiliary Solvent	None	
Water Hardness	0.15 mmol Ca^{2+} & Mg^{2+}/L	
Analytical Monitoring	HPLC was used for determination of the concentration of the test substance. Cell concentrations were determined by a Coulter® Multisizer Particle Counter.	
Remarks - Method	After a range-finding test, algae with a density of 1×10^4 cells per mL were exposed to WAFs (prepared as per C.2.1. Acute toxicity to fish) in triplicate with nominal loading rates of 10, 20, 40, 80 and 160 mg/L. Microscopic inspection of the WAFs showed no micro-dispersions or undissolved test material to be present. The test mixtures were irradiated 24 h/day at pH 7.4–8.4 and 24 ± 1°C for a period of 96 hours. After 96 h, a regrowth test was performed to determine the algicidal or algistatic effect of the test substance. A student's t-test incorporating Bartlett's test for homogeneity of variance was carried out on the data to determine any statistically significant differences between test and control groups. Endpoints and confidence limits were calculated using Xlfit software, and Litchfield and Wilcoxon method.	

RESULTS

Biomass		*Growth*	
E_bL_{50} mg/L at 96 h	*NOEL mg/L*	*E_rL_{50} mg/L at 96 h*	*NOEL mg/L*
15 (95% CI: 14 – 16)	10	25 (95% CI: 23 – 28)	10

Remarks - Results

Cell growth of the control increased 133-fold after 72 h, thus validating the test.

Regrowth was observed to occur in the control and the 10 mg/L loading rate WAF after 72 h, and in the 20 mg/L loading rate WAF test culture after 96 h. No regrowth was observed to occur in the 40, 80 and 160 mg/L loading rate WAF tests cultures after 216 h, indicating that the test substance was algicidal.

The actual concentrations of the test substance were found to range from 0.134 mg/L to 2.31 mg/L at 0 h, and < LOQ to 1.71 mg/L at 96 h. The measured concentrations (greater than the water solubility level) were considered to possibly be due to micro-emulsions of the test substance present, despite microscopic inspections indicating otherwise, and therefore these results should be treated with caution. A decrease in measured concentration over the period of 96 h may be attributed to long term adsorption of the test substance to algal cells.

Given that the toxicity of the test substance cannot be attributed to a single component, the results are based on nominal loading rates only. Hence, the test substance and, by inference, the notified polymer are harmful to algae.

CONCLUSION The notified polymer is harmful to algae.

TEST FACILITY Harlan Laboratories Limited (2009e)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE CP2490 (Neat)

METHOD OECD TG 209 Activated Sludge, Respiration Inhibition Test

Inoculum	Activated sewage sludge from a domestic sewage treatment plant	
Exposure Period	3 hours	
Concentration Range	Nominal:	1000 mg/L
	Actual:	1000 mg/L

Remarks – Method	After a range-finding test was conducted, tests (in triplicate) were conducted by exposing activated sewage sludge to 1000 mg/L dispersion of the test substance and synthetic sewage for a period of 3 h at 21°C. Reference material (3,5–dichlorophenol) at concentrations of 3.2, 10, and 32 mg/L was used in order to confirm the suitability of the inoculum. The test water had a total hardness of 140 mg $CaCO_3$/L.
RESULTS	
IC50	> 1000 mg/L
NOEC	1000 mg/L
Remarks – Results	Variation in respiration rates of the control after 3 h contact time was ±2%, and the 3 h IC50 for the reference material was 8.5 mg/L (95% CI: 6.7–11 mg/L), thus validating the test. Observations made throughout the duration of the test revealed that a dark brown dispersion of test substance coagulated on the flask and surface of the test media. The test substance and, by inference, the notified polymer are not expected to be harmful to microbial respiration.
CONCLUSION	The notified polymer is not harmful to microbial respiration.
TEST FACILITY	Harlan Laboratories Limited (2009f)

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