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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Polymer in Rhodafac PV-27

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

TABLE OF CONTENTS

FULL PUBLIC REPORT

Polymer in Rhodafac PV-27

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Rhodia Chemicals Pty Ltd (ABN: 80 004 449 870)
44 Real Avenue
Norman Park Qld 4170

and

BP Australia Pty Ltd (53 004 085 616)
360 Elizabeth 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; Other names; CAS number; Molecular and structural formula; Molecular weight; Purity; Impurities; Additives/adjuvants; Import volume; Identity of sites; Use details.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows:
All toxicological investigations were performed on analogues of the notified polymer.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Rhodafac PV-27

MOLECULAR WEIGHT
500 - 1000 Da

ANALYTICAL DATA
Reference NMR, IR, HPLC, GPC spectra were provided.

3. COMPOSITION

DEGREE OF PURITY 60-70 %

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Colourless to pale yellow liquid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	< 0 °C	Estimated
Boiling Point	From 47.5°C	Measured
Density	1103 kg/m^3	Measured
Vapour Pressure	Not determined	Not expected to be highly volatile based on molecular weight.
Water Solubility	200-300 g/L	Visual estimate
Hydrolysis as a Function of pH	Stable at 25°C	Measured: Definitive test not required based on the results of the preliminary test
Partition Coefficient (n-octanol/water)	Not measurable	The notified polymer is a surfactant and is expected to partition to phase boundaries rather than bioaccumulate
Adsorption/Desorption	Not determined	Argument: some adsorption to sediment and soil is expected based on the surface activity of the polymer
Dissociation Constant	Not determined	Literature: expected to be fully dissociated in the environmental pH range, with pK_{a1} and pK_{a2} values of approximately 1.5 and 6.58, respectively.
Particle Size	Not determined	Liquid
Flash Point	>100°C (closed cup)	MSDS
Autoignition Temperature	>100°C	Estimated, as the autoignition temperature will be greater than the flash point.
Explosive Properties	Not determined	Not expected to be explosive based on structure.

DISCUSSION OF PROPERTIES
For full details of tests on physical and chemical properties, please refer to Appendix A.

Reactivity
Stable under normal handling and storage conditions.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified polymer (35-50% concentration) will be imported. Alternatively, it may be imported as a finished hydraulic fluid product at concentrations of < 1%.

MAXIMUM INTRODUCTION VOLUME OF NOTIFIED CHEMICAL OVER NEXT 5 YEARS

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

PORT OF ENTRY
Melbourne

IDENTITY OF MANUFACTURER/RECIPIENTS
Offshore oil and gas industry

TRANSPORTATION AND PACKAGING
The notified polymer (35-50% concentration) will be imported in closed head 200 L plastic drums by ship and transported by road to the formulator's warehouse for reformulation. Finished products containing the notified

polymer (<1%) will be transported by road in 200 L plastic drums to customers. Over 50% of the finished product will be exported for use outside Australia.

USE
The notified polymer will be used as an additive in hydraulic control fluids (at concentrations of < 1%) on offshore oil platforms or vessels.

OPERATION DESCRIPTION
Reformulation
The imported product containing the notified polymer (35-50%) will be moved from the storage facility by forklift. It will be transferred to a blending tank via insertion of a dip tube and use of automated pumps. The mixing process will be automated and occur in a closed system under local exhaust ventilation. Quality control sampling will occur after mixing. The resulting product, containing the notified polymer at concentrations of <1%, will be dispensed into 200 L drums using an automated filling machine. The finished product will be transferred to the warehouse for storage until transported to end use sites. Over 50% of the finished product will be exported for use outside Australia.

End user operation in Australia – Oil and gas production
During end use, the finished product containing the notified polymer (<1%) will be transferred from the drums to the off-shore hydraulic system reservoir. This will involve pumping from the drum through a hose to the fill connection of the hydraulic system reservoir. The hydraulic fluid containing the notified polymer will be conducted through the deepwater subsea umbilical system and controlled from a platform or vessel. This process is fully computerised and automated.

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)*
Transportation from dock to formulator's site	3	2-3	10-15
Transportation to end users	6	2-3	10-15
Reformulation workers	5	2	20
End user sites			
Operators	5	2	150
Maintenance workers	2	1	75

EXPOSURE DETAILS
Reformulation
Workers may be exposed to the notified polymer at concentrations of 35-50% via the dermal and ocular routes due to drips, spills and splashes during charging of the mixer and blending. In addition, dermal and ocular exposure to the notified polymer at concentrations of <1% may occur during QC testing and drum filling processes. Such exposures are expected to be low as a result of the enclosed nature of the mixing vessel, the automated nature of many of the processes, the local exhaust ventilation utilised, and the wearing of personal protective equipment including coveralls, goggles and impervious gloves. Inhalation exposure of workers to the notified polymer (concentrations up to 35-50%) may also occur during blending, as aerosols may be released. However, the enclosed mixing and local exhaust ventilation system in place should minimise the potential for this to occur.

End user operations
Dermal and ocular exposure of workers to the notified polymer may occur due to residues, spills, and splashes when opening the containers, transferring to the off-shore hydraulic system reservoir, manually connecting and disconnecting lines and hoses, and handling of hydraulic system parts that may contain small amounts of the notified polymer (for example, during maintenance operations). Such exposure should be lowered by the use of engineering controls expected to be in place at the refineries (such as local exhaust ventilation), containment

procedures (such as catching pans), the personal protective equipment worn by workers (such as safety glasses, gloves and overalls), and the low concentration of the notified polymer in the products.

The MSDS for the end use product containing the notified polymer at concentrations <1% contains a warning regarding high pressure applications. Specifically, it is stated that injections through the skin may occur following contact with the product at high pressure, as the product may be forced considerable distances along tissue planes. Such exposure may occur in certain circumstances involving accidental release of the notified polymer, such as during building and testing of equipment prior to subsea deployment, or the ongoing operation of some equipment on the surface. High pressure exposure during such applications is expected to be lowered as workers will typically be separated and protected from hydraulic equipment during building and testing and when hydraulic pressurisation takes place.

6.1.2. Public exposure

The notified polymer is intended only for use in industry and as such, public exposure is expected to be negligible.

6.2. Human health effects assessment

The results from toxicological investigations conducted on analogues of the notified polymer 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 (1) (Analogue A, 87%)	low oral toxicity LD50 >2000 mg/kg bw
Rat, acute oral toxicity (2) (Analogue B, 60%)	low oral toxicity LD50 >2000 mg/kg bw
Rat, acute oral toxicity (3) (Analogue C, 89%)	low oral toxicity LD50 >2000 mg/kg bw
Rat, acute oral toxicity (4) (Analogue D, 89%)	low oral toxicity LD50 = 3950 mg/kg bw
Rat, acute oral toxicity (5) (Analogue E, 89%)	low oral toxicity LD50 >2000 mg/kg bw
Rat, acute oral toxicity (6) (Analogue E, 89%)	low oral toxicity LD50 = 14300 mg/kg bw
Rabbit, skin irritation (1) (Analogue F, 70%)	corrosive
Rabbit, skin irritation (2) (Analogue C, 89%)	moderately irritating
Rabbit, skin irritation (3) (Analogue E, 89%)	corrosive
Rabbit, skin irritation (4) (Analogue D, 89%)	severely irritating
Rabbit, skin irritation (5) (Analogue E, 89%)	moderately irritating
Rabbit, skin irritation (6) (Analogue E, 89%)	severely irritating
Rabbit, skin irritation (7) (Analogue A, 87%)	severely irritating
Rabbit, skin irritation (8) (Analogue B, 60%)	slightly irritating
Rabbit, eye irritation (1) (Analogue A, 87%)	serious eye damage
Rabbit, eye irritation (2) (Analogue B, 60%)	serious eye damage
Rabbit, eye irritation (3) (Analogue C, 89%)	severely irritating
Rabbit, eye irritation (4) (Analogue G, 87%)	irritating
Rabbit, eye irritation (5) (Analogue E, 89%)	irritating
Guinea pig, skin sensitisation – adjuvant test (1) (Analogue E, 89%)	no evidence of sensitisation
Guinea pig, skin sensitisation – adjuvant test (2) (Analogue C, 89%)	no evidence of sensitisation
Rat, repeat dose oral toxicity – 90 days a) Mix of analogues E, G and I b) Analogue J	a) NOEL 530 mg/kg bw/day (Mix of analogues E, G, I) b) NOEL could not be established for analogue J
Dog, repeat dose oral toxicity – 90 days a) Mix of analogues E, G and I b) Analogue J	a) NOEL 300 mg/kg bw/day b) NOEL could not be established for analogue J
Mutagenicity – bacterial reverse mutation (1) (Analogue C, 89%)	non mutagenic
Mutagenicity – bacterial reverse mutation (2) (Analogue B, 60%)	non mutagenic
Mutagenicity – bacterial reverse mutation (3) (Analogue D, 89%)	non mutagenic
Mutagenicity – bacterial reverse mutation (4) (Analogue E, 89%)	non mutagenic

Genotoxicity – in vivo mammalian erythrocyte micronucleus test (Analogue H, 25%)	non genotoxic

The analogues of the notified polymer used for toxicological testing contained similar functional groups to the notified polymer, though with varying chain lengths. In addition, one functional group of the notified polymer was not reflected in the structures of the analogues.

No information was available on the toxicokinetics of the notified polymer or its analogues. The notified polymer may be absorbed dermally following skin contact due to its surfactant properties.

Acute toxicity
The acute oral toxicity of several analogues of the notified polymer was determined. Each of the analogues was found to be of low acute oral toxicity. By analogy, the notified polymer is also considered to be of low acute oral toxicity.

Acute dermal and inhalation toxicity studies were not performed on the notified polymer or analogues.

Irritation and Sensitisation
The skin irritation potential of a number of analogues of the notified polymer was determined. Irritancy effects of varying degrees were observed in the different studies, including two that were corrosive, one that was possibly corrosive, and two that were severe irritants. As such, it is expected that the notified polymer may cause skin corrosion. Although the effects in one of the studies would attract the risk phrase R35 – causes severe burns, on a weight of evidence basis it is considered that the notified polymer should be classified as R34 – causes burns.

A number of analogues of the notified polymer were tested for eye irritation, with significant irritancy observed in all tests. As such, the notified polymer may cause serious eye damage.

The skin sensitisation potential of two analogues of the notified polymer was tested. Both of the tests displayed no evidence of sensitisation. Based on the available information, the notified polymer is not expected to cause skin sensitisation.

Repeated Dose Toxicity
A 13-week repeated dose oral study in rats using analogues of the notified polymer in the diet at concentrations of 0.5, 1.0 and 2.0% showed no adverse effects in haematopoietic function or clinical parameters. Growth rate was depressed in high dose animals, however absolute and relative increases in organ weight had no supportive histopathological abnormalities. The NOEL for the analogue mixture (mixture of E, G and I) used in the diet was 530 mg/kg bw/day (1%). A 15-week repeat dose oral study in dogs using analogues showed no adverse effects in haematopoietic function or clinical parameters. Absolute and relative increases in liver and kidney weights had no supportive histopathological abnormalities. High dose animals that exhibited a relative increase in adrenal weight showed adrenal cortical hyperplasia. The NOEL for the analogue mixture (mixture of E, G and I) used in the diet was 300 mg/kg bw/day (1%) based on this finding. The results of these studies are considered representative of the likely effects of the notified polymer.

Mutagenicity
Several analogues were non-mutagenic in bacterial reverse mutation assays. One analogue was tested in an in vivo mouse micronucleus test and was found to be non-clastogenic. Therefore, based on the information provided, the notified polymer is likely to be non-genotoxic.

Health hazard classification
Based on analogue data provided, the notified polymer is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004). The following risk phrase applies to the notified polymer:

C; R34 Causes burns

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
Toxicological studies on analogues of the notified polymer indicate that the notified polymer may be corrosive

to skin and cause serious damage to eyes. Lower concentrations such as those used in end-use products (<1%) are below the concentration cut-off for classification as a hazardous substance but may have some irritation potential.

Dermal, ocular and inhalation (aerosol) exposure of workers to the notified polymer at concentrations up to 35-50% may occur during handling of the notified polymer, particularly during charging of the mixer. At such concentrations, workers involved in these operations could potentially be at risk of skin corrosion and serious eye damage. However, the use of engineering controls and personal protective equipment is expected to minimise exposure and reduce the risk of such effects.

Dermal and ocular exposure of workers to the notified polymer at concentrations up to 1% may occur mainly during handling of the hydraulic products containing the notified polymer. Skin and eye irritation effects are not expected at these concentrations.

High pressure application of the notified polymer at concentrations <1% into the skin may occur during building and testing of equipment prior to subsea deployment, or the ongoing operation of some equipment on the surface. The use of safe work practices and separation/protection of workers from equipment during such processes is expected to minimise exposure and reduce the risk of such effects.

In conclusion, the occupational health and safety risk associated with the notified polymer is not considered to be unacceptable when engineering controls and PPE are used.

6.3.2. Public health

As the public are not expected to be exposed to the notified polymer, except in the case of accidents during transport, the risk to public health is considered to be very low.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified polymer will not be manufactured in Australia, hence there are no environmental releases associated with this process. The notified polymer at 35-50% concentration will be imported into Australia and transported from the wharf to formulators. The formulation process is fully automated, with dip tubes and automated pumps used in transferring the notified polymer into closed system blending tanks where dilution with other ingredients occurs. Likewise, the formulated product is dispensed into 200 L drums with an automated filling machine. The notified polymer (n.p.) will be present at < 1% by weight in the formulated product, resulting in a minimum of 272,700 L (=(3000 kg n.p./year ÷ < 0.01) × 0.909 L/kg) hydraulic control fluid per year.

The losses of notified polymer during reformulation are limited to the filling lance (100 L formulated product/year, equivalent to 1.1 kg notified polymer, or < 0.05% annually). This waste, along with import container residues will be reworked into subsequent batches. If the waste becomes soiled, it will be removed from site along with any waste (e.g. spillage) from reformulation according to State and Territory Regulations using licensed contractors for landfill disposal. As the reformulation facility is bunded, release to sewer of the notified polymer is not expected.

Environmental release of the notified polymer is unlikely during importation, storage and transportation. Emergency clean-up procedures (i.e. spill response instructions on the Material Safety Data Sheet) will limit the impact on the environment in the unlikely event of such incidents.

RELEASE OF CHEMICAL FROM USE

Over 50% of the formulated product will be exported. Product remaining in Australia will be primarily used in equipment fill where it will remain confined within the installed hydraulic equipment for the duration of the field life, typically 20 years. Small amounts of waste are expected from breaking hose connections during functional testing, however, this will typically take place on-shore at various hydraulic equipment manufacturers across Australia, which typically are capable of managing and containing spills. The formulated product will be automatically transferred by professional engineers to hydraulic system reservoirs in offshore oil and gas facilities.

The formulated product will also be added to subsea production control systems. In this application total loss of hydraulic fluid to the marine environment through venting is expected from actuation of the subsea Christmas tree valves which are used to control oil and gas production from sub-sea wells. The import volume for domestic use is expected to cover up to 43 subsea wells. Each well will typically use 1000 L of formulated product during a full year of operation which is equivalent to a maximum of 11 kg n.p./well/year (=1000 L × 1.1 kg/L × 0.01). The amount of notified polymer released into the marine environment across all wells is 473 kg (=11 kg × 43), or 16% of the import volume. Leakage is expected to be gradual/periodic rather than bulk discharges across multiple off-shore oil and gas production locations around Australia.

Domestic use is expected to comprise ~1500 kg/y of the notified polymer (50% of import volume of 3000 kg/y is destined for re-export). Therefore, the yearly amount of notified polymer used as equipment fill is 1027 kg (1500 kg – 473 kg) across all sites. This is the expected amount of the notified polymer remaining in equipment at decommissioning that would require disposal or re-use.

RELEASE OF CHEMICAL FROM DISPOSAL

Washings from empty import containers and formulation equipment are re-used in subsequent reformulation batches. Liquid waste from reformulation will be collected and safe disposal managed according to State and Territory Regulations, most likely to landfill. Empty containers of the formulated product will be collected by licensed waste collectors and are likely to be disposed of by incineration.

During decommissioning, hydraulic equipment is recovered from the seabed and a disposal plan will be implemented according to State and Territory regulations for landfill disposal. Alternatively, the equipment may be shipped overseas for re-use.

7.1.2 Environmental fate

Measures for containing and disposing of spillages, washings from the cleaning of formulation equipment and container residues provide a high level of confidence that none of the imported notified polymer will be released to the sewer during the formulation process. Releases are expected to be collected for use in subsequent batches or disposed of to landfill. Similarly, measures for containing and disposing of used hydraulic fluid and product container residues are likely to ensure release to landfill. Approximately 16% of the annual import volume of the notified polymer, integrated over all sites, will be released to the marine environment during normal use.

Fate in the marine environment

The notified polymer has very high water solubility and is expected to dissociate within marine environments, indicating a preference for the water phase. However, some adsorption to sediment and suspended organic material is expected based on the surface activity of the polymer. The high water solubility and relatively high molecular weight of the notified polymer indicate low potential for bioaccumulation, which is further reduced by biodegradation in marine environments.

Fate in landfill

The notified polymer is highly water soluble and therefore potentially mobile through soil. However, some adsorption to soil and metal surfaces is expected based on the notified polymers properties as a surfactant and corrosion inhibitor, respectively. In addition, the notified polymer is likely to be degraded over time through abiotic and biotic processes.

7.1.3 Predicted Environmental Concentration (PEC)

Off-shore release

It is expected that all of the notified polymer that is imported as additive concentrates such as Rhodafac PV-27 will be reformulated, with ~50% used in off-shore hydraulic control fluids in Australian waters. It is expected that ~16% of the total import volume of the notified polymer used in this manner will be released directly to the marine environment annually. The yearly release of hydraulic control fluid is 1000 L (11 kg) per well, but as this release may be infrequent, a worst case scenario of this amount being released in one event will be considered. The concentration of the notified polymer at the point of discharge is expected to be < 11 kg/1000 L = 11 g/L. The formulated product does not fit into one of the four application groups for which PECs in water and sediment in the vicinity of off-shore oil and gas production sites can be determined by the CHARM model (Thatcher *et al.*, 2005). An alternative approach is to determine the volume of water and sediment required to obtain acceptable Q values. This approach is described under Section 7.3.

On-shore release

The notifier has stated that the hydraulic fluid remaining in equipment following decommissioning will be disposed of to landfill or sent overseas for re-use. The frequency of on-shore disposal of used umbilical hydraulic fluids is currently unknown. However, given a typical field life of 20 years or more, on-shore disposal is not expected to be frequent in Australia. It is not considered meaningful to calculate a terrestrial PEC for the notified polymer following on-shore disposal in landfill given that these events are likely to be infrequent and distributed around Australia.

7.2. Environmental effects assessment

The results from ecotoxicological investigations conducted on the additive concentrate, Rhodafac PV-27, with marine species are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity (*S. maximus*)	LC50 13.3 mg/L	Harmful
Invertebrate Toxicity (*A. tonsa*)	LC50 9.2 mg/L	Toxic
Algal Toxicity (*S. costatum*)	E_rC50 6.5 mg/L	Toxic
Corophium volutator toxicity	LC50 151.3 mg/kg (dry wt.)	Unclassifiable

The marine diatom (*Skeletonema costatum*) was the most sensitive organism to an additive concentrate containing 60-70% of the notified polymer. The zooplankton (*Acartia tonsa*) and juvenile turbot (*Scophthalmus maximus*) were less sensitive, but the notified polymer remains toxic or harmful to these species. Based on these results, marine primary producers appear most sensitive to acute toxic effects of the notified polymer. The acute LC50 value of 151.3 mg/kg for the sediment dweller, *C. volutator*, will be used for the sediment compartment.

7.2.1 Predicted No-Effect Concentration

The acute toxicity of the notified polymer towards species of each of the three trophic levels of marine ecosystems was tested. The predicted no-effect concentration for the water compartment of marine ecosystems is calculated by dividing the lowest endpoint (marine diatom) by a safety factor of 100. As only one sediment ecotoxicology study was submitted, the endpoint for this species is divided by a safety factor of 1000.

Water PNEC

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (*Skeletonema costatum*)	6.5	mg/L
Assessment Factor	100	
Mitigation Factor	1.00	
PNEC:	65	µg/L

Sediment PNEC

Predicted No-Effect Concentration (PNEC) for the Sediment Compartment		
LC50 (*Corophium volutator*)	151.3	mg/kg
Assessment Factor	1000	
Mitigation Factor	1.00	
PNEC:	151.3	µg/kg

7.3. Environmental risk assessment

Aquatic risk

The release of 1000 L of the formulated product directly into the marine environment is equivalent to 11 kg of the notified polymer. The volume of water required for the dilution of this amount of the notified polymer to an acceptable level (i.e. PEC < 65 µg/L and PEC/PNEC < 1) can be estimated as 169,230,769 L (=11 × 10^9 µg/65 µg/L), or approximately 170,000 m^3 of seawater. Release is expected from equipment located at the bottom of the sea floor, and if a hemispherical impact volume is assumed (with an affected volume of $[2/3]\pi r^3$), then the use of this notified polymer may have the potential to have an adverse effect on pelagic biota within a radius of 43 m ($r = \sqrt[3]{(170{,}000 \times 3/2\pi)}$) from the release point. However, it is very unlikely that the notified polymer will be concentrated within this volume of water, with dilution in ocean water by currents and diffusion, as well as association with sediment expected to occur. The notified polymer has also shown potential for biodegradation in the marine environment. In addition, this scenario assumes release occurs at one point in time, whereas in reality, release is likely to be infrequently staggered over a year. These arguments indicate that sensitive biota in the vicinity of the discharge point are only likely to be exposed to potentially toxic peak concentrations of the notified polymer for a short period following discharge of the hydraulic control fluid before currents act to disperse the soluble components of the substance to well below potentially toxic levels.

Sediment risk

Based on a release of 1000 L of hydraulic fluid, up to 11 kg of the notified polymer may settle and associate with sediment annually around each well. It is generally assumed that the particle density of soil and sediment is 2.65 g/cm^3. Marine sediment in off-shore regions around Australia have been characterised into gravel, sand and mud on the basis of grain size (Passlow et al., 2005). Depending on the specific location, these regions may be wholly mud, gravel or sand, or combinations thereof. Mud, containing the smallest grains (clays and silts) has the highest porosity and therefore the lowest bulk density (dry weight mass/saturated volume). The porosity range of terrestrial clay soils typically ranges from 50-60%, which is equivalent to a bulk density range of ~1.1-1.3 g/cm^3. Bulk densities in marine environments may be even lower, ranging from 0.5 g/cm^3 for a muddy sediment layer to 1.5 g/cm^3 for a sandy sediment layer (van Hattum *et al.*, 2002). The lower values found in sediment are most likely represented by the effects of organic matter and water movement causing sediment lifting (an increase in water volume not attributable to the sediment porosity). This is consistent with the findings of Flemming and Delafontaine (2000) in Eleftheriou and McIntyre (eds.) who obtained a highly correlated relationship between dry bulk density and water content (based on sediments from a wide range of environments) where the former is lower that would be predicted based on the water content or porosity. Water contents ranging from 20–80% represented bulk density values of 1.7–0.3, respectively. A worst case scenario is represented by the lower bulk density value, as a greater volume is required to reduce the risk quotient (Q) value to an acceptable level.

A sediment bulk density value of 1 g/cm^3 is the default value in the marine antifouling model to predict environmental concentrations (MAM-PEC) (van Hattum *et al.*, 2002), which is used in Australia to predict marine environmental concentrations of biocides arising from antifoulant use. If this value is used, then the mass of sediment required to reduce the Q value to an acceptable level is 72,703,238 kg (11 × 10^9 µg ÷ 151 µg/kg), and the volume would be 72,703 m^3 (72,703,238 kg ÷ 1000 kg/m^3). If the aerobic zone of the sediment is assumed to be 5 cm and the release pattern cylindrical into the sediment (with an affected volume of $\pi r^2 h$), then the use of this notified polymer may have the potential to have an adverse effect on sediment-dwelling organisms within a radius of 680 m ($\sqrt{(72{,}703\ m^3/0.05\pi)}$) from the point of release.

The potential impact radius in the aerobic zone of sediment for the notified polymer is comparable to the 500 m radius from an off-shore drilling location assumed in the CHARM model. However, it is noted that this calculation is based on conservative estimates for both discharge volumes and sediment toxicity. Moreover, although the surface-active nature of the notified polymer indicates some association with sediment is to be expected, its very high water solubility indicates that most will remain solubilised in water, and with a high average molecular weight, is unlikely to bioaccumulate. The notified polymer is also expected to biodegrade in marine environments. In addition, it would be unlikely for the notified polymer to be concentrated within this

volume, with diffusion and current drift expected to move the notified polymer some distance prior to settling on and associating with sediment.

Conclusion
Based on conservative risk analyses for aquatic and sediment organisms, the notified polymer is not expected to have adverse effects on pelagic or benthic biota outside the immediate vicinity of off-shore oil and gas sites following a worst-case discharge of hydraulic control fluid. Some transient toxic effects may occur close to the discharge point, but these are not likely to be significant or persistent. The environmental risks associated with the introduction and intended use of the notified polymer are therefore acceptable.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on analogue data, the notified polymer is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)]. The following risk phrase applies to the notified polymer:

C; R34 Causes burns

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 2003) 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***
Corrosive	1C	Causes severe skin burns and eye damage
Acute hazards to the aquatic environment	2*	Toxic to aquatic life

* No chronic classification has been applied as the notified polymer is not expected to be persistent or bioaccumulative in the marine environment.

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 proposed use pattern, the notified polymer is not expected to pose an unacceptable risk to the environment.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- The Office of the ASCC, Department of Employment and Workplace Relations (DEWR), should consider the following health hazard classification for the notified polymer:
  - C; R34 Causes burns

- Use the following risk phrases for products/mixtures containing the notified polymer:
  - ≥10%: R34
  - ≥ 5%; < 10%: R36/38

- The imported product containing the notified polymer at 35-50% concentration should be classified as Class 8 (Corrosive) Packing Group III under the Australian Dangerous Goods Code (NTC, 2007).

Material Safety Data Sheet

- The MSDS provided by the notifier for the imported product containing the notified polymer at 35-50% concentration should be amended as follows:
  - Disclosure of the full chemical name of the notified polymer.
  - Inclusion of the appropriate risk phrase (C; R34 Causes burns) and classification according to the Australian Dangerous Goods Code (Class 8, Packing Group III).

CONTROL MEASURES

Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified polymer as introduced (35-50% concentration):
  - Local exhaust ventilation during reformulation operations
  - Enclosed mixing vessels for reformulation

- Employers should implement the following isolation or engineering controls to minimise occupational exposure to the notified polymer in end use products (< 1% concentration):
  - Where possible, workers should be isolated from the end products used under high pressure, particularly in situations where release may occur, such as during hydraulic pressurisation of equipment.

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified polymer as introduced (35-50% concentration):
  - Avoid contact with skin and eyes
  - Avoid generation of aerosols
  - Ready access to emergency shower and eye wash facilities

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified polymer in end use products (<1% concentration):
  - Avoid contact with skin and eyes
  - Avoid contacting the skin with the product at high pressure and seek medical attention if such contact occurs

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified polymer as introduced (35-50% concentration) and in end use products (<1% concentration):
  - Impervious gloves and overalls
  - Eye protection e.g. Safety glasses/face mask
  - Respiratory protection if aerosols or mists are generated

  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 chemical 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 chemical should be disposed of to landfill.

Emergency procedures

- Spills or accidental release of the notified polymer should be handled by containment through bunding systems, collection with absorbent material and disposal in accordance with local regulations.

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if
  - the method of disposal of the notified polymer during formulation, refill, or following decommissioning has changed;

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from hydraulic fluid additive for off-shore oil and gas production, or is likely to change significantly;
  - the amount of chemical being introduced has increased from 3 tonnes per annum, or is likely to increase, significantly;
  - the chemical has begun to be manufactured in Australia;
  - additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

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

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

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Boiling Point 47.5°C

Method	ASTM D1078 Determined by visual inspection (rather than by the distillation method) due to the viscosity of the sample.
Remarks	Value is lower than expected and may not be a valid result. However, it is noted that the notified polymer contains impurities of varying boiling points, though none of which are known or expected to be within the range of the measured result.
Test Facility	Probe Intertek (2008a)

Density 1103 kg/m^3

Method	ASTM D4052
Test Facility	Probe Intertek (2008a)

Vapour Pressure Could not be determined

Method	ASTM D5191
Remarks	Determination could not be made due to the viscosity of the sample
Test Facility	Probe Intertek (2008a)

Water Solubility 200 - 300 g/L at 20°C

Method	ASTM D1722 (modified)
Remarks	Determined by visual inspection. A 20% solution in water yielded complete dissolution (clear solution), while a 30% solution in water remained hazy and began to separate. The results indicate that the limit of solubility is between 200–300 g/L.
Test Facility	Probe Intertek (2008a)

Hydrolysis as a Function of pH Stable at 25°C (pH 4-9)

Method	OECD TG 111 Hydrolysis as a Function of pH
Remarks	A preliminary test was conducted at pH 4, 7 and 9.2. The temperature at which this test was conducted was not specified, but is presumed to be 50°C in accordance with the test guideline. As less than 10% hydrolysis was observed after 5 days by ^{1}H NMR, the test substance is considered hydrolytically stable. However, hydrolysis is expected to occur over time, based on the presence of the phosphate ester group.
Test Facility	Probe Intertek (2008b)

Partition Coefficient (n-octanol/water) Not measurable

Method	OECD TG 117 Partition Coefficient (n-octanol/water)
Remarks	The HPLC method was used for the attempted determination of the partition coefficient. The results of the test are not considered reliable due to the poor resolution of the HPLC chromatograph. The notified polymer is a surfactant which will tend to partition to phase boundaries rather than between phases.
Test Facility	Probe Intertek (2008a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral (1)

TEST SUBSTANCE: Analogue A (87%)

METHOD: OECD TG 401 Acute Oral Toxicity – Limit Test.
EC Directive 92/69/EEC B.1 Acute Toxicity (Oral) – Limit Test.

Species/Strain: Rat/Sprague-Dawley
Vehicle: None
Remarks - Method: No significant protocol deviations.

RESULTS

Dose mg/kg bw	*Number and Sex of Animals*	*Mortality*
2000	5 males	0
2000	5 females	0

LD50: >2000 mg/kg bw
Remarks - Results: There were no clinical signs of toxicity, any abnormal effects in organs at necropsy, or adverse bodyweight effects.

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

TEST FACILITY: Centre International de Toxicologie (CIT) (1996a)

B.2. Acute toxicity – oral (2)

TEST SUBSTANCE: Analogue B (60%)

METHOD: OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
EC Directive 92/69/EEC B.1tris Acute Oral Toxicity – Acute Toxic Class Method.

Species/Strain: Rat/Sprague-Dawley
Vehicle: Purified water
Remarks - Method: No significant protocol deviations.

RESULTS

Dose mg/kg bw	*Number and Sex of Animals*	*Mortality*
200	3 males	0
2000	3 males	0
2000	3 males	0

LD50: >2000 mg/kg bw
Signs of Toxicity: No clinical signs were observed in the animals treated with 200 mg/kg.

At the 2000 mg/kg dose-level, hypoactivity was observed in one male 4 hour after treatment, with no signs observed in the other animals.

The body weight gain of females given 2000 mg/kg was reduced during the second week of the observation period compared to historical control animals. The body weight gain of other treated animals was not affected by treatment.

Effects in Organs: None

CONCLUSION	The test substance is of low toxicity via the oral route.
TEST FACILITY	Centre International de Toxicologie (CIT) (2002a)

B.3. Acute toxicity – oral (3)

TEST SUBSTANCE	Analogue C (89%) NOTE: The study report was written in French. The following summary was provided by the notifier.
METHOD	OECD TG 401 Acute Oral Toxicity. EC Directive 92/69/EEC B.1 Acute Toxicity (Oral).
Species/Strain	Rat/ Sprague-Dawley
Vehicle	Distilled water
Remarks - Method	No significant protocol deviations.

RESULTS

Dose mg/kg bw	*Number and Sex of Animals*	*Mortality*
2000	5 males	0
2000	5 females	0

LD50	> 2000 mg/kg bw
Signs of Toxicity	The general behaviour and body weight gain of the animals were not affected by treatment with the test substance.
Effects in Organs	Macroscopic examination revealed no abnormalities in the animals killed at the end of the study.
CONCLUSION	The test substance is of low toxicity via the oral route.
TEST FACILITY	Centre International de Toxicologie (CIT) (1995a)

B.4. Acute toxicity – oral (4)

TEST SUBSTANCE	Analogue D (89%)
METHOD	Similar to OECD TG 401 Acute Oral Toxicity.
Species/Strain	Rat/Wistar Strain Albino
Vehicle	None
Remarks - Method	Only three animals were used at each dose level.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3M; 3F	3140	2 (1M & 1F) 33%
2	3M; 3F	3520	3 (1M & 2F) 50%
3	3M; 3F	3970	3 (1M & 2F) 50%
4	3M; 3F	4450	1 (1M) 17%
5	3M; 3F	5000	5 (2M &3F) 83%

LD50	3950 mg/kg bw
Signs of Toxicity	At the 3520 mg/kg dose level, three animals appeared dehydrated early during the observation period.
Effects in Organs	<u>*Dose level 3140 mg/kg*</u> One male showed fibrous tissue encasing the heart and lungs. In one

female all internal organs were cannibalised except for the stomach and part of the intestine. The stomach was also filled with red fluid.

Dose Level 3520 mg/kg
One male was partially cannibalised and the gastric mucosa was severely reddened. Two females had loose faeces; one of which also had gastric mucosa severely reddened, and the other also had severely reddened gastrointestinal mucosa.

Dose level 3970 mg/kg
One male had moderately reddened pyloric mucosa. Two females had severely reddened gastrointestinal mucosa.

Dose level 4450 mg/kg
No gross changes observed in any of the animals.

Dose level 5000mg/kg
Two males showed severely reddened gastrointestinal mucosa, with one of them also showing blanching of the liver and kidney. Three females showed severely reddened gastrointestinal mucosa and blanching of the liver.

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

TEST FACILITY Consumer Product Testing (1981)

B.5. Acute toxicity – oral (5)

TEST SUBSTANCE Analogue E (89%)

METHOD Similar to OECD TG 401 Acute Oral Toxicity.
Species/Strain Rat/Sprague-Dawley
Vehicle Methocel 0.5% in sterile water
Remarks - Method Only brief details provided.

RESULTS

Dose mg/kg bw	*Number and Sex of Animals*	*Mortality*
500	3M	0
2000	3F	0

LD50 >2000 mg/kg bw

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

TEST FACILITY Research Toxicology Centre (2004)

B.6. Acute toxicity – oral (6)

TEST SUBSTANCE Analogue E (89%)

METHOD Not specified .
Species/Strain Rat/Sherman-Wistar strain
Vehicle None
Remarks - Method Doses were administered directly into stomach by means of syringe and stomach tube.

RESULTS

Dose mg/kg bw	*Number and Sex of Animals*	*Mortality*
7500	5	0/5
10500	5	1/5
15000	5	3/5
21000	5	4/5
30000	5	5/5

LD50 14300 mg/kg bw
Signs of Toxicity Not specified
Effects in Organs Not specified

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

TEST FACILITY Industrial Biology Laboratories, Incorporated (1965)

B.7. Irritation – skin (1)

TEST SUBSTANCE Analogue F (70%)

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).
Species/Strain Rabbit/New Zealand White
Number of Animals 1 male
Vehicle None
Observation Period 6 days
Type of Dressing Semi-occlusive.
Remarks - Method No significant protocol deviations.
The test substance was applied to one flank of the test animal for 3 minutes and to the other flank for 4 hours.

RESULTS

Lesion	*Mean Score* Contact time*		*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	3 min	4 hr			
Erythema/Eschar	3.7	NC	4	> 5 days	4
Oedema	0.7	NC	4	> 48 hr	2

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH flank of the test animal.
NC = not calculated as the 72 hr scoring was masked by crusting.

Remarks - Results

<u>After a 3-minute exposure</u>
A well-defined erythema (grade 2) was noted on day 1, a moderate to severe erythema (grade 3) on day 2, then a severe erythema (grade 4) associated with cutaneous necrosis was recorded from day 3. A slight oedema (grade 2) was also observed on day 2.

<u>After a 4-hour exposure</u>
A well-defined erythema (grade 2) was noted on day 1, then on day 2 a severe erythema (grade 4), severe oedema (grade 4) and crusts were noted. These lesions turned into cutaneous necrosis, and the animal was killed on day 6 for ethical reasons.

CONCLUSION The test substance is corrosive to the skin.

TEST FACILITY Centre International de Toxicologie (CIT) (2002b)

B.8. Irritation – skin (2)

TEST SUBSTANCE	Analogue C (89%) NOTE: The study report was written in French with some summary sections in English.
METHOD	Similar to OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	None
Observation Period	15 days
Type of Dressing	Semi-occlusive.
Remarks – Method	No deviations from standard protocol

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	4.0	4.0	3.3	4	11 days	0
Oedema	2.7	1.3	2.0	4	6 days	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal following 4 hours exposure.

Remarks – Results	<u>*After exposure of 3 minutes and 1 hour*</u> Moderate to severe cutaneous reactions were observed for 6 to 7 days. <u>*After exposure of 4 hours*</u> Moderate to severe cutaneous reaction were observed in all animals for 7 to 8 days.
CONCLUSION	The test substance is severely irritating to skin.
TEST FACILITY	Centre International de Toxicologie (CIT) (1995b)

B.9. Irritation – skin (3)

TEST SUBSTANCE	Analogue E (89%)
METHOD	Similar to OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	6
Vehicle	None
Observation Period	7 days
Type of Dressing	Occlusive
Remarks - Method	Observations were made at 4, 24, 48 hours and 7 days after application. Following the 4 hour observation, any remaining test substance was removed from the test site.

RESULTS

Lesion	*Mean Score**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Erythema/Eschar	2.6	4	> 7 days	4
Oedema	3.4	4	> 7 days	4

*Calculated on the basis of the scores at 24 and 48 hours for ALL animals.

Remarks - Results	Crust, scar or scale formation was noted on all animals at 7 days.
CONCLUSION	The test substance is corrosive to the skin.
TEST FACILITY	Consumer Product Testing (1983a)

B.10. Irritation – skin (4)

TEST SUBSTANCE	Analogue D (89%)
METHOD	Similar to OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	6
Vehicle	None
Observation Period	7 days
Type of Dressing	Occlusive
Remarks - Method	Observations were made at 4, 24, 48 hours and 7 days after application. Following the 4 hour observation, any remaining test substance was removed from the test site.

RESULTS

Lesion	*Mean Score**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Erythema/Eschar	3.9	4	> 7 days	4
Oedema	3.8	4	> 7 days	4

*Calculated on the basis of the scores at 24 and 48 hours for ALL animals.

Remarks - Results	Crust formation was noted on all animals at 7 days. The observation period was not sufficient to determine if the responses were indicative of skin corrosion, however, the study authors considered the test substance to be corrosive to rabbit skin.
CONCLUSION	The test substance is severely irritating to the skin.
TEST FACILITY	Consumer Product Testing (1983b)

B.11. Irritation – skin (5)

TEST SUBSTANCE	Analogue E (89%)
METHOD	Not specified Section 191.11 of the Final Order, Enforcement Regulations, Federal Register, Vol. 26, No. 155, P. 7336, 12th August 1961
Species/Strain	Rabbit/Albino
Number of Animals	6
Vehicle	-
Observation Period	72 hr
Type of Dressing	Not specified
Remarks - Method	Reactions of intact and abraded skin were evaluated at 24 and 72 hours.

RESULTS

Lesion (intact skin only)	*Mean Score**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Erythema/Eschar	2.8	3	>72 hr	2
Oedema	0.9	1	>72 hr	0

*Calculated on the basis of the scores at 24 and 72 hours for ALL animals.

Remarks - Results	The primary irritation index was calculated to be 3.7. The recorded information cannot be used for classification purposes under the *Approved Criteria for Classifying Hazardous Substances*.
CONCLUSION	The test substance is moderately irritating to the skin.
TEST FACILITY	Industrial Biology Laboratories, Incorporated (1966b)

B.12. Irritation – skin (6)

TEST SUBSTANCE	Analogue E (89%)
METHOD	Similar to OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	1
Vehicle	None
Observation Period	14 days
Type of Dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations. The test substance was applied to three separate sections of the dorsal surfaces of the trunk of the animal. Such patches were held in place for three minutes, one hour and four hours and subsequent observations made at 1, 24, 48, 72 hr, 7 and 14 days after dosing.

RESULTS

Lesion	*Mean Score**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Erythema/Eschar	3	4	< 7 days	0
Oedema	0.3	1	< 4 days	0

*Calculated on the basis of the scores at 24, 48 and 72 hours for the test animal following 4 hours exposure.

Remarks - Results

<u>*After exposure of 3 minutes*</u>
No skin reactions were observed until the 24 hour examination. At the 24 and 48 hour observation, very slight erythema were observed, whilst at the 72 hr and 7 day observation this had become severe erythema. Well-defined oedema were observed at the 72 hour and 7 day examinations. Necrosis was also observed at 7 days.

<u>*After exposure of 1 hour*</u>
Severe erythema were present from the 4 hour to the 7 day observation time. This was accompanied by moderate oedema from 24 to 72 hours, which then reduced in severity to slight at the 7 day observation. Necrosis was also observed between 24 hr and 7 days, and scabs and desquamation was also noted at the end of the observation period (14 days).

<u>*After exposure of 4 hours*</u>
Moderate to severe erythema were observed up to 72 hours, with very slight oedema at 72 hours and desquamation at 7 days and 14 days.

CONCLUSION	The test substance is severely irritating to the skin.
TEST FACILITY	Research Toxicology Centre (2004)

B.13. Irritation – skin (7)

TEST SUBSTANCE	Analogue A (87%)

METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	Three
Vehicle	None
Observation Period	Up to 72 hours after removal of the dressing and then daily for 11 days
Type of Dressing	Semi-occlusive.
Remarks – Method	No deviation from standard protocol. A single dose of 0.5 mL of the undiluted test substance was applied for 4 hours. The test substance was applied initially for a period of 3 minutes and 4 hours to one male and for 4 hours to two other males. The pH of the test substance at a concentration of 10g/100 mL was 1.5.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	3.3	3.3	3.0	4	9 days	0
Oedema	3.3	2.0	1.3	4	72 h	0

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

Remarks - Results	<u>*After 3- minute exposure (1 animal)*</u> A very slight to moderate erythema was observed form day 1 up to day 5. It was accompanied by a slight oedema on days 2, 3 and 4. Dryness of the skin was noted on day 4 and it persisted until the end of the observation period (day 15). The dryness of the skin masked the scoring of erythema from day 6 up to day 9. <u>*After 4-hour exposure (3 animals)*</u> Well-defined to severe erythema was noted in 2 animals from day 1 to day 5 or 6. The scoring of erythema was then masked by dryness of the skin until day 9. A slight to severe oedema was also observed in these animals from day 1 to day 3 or 4. In the remaining animal, a well-defined to moderate erythema was noted from day 1 up to day 6. It was accompanied by a slight oedema on days 1, 2 and 3. Dryness of the skin was noted on day 4 in all animals, and it persisted until the end of the observation period (day 15).
CONCLUSION	The test substance is severely irritating to the skin.
TEST FACILITY	Centre International de Toxicologie (CIT) (1996b)

B.14. Irritation – skin (8)

TEST SUBSTANCE	Analogue B (60%)
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	Three
Vehicle	None
Observation Period	Up to 72 hours after removal of the dressing and then daily for 9 days.
Type of Dressing	Semi-occlusive.
Remarks – Method	No deviation from standard protocol. A single dose of 0.5 mL of the undiluted test substance was applied for 4 hours. The test substance was applied initially for a period of 3 minutes and 4 hours to one male and for 4 hours to two other males.

The pH of the test substance at the concentration of 10% in water was 2.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	2.0	1.0	0.3	2	72h	2
Oedema	0.0	0.0	0.0	0	-	0

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

Remarks – Results

After a 3-minute exposure (one animal)
A very slight or well-defined erythema was noted from day 1 (1hour) up to day 4 (72hour). A slight oedema was only observed on day 2. Dryness of the skin was recorded on days 5 and 6 only.

After a 4-hour exposure (3 animals)
A well-defined erythema was noted in all animals on day 1 (1hour) and a very slight or well-defined erythema persisted up to day 2, 4 or 8. A slight oedema was noted in all animals on day 1 only.

CONCLUSION The test substance is slightly irritating to the skin.

TEST FACILITY Centre International de Toxicologie (CIT) (2002c)

B.15. Irritation – eye (1)

TEST SUBSTANCE Analogue A (87%)

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).

Species/Strain Rabbit/New Zealand White
Number of Animals 3
Observation Period Up to 22 days.
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	2.3	2.0	NC	3	< 14 days	0
Conjunctiva: chemosis	3.3	3.0	3.7	4	< 15 days	4
Conjunctiva: discharge	NC	NC	NC	S	< 12 days	0
Corneal opacity, intensity	2.7	2.0	NC	4	> 22 days	4
Corneal opacity, area	2.7	4.0	NC	4	> 22 days	4
Iridial inflammation	1.0	1.0	NC	1	< 14 days	1

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.
NC = not calculated due to effects that obscured scoring
S = Whitish purulent discharge

Remarks - Results

Conjunctival reactions were noted in all animals, including marked chemosis, marked conjunctival redness and whitish purulent discharge. The chemosis and redness were observed from day 1 up to days 14 or 16, and the discharge up to day 11 (at the longest).

Very slight iritis was observed in all animals from day 1 to days 10 or 13.

Marked corneal opacity was also noted in all animals on day 1. It persisted up to day 11 in one animal, and until the end of the observation period (day 22) in another one. In the remaining animal, local haemorrhage occurred on day 5, and the animal was killed for welfare reasons.

Alopecia around the eye and neovasularisation were also observed in 2 animals, with neovasularisation persisting in one animal until the end of the observation period (day 22).

CONCLUSION The test substance causes serious damage to the eyes.

TEST FACILITY Centre International de Toxicologie (CIT) (1996c)

B.16. Irritation – eye (2)

TEST SUBSTANCE Analogue B (60%)

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).

Species/Strain Rabbit/New Zealand White
Number of Animals 1
Observation Period 22 days
Remarks - Method No significant protocol deviations. As the results indicated severe effects on the first animal tested, no other animals were used.

RESULTS

Lesion	*Mean Score* Animal No.*	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1			
Conjunctiva: redness	2.0	2	> 22 days	1
Conjunctiva: chemosis	3.0	3	> 22 days	2
Conjunctiva: discharge	S	2	< 13 days	0
Corneal opacity, intensity	2.0	2	< 16 days	0
Corneal opacity, area	2.0	2	< 16 days	0
Iridial inflammation	1.0	1	< 10 days	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.
S = Whitish purulent discharge, not calculated

Remarks - Results Very slight to moderate conjunctival reactions (very slight to moderate chemosis, very slight or slight redness of the conjunctiva and clear to whitish purulent discharge) were observed in the animal from day 1: some of these reactions persisted up to the end of the observation period (day 22).

A slight iritis was noted on day 2, it persisted up to day 9. A slight corneal opacity was recorded between day 2 and day 15. Alopecia around the eye was noted from day 3 up to day 17 and neovascularisation was recorded from day 18 up to the end of the observation period (day 22).

CONCLUSION The test substance causes serious damage to the eyes.

TEST FACILITY Centre International de Toxicologie (CIT) (2002d)

B.17. Irritation – eye (3)

TEST SUBSTANCE	Analogue C (89%)
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation). NOTE: The study report was written in French with some summary sections in English.
Species/Strain	Rabbit/New Zealand White
Number of Animals	1 male
Observation Period	72 h
Remarks – Method	No significant deviations from standard protocol. As the results obtained on the first animal tested revealed severe effects, no further animals were used.

RESULTS

Lesion	*Mean Score**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Conjunctiva: redness	3.0	3	> 72 hr	3
Conjunctiva: chemosis	2.7	3	> 72 hr	2
Conjunctiva: discharge	NC	-	-	-
Corneal opacity, intensity	2.7	3	> 72 hr	3
Corneal opacity, area	3.3	4	> 72 hr	3
Iridial inflammation	NC	1	-	-

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.
NC = Mean score for iris lesions could not be calculated.

Remarks – Results	Severe ocular reactions were observed throughout the study. The animal was killed on humane ground 72 hours after treatment.
CONCLUSION	The test substance is severely irritating to the eye.
TEST FACILITY	Centre International de Toxicologie (CIT) (1995c)

B.18. Irritation – eye (4)

TEST SUBSTANCE	Analogue G (87%)
METHOD	Procedure of Draize, Woodard and Calvery in the Food Drug Cosmetic Law Journal, Vol 10(10), p 724, 1955
Species/Strain	Rabbit/Albino
Number of Animals	3
Observation Period	7 days
Remarks – Method	Three albino rabbits were used to screen this material at 3 concentrations, one rabbit being used for each concentration (10%, 15% and 20% in water). Based on the results, the study was continued at 20% concentration and tested in three additional. A further study was performed on the concentrated material as supplied using one animal. The scoring scale was not described in the study report.

RESULTS

Remarks – Results	Moderate to severe irritation to the conjunctiva was observed following testing of the substance at 20% concentration in water. This cleared by

7 days.

CONCLUSION The test substance at 20% is irritating to the eye.

TEST FACILITY Industrial Biology Laboratories Incorporated (1961)

B.19. Irritation – eye (5)

TEST SUBSTANCE Analogue E (89%)

METHOD Section 191.12 of the Fianl Order, Enforcement Regulations, Federal Register, Vol. 29, No. 182, p. 13007, 17 Sept 1964

Species/Strain Rabbit/Albino
Number of Animals 6
Observation Period 7 days
Remarks – Method None

RESULTS

Remarks – Results The test material produced corneal opacity and conjunctivitis in all animals which had not cleared by the 7th day of observation. No further detail was provided.

CONCLUSION The test substance is irritating to the eye.

TEST FACILITY Industrial Biology Laboratories Incorporated (1966b)

B.20. Skin sensitisation (1)

TEST SUBSTANCE Analogue E (89%)

METHOD OECD TG 406 Skin Sensitisation - Guinea Pig Maximisation Test.

Species/Strain Guinea pig/Dunkin-Hartley
PRELIMINARY STUDY Maximum Non-irritating Concentration:
intradermal: 0.1%
topical: 10%

MAIN STUDY
Number of Animals Test Group: 5 Control Group: 3
INDUCTION PHASE Induction Concentration:
intradermal: 0.1% in FCA emulsion
topical: 10% in sterile water

CHALLENGE PHASE
1st challenge topical: 1% sterile water
2nd challenge Not performed
Remarks - Method Topical sites were pretreated with sodium lauryl sulphate. It is noted that only 5 and 3 animals were used in the treatment and control groups, respectively. There is no information about the positive control group.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: 1st challenge*	
		24 h	*48 h*
Test Group	1% in sterile water	0/5	0/5
Control Group	Sterile water	0/3	0/3

Remarks - Results

CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the test substance under the conditions of the test.
TEST FACILITY	Research Toxicology Centre (2004)

B.21. Skin sensitisation (2)

TEST SUBSTANCE	Analogue C (89%)
METHOD	OECD TG 406 Skin Sensitisation – Guinea Pig Maximisation Test. EC Directive 96/54/EC B.6 Skin Sensitisation - Guinea Pig Maximisation Test.
Species/Strain	Guinea pig/Hartley Crl: (HA) BR
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: <0.1% topical: 10% Maximum concentration to cause mild-moderate irritation: intradermal: 1% topical: 20%
MAIN STUDY	
Number of Animals	Test Group: 10 Control Group: 5
INDUCTION PHASE	Induction Concentration: intradermal: 1% in FCA topical: 20% in sterile saline
CHALLENGE PHASE	
1st challenge	topical: 10% in sterile saline
2nd challenge	topical: 5% in sterile saline
Remarks - Method	There were no significant protocol deviations.

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	10% for the 1st challenge 5% for the 2nd challenge	9/9	1/9	4/9	1/9
Control Group	Sterile saline	0/5	0/5	0/5	0/5

Remarks – Results

One animal of the treated group was found dead on day 12. No clinical signs were observed prior to death and spontaneous mortality is sometimes observed in this species. It was not attributed to treatment with test substance.

First challenge
No cutaneous reactions were observed in the animals of the control group.
In the treated group, a discrete or moderate erythema (grade 1 or 2) was noted in all animals at the 24-hour. A discrete erythema (grade 1) persisted in only 1/9 animals at the 48-hour.
Dryness of the skin was recorded in 5/9 animals at the 48-hour.

Second Challenge
No cutaneous reactions were observed in the animals of the control group.
In the treated group, a discrete erythema (grade 1) was noted in 4/9 animals at the 24-hour. It persisted in only 1/9 animals at the 48-hour.
Dryness of the skin was recorded in a 3/9 animals at the 48-hour.

The cutaneous reactions observed in the animals of the treated group, which were of

low intensity and mainly non persistent, are most probably attributable to the irritant properties of the test item but not to delayed contact hypersensitivity.

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

TEST FACILITY Centre International de Toxicologie (CIT) (2002e)

B.22. Repeat dose toxicity (1)

TEST SUBSTANCE Test substance 1: Mixture of Analogues E, G, I (mix ratio unknown)
Test substance 2: Analogue J (concentration unknown)

METHOD Internal protocol
Species/Strain FDRL Strain Albino Rats
Route of Administration Oral – diet
Exposure Information Total exposure days: 13 weeks
Dose regimen: Not stated
Post-exposure observation period: None
Vehicle None
Remarks – Method No standard guideline was followed.
The control group received the basal diet only consisted of nutritionally adequate commercial meal. The others received the basal diet mixed with sufficient test material to provide animals with 250, 500 and 1000mg/kg bw/day Test substance 1 or Test substance 2 (1000mg/kg bw/day), respectively. In order to compensate for the changing ratio of food intake to body weight in young rats during the early period of rapid growth, the concentration of the test materials in the diet was adjusted periodically during the first 10 weeks of the experiment. At that time, voluntary food intake of each rat was stabilised at approximately 50g/kg bw/day. The amount of test material in the diet was then equivalent to 0.5, 1.0 and 2.0 % of Test substance 1, and 2% of Test substance 2.
Appearance, behaviour and survival were observed daily. Body weights and food intake were recorded weekly for 12 weeks. During the 13th week, clinical and biochemical parameters were determined in five rats of each sex in each group and rats were then sacrificed.

RESULTS

Group	*Number and Sex of Animals*	*Dose % in diet (mg/kg bw/day)*	*Mortality*
control	22/sex	0	Nil
low dose (substance 1)	22/sex	0.5% (254)	Nil
mid dose (substance 1)	22/sex	1% (530)	Nil
high dose (substance 1)	22/sex	2% (1080)	Nil
high dose (substance 2)	22/sex	2% (1080)	Nil

Mortality and Time to Death
All rats survived the study.

Clinical Observations
There was no difference in behaviour or appearance of the control rats and the treated rats.
At 12 weeks, no significant difference was found in body weight gain of animals of either sex given 0.5, 1.0 or 2.0 % of test substance 1 compared with respective control groups. However, both male and female rats fed the 2.0% test substance 2 gained significantly less weight than the control animals.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
The test substance has no effect on haematology, blood urea nitrogen, and blood glucose values or on the urine

analytical data.

Effects in Organs

The mean organ weight data indicate a dose-related enlargement of the liver in all male animals fed both test substances in terms of the proportion to body weight as well as absolute weight. The male rats fed 1.0 or 2.0% test substance 1 exhibited increased kidney weights both on an absolute basis and as ratios to body weight, whereas the kidney weights of animals that received 2.0% test substance 2 were increased on a relative basis only. The latter finding may reflect lower absolute body weights of the animals in this group as compared to the control group. A similar finding was noted for the testes in this group of animals.

Both the absolute and relative liver weight in the female rats given 2% test substance 1 was increased compared to control females. The increase in relative liver weight found in females given 2% test substance 2 was related to the decrease in absolute body weight compared to that of the comparable controls.

The mean absolute weight of the adrenals was significantly less for the male animals fed the 1% test substance 1 or the 2.0% test substance 2 whereas as a ratio to body weight, only the former group exhibited a significant decrease in adrenal weight compared to control animals.

The absolute adrenal weights of the females that received 1.0 or 2.0% test substance 1 were increased but the ratio of adrenal to body weights was not statistically significant ($p>0.05$). A significant decrease in relative ovarian weights was found in female rats that received 1.0% test substance 1. This response was not dose related. No other significant variations in organ weights were found in these animals.

Gross examination of all rats at necropsy revealed only hydropelvis as a significant finding. Although hydropelvis was not found on gross examination in the control rats in this study, it is common in Wistar-derived strains. It is therefore not considered to be related to treatment with either of the test substances.

Microscopic examinations revealed the usual scatter of changes commonly seen in laboratory rats. Hydropelvis and interstitial nephritis were found in both control and treated animals. The significance of the gross findings of hydropelvis in treated animals only mitigated by microscopic confirmation of hydropelvis in control studies.

No significant changes in histomorphologic structures were found in livers or adrenals which could be correlated with the changes in the weights of these glands. It is possible that the increased liver weights may be the result of increased metabolic activity in this organ. Measurements of the width of the zona glomerulosa revealed no significant differences between the adrenals of the control group and the 2.0% test substance 1 or the test substance 2 treatment groups.

Remarks – Results

Although several organs of the rats fed the test substances were increased in terms of either absolute weight or ratio of body weights compared to those of the controls, the significance of these findings is mitigated by the absence of any histopathologic changes that could be correlated with such variations.

CONCLUSION

It was concluded that 1% of test substance 1 in the diet (530 mg/kg bw/day) was the NOEL in rats, based on some depression in body weight, and increases in organ weights seen at the higher dose level. A NOEL could not be determined for test substance 2 as growth of test animals was significantly retarded compared to control animals at the dose tested.

TEST FACILITY Food and Drug Research Laboratories (1967a)

B.23. Repeat dose toxicity (2)

TEST SUBSTANCE	Test substance 1: Mixture of Analogues E, G, I (mix ratio unknown) Test substance 2: Analogue J (concentration unknown)
METHOD	Internal protocol
Species/Strain	Dogs/beagles
Route of Administration	Oral – diet

Exposure Information	Total exposure days: 15 weeks Dose regimen: 6 days per week and 1 h daily Post-exposure observation period: None
Vehicle	None
Remarks – Method	No standard guideline was followed. The control group received the basal diet consisting of a nutritionally adequate commercial dog meal with no additive. The test group received the same diet into which 0.5, 1.0, and 2.0% of the test substance was thoroughly mixed. Appearance, behaviour and survival were observed daily. Body weights and food intake were recorded weekly. The clinical examinations were repeated after the 12th week. After 15th week, the dogs were sacrificed.

RESULTS

Group	*Number and Sex of Animals*	*Dose % in diet (mg/kg bw/day)*	*Mortality*
control	4/sex	0.0	Nil
low dose (substance 1)	3/sex	0.5 (150)	Nil
mid dose (substance 1)	3/sex	1.0 (300)	Nil
high dose (substance 1)	4/sex	2.0 (600)	Nil
high dose (substance 2)	4/sex	2.0 (600)	Nil

Mortality and Time to Death

All dogs survived the study and were sacrificed during the 15th week.

Clinical Observations

There were no significant differences in behaviour or appearance of the control dogs and the treated dogs. The only significant losses in body weight which appear to be significant occurred in the group fed 2.0% of the test substances. They paralleled reductions in voluntary food intake and were most consistent in the mixture group.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Heamoglobin, hematocirt, total and differential leukocyte counts of the controls and test animals were comparable and within normal limits. Similarly, the blood urea nitrogen and blood glucose values as well as the urine analytical data failed to suggest any effect related to dosage of the test substances.

Effects in Organs

The ratio of liver weight to body weight was significantly increased in male dogs fed 2% test substance 2 and in female dogs fed 2% of test substance 1 and 2. This increased was attributed to the lower body weights in these animals compared to control animals.

The absolute kidney weight of female dogs given test substance 2 was less than that of control females. On a relative basis, however, the kidney weights were elevated in the females that were fed 0.5 and 1.0% of the mixture and in the male dogs that received 2% of either test substance. As with the liver the increased ratios of kidneys to body weight of these animals as compared to those of the respective control animals may be a reflection of poorer growth.

Ovarian weight was elevated on an absolute basis in the females fed 1% of test substance 1, and on a relative basis in animals given 0.5, 1.0 or 2%. No significant increases were found in adrenal gland weights on an absolute basis. However, male and female dogs fed 0.5% of test substance 1 or 2.0% of test substance 2 exhibited relative increases in adrenal weight compared to control dogs. Male dogs given 2% of the test substance 2 demonstrated a relative increase in thyroid weight while female dogs fed 2% test substance 2 exhibited an absolute decrease in brain weight.

At necropsy, no significant changes were found in these animals following gross examination. Chronic inflammatory cell accumulation in the submucosa of the gall bladder was found with greater frequency in dogs given 0.5, 1.0 and 2.0% test substance 1 than in control dogs or dogs given 2% test substance 2.

Adrenal cortical hyperplasia was found in 2, 4 and 4 animals of the 1% test substance 1, 2.0% test substance 1 and 2.0% test substance 2 groups, respectively.

Remarks – Results

Losses in body weight paralleled reductions in voluntary food intake and may have involved a palatability factor.

Although some changes in organ weights were noted to be statistically significant in comparison with the controls, the magnitude of the variations was small and scattered among the dosage levels to the extent that no consistent dose-related effects were apparent. Furthermore, changes in organ weights in the animals with respect to control animals appears to reflect the decreases in absolute body weight of the various groups since no significant gross nor histological findings were seen to account for the variations reports.
Chronic gall bladder inflammation was noted in all the groups which were found to be of a grade and type common to normal untreated dogs of this species.

CONCLUSION
It was concluded that 1% of test substance 1 in the diet (300 mg/kg bw/day) was the NOEL in rats, based on some depression in body weight, and increases in organ weights at the higher dose level. A NOEL could not be determined for test substance 2 as effects were observed at the only dose tested (2%, 600 mg/kg bw/day).

TEST FACILITY Food and Drug Research Laboratories (1967b)

B.24. Genotoxicity – bacteria (summary of genotoxicity studies provided)

Test Substance	**(1) Analogue C** (89%)	**(2) Analogue B** (60%)	**(3) Analogue D** (89%)	**(4) Analogue E** (89%)
Method	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.			Not stated
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, TA102.	*S. typhimurium*: TA1535, TA1537, TA98, TA100, TA102	*S. typhimurium*: TA1535, TA1537, TA98, TA100, TA102	*S. typhimurium*: TA98, TA100
Metabolic Activation System	Aroclor induced rat liver S9 is used as the metabolic activation system.			Phenobarbital and betanaphthoflavone induced rat liver S9
Concentration Range in Main Test	a) With metabolic activation: 78.125-5000 µg/plate. b) Without metabolic activation: 31.25-5000µg/plate.	a) With metabolic activation: 312.5 - 5000 µg/plate. b) Without metabolic activation: 312.5 -5000 µg/plate.	a) With metabolic activation:0-5000 µg/plate. b) Without metabolic activation: 0-1000 µg/plate.	a) With metabolic activation: 61.7 - 5000 µg/plate. b) Without metabolic activation: 61.7 - 5000 µg/plate.
Vehicle	Dimethylsulfoxide (DMSO)	Distilled water		DMSO
Method	Plate incorporation (for majority of tests)	Plate incorporation	Plate incorporation (for majority of tests)	Plate incorporation
Remarks – Method	No deviation from standard protocol			Based on those described in the OECD guidelines
Remarks – Results	Nil	Nil	Nil	Nil
Conclusion	The test substances were not mutagenic to bacteria under the conditions of the test.			
Test Facility	Centre International de Toxicologie (CIT) (2002f)	Centre International deToxicologie (CIT) (2001a)	Centre International deToxicologie (CIT) (2001b)	Research Toxicology Centre (RTC) (2004)

B.25. Genotoxicity – in vivo

TEST SUBSTANCE	Analogue H (25% aqueous emulsion)
METHOD	OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
Species/Strain	mouse/Crl:CD-1®(ICR)BR
Route of Administration	Oral – gavage.
Vehicle	Water.

Remarks - Method 6 mice received 0, 500, 1 000 or 2 000 mg/kg notified polymer in distilled water by gavage; bone marrow was sampled 24 and 48 hours after dosing

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
1	6 males	0	24, 48
2	"	500	24, 48
3	"	1000	24, 48
4	"	2000	24, 48

RESULTS

Remarks - Results The notified polymer did not induce an increase in the frequency of micronucleated polychromatic erythrocytes; cyclophosphamide, the positive control, demonstrated the sensitivity of the test; there was no statistically significant decrease in the PCE:NCE ratio, demonstrating that the test article was not cytotoxic to the bone marrow.

CONCLUSION The notified polymer was not clastogenic under the conditions of this test.

TEST FACILITY Covance Laboratories (1998).

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Biodegradability

TEST SUBSTANCE	Rhodafac PV-27 (containing up to 70% notified polymer)
METHOD	OECD TG 306 Biodegradability in Seawater: Closed Bottle Test
Inoculum	None added
Exposure Period	28 days
Auxiliary Solvent	None reported
Analytical Monitoring	Not reported
Remarks - Method	The seawater was collected from a depth of ~80 m at Byfjord and aged under aerobic conditions at 20°C for at least 1 week. Particles in the seawater were separated by a settling and decanting method. The test was also performed at 20°C with 2.55 mg test substance/L. Dissolved oxygen in the water of the test flasks was determined electrochemically. A nitrogen inhibitor (allylthiourea) was added to the test system to prevent oxygen consumption by nitrification. Tests were also conducted on the reference substance sodium benzoate.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	37	7	81
14	43	14	86
21	50	21	87
28	61	28	91

Remarks - Results

The salinity of the seawater was 34‰ and the dissolved organic carbon (DOC) was low. The biochemical oxygen demand (BOD) was calculated as a percentage of the chemical oxygen demand (COD) and the theoretical oxygen demand (ThOD) for the test and reference substances, respectively.

The COD of the test substance was 1.761 mg COD/mg sample with a day 28 specific oxygen demand of 1.08 mg BOD/mg sample. The results indicate a potential for biodegradation in the marine environment. Oxidation of the reference substance was 82% in a test with both the test and reference substances, indicating that the test substance does not inhibit bacterial growth at the concentration tested.

CONCLUSION The results indicate a potential for biodegradation in the marine environment

TEST FACILITY RF-Miljølab (2000)

C.1.2. Bioaccumulation

Remarks - Results

The notified polymer is not expected to bioaccumulate because it is an anionic surfactant with very high water solubility and a relatively high average molecular weight. The potential for this polymer to bioaccumulate will be further reduced by biodegradation in marine environments.

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Rhodafac PV-27 (containing up to 70% notified polymer)
METHOD	PARCOM Method 1995 OECD TG 203 Fish, Acute Toxicity Test – semi static. STL Runcorn SOP III.6.
Species	Juvenile turbot (*Scophthalmus maximus*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	Not reported
Remarks – Method	The fish were fed and acclimatised to the test conditions by being held in artificial seawater at 15±2°C. All fish at each test concentration were held in single 10 L tanks. The artificial seawater was maintained at pH values ranging from 7.87-8.10, a temperature of 16°C, 92.7-99.5% dissolved oxygen concentration, and 32.3-33.4‰ salinity. The test media were replaced at 48 hours.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality (%)*				
Nominal	*Actual*		*0 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	n.d.	7	0	0	0	0	0
5.6	n.d.	7	0	0	0	0	0
10.0	n.d.	7	0	0	0	0	0
17.8	n.d.	7	0	100	100	100	100
31.6	n.d.	7	0	100	100	100	100
56.2	n.d.	7	0	100	100	100	100

n.d. = not determined

LC50	13.3 mg/L at 96 hours
NOEC	10.0 mg/L at 96 hours
Remarks – Results	No analysis of test solutions was reported, however, based on the reported water solubility, the actual concentration of Rhodafac PV-27 is expected to be close to the nominal concentration. No non-lethal toxic effects were reported. The mortality data were analysed with the statistical programme Toxcalc and the calculated end-points are not adjusted to the nominal concentration of the notified polymer in Rhodafac PV-27. There was clear evidence of a rapid increase in acute toxicity to turbot at nominal concentrations greater than the NOEC under the conditions of this test.
CONCLUSION	The notified polymer is harmful to marine fish
TEST FACILITY	STL (2004a)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Rhodafac PV-27
METHOD	ISO 14669:1999(E) STL Runcorn SOP III.14
Species	*Acartia tonsa*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	Not reported

Analytical Monitoring	None reported
Remarks - Method	Water Accommodated Fractions (WAFs) were prepared by adding appropriate amounts of Rhodafac PV-27 to 25 µm filtered seawater, which was then spun overnight and allowed to settle for 4 hours. The test organisms were exposed to solutions prepared from the clear central portion. Initially, the seawater was ~15°C, had a pH value of 8.11, salinity of 31.0 g/L, and dissolved oxygen level of 92.7%. Organisms were also exposed to seawater as the control and to 3,5-dichlorophenol as the toxic reference. The number of replicates was not reported.

RESULTS

Concentration mg/L Nominal	*Number of A. tonsa*	*Number Immobilised (%)*	
		24 h	*48 h*
0	Not reported	7	3
3.56	Not reported	0	30
6.32	Not reported	10	35
11.25	Not reported	15	50
20.0	Not reported	25	70
35.6	Not reported	100	100
63.2	Not reported	100	100
3,5-dichlorophenol	Not reported	0	80

LC50	9.2 mg/L at 48 hours (95% C.I.: 6.4-12.3 mg/L)
LOEC	3.56 mg/L at 48 hours
Remarks - Results	No analysis of test solutions was reported. However, based on the reported solubility, the actual concentration of the notified polymer is expected to be close to the nominal concentration despite the unexplained use of WAFs in this test. The mortality data were analysed with Toxcalc based on the nominal concentration of Rhodafac PV-27. There was clear evidence of a dose response in this study. The study report does not explain why the immobilisation in the control decreased from 24-48 h.

CONCLUSION The notified polymer is toxic to marine zooplankton

TEST FACILITY STL (2004b)

C.2.3. Toxicity to sediment swelling organisms

TEST SUBSTANCE Rhodafac PV-27

METHOD PARCOM 1995
STL Runcorn SOP III.33

Species	*Corophium volutator*
Exposure Period	10 days
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	None reported
Remarks - Method	The study was carried out as a screening level test. The notified polymer (as Rhodafac PV-27) was added to sieved marine sediment from the same collection site as the amphipods and mixed thoroughly with 100 mL of artificial seawater with 25-35‰ salinity. Two replicate vessels for each treatment and 5 for the control were prepared and test organisms added at a density of 20 per vessel. The vessels were aerated for 12 hours prior to and during the test period. The test was carried out under static conditions with continuous aeration.

RESULTS

Concentration mg/kg	*Number of C. volutator*	*Number of dead C. volutator*	*Mortality*
Nominal	*0 d*	*10 d*	*(%)*
0	100	4	4
10	40	12	30
100	40	17	43
1000	40	29	73

LC50	151.3 mg/kg sediment (95% C.I.: 51.3-531.4 mg/L) at 10 days
LOEC	10 mg/kg sediment at 10 days
Remarks - Results	No analysis of test solutions was reported. Results are expressed as dry weight of sediment based on nominal applied loading of Rhodafac PV-27. Organisms which became opaque and were lying on the sediment surface, or exhibited no movement or response to stimuli at the end of the study were recorded as mortalities. The mortality data were analysed with Toxcalc and there was clear evidence of a dose response.
CONCLUSION	The notified polymer has some toxicity to the sand dwelling marine amphipod, *Corophium volutator,* at high nominal loadings, but the toxicity cannot be classified.
TEST FACILITY	STL (2007a)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE	Rhodafac PV-27
METHOD	EN ISO 10253 (1998) STL Runcorn III.19.
Species	*Skeletonema costatum*
Exposure Period	72 hours
Concentration Range	Nominal: 1.0, 1.78, 3.16, 5.62 and 10 mg/L
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	Not reported
Remarks - Method	Water Accommodated Fractions (WAFs) were prepared by adding appropriate amounts of Rhodafac PV-27 to Guillard's f/2 + Si test media, followed by spinning and settling. The test organisms were exposed to solutions prepared from the clear central portion. Six replicate control flasks and 3 replicate flasks at each test concentration including the reference material (3,5-dichlorophenol, 1.5 mg/L) were prepared, giving initial algal cell densities of 11,280 cells/mL. These were incubated at 20±2°C under continuous white light. Although no lux readings were taken due to an error, the healthy control growth indicates that the light conditions were acceptable. The test was carried out under static conditions.

RESULTS

Biomass		*Growth*	
E_bC50	NOEC	E_rC50	NOEC
mg/L at 72 h	mg/L	mg/L at 72 h	mg/L
Not calculated	Not calculated	6.5 (95% C.I.: 4.0-10.7	1.0

Remarks - Results	No analysis of test solutions was reported, however, the actual concentration of the notified polymer is expected to be close to the nominal concentration, at least initially. However, as the notified polymer is a surfactant some adsorption on algal growth mass would be expected. No unusual cell growth or deformities were observed microscopically.

	Slow initial cell growth in the controls rather than a direct toxic effect was considered responsible for the negative inhibition and 100% inhibition values observed at 24 hours. The cell density in the control vessels increased overall by a factor of 24.8 times over the 72-hour period of the test. A clear dose response was observed at 48 and 72 h, with the exception of the 5.62 mg/L treatment group, where inhibition was minimal.
CONCLUSION	The notified polymer is toxic to marine algae
TEST FACILITY	STL (2004c)

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