

**NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT  
SCHEME**

**FULL PUBLIC REPORT**

**Chemical in OLOA 270**

Under subsection 38(5) of the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act), the Director of Chemicals Notification and Assessment publishes this assessment report by giving a copy of it to the:

- Chief Executive Officer of the National Occupational Health and Safety Commission;
- Secretary of the Department of Environment and Heritage; and
- Secretary of the Department of Health and Aged Care.

This assessment report will not be available for inspection by the public.

Director  
Chemicals Notification and Assessment

29 March 2001

## TABLE OF CONTENTS

FULL PUBLIC REPORT.....	3
1. APPLICANT.....	3
2. IDENTITY OF THE CHEMICAL.....	3
3. PHYSICAL AND CHEMICAL PROPERTIES.....	3
3.1 Comments on Physico-Chemical Properties.....	4
4. PURITY OF THE CHEMICAL.....	5
5. USE, VOLUME AND FORMULATION.....	7
6. OCCUPATIONAL EXPOSURE.....	7
7. PUBLIC EXPOSURE.....	10
8. ENVIRONMENTAL EXPOSURE.....	10
8.1 Release.....	10
8.2 Fate.....	11
9. EVALUATION OF TOXICOLOGICAL DATA.....	12
9.1 Acute Toxicity.....	12
9.2 Repeated Dose Toxicity of the Analogue Chemical.....	23
9.3 Genotoxicity of the Analogue Chemical.....	28
9.4 Skin Absorption of the Analogue Chemical.....	30
9.5 Overall Assessment of Toxicological Data.....	31
10. ASSESSMENT OF ENVIRONMENTAL EFFECTS.....	32
11. ASSESSMENT OF ENVIRONMENTAL HAZARD.....	33
12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS.....	34
Assessment of Toxicological Hazard.....	34
Occupational Health and Safety.....	34
Public Health.....	36
13. RECOMMENDATIONS.....	36
14. MATERIAL SAFETY DATA SHEET.....	37
15. REQUIREMENTS FOR SECONDARY NOTIFICATION.....	38
16. REFERENCES.....	38

## FULL PUBLIC REPORT

### Chemical in OLOA 270

#### 1. APPLICANT

Chevron Chemical Australia of Level 22, 385 Bourke Street, Melbourne, Victoria 3000 (ABN 001 010 037) has submitted a standard notification statement in support of their application for an assessment certificate for 'Chemical in OLOA 270'.

#### 2. IDENTITY OF THE CHEMICAL

**Marketing Name:** OLOA 270

The chemical name, CAS number, molecular and structural formulae, molecular weight and spectral data have been exempted from publication in the Full Public Report and the Summary Report.

'Chemical in OLOA270' is referred to as 'OLOA 270' throughout the report.

#### 3. PHYSICAL AND CHEMICAL PROPERTIES

All tests were conducted using a structural analogue of the notified chemical. The analogue was previously assessed by NICNAS as NA/253.

<b>Appearance at 20°C &amp; 101.3 kPa:</b>	Dark brown viscous liquid
<b>Boiling Point:</b>	Decomposes before boiling
<b>Specific Gravity:</b>	1.093 g/mL at 15°C
<b>Vapour Pressure:</b>	$0.49 \times 10^{-4}$ KPa at 25°C - see comments
<b>Water Solubility:</b>	83 ppm - see comments
<b>Partition Co-efficient (n-octanol/water):</b>	Expected to be > 8 - see comments
<b>Hydrolysis as a Function of pH:</b>	Does not contain hydrolysable functional groups
<b>Adsorption/Desorption:</b>	Expected to strongly adsorb to soil - see comments

<b>Dissociation Constant:</b>	Some dissociation is expected in the pH range 4 to 9 - see comments
<b>Particle Size:</b>	Not applicable, chemical exists as a liquid
<b>Flash Point:</b>	> 200°C
<b>Flammability Limits:</b>	Will burn in the presence of sufficient heat and oxygen
<b>Autoignition Temperature:</b>	Not expected to auto-ignite
<b>Explosive Properties:</b>	Not known to be explosive
<b>Reactivity/Stability:</b>	Will react in the presence of strong oxidising agents. Stable to acid and base.

### 3.1 Comments on Physico-Chemical Properties

The vapour pressure is that of the refined lube oil in which the notified chemical is dissolved.

In Rausina et al (1996), the water solubility of the notified chemical is stated to be 83 ppm using Semipermeable Membrane Devices (SPMDs). This value is based on the water solubility determined for oil additive detergents that are stated to be similar in structure. The stated value is consistent with the notified chemical containing long hydrophobic alkyl chains. The calcium salt of the notified chemical would be expected to be insoluble in water (as illustrated by soap binding with calcium in hard water as soap scum). The water solubility for the analogue to the notified chemical was determined, via the shake flask method, to be 40.9 mg/L (40.9 ppm) (Robson, 1993).

Measurement of the n-octanol/water partition coefficient of the analogue chemical was attempted using an HPLC method which was briefly reported. Only 1.9% of the compound could be dissolved in acetonitrile and this had a log  $P_{OW}$  of 7.1. The insoluble material can be assumed to have a log  $P_{OW}$  > 8. Based on this information, the notified chemical is expected to have a log  $P_{OW}$  > 8. The n-octanol/water partition coefficient for the notified chemical was determined by TLC to be greater than 6 (Robson, 1993).

No adsorption/desorption data were provided. However, the high log  $P_{OW}$ , high hydrocarbon content and strong dispersant nature of the notified chemical indicate that the material would have a large  $K_{OC}$  and adsorb strongly to the organic component of soils and sediments. The following results were provided for the analogue chemical (Heim, 1995).

SOIL MATRIX	% OC	$K_{oc}$ Adsorption	$K_{oc}$ Desorption
Loam 161	3.78	$3.14 \times 10^4$	$4.03 \times 10^4$
Silt loam 165	1.74	$6.26 \times 10^4$	$1.84 \times 10^5$
Sandy Loam G595	0.93	$1.72 \times 10^5$	$1.10 \times 10^5$

As these results are consistently around 5000 or greater it is assumed that the notified chemical would be immobile in soil.

No dissociation data were provided. However, the notified chemical is a substituted phenol, which are weakly acidic (Morrison and Boyd, 1976). Therefore it is possible that some dissociation will occur in the environmental pH range of 4 to 9.

#### 4. PURITY OF THE CHEMICAL

**Degree of Purity:** 70-80% in lubricating oil solvent  
**Comment :** The lubricating oil is necessary to reduce the viscosity of the product to a level that enables the product to be pumped and stored.

#### Hazardous Impurities:

*Chemical name:* Calcium hydroxide  
*CAS No.:* 1305-62-0  
*Weight percentage:* 0.5% max  
*Toxic properties:* Chemical is a severe eye irritant and a skin and respiratory irritant, causing dermatitis. It is mildly toxic by ingestion (Sax and Lewis, 1996). The American Conference of Governmental of Industrial Hygienists (ACGIH) has allocated a Time Weighted Average (TWA) exposure standard of 5 mg/m<sup>3</sup> (Sax and Lewis, 1996).

*Chemical name:* Ethylene glycol  
*CAS No.:* 107-21-1  
*Weight percentage:* 0.1% max  
*Toxic properties:* Chemical is harmful if swallowed, when contained in mixtures at >25% (NOHSC, 1999).

*Chemical name:* Phenol, (tetrapropenyl) derivatives  
*CAS No.:* 74499-35-7  
*Weight percentage:* 10% max  
*Toxic properties:* Chemical is a skin and eye irritant (Biosearch Inc, EPA Doc 86-980000160, 1998; Biosearch Inc. EPA Doc 86-980000159, 1998). Toxicity also assumed by analogy with phenol.

*Chemical name:* Phenol, C<sub>20-30</sub>-alkyl derivatives  
*CAS No.:* Not assigned  
*EPA Number:* P 96-952, -953, -954, -955, -956, -957  
*Weight percentage:* 4.5% max  
*Toxic properties:* Toxicity assumed by analogy with phenol.

*Chemical name:* Phenol, (tetrapropenyl) derivatives, manufactured of distillation residues  
*CAS No.:* 220794-73-0  
*Weight percentage:* 1% max  
*Toxic properties:* Toxicity also assumed by analogy with phenol.

**Non-hazardous Impurities  
(> 1% by weight):**

*Chemical name:* Phenol, (tetrapropenyl) derivatives, calcium salts  
*Weight percentage:* 5% max  
*CAS No.:* Not assigned

*Chemical name:* Phenol, C<sub>20-30</sub>-akyl derivatives, calcium salts  
*Weight percentage:* 5% max  
*CAS No.:* Not assigned

*Chemical name:* Sulfurized 2-hydroxybenzoic acid, C<sub>20-30</sub>-akyl derivatives, tetrapropenyl derivatives and (tetrapropenyl) derivatives, reaction products with distillation residues from manufacture of, derivatives  
*Weight percentage:* < 2%  
*CAS No.:* Not assigned

**Additives/Adjuvants:**

*Chemical name:* Lubricating oil solvent : petroleum distillates, hydrotreated heavy paraffinic, DMSO content <2.5%.  
*CAS No.:* 64742-54-7  
*Weight percentage:* Up to 30 %  
*Toxic properties:* Classification as a carcinogen applies when the chemical is present in mixtures at greater than 0.1 % and the mixture contains > 3% DMSO.

<i>Chemical name:</i>	Lubricating oil solvent : petroleum distillates, solvent refined heavy paraffinic, DMSO content <2.5%.
<i>CAS No.:</i>	64741-88-4
<i>Weight percentage:</i>	Up to 30 %
<i>Toxic properties:</i>	Classification as a carcinogen applies when the chemical is present in mixtures at greater than 0.1 % and the mixture contains > 3% DMSO.

## **5. USE, VOLUME AND FORMULATION**

The notified chemical will not be manufactured in Australia.

Up to 210 tons will be imported in the first year either as a 70-80% solution in a highly refined lubricating oil solvent (termed a “component”), or at 10-80% as part of an additive mixture (termed a “package”) with other lubricating oil components. Up to three different packages will be imported. It is anticipated that importation will increase by 2-3% in subsequent years. Both the component and the packages will be blended into finished oils in Australia.

The primary use is as one of the ingredients in lubricants used by the marine diesel engine lubricant market where the final concentration of the notified chemical will be 0.75-12% (1-15% component). The function of the notified chemical in the lubricant is to reduce piston and crankcase deposits and to control oxidation of the lubricant.

A small amount (< 0.5%) may also be used for high performance hydraulic oils for closed hydraulic systems in large construction equipment such as earth movers, road graders and power scoop shovels. Typically the hydraulic oils contain 0.1-0.2% OLOA 270 component.

## **6. OCCUPATIONAL EXPOSURE**

The notified chemical, as a component or as part of a package, is imported into two marine terminals in Australia by bulk shipment, in marine isotanks or in drums. Bulk shipments are unloaded into tankers. The tankers, isotanks and drums are transported to blending plants where the components or packages are blended into finished lubricant oils for onward transport to end users.

The table identifies the nature of work done where occupational exposure to the notified chemical in a component, package or finished oil may occur at marine terminals or blending plants.

<i>Nature of Activity &amp; (Number of Workers)</i>	<i>% OLOA 270 as manufactured in Lube oil (ie. as a component)</i>	<i>Maximum Potential Exposure Duration</i>
<u>Marine Terminal</u>		
Unloading (1 or 2)	10 – 100	30 min/day; 2 days/year.
Sampling (1 or 2)	10 – 100	30 min/day; 2 days/year.
Analysis (1 or 2)	10 – 100	30 min/day; 2 days/year.
Loading tanker trucks (1 or 2)	10 – 100	30 min/day; 4 days/year.
Equipment Cleaning (1 or 2)	<1	4 hours/day; 1 day/year.
<u>Blending Facility</u>		
Unloading to Storage Tanks (1 or 2)	10-100	1 hour/day; 4 days/year.
Delivery vessel cleaning (1 or 2)	<1	2-3 hours/day; 4 days/year.
Sampling (2 or 4)	10-100	30 mins/day; 11 days/year.
Analysis (1 to 2)	10-100	30 mins/day; 11 days/year.
Loading into Tanks (1 or 2)	1-15	2 hours/day; 13 days/year.
Loading into drums (1 or 2)	1-15	8 hours/day; 3 days/year.
Equipment Cleaning (1 or 2)	<1	3 hours/day; 2 day/year.

### *Marine Terminals*

Components and packages arriving via marine bulk tank will be transferred to storage tanks via hard piping, for subsequent transfer into road tankers. During transfer operations skin and eye contact may occur when workers connect and disconnect pump lines between the ship and storage tank and between the storage tank and road tanker. Ship tanks are washed via automated procedures and worker exposure will not occur. Storage tanks are flushed through with solvent before cleaning personnel enter the tanks.

Skin contact may also occur when sampling and analysis is being conducted of the component and package in the storage tanks, isotanks or drums. Apart from those involved in sampling and analysis, occupational exposure to the contents of the isotanks and drums is not likely except in the event of a spill.

The notified chemical is of low volatility and inhalation exposure is unlikely.

### *Lubricant Blending Plant*

The notified chemical, as a component or in packages, arriving in road tankers and isotanks will be unloaded and transferred to storage tanks at 4 to 7 blending facilities via four inch hosing which workers will fasten. Fastening and unfastening takes approximately 10 minutes. A special air back flush system is used to prevent spillage during transfer. For unloading of drums workers will connect a pump line to the drum. Delivery tanks and drums are typically steam cleaned. For unloading from the three types of containers incidental skin contact to splashes, drips and spills may occur as pump lines are connected or disconnected and samples are handled.

Blending into finished lubricant oil occurs on-line and is computer controlled, thereby excluding the potential for occupational exposure. Sampling occurs from the blend tank and analysis by workers takes a few minutes. The blended lubricant is packaged into 200 L drums or sold in bulk in tank trucks. Drum filling is an automated process and worker intervention is not required unless the filling line operation requires adjustment. However, workers are required to insert bungs and label the drums. Bulk loading involves connecting 4 inch lines from the tank to the truck and subsequent disconnection. Occasionally equipment has to be entered for maintenance and repair operations. In such instances the tanks are flushed through with lube oil and then water before being entered by personnel.

### *Marine Vessels*

Ship workers may receive skin and eye contact to the finished lubricant containing the notified polymer as the lubricant is transferred to the ship and during drum cleaning. Although specific details are not known, the frequency of tasks are likely to be comparable to those at marine terminals and blending facilities. Ship mechanics may be exposed to the finished lubricant during their normal work. It is inevitable that mechanics will receive skin contact given the nature of the job, the scale of operations and the fact that protective gloves are not widely used. Up to several hundred workers may be involved in these tasks.

### *Closed Hydraulic Systems*

There is potential for exposure when oils are added to and drained from systems. Such operations are carried out by skilled workers and are likely to occur once a year. The number of workers is not known but is estimated to be up to 40% of Australian workers involved in such activities.

### Control Measures and Worker Education and Training

Workers at marine terminals, lubricant blending plants and ship workers will wear coveralls, gloves & eye protection. The notifier states that inspections of their customers' sites have found that the blending facilities are well ventilated, with control systems for accidental spills and wastewater treatment. The notified chemical will be handled by employees of major Australian lubricant manufacturers. Workers involved in the blending activities are reported to have received training in the handling of additive packages.

## **7. PUBLIC EXPOSURE**

The notified chemical will not be sold to the public. However, exposure of the public to the notified chemical may occur in the event of an accidental spill during transport of the notified chemical. According to the material safety data sheets (MSDS) provided for the notified chemical, a spill should be contained with absorbent (soil, sand or other inert material) material and sealed in properly labelled drums for disposal. Runoff should be prevented from entering drains and waterways.

The notified chemical is blended into finished lubricant oils, intended for use on large marine vessels, with other hydraulic uses (in industrial/farm equipment). The potential for public exposure is only likely in the event of an accidental or inadvertent release.

## **8. ENVIRONMENTAL EXPOSURE**

### **8.1 Release**

The blending operations are performed at specially constructed sites owned and operated by petroleum companies. Up to seven sites in Australia may be involved in producing the marine diesel engine lubricants and hydraulic oils. Release to the environment is expected to occur only in the unlikely event of an accident during transport or an accidental leak.

The additive packages (for both uses) containing the notified chemical will be delivered to and stored at the blending facilities in isotanks or drums. It is anticipated that there will be minimal release of the notified chemical during transfer from the storage containers to the blending tanks, as a special air back flush system prevents any spillage. Blending occurs in fully enclosed automated systems. Blending tanks will be cleaned with lube oil, which will typically be recycled during subsequent blending or incinerated. Any spills incurred in the blending operations will be contained within concrete bunds and either reclaimed or sent to on-site waste-water treatment facilities. At the treatment facilities residual hydrocarbon based products will be separated from the aqueous stream by the Australian Petroleum Industry (API) process. Removal of greater than 95% hydrocarbon is claimed. Before being released to the sewage system, the aqueous waste undergoes further treatment involving pond aeration and sand filtration. The remaining oily waste will be incinerated.

The empty drums containing residual notified polymer 270 will be steam cleaned. The resultant aqueous waste is sent to on-site waste-water treatment facilities.

At marine terminals ISO procedures are in place to minimise spills. The finished lubricant containing the notified chemical is transferred to the ship-board storage tank by hoses from the delivery container. Aboard ship, the oil is pumped through hard piping to the engine. Containers holding residual notified chemical will be cleaned by steam with the waste-water entering a treatment facility at the receiving terminal. The waste will be treated in a similar fashion to that at blending facilities.

In both uses skilled tradesmen will be undertaking all maintenance of the equipment so spills and leaks will be kept to a minimum and cleaned up immediately. Fresh oil may be added to the engines/hydraulics over time to keep levels constant and to maintain the effectiveness of the oil but generally the machinery will undergo a major service once a year. Since this will involve skilled tradesmen, used oil generated from the draining of the oil or engine repair will be incinerated or sent for recycling.

## **8.2 Fate**

In the case of accidental release to land, the anticipated high adsorption/desorption property of the notified chemical indicates that it would not be mobile, but would adsorb onto and become strongly associated with the organic component of soils and sediments. Similarly, in the event of accidental release into the water compartment, it is likely to become associated with suspended organic material, and eventually be incorporated into sediments. It is expected that if placed into landfill (for example adsorbed onto sawdust after accidental spills) the material would be very slowly degraded through the biological and abiotic processes operating in these facilities.

No information on the capacity of the notified chemical to undergo biodegradation was provided, however biodegradation results for the analogue chemical are available. A ready biodegradability study on the analogue according to OECD TG 301D (Closed Bottle) Method (Douglas, 1993a). A piece of glass filter paper impregnated with the notified chemical was placed into a test bottle which was then filled with activated sewage sludge bacteria inoculated inorganic nutrient medium, and sealed. The study was conducted in duplicate so that dissolved oxygen measurements could be done at 0, 5, 15 and 28 days. Sodium benzoate was used as the standard substance. After 28 days, only 12% of the analogue chemical had degraded after 28 days. This indicates that it is not readily biodegradable, while the sodium benzoate reached 88% degradation.

The expected high log  $P_{OW}$ , molecular weight and low rate of biodegradation of the notified chemical, indicate the potential for bioaccumulation (Connell, 1989). However, direct exposure to the water compartment is considered to be unlikely and will limit the potential for bioaccumulation.

Incineration of waste oil containing the notified chemical will destroy the substance with evolution of water vapour and oxides of carbon and sulphur, together with production of calcium compounds that would be assimilated with the ash. Sludge from waste treatment plants or oil recycling facilities could also be incinerated.

## 9. EVALUATION OF TOXICOLOGICAL DATA

All testing was conducted using the closely related structural analogue. The analogue was previously assessed by NICNAS as NA/253. The results obtained for the structural analogue are considered to be equivalent to those which would be obtained with the notified chemical.

The test chemical was administered as a 70-80% solution in a lubricating oil solvent. The doses presented refer to the solvent mixture.

All tests were performed according to OECD/EEC guidelines and in facilities that comply with GLP.

### 9.1 Acute Toxicity

#### Summary of the acute toxicity of the analogue chemical

<i>Test</i>	<i>Species</i>	<i>Outcome</i>	<i>Reference</i>
acute oral toxicity	rat	LD <sub>50</sub> >5000 mg/kg	Glaza, 1993a
acute dermal toxicity	rat	LD <sub>50</sub> >2000 mg/kg	Glaza, 1993b
skin irritation	rabbit	Non-irritant	Glaza, 1993c
eye irritation	rabbit	Slight irritant	Glaza, 1993d
skin sensitisation	guinea pig	Skin sensitiser	numerous

#### 9.1.1 Oral Toxicity (Glaza, 1993a)

<i>Species/strain:</i>	Rat / Crl: CDrBR
<i>Number/sex of animals:</i>	5 males and 5 females
<i>Observation period:</i>	14 days
<i>Method of administration:</i>	Gavage, 5000 mg/kg, undiluted
<i>Test method:</i>	OECD TG 401 (Limit Study)
<i>Mortality:</i>	None
<i>Clinical observations:</i>	Soft stools were noted in 7 animals within 4 hours of treatment. Dark staining of the urogenital region was observed in all animals on days 1 and 2. No other signs of toxicity were observed.
<i>Morphological findings:</i>	No treatment-related macroscopic findings were observed.
<i>LD<sub>50</sub>:</i>	> 5000 mg/kg
<i>Result:</i>	The test chemical is of very low acute oral toxicity in rats.

### 9.1.2 Dermal Toxicity (Glaza, 1993b)

<i>Species/strain:</i>	Rat / Crl: CDrBR
<i>Number/sex of animals:</i>	5 males and 5 females
<i>Observation period:</i>	14 days
<i>Method of administration:</i>	2000 mg/kg liquid chemical applied undiluted under occlusive dressing.
<i>Test method:</i>	OECD TG 402 (Limit Study)
<i>Mortality:</i>	None
<i>Clinical observations:</i>	No signs of local or systemic toxicity were observed.
<i>Morphological findings:</i>	No treatment-related macroscopic findings were observed.
<i>LD<sub>50</sub>:</i>	> 2000 mg/kg
<i>Result:</i>	The test chemical is of low dermal toxicity in rats.

### 9.1.3 Inhalation Toxicity

Study not conducted for this viscous liquid of low volatility.

### 9.1.4 Skin Irritation (Glaza, 1993c)

<i>Species/strain:</i>	Rabbit / Hra: (NZW) SPF
<i>Number/sex of animals:</i>	3 males and 3 females
<i>Observation period:</i>	Up to 96 hours
<i>Method of administration:</i>	Undiluted liquid chemical applied for four hours under semi-occlusive dressing.
<i>Test method:</i>	OECD TG 404

*Draize scores:*

<i>Time after treatment (days)</i>	<i>Animal #</i>					
	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>
<hr/>						
<i>Erythema</i>						
0	0					
1	1					
2	1	Zero scores were recorded for 5/6 animals at all time points				
3	0					
4	0					
<hr/>						
<i>Oedema</i>						
0						
1						
2		Zero scores were recorded in all animals at all time points.				
3						
4						

<sup>a</sup> see Attachment 1 for Draize scales

*Comment:* Mean score for all six animals over the 24, 48 and 72 hour time points : Erythema = 0.1 ; Oedema = 0

*Result:* The test chemical is very slightly irritating to the skin of rabbits.

### 9.1.5 Eye Irritation (Glaza, 1993d)

*Species/strain:* Rabbit / Hra: (NZW) SPF

*Number/sex of animals:* 3 males and 6 females

*Observation period:* Up to 96 hours

*Method of administration:* 0.1mL liquid test chemical was dropped into the conjunctival sac of one eye in each animal. The eyes of 3 males and 3 females remained unwashed. The eyes of another 3 females were irrigated approximately 30 seconds after instillation.

*Test method:* OECD TG 405

*Draize scores of unirrigated eyes:*

<i>Animal</i>	<i>Time after instillation</i>														
	<i>0 day</i>		<i>1 days</i>		<i>2 days</i>		<i>3 days</i>		<i>4 days</i>						
<i>Cornea</i>	<i>o</i>	<i>a</i>	<i>o</i>	<i>a</i>	<i>o</i>	<i>a</i>	<i>o</i>	<i>a</i>	<i>o</i>	<i>a</i>					
1															
2															
3	Zero scores were recorded in all animals at all time points.														
4															
5															
6															
<i>Iris</i>															
1															
2															
3	Zero scores were recorded in all animals at all time points.														
4															
5															
6															
<i>Conjunctiva</i>	<i>r</i>	<i>c</i>	<i>d</i>	<i>r</i>	<i>c</i>	<i>d</i>	<i>r</i>	<i>c</i>	<i>d</i>	<i>r</i>	<i>c</i>	<i>d</i>	<i>r</i>	<i>c</i>	<i>d</i>
1	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0
2	1	1	1	1	1	0	1	1	0	1	0	0	0	0	0
3	2	1	2	1	1	0	1	0	0	0	0	0	0	0	0
4	2	1	2	2	1	0	1	0	0	0	0	0	0	0	0
5	2	1	1	1	1	0	1	1	0	1	0	0	0	0	0
6	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0

<sup>1</sup> see Attachment 1 for Draize scales

o = opacity   a = area   r = redness   c = chemosis   d = discharge

*Irrigated eyes:*

As with the unwashed eyes zero scores were recorded for corneal and iris effects throughout the study. Grade 1 scores for conjunctival redness and chemosis were recorded in 1 to 3 animals at 24 hours, but this had resolved by the 48 hour observation period.

*Comment:*

Mean score for all six animals with unirrigated eyes over the 24, 48 and 72 hour time points : Cornea = 0 ; Iris = 0 ; Conjunctival redness = 0.84 ; conjunctival chemosis = 0.33

*Result:*

The test chemical is slightly irritating to the eyes of rabbits.

### 9.1.6 Skin Sensitisation

Several skin sensitisation studies have been conducted. Five studies were conducted using guineapigs, three testing the analogue chemical as manufactured (that is, at 70-80% in lube oil), and two testing different finished oil products. Four studies were conducted in humans, one using the analogue substance at 70-80% in lube oil, and three using different finished oil products.

#### 9.1.6.1 Skin Sensitisation in Guineapigs, using the Analogue Chemical (Morris, 1993)

<i>Species/strain:</i>	Guineapig / Hartley
<i>Number of animals:</i>	10 male and 10 female test animals and 5 male and 5 female control animals.
<i>Induction procedure:</i> days, 0, 7 and 14	0.3 mL of 100% test chemical chemical, applied to intact skin, under occlusive dressing for 6 hours. Control animals received Spectrum Mineral Oil Light.
<i>Challenge procedure:</i> day 28	Test and control animals were treated with 0.3 mL of 0.5% test chemical in Spectrum Mineral Oil Light, applied to intact skin of a previously untreated site, under occlusive dressing for 6 hours.
<i>Test method:</i>	OECD TG 406 (Buehler)

#### *Challenge outcome:*

<i>Challenge concentration</i>	<i>Test animals</i>		<i>Control animals</i>	
	<i>24 hours*</i>	<i>48 hours*</i>	<i>24 hours</i>	<i>48 hours</i>
0.5%	1/20**	5/20	0/10	0/10

\* time after patch removal

\*\* number of animals exhibiting responses greater than those seen in controls

*Comment:* All control animals showed grade +/- or 1 skin reactions at 24 and/or 48 hours after challenge. Animals with grade 2 skin reactions are recorded in the table. 25% of test animals showed skin reactions greater than those seen in control animals.

*Result:* The test chemical is sensitising to the skin of guinea pigs.

**9.1.6.2 Skin Sensitisation in Guinea pigs, using the Analogue Chemical (Kreuzmann, 1993)**

*Species/strain:* Guinea pig / Hartley

*Number of animals:* 10 male and 10 female test animals and 5 male and 5 female control animals.

*Induction procedure:* days, 0, 7 and 14  
0.3 mL of 25% test chemical in Spectrum Mineral Oil Light, applied to intact skin, under occlusive dressing for 6 hours. Control animals received Spectrum Mineral Oil Light.

*Challenge procedure:* day 28  
Test and control animals were treated with 0.3 mL of 2.5% test chemical in Spectrum Mineral Oil Light, applied to intact skin of a previously untreated site, under occlusive dressing for 6 hours.

*Test method:* OECD TG 406 (Buehler)

*Challenge outcome:*

<i>Challenge concentration</i>	<i>Test animals</i>		<i>Control animals</i>	
	<i>24 hours*</i>	<i>48 hours*</i>	<i>24 hours</i>	<i>48 hours</i>
2.5%	2/20**	5/20	0/10	0/10

\* time after patch removal

\*\* number of animals exhibiting responses greater than those seen in controls

*Comment:* All control animals showed grade +/- or 1 skin reactions at 24 and/or 48 hours after challenge. Animals with grade 2 skin reactions are recorded in the table. Oedema was also noted in 4 of the animals showing grade 2 reactions. 25% of test animals showed skin reactions greater than those seen in control animals.

*Result:* The test chemical is sensitising to the skin of guinea pigs.

### 9.1.6.3 Skin Sensitisation in Guinea pigs, using the Analogue Chemical (Morris, 1994a)

<i>Species/strain:</i>	Guineapig / Hartley
<i>Number of animals:</i>	10 male and 10 female test animals and 5 male and 5 female control animals.
<i>Induction procedure:</i> days, 0, 7 and 14	0.3 mL of 25% test chemical in Spectrum Mineral Oil Light, applied to intact skin, under occlusive dressing for 6 hours. Control animals received Spectrum Mineral Oil Light.
<i>Challenge procedure:</i> Primary challenge day 28	Test and control animals were treated with 0.3 mL of 2.5% test chemical in Spectrum Mineral Oil Light, applied to intact skin of a previously untreated site, under occlusive dressing for 6 hours.
Re-challenge day 34	Test and control animals were treated with 0.3 mL of 2.5% test chemical in Spectrum Mineral Oil Light, applied under occlusive dressing for 6 hours.
Cross-challenge day not specified	Test and control animals were treated with 0.3 mL of 100% test chemical in Spectrum Mineral Oil Light, applied under occlusive dressing for 6 hours.
<i>Test method:</i>	OECD TG 406 (Buehler)

*Challenge outcome:*

<b>Challenge concentration</b>	<b>Test animals</b>		<b>Control animals</b>	
	<b>24 hours*</b>	<b>48 hours*</b>	<b>24 hours</b>	<b>48 hours</b>
2.5%	2/20**	4/20	0/10	1/10
<b>Rechallenge concentration</b>				
2.5%	4/20	4/20	0/10	0/10
<b>Cross-challenge</b>				
100%	9/19	4/19	0/10	0/10

\* time after patch removal

\*\* number of animals exhibiting responses greater than those seen in controls

*Comment:* All control animals showed grade +/- or 1 skin reactions at 24 and/or 48 hours after challenge. Animals with grade 2 or 3 skin reactions are recorded in the table. Oedema was also noted in 3 of the animals showing grade 2 reactions at rechallenge, in 8 of the animals showing grade 2 or 3 reactions at cross-challenge and in one test animal showing grade 1 skin reaction at cross-challenge. 15%, 20%

and 47% of test animals showed skin reactions greater than those seen in control animals at challenge, rechallenge and cross-challenge, respectively.

*Result:* The test chemical was sensitising to the skin of guinea pigs.

#### **9.1.6.4 Skin Sensitisation in Humans, using the Analogue Chemical (Boisits et al, 1993)**

*Study Group* 105 individuals, predominantly female, aged 21-60 years

*Test Substance* 100% analogue chemical

*Induction procedure:* Test chemical was applied under occlusive dressing to the infrascapular region of the back for 24 hours, on Mondays, Wednesdays and Fridays for 6 applications. Due to skin reactions, the remaining 2 applications were made under semi-occlusive patches. Treated sites were evaluated before reapplication of test chemical.

*Challenge procedure:* Not conducted

*Test method:* Repeated Insult Patch Test in Humans

*Challenge outcome:* Due to intensity of skin reactions produced during induction, the challenge phase of the study was not conducted.

*Comment:* 19 individuals experienced intense reactions either during the course or on completion of the induction phase of the study. 16 of these were considered to be sensitisation reactions. Subjects with severe reactions needed medical treatment to help relieve symptoms, taking up to 6 weeks in the 3 most severe cases.

*Result:* The test chemical was sensitising to the skin of humans.

#### **9.1.6.5 Skin Sensitisation in Guinea pigs, using Finished Oil containing 16% Analogue Chemical (Morris, 1994b)**

*Species/strain:* Guinea pig / Hartley

*Number of animals:* 10 male and 10 female test animals and 5 male and 5 female control animals.

*Induction procedure:*  
days, 0, 7 and 14 0.3 mL of 100% finished oil (containing 16% analogue chemical), applied to intact skin, under occlusive dressing

for 6 hours.  
Control animals received Spectrum Mineral Oil Light.

*Challenge procedure:*  
day 28

Test and control animals were treated with 0.3 mL of 100% test chemical (16% analogue chemical), to intact skin of a previously untreated site, under occlusive dressing for 6 hours.

*Test method:* OECD TG 406 (Buehler)

*Challenge outcome:*

<i>Challenge Concentration of OLOA 270</i>	<i>Test animals</i>		<i>Control animals</i>	
	<i>24 hours*</i>	<i>48 hours*</i>	<i>24 hours</i>	<i>48 hours</i>
16%	11/18**	3/18	5/10	1/10

\* time after patch removal

\*\* number of animals exhibiting grade 1 or greater responses

*Comment:*

All test and control animals showed grade +/- or 1 skin reactions at 24 and/or 48 hours after challenge. Animals with grade 1 skin reactions are recorded in the table. 61% and 51% of test and control animals, respectively, showed grade 1 skin reactions. A difference in response between test and control animals of 10% does not constitute a positive result.

*Result:*

The finished oil containing 16% analogue chemical was not sensitising to the skin of guinea pigs.

#### **9.1.6.6 Skin Sensitisation in Guinea pigs, using Finished Oil containing 7.6% Analogue Chemical (Morris, 1996)**

*Species/strain:* Guinea pig / Hartley

*Number of animals:* 10 male and 10 female test animals and 10 male and 10 female control animals.

*Induction procedure:*  
days, 0, 7 and 14

0.3 mL of 100% finished oil (containing 7.6% analogue chemical), applied to intact skin, under occlusive dressing for 6 hours.  
Control animals received Spectrum Mineral Oil Light.

*Challenge procedure:*  
day 28

Test and control animals were treated with 0.3 mL of 100% finished oil (containing 7.6% analogue chemical), applied to intact skin of a previously untreated site, under occlusive dressing for 6 hours.

*Test method:* OECD TG 406 (Buehler)

*Challenge outcome:*

<i>Challenge concentration of OLOA 270</i>	<i>Test animals</i>		<i>Control animals</i>	
	<i>24 hours*</i>	<i>48 hours*</i>	<i>24 hours</i>	<i>48 hours</i>
7.6%	0/20**	1/20	0/20	0/20
7.6%	15/20***	8/20	12/20	10/20

\* time after patch removal

\*\* number of animals exhibiting grade 2 skin response

\*\*\* number of animals exhibiting grade 1 skin response

*Comment:* One test animal showed a skin reaction higher than that seen in control animals. 75% and 60% of test and control animals, respectively, showed grade 1 skin reactions, with 15% more test animals showing grade 1 compared to controls. A difference in response between test and control animals of 15% indicates a positive result in a non-adjuvant test.

*Result:* The finished oil containing 7.6% analogue chemical was sensitising to the skin of guinea pigs.

#### **9.1.6.7 Skin Sensitisation in Humans, using Finished Oil containing 16% Analogue Chemical (Harper et al, 1995)**

*Study Group* 89 subjects

*Test Substance* 100% finished oil (containing 16% analogue chemical)

*Induction procedure:* Test chemical was applied under semi-occlusive dressing to the upper arm for 24 hours, on Mondays, Wednesdays and Fridays for 3 consecutive weeks. Treated sites were evaluated immediately before reapplication of test chemical.

*Challenge procedure:* 14 days after the end of the induction phase, test chemical was applied to previously untreated sites, under semi-occlusive dressing for 24 hours. A confirmatory rechallenge was conducted in one subject, approximately 7 weeks after the primary challenge.

*Test method:* Repeated Insult Patch Test in Humans

*Challenge outcome:* 1/89 subjects showed a reaction indicative of sensitisation at the induction site immediately before the challenge

application was made. This subject also showed sensitisation reactions 96 hours after the primary challenge and 96 hours after rechallenge.

*Comment:* 2 subjects exhibited mild erythema at the second and eighth induction visit, respectively. The responses resolved within the following two visits.

*Result:* The finished oil containing 16% OLOA 270 was sensitising to the skin of humans.

#### **9.1.6.8 Skin Sensitisation in Humans, using Finished Oils containing 7.6% Analogue Chemical (Buehler et al, 1997a)**

*Study Group* 24 subjects, 20 females and 4 males, aged 25-60 years

*Test Substance* 100% XF-2229 and XF-2235 (both containing 7.6% analogue chemical)

*Induction procedure:* Nine repeated applications of test chemical were made over 3 weeks, under semi-occlusive dressing to the upper arm for 24 hours. Treated sites were evaluated before reapplication of test chemical.

*Challenge procedure:* Approximately 2 weeks after the end of the induction phase, test chemical was applied to previously untreated sites, under semi-occlusive dressing for 24 hours.

*Test method:* Repeated Insult Patch Test in Humans

*Challenge outcome:* Two subjects showing moderate erythema or mild erythema with papules during the rest period between induction and challenge phases showed sensitisation reactions following challenge with XF-2229. There was no evidence of sensitisation with XF-2235.

*Comment:* Responses to both test oils were mild, with an occasional mild erythema being observed during induction and challenge phases.

*Result:* A finished oil containing 7.6% analogue chemical was sensitising to the skin of humans

### **9.1.6.9 Skin Sensitisation in Humans, using Finished Oils containing 5.8% Analogue Chemical (Buehler et al, 1993b)**

<i>Study Group</i>	111 subjects, 85 females and 26 males, aged 21-60 years
<i>Test Substance</i>	Two finished oils (both containing 5.8% analogue chemical)
<i>Induction procedure:</i>	Nine repeated applications of test chemical were made over 3 weeks, under semi-occlusive dressing to the upper arm for 24 hours. Treated sites were evaluated before reapplication of test chemical.
<i>Challenge procedure:</i>	Approximately 2 weeks after the end of the induction phase, test chemical was applied to previously untreated sites, under semi-occlusive dressing for 24 hours.
<i>Test method:</i>	Repeated Insult Patch Test in Humans
<i>Challenge outcome:</i>	There was no evidence of sensitisation reactions in any subjects following challenge with either test chemical.
<i>Comment:</i>	Responses to both test oils were generally mild, with transient mild erythema being observed during induction and challenge phases.
<i>Result:</i>	The finished oil containing 5.8% OLOA 270 was not sensitising to the skin of humans

## **9.2 Repeated Dose Toxicity of the Analogue Chemical**

### **9.2.1 28 Day Repeated Dose Toxicity in Rats (Shour et al, 1993)**

<i>Species/strain:</i>	Rat / Sprague-Dawley
<i>Number/sex of animals:</i>	6 males and 6 females per group
<i>Method of administration:</i>	Orally by gavage
<i>Dose/Study duration:</i>	0, 100, 500 and 1000 mg/kg/day test chemical in peanut oil on 28 consecutive days (designated; control, low, mid and top-dose groups, respectively). An extra control and top-dose group were included and allowed a 14-day recovery period.
<i>Test method:</i>	OECD TG 407 (with a 14 day recovery period)

### *Clinical observations*

One top-dose female died in week 2 due to gavage error. No other deaths occurred. Top-dose females showed very slight but statistically significant lower body weight gains compared to controls after the first week of dosing. However body weight gains were comparable with controls for the remainder of the study. There were no treatment-related differences in body weight gain in the top-dose males or in the lower-dose groups throughout the study.

General signs of toxicity, including brown staining or matting on various body surfaces, tan staining around the mouth with increased salivation, were seen within an hour of dosing, primarily in top-dose males and females but also, usually at a lower incidence, in animals treated with 500 and 100 mg/kg/day.

### *Clinical chemistry/Haematology*

Treated males showed a statistically significant decrease in reticulocyte count of 44, 42 and 44 % in 100, 500 and 1000 mg/kg/day treated groups, respectively, compared to controls. This decrease was not evident in the recovery group and due to the lack of a dose-response relationship was not considered to be of toxicological significance.

Top-dose males showed a statistically significant increase of 41% compared to controls in alanine aminotransferase. The value was comparable with controls by the end of the recovery period.

No other treatment-related findings were observed.

### *Effects on the Organs*

A slight but statistically significant increase (18% compared to controls) in absolute liver weight was noted in top-dose males at the end of the treatment period. The increase was no longer evident at the end of the recovery period. Corresponding increases were also noted in liver weight relative to body (19%) and brain (16%) weights. Mid and top-dose females also showed statistically significant increases in liver weight, relative to body weight (10 and 18 %, respectively). However increases in absolute liver weight, and relative to brain weight, were not noted in females. Also, as no corresponding clinical findings were noted, the liver weight findings in females were considered to be of doubtful toxicological significance.

The only treatment-related macroscopic findings were those associated with gavage error in the female that died. No treatment-related microscopic findings were observed.

### *Result*

Based on liver toxicity with 1000 mg/kg/day in male rats, a No Observed Adverse Effect Level of 500 mg/kg/day was identified.

## **9.2.2 90 Day Repeated Dose Toxicity in Rats (Chengelis et al, 1995)**

<i>Species/strain:</i>	Rat / Sprague-Dawley
<i>Number/sex of animals:</i>	12 males and 12 females per group
<i>Method of administration:</i>	Orally by gavage

*Dose/Study duration:* 0, 100, 500, 1000 mg/kg/day (designated; control, low, mid and top-dose groups, respectively), in peanut oil, daily for 91 or 92 consecutive days, with extra control and top-dose groups given a 28 day recovery period

*Test method:* OECD TG 408 (with 28 day recovery period)

#### *Clinical observations*

Two top-dose females died in weeks 2 and 4 respectively. One low and one mid-dose male died in weeks 2 and 3 respectively. Cause of death was not determined due to excessive autolysis in these 4 animals. Another two top-dose females died in week 6 due to gavage error.

General signs of toxicity such as increased salivation and red matting around the mouth, were observed, usually 1 hour after dosing, in a dose-dependant manner in all treated groups. Rales were also noted in 3 top-dose animals, one of which died. No clinical signs were seen during the recovery period.

Mean body weight gain was reduced in all male treated groups and was statistically significant in mid and top-dose males. Body weight at the end of the treatment period was 5, 11 and 12 % lower than control values in low, mid and top-dose males, respectively. The decreases corresponded with statistically significant decreases in food consumption. Similar trends were not observed for mean body weight, body weight gain or food consumption in females.

#### *Clinical chemistry/Haematology*

No treatment-related changes were observed.

#### *Ophthalmology*

Bilateral corneal crystals were observed at an incidence of 0/24, 2/11, 4/11 and 10/24 in control, low, mid and top-dose males, respectively, at the 13 week observation time. At the end of the recovery period, the effect was seen in 3/12 and 6/12 control and top-dose males, respectively. The effect was not seen in females and is noted to commonly occur in Sprague-Dawley rats, particularly males and is therefore considered unlikely to be due to treatment.

#### *Effects on the organs*

Mid and top-dose females showed a statistically significant increase in adrenal weight relative to body weight (24 and 28%, respectively) at the 13-week necropsy. Mid and top-dose females also showed a statistically significant increase in relative liver weight (17 and 26%, respectively). The statistically significant increase in top-dose females was also seen for absolute liver weight (19%). No corresponding macro- or microscopic findings were observed in the adrenals or liver and no changes were seen at the end of the recovery period.

Mid and top-dose males showed statistically significant increases in relative to body weight brain (11 and 11%, respectively), liver (11 and 18%, respectively) and kidney (14%, top-dose only) weights at the 13-week necropsy. Changes were not seen in absolute, or relative to brain weight and were considered unlikely to be due to treatment.

No treatment-related macro or microscopic findings were reported.

### *Result*

Based principally on general signs of toxicity as well as adrenal toxicity, a No Observed Adverse Effect Level of 100 mg/kg/day was identified.

### **9.2.3 Combined Repeated Dose Toxicity and Reproductive Screen in Rats (Shour et al, 1993)**

<i>Species/strain:</i>	Rat / Sprague-Dawley
<i>Number/sex of animals:</i>	12 males and 12 females per group
<i>Method of administration:</i>	Orally by gavage
<i>Dose/Study duration:</i>	Males : 0, 100, 500 and 1000 mg/kg/day (designated; control, low, mid and top-dose groups, respectively) test chemical in peanut oil for at least 28 consecutive days prior to mating, during mating, and post mating for a total minimum of 70 days. Females : 0, 100, 500 and 1000 mg/kg (designated; control, low, mid and top-dose groups, respectively) test chemical in peanut oil for at least 28 consecutive days prior to mating and until lactation day 4 or until post-mating day 25 for those which did not deliver a litter.
<i>Test method:</i>	OECD TG 422

#### *Clinical observations*

No deaths occurred in F<sub>0</sub> generation. Top-dose F<sub>0</sub> males showed a statistically significant reduction (25% compared to controls) in body weight gain during weeks 4-5. However this was not observed at any other interval and was considered to be transitory. There were no other changes in body weight or body weight gain in any F<sub>0</sub> dose group at any time of the study including through gestation and lactation.

General signs of toxicity, including brown staining or matting on various body surfaces, tan staining or clear matting around the mouth with increased salivation, were seen within an hour of dosing in top-dose F<sub>0</sub> males and females. On occasion, the signs persisted to the daily observations. Some of these clinical signs were also observed at a lower incidence in the mid-dose F<sub>0</sub> animals. Similar signs were also observed with low-dose F<sub>0</sub> females, occurring only as single incidents. An increased incidence of soft stools was also noted in top-dose F<sub>0</sub> animals, but not in the other treatment groups.

Physical condition and body weights of pups were unaffected by treatment.

#### *Clinical chemistry/Haematology*

Testing not conducted.

### *Effects on the Organs*

Top-dose F<sub>0</sub> females showed a statistically significant increase (10% compared to controls) in absolute liver weight. No corresponding increases were noted in liver weights relative to body or brain weight. No such increase was seen in males. No treatment-related micro- or macroscopic findings were observed in F<sub>0</sub> males or females in any treatment group.

No treatment-related micro- or macroscopic findings were observed in males or female pups in any treatment group.

### *Reproductive Performance*

No treatment-related changes were noted in the numbers of successful matings, pregnancies, litters delivered or stillborn and viable pups. The mean number of days between pairing and coitus, the length of gestation and the sex ratio of the pups were unaffected by treatment.

### *Result*

A No Observed Adverse Effect Level of 1000 mg/kg/day, the highest dose tested, was identified for general toxicity and reprotoxicity in this 70 day (approximately) reprotoxicity screening study.

## **9.2.4 Developmental Toxicity Study in Rats (Nemec et al, 1995)**

*Species/strain:* Rat / Sprague-Dawley

*Number/sex of animals:* 25 females per group

*Method of administration:* Orally by gavage

*Dose/Study duration:* 0, 100, 500 and 1000 mg/kg/day (designated; control, low, mid and top-dose groups, respectively) test chemical in peanut oil from gestation days 6-15. Hysterectomies were performed on all animals on gestation day 20.

*Test method:* OECD TG 414

### *Clinical observations*

No deaths occurred. Mid- and top-dose dams showed slight to severe hair loss within (2 days of treatment) on the hindlimbs, ventral-abdominal area and at the base of the tail. Mid- and top-dose dams also showed tan staining around the mouth at one hour after treatment.

Mid- and top-dose dams showed body weight loss during gestation days 6-7. This resulted in statistically significant reductions in body weight gain on days 6-9 (both groups) and 9-12 (top-dose only). Compensatory increases in body weight gain occurred in both groups post treatment (gestation days 16-20). The decreases and increases in body weight gain reflected changes in food consumption.

### *Histopathology*

No treatment-related macroscopic findings were observed in the dams and the mean gravid uterine weights were unaffected by treatment.

Intrauterine growth and survival were unaffected by treatment. There were no changes in the number of corpora lutea or implantation sites, postimplantation loss, the number of viable fetuses, foetal body weight or foetal sex ratio.

Malformations were seen in 0/373, 3/357, 2/378 and 1/345 fetuses in the 0, 100, 500 and 1000 mg/kg/day dose groups. Malformations included external, internal visceral and skeletal but did not show any consistency or dose-relationship to indicate that they were related to exposure.

### *Result*

No signs of developmental toxicity were observed with 1000 mg/kg/day, the highest dose tested. Mild maternal toxicity was observed and a No Observed Adverse Effect Level of 100 mg/kg/day was identified in the dams.

## **9.3 Genotoxicity of the Analogue Chemical**

### **9.3.1 *Salmonella typhimurium* Reverse Mutation Assay (Lawler, 1993)**

<i>Strains:</i>	<i>Salmonella typhimurium</i> : TA98; TA100; TA1535; TA1537 <i>Escherichia coli</i> : WP2 <i>uvrA</i>
<i>Metabolic activation:</i>	Aroclor-induced rat liver S9
<i>Concentration range:</i>	33.3, 100, 333, 1000, 3330, 10000 µg/plate,
<i>Test method:</i>	OECD TG 471
<i>Comment:</i>	There were no increases in the number of revertants in any of the test strains, with or without exogenous activation. There was no evidence of cytotoxicity in any of the test strains, with or without activation. Slight precipitation was observed with 333 µg/plate and above. Positive and negative controls produced results in the expected ranges.
<i>Result:</i>	The test chemical was not mutagenic under the conditions of the test.

### 9.3.2 Chromosomal Aberration Assay in Chinese Hamster Ovary Cells (Murli, 1993)

*Cells:* Chinese Hamster Ovary Cells

*Metabolic activation system:* Aroclor-induced rat liver S9

*Dosing schedule:*

Metabolic Activation	Experiment/ Number	Test concentration (µg/mL)	Controls
-S9	1	treatment time = 21 hours harvest time = 24 hours test concentrations = 50*, 125*, 250*, 500*, 750, 1000 µg/mL With the 24 hour harvest time, severe toxicity was evident with 500 µg/mL (with a reduction in MI > 50%) and complete toxicity with 750 and 1000 µg/mL.	Positive : MMC Negative: PL
	2	treatment time = 45 hours harvest time = 48 hours test concentrations = 50*, 125*, 250*, 500, 750, 1000 µg/mL With the 48 hour harvest time, severe toxicity was evident with 250 µg/mL (with a reduction in MI > 50%) and complete toxicity with 500 µg/mL and above.	
+S9	3	treatment time = 6 hours harvest time = 24 hours test concentrations = 10, 50*, 150*, 325*, 750*, 1500, 2250, 2290 µg/mL Severe toxicity was evident with 750 µg/mL (with a reduction in MI > 50%) and complete toxicity with 1500 µg/mL and above.	Positive: CP Negative: PL

MMC – mitomycin

PL – 10% pluronic in corn oil

CP - cyclophosphamide

\* - cultures selected for metaphase analysis

*Test method:* OECD TG

*Comment:* There were no increases in the frequency of cells with aberrations, with or without exogenous activation. Positive and negative controls produced results in the

expected ranges.  
Harvest times were reportedly chosen to take into account cell cycle delays observed in the range-finding studies.

*Result:* The test chemical was not clastogenic under the conditions of the test.

### 9.3.3 Micronucleus Assay in the Bone Marrow Cells of the Mouse (Murli, 1992)

*Species/strain:* Mice / ICR

*Number and sex of animals:* 5 males and 5 females per group

*Doses:* 1250, 2500, 5000 mg/kg

*Method of administration:* Intraperitoneal

*Test method:* OECD TG 474 (with additional sampling times)

*Comment:* There were no increases in the frequency of micronucleated cells. Positive and negative controls produced results in the expected ranges.  
One low-dose animal (from 24 hour sample group) was found dead approximately 15 hours after treatment. All top-dose animals showed rough hair coats. Decreases in polychromatic to normochromatic cell ratio were noted in all the 48-hour sample male groups which did not reach statistical significance.

*Result:* The test chemical was not clastogenic under the conditions of the test.

## 9.4 Skin Absorption of the Analogue Chemical

### 9.4.1 Skin Absorption *In Vitro* (Krueger et al, 1995a)

*Skin type:* Dorsal rat (Sprague-Dawley) skin from abdominal and thoracic regions.  
Dorsal human cadaver skin (from one black male and one caucasian female).

*Test method:* Sliced sections of rat and human stratum corneum, of 330-370 and 370-410  $\mu\text{m}$  thickness, respectively, were mounted onto foam blocks.  $^{14}\text{C}$ -ring labelled test chemical was applied at  $10 \text{ mg/cm}^2$  in 6% aqueous solution of Volpo-20 in diffusion cells. Receptor fluid was sampled at 2, 4, 6, 8, 24 and 30 hours from the rodent skin experiments and at 2, 4, 6,

8, 24, 30, 48, 54 and 72 hours from the human skin experiment.

Radioactivity was quantified using liquid scintillation counting.

*Result:* In the rat skin experiments 0.22% and 0.5% of the applied dose was recovered in the receptor fluid 24 and 30 hours after 8 hours exposure. A flux rate of  $1.3\mu\text{g}/\text{cm}^2/\text{hour}$  was calculated following 30 hours exposure. In the human skin experiment 0.09% of the applied dose was recovered in the receptor fluid following 72 hours exposure. A flux rate of  $0.1\mu\text{g}/\text{cm}^2/\text{hour}$  was calculated. The mass balance was 92-96%.

*Comment:* OLOA 270 is minimally absorbed through rat and human skin *in vitro*.

#### 9.4.2 Skin Absorption *In Vivo* (Kreuger et al, 1995b)

*Species / strain:* Rat / Sprague-Dawley

*Number/sex of animals:* 5 females per group

*Test method:*  $^{14}\text{C}$ -ring labelled test chemical was applied at  $10\text{ mg}/\text{cm}^2$  to dorsal skin, under a non-occlusive cell for 8 hours. Animals were sampled at 8, 24 and 72 hours. Radioactivity in urine, faeces,  $\text{CO}_2$  and body tissues was quantified using liquid scintillation.

*Result:* 0.4-0.6% of the applied dose was systemically absorbed at the 8, 24 and 72 hour sample times. An average absorption rate of approximately  $6\mu\text{g}/\text{cm}^2/\text{hour}$  was calculated. The mass balance was 86-100%.

*Comment:* OLOA 270 is minimally absorbed through rat skin *in vivo*.

### 9.5 Overall Assessment of Toxicological Data

All testing was conducted using a closely related structural analogue. The results obtained for the structural analogue are considered to be equivalent to those which would be obtained with the notified chemical.

Based on the results with the analogue, the notified chemical is considered to be of very low acute oral and low acute dermal toxicity.  $\text{LD}_{50}$  values of  $>5000$  and  $>2000\text{ mg}/\text{kg}$ , respectively, were obtained in rodents for the analogue chemical tested as the component, that is, at 70-80% in lubricating oil. There was no assessment of the inhalation toxicity of the notified chemical or the analogue. It is a very slight skin irritant and a slight eye irritant.

Several studies, conducted in animals and humans, have demonstrated that the notified chemical is a skin sensitiser. One human study indicated that a finished oil containing 5.8% component was not a skin sensitiser while finished oils containing 7.6% and more, were skin sensitisers.

NOAEL values of 500 and 100 mg/kg/day were identified in 28 and 90 day repeat dose oral studies, respectively for the analogue component. NOAEL values were based on liver, adrenal and general toxicity. A NOAEL of 1000 mg/kg/day, the highest dose tested, was identified for reprotoxicity and developmental toxicity. It should be noted that the reprotoxicity study was a screening study.

Results from a reverse mutation assay in bacterial cells, a chromosome aberration assay in mammalian cells and a micronucleus study in mice were negative, indicating that the notified chemical is not a genotoxicant.

Minimal skin absorption has been demonstrated in *in vitro* and *in vivo* studies, with less than 1% being absorbed upto 72 hours after 8 hour exposures.

Extrapolating from the submitted information, the notified chemical should be classified as a skin sensitiser (R43) according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC 1999).

## 10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

All testing was conducted using the closely related structural analogue. The analogue was previously assessed by NICNAS as NA/253. The results obtained for the structural analogue are considered to be equivalent to those which would be obtained with the notified chemical.

The following were carried out according to OECD Test Methods.

Species	Test	Concentrations* (mg/L) (as WAF)	Result (mg/L) (as WAF)
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	96 hour acute	0 and 1000	NOEC ≥ 1000 (WAF)
Water Flea ( <i>Daphnia magna</i> )	48 hour acute	0 and 1000	NOEC ≥ 1000 (WAF)
Algae ( <i>Selenastrum capricornutum</i> )	96 hour growth	0, 62.5, 125, 250, 500 and 1000	ER50 > 370 EB50 > 340 NOEC = 250

\* In the studies the water accommodated fraction (WAF) was prepared by stirring the mixtures of test chemical in water for 24 hours, settling the mixtures for 1 hour and siphoning off the water phase containing the WAF, while ensuring that no settled or surface floating test substance was transferred. None of the ecotoxicity studies made reference to the observation of precipitated material or oil in any of the test vessels.

An acute toxicity study of the analogue chemical on rainbow trout was conducted according to OECD TG 203 (Douglas, 1993b). The study was conducted in duplicate with ten fish in each test vessel, at a 100% water accommodated fraction (WAF), ie 1000 mg/L, and a control. No mortality or sub-lethal effects were observed throughout the 96 hour observation period. A no-observed effect concentration equal to or greater than 1000 mg/L (WAF) was identified.

An acute toxicity study of the analogue chemical on *Daphnia magna* was conducted according to OECD TG 202 (Douglas, 1993c). The study was conducted with a duplicate, control and in quadruplicate for the test concentration of 100% WAF, ie 1000 mg/L prepared as above, with ten daphnia in each test vessel. No immobilisation was observed throughout the 48 hour observation period. A no-observed effect concentration equal to or greater than 1000 mg/L (WAF) was identified.

An algal growth inhibition study of the analogue chemical on unicellular green algae was conducted according to OECD TG 201 (Douglas, 1993d). Five concentrations (62.5, 125, 250, 500 and 1000 mg/L WAF) were tested in triplicate with one control (6 replicates). An aliquot of concentrated algal suspension was added to each test vessel. The vessels were loosely stoppered and incubated for 72 hours under a continuous illumination of 7000 lux, at 24°C and an oscillation of 100 cycles per minute. To determine growth, samples were taken at 0, 24, 28 and 72 hours and the absorbance measured at 665 nm. The  $E_bC_{50}$  (72 hr) was 340 mg/L (WAF) and the  $E_rC_{50}$ (24-48 hr) was 370 mg/L (WAF). A no-observed effect concentration of 250 mg/L (WAF) was identified.

The ecotoxicity data for the analogue chemical indicate that the notified chemical it is not toxic to fish and daphnia up to the limit of its water solubility, but does show some toxicity to algae below this limit.

## **11. ASSESSMENT OF ENVIRONMENTAL HAZARD**

The environmental risk from the notified chemical is considered to be low provided that the material is used as a component of marine diesel engine lubricants and in hydraulic oils. Release to the environment is expected only in the unlikely event of an accident during transport or an accidental leak. It is expected that minimal waste will be generated from lubricant formulation and use, and this waste will either be incinerated or placed into landfill.

Very little release is anticipated from maintenance activities with any used oil, generated from the draining of the oil or engine repair, being incinerated or sent for recycling.

The chemical is expected to have a high log  $P_{OW}$  value and if released to the soil compartment will become strongly associated with the organic component of soils and sediments and is not expected to be mobile in these media.

The notified chemical is not readily biodegradable. However, if released to landfill or associated with soil, it is expected to slowly degrade through biotic and abiotic processes. This will result in the formation of water, sulphides and oxides of carbon, with the calcium component associating with soil minerals. Incineration would lead to water vapour and oxides of carbon and sulphur, with the calcium being assimilated into ash.

Based on the ecotoxicity data provided the notified chemical is not expected to be toxic to fish or daphnia up to the limit of its water solubility. Some toxicity to algae will occur below this limit. The expected high partition coefficient and low biodegradability of the notified chemical indicate the potential for bioaccumulation if spilt into waterways. However, very little of the chemical is likely to reach the aquatic compartment and a hazard to aquatic organisms is not considered likely.

## **12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS**

### **Assessment of Toxicological Hazard**

Based on the results of a closely related analogue, the notified chemical is considered to be of very low acute oral and low acute dermal toxicity. There was no assessment of the inhalation toxicity of the notified chemical or the analogue. The notified chemical is taken as a very slight skin irritant, a slight eye irritant and a skin sensitiser.

NOAEL values of 500 and 100 mg/kg/day were identified in 28 and 90 day studies, respectively, for the analogue component. A NOAEL of 1000 mg/kg/day, the highest dose tested, was identified for reprotoxicity and developmental toxicity. It should be noted that the reprotoxicity study was a screening study.

Results from *in vitro* and *in vivo* assays indicate that the notified chemical is not a genotoxicant. Minimal skin absorption has been demonstrated.

Extrapolating from the submitted information, the notified chemical should be classified as a skin sensitiser (R43) according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC 1999).

### **Occupational Health and Safety**

The notified chemical is not manufactured in Australia, but will be imported as a 'component' in lube oil or in 'additive packages' with other chemicals at maximum concentrations of 80% notified chemical or 100% component. The notified chemical will be introduced in the form of a viscous liquid of low volatility. Therefore it is unlikely to form vapours or mists and inhalation exposure is not of concern with the exception of one scenario at the lubricant blending plant, see (2) below. All workers handling the notified chemical are reported to be skilled refinery workers and mechanics.

#### **1. Marine Terminals**

Imported isotanks and drums will not normally be opened until arrival at blending facilities. Therefore waterside and transport workers will not be directly exposed to the notified chemical from these containers except in the event of spills. Skin and eye contact with up to 80% notified chemical (100% component) may occur when bulk tanks are unloaded to storage tanks and storage tanks unloaded to road tankers. Exposure may also occur during sampling and analysis activities. All tasks are of short duration (30 minutes/day) and infrequent (2 to 4 days/year) and it is reported that workers will wear coveralls, gloves and eye protection. Overall, if handled as described by the notifier, exposure is likely to be

negligible and the risk of adverse effects such as systemic toxicity and eye irritation is considered to be low. However, exposure must be prevented to protect against skin sensitisation.

Cleaning operations are automated and exposure will not occur.

## **2. Lubricant Blending Plant**

Skin and eye contact with up to 80% notified chemical (100% component) may occur via splashes, drips or spills when road tanks, isotanks and drums are unloaded. Exposure to up to 80% notified chemical may also occur during sampling and analysis activities. Tasks are of short duration (up to 1 hour/day) and infrequent (up to 11 days/year).

Loading of finished oils for onward transport to end users may lead to exposure to up to 15% component. Tasks are of longer duration than the unloading activities, taking up to 8 hours/day but are also infrequent, occurring at a maximum of 13 days/year.

Whole body exposure to a mist containing the notified chemical may occur when delivery containers are being steam cleaned. Cleaning is of intermediate duration (up to 3 hours/day) and is infrequent (4 days per/year) The concentration of the notified chemical in the mist is less than 1%.

It is reported that workers will wear coveralls, gloves and eye protection in all scenarios at the blending plant. Overall, exposure to the notified chemical is likely to be negligible and the risk of adverse effects such as systemic toxicity and eye irritation is considered to be low. However, exposure to the liquid and mist must be prevented to protect against skin and respiratory sensitisation.

## **3. End Users : Marine Vessels**

Skin and eye contact with up to 12% notified chemical (15% component) may occur via splashes, drips or spills when transferring the finished oils to the ship and during drum cleaning operations. Duration and frequency of tasks are likely to be comparable to those at marine terminals and blending plants. Overall exposure is likely to be negligible and the risk of adverse effects such as systemic toxicity and eye irritation is considered to be low. However, exposure to the liquid must be prevented to protect against skin sensitisation.

Skin exposure to up to 12% notified chemical is reported to be inevitable for mechanics. Duration and frequency of activities has not been stated but it is reported that mechanics are unlikely to wear gloves. Noting that skin sensitisation has been produced in humans when exposed to a finished oil containing 7.6% component, it is possible that individuals may become sensitised to the notified chemical. Employers will need to ensure that mechanics handling the notified chemical are given the required health effects information and have the means to avoid repeated contamination with the chemical.

Taking into account the concentration of the notified chemical and the low systemic toxicity, eye irritation and systemic toxicity is not of concern to mechanics.

## **4. End Users : Closed Hydraulic Systems**

There is potential for exposure to up to 0.2% component when oils are added to and drained from systems. Such tasks are infrequent, being carried out once a year. It is also noted that the

high cost of the hydraulic oils will help to ensure that waste, spills and exposure is kept to a minimum. Overall exposure is likely to be negligible and the risk of adverse effects low.

Overall, there is no concern of adverse effects occurring to workers for most of the scenarios involving exposure to the notified chemical as the risk can be controlled as described by the notifier. However, there is a high concern for skin sensitisation in mechanics working on marine vessels.

### **Public Health**

The notified chemical is not available for sale to the public and will be used as a lubricant additive, primarily for use in marine vessels. Since the notified chemical will be used in engines not handled by the public, the risk of exposure of the public to the notified substance is considered to be low.

## **13. RECOMMENDATIONS**

To minimise occupational exposure to the Chemical in OLOA 270 the following guidelines and precautions should be observed:

It is recommended that the notified Chemical in OLOA 270 be labelled as a Skin Sensitiser with the following R and S phrases :

R43	May cause sensitisation by skin contact
S24	Avoid contact with skin
S27	Wear suitable gloves

- The notifier's MSDS be provided to the occupational health and safety officer during the workplace assessment process and to the authorised medical practitioner responsible for health surveillance in the workplace to alert them to the potential for skin sensitisation;
- Workers should receive regular instruction on good occupational hygiene practices in order to minimise personal contact, and contamination of the work environment with formulations that contain OLOA 270. In particular, contaminated clothing should be removed without delay. The affected skin area should be decontaminated with a waterless hand cleaner, mineral oil, petroleum jelly, then washed with soap and water.
- Workers should be advised of the potential for occupational dermatoses following repeated skin exposure to OLOA 270 and to report any skin changes to the occupational health and safety officer at their workplace. When an occupational skin disease occurs, work practices and opportunities for contact with the substance should be reviewed and preventive measures instigated to ensure other workers do not develop the same condition. Further guidance on preventing the occurrence of occupational skin diseases can be found in the NOHSC guide *Occupational Diseases of the Skin* (NOHSC 1990).
- Personal protective equipment (PPE) should be used on all occasions where exposure to additive packages containing OLOA 270 occurs. Chemical impervious gloves and

clothing is necessary to prevent skin contact. Consideration should be given to the ambient environment, physical requirements and other substances present when selecting protective clothing and gloves. Workers should be trained in the proper fit, correct use and maintenance of their protective gear. PPE guidance in the selection, personal fit and maintenance of personal protective equipment can be obtained from:

Protective eyewear:	AS 1336 (SAA 1994); AS/NZS 1337 (SAA/SNZ 1992).
Chemical impermeable clothing:	AS 3765.2 (SAA 1990).
Impermeable gloves:	AS 2161.2 (SAA/SNZ 1998).
Occupational footwear:	AS/NZS 2210 (SAA/SNZ 1994);

- Workplace practices and control procedures consistent with provisions of State, Territory and Commonwealth legislation based on the *National Model Regulations for the Control of Workplace Hazardous Substances* must be in operation if products containing OGA 499 are determined to be hazardous.
- A copy of the MSDS should be easily accessible to employees.

If the conditions of use are varied from its use as a component of lubricant oil to be used in industrial engines, then further assessment may be required to assess the hazards to public health. In particular, should products become available to be added to car engines by members of the public, further consideration of the skin sensitisation effects would be required.

#### **14. MATERIAL SAFETY DATA SHEET**

An MSDS for the notified chemical in OLOA 270 and for an example additive package containing the notified chemical in OLOA 270 were provided in a format consistent with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 1994).

This MSDS were provided by the applicant as part of the notification statement. They are reproduced here as a matter of public record. The accuracy of this information remains the responsibility of the applicant.

## 15. REQUIREMENTS FOR SECONDARY NOTIFICATION

Under the Act, the director must be informed if any of the circumstances stipulated under subsection 64(2) of the Act arise, and secondary notification of the notified chemical may be required. No other specific conditions are prescribed.

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## Attachment 1

The Draize Scale (Draize, 1959) for evaluation of skin reactions is as follows:

<b><i>Erythema Formation</i></b>	<b><i>Rating</i></b>	<b><i>Oedema Formation</i></b>	<b><i>Rating</i></b>
No erythema	0	No oedema	0
Very slight erythema (barely perceptible)	1	Very slight oedema (barely perceptible)	1
Well-defined erythema	2	Slight oedema (edges of area well-defined by definite raising)	2
Moderate to severe erythema	3	Moderate oedema (raised approx. 1 mm)	3
Severe erythema (beet redness)	4	Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

The Draize scale (Draize *et al.*, 1944) for evaluation of eye reactions is as follows:

### ***CORNEA***

<b><i>Opacity</i></b>	<b><i>Rating</i></b>	<b><i>Area of Cornea involved</i></b>	<b><i>Rating</i></b>
No opacity	0 none	25% or less (not zero)	1
Diffuse area, details of iris clearly visible	1 slight	25% to 50%	2
Easily visible translucent areas, details of iris slightly obscure	2 mild	50% to 75%	3
Opalescent areas, no details of iris visible, size of pupil barely discernible	3 moderate	Greater than 75%	4
Opaque, iris invisible	4 severe		

### ***CONJUNCTIVAE***

<b><i>Redness</i></b>	<b><i>Rating</i></b>	<b><i>Chemosis</i></b>	<b><i>Rating</i></b>	<b><i>Discharge</i></b>	<b><i>Rating</i></b>
Vessels normal	0 none	No swelling	0 none	No discharge	0 none
Vessels definitely injected above normal	1 slight	Any swelling above normal	1 slight	Any amount different from normal	1 slight
More diffuse, deeper crimson red with individual vessels not easily discernible	2 mod.	Obvious swelling with partial eversion of lids	2 mild	Discharge with moistening of lids and adjacent hairs	2 mod.
Diffuse beefy red	3 severe	Swelling with lids half-closed	3 mod.	Discharge with moistening of lids and hairs and considerable area around eye	3 severe
		Swelling with lids half-closed to completely closed	4 severe		

### ***IRIS***

<b><i>Values</i></b>	<b><i>Rating</i></b>
Normal	0 none
Folds above normal, congestion, swelling, circumcorneal injection, iris reacts to light	1 slight
No reaction to light, haemorrhage, gross destruction	2 severe

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