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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

CHEMICAL IN NEW OLOA 216C, 216Q, 218A, 219, 219C and 219M

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Street Address:	334 - 336 Illawarra Road MARRICKVILLE NSW 2204, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX	+ 61 2 8577 8888
Website:	www.nicnas.gov.au

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FULL PUBLIC REPORT

CHEMICAL IN NEW OLOA 216C, 216Q, 218A, 219, 219C and 219M

1. APPLICANT

Holder of the original assessment certificate (No. 1281, NA/890):
Chevron Chemical Australia (ARBN 001 010 037) of 385 Bourke Street MELBOURNE VIC 3000.

Applicant for an extension of the original assessment certificate:
Mobil Oil Australia Pty Ltd (ABN 88 004 052 984) of 29 Francis Street, Yarraville, Victoria 3013.

2. IDENTITY OF THE CHEMICAL

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

Marketing Name:	New OLOA 216C (containing 60% notified chemical), New OLOA 216Q (containing 58% notified chemical), New OLOA 218A (containing 58% notified chemical), New OLOA 219 (containing 69% notified chemical), New OLOA 219C (containing 72% notified chemical) and New OLOA 219M (containing 69% notified chemical).
Molecular Weight:	Approximately 664 for trisulfide.
Method of Detection and Determination:	IR and NMR. Calcium and sulfur were analysed by Inductively Coupled Plasma Atomic Emission Spectroscopy or Atomic absorption sensitive to 20 ppb.
Spectral Data:	IR and NMR spectra were provided.

3. PHYSICAL AND CHEMICAL PROPERTIES

The physical and chemical property data provided by the notifier were determined based on 2 similar existing commercial products, OLOA 216Q and OLOA 219, which differ only in the length of the alkyl side chain of the molecule. Due to their structural similarity, the physical and chemical properties of OLOA 216Q and OLOA 219 are expected to be similar to the notified chemicals.

Appearance at 20°C & 101.3 kPa:	Dark brown or black viscous liquid.
Boiling Point:	Decomposes before boiling.
Specific Gravity:	1 000 kg/m^3 at 15°C (OLOA 216Q); 1 075 kg/m^3 at 15°C (OLOA 219).
Vapour Pressure:	4.77x10^{-5} kPa at 25°C (lube oil).
Water Solubility:	< 100 ppb (see comments below).
Partition Co-efficient (n-octanol/water):	Log P_{OW}>8 (see comments below).

Hydrolysis as a Function of pH:	Stable under all conditions.
Adsorption/Desorption:	See comments below.
Dissociation Constant:	Not determined.
Particle Size:	The viscous liquid will not form particles.
Flash Point:	>200°C
Flammability Limits:	Will burn in the presence of enough heat and oxygen.
Autoignition Temperature:	>200°C
Explosive Properties:	Not known to be explosive.
Reactivity/Stability:	Stable to acid and base but will react with strong oxidising agents.

3.1 Comments on Physico-Chemical Properties

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

The water solubility is stated as <100 parts per billion and is based on the water solubility determined for oil additive detergents that are stated to be similar in structure (Rausina et al., 1996). The level stated is consistent with the chemical containing hydrophobic alkyl chains. In water, the calcium salt of the notified chemical would be expected to be insoluble, as illustrated by soap binding with calcium in hard water as soap scum.

Measurement of the n-octanol/water partition coefficient was attempted using an HPLC method and only brief details were provided. However, only 3.9% of OLOA 219 could be dissolved in acetonitrile, and this had a log P_{OW} of 5.5. The insoluble material can be assumed to have a log P_{OW} > 8, and on the basis of this, the notified chemical is expected to have a log P_{OW} > 8.

No adsorption/desorption data were provided, but the high log P_{OW}, high hydrocarbon content and strong dispersant nature of the chemical indicate that the notified chemical would have a large K_{OC} and adsorb strongly to the organic component of soils and sediments.

Dissociation data for the new chemical, which is a substituted phenol, were not supplied. Substituted phenols are weakly acidic (Morrison and Boyd, 1976) and 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: 58-72%, depending on the individual commercial product.

Hazardous Impurities:

Chemical name:	Phenol, (tetrapropenyl) derivatives
CAS No.:	74499-35-7
Weight percentage:	1.0
Toxic properties:	Expected to be a skin irritant.
Chemical name:	Phenol, (tetrapropenyl) derivatives, manufacture of distillation residues
CAS No.:	220794-73-0

Weight percentage:	0.1
Toxic properties:	Expected to be a skin irritant.

Chemical name:	Lime
Synonyms:	Calcium hydroxide
CAS No.:	1305-62-0
Weight percentage:	0.5
Toxic properties:	On the NOHSC List of Designated Hazardous Substances (NOHSC, 1999a) with a NOHSC Exposure Standard of 5 mg/m^3 (TWA) (NOHSC, 1995).

The following impurity is present only in the high-calcium products.

Chemical name:	Ethylene glycol
CAS No.:	107-21-1
Weight percentage:	<0.1
Toxic properties:	On the NOHSC List of Designated Hazardous Substances (NOHSC, 1999a) with a NOHSC Exposure Standard of 60 mg/m^3 (TWA) and 120 mg/m^3 (STEL) (NOHSC, 1995).

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

The following impurities are present only in the high-calcium products.

Chemical name	***CAS number***	***Percent by weight***
Calcium carbonate	471-34-1	<15
Alcohols, C_{9-11}-iso, C_{10} rich	6852-85-2	<2
Calcium long chain alkylbenzene sulfonates	Accession numbers 155454 and 178644	5

Additives/Adjuvants:

Chemical name:	Distillates (petroleum) solvent refined light paraffinic & Distillates (petroleum) hydrotreated heavy paraffinic.
Synonyms:	Lube
CAS No.:	64742-54-7 & 64741-88-4
Weight percentage:	26-31%
Toxic properties:	Category 2 carcinogens, with concentration cut-offs of 0.1 %, unless it contains less than 3 % DMSO extract as measured by IP 346 (NOHSC, 1999a).

Chemical name:	Silicon based foam inhibitor
CAS No.:	Not provided.

Weight percentage: 30-45 ppm

5. USE, VOLUME AND FORMULATION

Original assessment

The notified chemical is one of the components used in formulating additive mixtures for finished lubricants such as marine, railroad and heavy-duty vehicle diesel oils, and passenger car gasoline engine oils. The notified chemical as a sulfurized calcium phenate detergent provides corrosion and oxidation protection to lubricating oils. It can also control piston deposit formation to prevent engine wear.

Extension application

The notified chemical will be imported as an additive component in transmission, engine and industrial lubricant ready to use finished lubricant products.

Original assessment

The notifier did not provide the volume of importation in the first 5 years. However, they estimate that import volumes for the formulated additive packages will be up to 5 000 tonnes/annum, which is equivalent to approximately 3 000 tonnes/annum of the notified chemical, calculated using an active ingredient percentage of 60%.

Extension application

Up to one tonne of the notified chemical will be imported per annum by each of first five years.

The active levels of the notified chemical in the low calcium versions, New OLOA 216C, 216Q and 218A, are 60, 58 and 58%, respectively in highly refined lubricating oil solvents. The active levels of the notified chemical in the high calcium versions, New OLOA 219, 219M and 219C are 69, 69 and 72%, respectively. These OLOA products are present in finished oil at 1-5% (0.5-3.5% notified chemical) but may be as high as 18% (about 12.5% notified chemical) in some marine lubricants.

Original assessment

The OLOA products will be imported either as a component of formulated additive packages (5-65% OLOA, 3-47% active ingredient) or neat (100% OLOA, 58-72% active ingredient) by ship in bulk, marine iso-tanks and 200 L drums. Drums are 16 gauge steel and iso-tanks are rigid steel containers. The finished engine oil products will be packaged in litre bottles, 200 L drums, iso-tanks or in bulk shipments.

Extension application

The notified chemical in finished lubricants will be shipped by sea in isotainers, 205 L steel drums or small plastic containers (1-5 L capacity). All goods will be transported directly by road to storage facility at Mobil Oil Australia blending facility at Yarraville.

6. OCCUPATIONAL EXPOSURE

The table identifies the nature of work done where occupational exposure to the notified chemical, in additive package, may occur during transport, storage and reformulation.

Nature of Activity & (Number of Workers)	***% NEW OLOA (% Notified chemical)***	**Maximum Potential *Exposure Duration***
Transport and storage		
Unloading	5-100	0.5 hour/day; 7 days/year.
(1-2)	(3-72)	
Sampling	5-100	0.5 hour/day; 22 days/year.
(1-2)	(3-72)	

Analysing	5-100	0.5 hour/day; 22 days/year.
(1-2)	(3-72)	
Loading	5-100	0.5 hour/day; 50 days/year.
(1-2)	(3-72)	
Cleaning	5-100	4 hours/day; 2 day/year.
(1-2)	(3-72)	
Reformulation		
Sampling	1-100	0.5 hour/day; 35 days/year.
(2-4)	(0.5-72)	
Analysing	1-100	0.5 hour/day; 35 days/year.
(1-2)	(0.5-72)	
Unloading	1-100	1 hour/day; 9 days/year.
(1-2)	(0.5-72)	
Tank car cleaning	1-100	2-3 hours/day; 9 days/year.
(1-2)	(0.5-72)	
Loading	1-18	1 hour/day; 24 days/year.
(1-2)	(0.5-12.5)	
Facility cleaning	1-18	1 hours/day; 3 day/year.
(1-2)	(0.5-12.5)	
Drumming/bottling	1-18	8 hours/day; 18 days/year.
(1-2)	(0.5-12.5)	

TRANSPORT AND STORAGE

Transport and storage workers are not expected to be exposed to the additive packages containing the notified chemical during shipment in isotanks or drums except in the case of an accident involving spillage. The formulated additive package together with New OLOA products will also be imported in bulk, and transferred from the ship to a holding tank, then to road tankers. Dermal and ocular exposure of the waterfront and transport workers to drips and spills of the notified chemical (3-72%) in either neat or formulated additive packages is possible during the connection and disconnection of the transfer hoses during these procedures. New OLOA products have a very low vapour pressure and, as mineral oil based products, a high viscosity, minimising the possibility of vapour and aerosol formation. Inhalation is therefore not expected to be an important route of exposure.

During sampling and analysis of the additive package there may be skin contact as sampling devices and analytical equipment are manipulated.

New OLOA products (58-72% notified chemical) and the formulated additive package (3-47% notified chemical) that delivered to the customer site in bulk tankers or in isotanks will be transferred to holding tanks by pumps. The delivery system will be equipped with an air back-flush system to minimise any spillage on disconnection. The tanker or isotank will be steam cleaned. Dermal and ocular exposure to drips and spills of the notified chemical is possible during these operations. Workers involved in hose transfer wear gloves, eye protection, protective clothing and hard hats.

REFORMULATION

At reformulation sites, New OLOA products shipped in drums will be transferred into the blend tank by drum pump. The notifier states that it takes 10 minutes for a worker to place a drum pump and transfer drum contents. During the connection and disconnection of lines, incidental skin contact from splashes, drips and spills is possible.

OLOA products arriving in either isotanks or road tanker will be unloaded and transferred to storage tanks via 10 cm hosing. Fastening a hose line takes about 10 minutes for a worker. A special air back flush system is used to prevent spillage during transfer. The notifier estimates that by adhering to ISO 9001 procedures, spills and leaks will be minimised. Transfer from storage tanks to the blend tank will be automated, using computer-controlled valves.

OLOA products in storage tanks, and blended lubricant will be sampled for laboratory analysis. Dermal exposure to the notified chemical as part of the blended lubricant is possible during the sampling. The notifier states that minimal exposure will occur during the laboratory testing, which will occupy several minutes per blend.

Blending of the OLOA products into finished lubricant occurs in a closed system at 60°C and is computer controlled, thereby excluding the potential for occupational exposure. The blended lubricant (0.5-12.5% notified chemical) is transferred automatically to a storage tank. From there it can either be dispensed directly into tanker trucks via 10 cm pump lines or packaged into 200 L drums. 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 and skin contact with contaminated drum surfaces may occur.

Bulk road tanker filling is performed by a transfer hose. Dermal exposure to drips and spills of blended lubricant is possible during the connection and disconnection of transfer hoses during the filling of bulk tankers.

The blend tank and the packaging lines are cleaned by rinsing with clean lubricating oil. Maintenance workers handling the equipment used for blending and filling may also come into dermal contact with residues containing the notified chemical.

The notified chemical will be handled by employees of major Australian lubricant manufacturers. The notifier states that inspections of their customer sites have found that their blending facilities are well ventilated, with control systems for accidental spills and wastewater treatment. Workers involved in the blending activities receive training in the handling of additive packages, and wear personal protective equipment such as gloves, eye protection, protective clothing and hard hats. Laboratory staff wear lab coats and eye protection.

DISTRIBUTION

The finished lubricant will have widespread use, and will be used by both professional and home motor mechanics. The transport, storage and retail sale of the lubricants will involve a large number of workers, but should involve little risk of exposure to the notified polymer, except in the case of an accidental spill.

END USERS

The final lubricant products containing 0.5-12.5% notified chemical will be sold in bottles, drums or bulk to customers and used as marine diesel engine lubricating oils, railroad diesel engine oils, heavy-duty vehicle diesel oils and passenger car gasoline engine oils.

Ship or dockside workers, railway workers and heavy-duty vehicle workers may receive skin and eye contact to the finished lubricant containing the notified chemical when they transfer the formulated lubricant from trucks to the fuel containers of ship, railway vehicle or heavy-duty vehicle engines and during drum cleaning. Exposure to the notified chemical during engine operation is unlikely as the lubricant oil is burnt.

Occupational exposure to the products containing the notified chemical will also occur at motor manufacturing and repair facilities throughout Australia. A large number of motor mechanics will be exposed to the products under a wide range of conditions. The motor oils are used to top up reservoirs or, less frequently, as a complete

lubricant change in engines. There is potential for exposure when oils are added to and drained from systems. Exposure of the hands may be significant as it is uncommon for gloves to be worn during addition of these products to engines

7. PUBLIC EXPOSURE

The finished commercial oils are packaged into 1 L, 4 L bottles and 200 L drums and can be sold to the public. Hence, potentially exposed populations include do-it-yourself (DIY) mechanics who add the finished oil product to internal combustion engines. Additionally, 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 Sheet (MSDS) provided for the notified substance, a spill should be contained with absorbent material (soil, sand or other inert material) and sealed in properly labelled drums for disposal. Runoff should be prevented from entering drains and waterways.

8. ENVIRONMENTAL EXPOSURE

8.1 Release

The blending operations are performed at specially constructed sites owned and operated by lubricant manufacturers, and up to twenty sites in Australia may be involved in producing lubricants which contain the notified chemical. Release to the environment prior to end use is expected to occur only in the unlikely event of an accident during transport or an accidental leak. The additive packages containing the notified chemical will be delivered to the blending facilities in bulk, isotanks (20,000 L) or steel drums and transferred to storage tanks. 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 where residual hydrocarbon based products will be separated from the aqueous stream by the Australian Petroleum Industry (API) process, with a claimed removal of greater than 95%. 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. Empty drums containing residual notified chemical would be steam cleaned, with the resultant aqueous waste sent to on-site waste-water treatment facilities.

The finished lubricants will be sold in drums and bulk to industrial and commercial customers and bottles to the domestic market. At marine customer sites, containers holding residual notified chemical would be steam-cleaned with the waste-water entering a treatment facility at the site and treated in a similar fashion to that at blending facilities. No further information was provided on release. If the worst case is assumed, where containers are disposed of to landfill and not recycled, and a maximum of 1% of the import volume remains in containers after transfer to engines, up to 30 tonnes of the notified chemical would be released to landfill.

The notified chemical is not substantially altered during use and does not decompose in crankcases because it is thermally stable. However, it is burned in the engine during oil consumption, with most of the ash remaining after combustion returning to the sump as insolubles, or emitted as particulate matter in the exhaust. The notified chemical will be attracted to and coat insoluble materials (soot particles, insoluble resins) and can be filtered or centrifuged out of the oil. Over time, fresh oil containing the notified chemical may be added to keep sump levels constant or during maintenance, the oil may be drained completely and replaced with new oil. In cases where specialised technicians perform oil changes or repairs, the used oil will be incinerated or sent for recycling. However, in the case of passenger vehicles where DIY enthusiasts perform at home oil changes, a significant percentage of the oil sold for use in these vehicles is released inappropriately. Information presented at an API 1997 Conference, showed that of oil sold for use in the automotive market, 14% was sold to DIY enthusiasts (Snow, 1997). Of this oil, approximately 13% was collected for recycling, 32% was lost or consumed during use, and the remaining 55% was released inappropriately eg buried, tipped into landfill, used for weed control, tipped into stormwater, or stored. A market breakdown for usage of the notified chemical was not provided, however in the worst case where all of the notified chemical will be sold for use in the automotive market, 14% will be sold to DIY enthusiasts and about half of this disposed of inappropriately. In reality, the amount of notified chemical disposed of inappropriately will be less than predicted in the worst case scenario, as notified chemical used in marine and railroad diesel engines is consumed during use.

Since the use of the lubricating oils will occur throughout Australia, all releases resulting from use or disposal of old oil will be very diffuse, and release of the notified material in high concentrations is unlikely except as a result of accidents during transport.

8.2 Fate

A CO_2 evolution test (EC Method C. 4-C) was conducted on a surrogate for the notified chemical, "old" OLOA 216Q (Edwards, 1997). The substance was added directly to chemically defined liquid medium and inoculated with activated sewage sludge bacteria, providing a final concentration equivalent to 10 mg carbon/L. The test flasks were purged of background CO_2 prior to connection, in the exit air line of each test flask, of CO_2 absorber bottles filled with $Ba(OH)_2$. Each test flask was placed on a rotary shaker for the test duration of 28 days. Unreacted $Ba(OH)_2$ was titrated against acid to determine the amount of CO_2 produced. Results indicated that OLOA 216Q is not readily biodegradable as the requirement for 60% biodegradation within 28 days, achieved within 10 days of reaching 10% biodegradation was not met. There was insufficient biodegradation of OLOA 216Q to allow statistical analysis. The CO_2 production from the reference chemical, performed under identical test conditions with sodium benzoate as substrate, indicated 66% biodegradation of the benzoate moiety after 2 days, and 89% biodegradation after 28 days, thereby validating the test. In addition, results from testing a toxicity control, consisting of OLOA 216Q at 10 mg carbon/L combined with sodium benzoate at 10 mg carbon/L, showed that at the concentration tested, OLOA 216Q is not inhibitory to respiration of the sewage bacteria. Based on the results for this surrogate, the notified chemical is not expected to be readily biodegradable.

Although not readily biodegradable, the material in landfill would be very slowly degraded through biological and abiotic processes.

In the case of accidental release to land, the material would not be mobile because of the anticipated high K_{OC} (see physico-chemical properties) and would adsorb onto and strongly associate with the organic component of soils and sediments. Similarly, in the event of accidental release into the water compartment or inappropriate disposal, it is likely to associate with suspended organic material, and eventually be incorporated into sediments.

The high log P_{OW}, modest molecular weight (664 g/mol for the 3-sulphur bridge) and anticipated 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 thereby limiting the potential for bioaccumulation.

Incineration of waste oil containing the notified material would destroy the substance with evolution of water vapour and oxides of carbon and sulphur, and 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

The toxicological data provided by the notifier were determined based on 2 similar existing commercial products, OLOA 216Q and OLOA 219, which differ only in the length of the alkyl side chain of the molecule. Due to their structural similarity, the toxicological profiles of OLOA 216Q and OLOA 219 are expected to be similar to the notified chemical.

Most of the toxicological studies were carried out in the laboratories compliance with Good laboratory Practice (GLP) standards and Quality Assurance (QA) regulations.

9.1 Acute Toxicity of OLOA 216Q

Summary reports (only) were provided for assessment in some instances as indicated in the table below.

Summary of the acute toxicity of OLOA 216Q

Test	Species	Outcome	*Reference*
acute oral toxicity 1*	rat	LD_{50} > 5 000 mg/kg	Rittenhouse & Narcisse, 1975
acute oral toxicity 2	rat	LD_{50} > 5 000 mg/kg	Glaza, 1997a
acute dermal toxicity 1*	rabbit	LD_{50} > 5 000 mg/kg	Rittenhouse & Narcisse, 1975
acute dermal toxicity 2	rat	LD_{50} > 2 000 mg/kg	Glaza, 1997b
skin irritation 1*	rabbit	A moderate skin irritant	Rittenhouse & Narcisse, 1975
skin irritation 2	rabbit	A slight to moderate skin irritant	Glaza, 1997c
eye irritation 1*	rabbit	A slight to moderate eye irritant	Rittenhouse & Narcisse, 1975
eye irritation 2	rabbit	A slight to moderate eye irritant	Glaza, 1997d
skin sensitisation	guinea pig	Inconclusive.	Morris, 1997

* summary reports only provided.

9.1.1 Oral Toxicity 1 (Rittenhouse & Narcisse, 1975)

Species/strain:	Rat/Sprague-Dawley
Number/sex of animals:	Test group: 10 males; Control group: 8 males.
Observation period:	14 days.
Method of administration:	A single oral dose of 5 g/kg (undiluted) was given by gavage.
Test method:	Similar to OECD TG 401
Mortality:	None.
Clinical observations:	None.
Morphological findings:	None.
Comment:	None.
LD_{50}:	> 5 000 mg/kg
Result:	OLOA 216Q was of very low acute oral toxicity in rats.

9.1.2 Oral Toxicity 2 (Glaza, 1997a)

Species/strain:	Rat/Sprague-Dawley
Number/sex of animals:	5/sex.
Observation period:	14 days.
Method of administration:	A single oral dose of 5 g/kg in corn oil was given by gavage.
Test method:	OECD TG 401

Mortality:	None.
Clinical observations:	Some animals had red-stained face, soft stool and dark/yellow urogenital area. All animals returned to normal by day 7.
Morphological findings:	None.
Comment:	None.
LD_{50}:	> 5 000 mg/kg.
Result:	OLOA 216Q was of very low acute oral toxicity in rats.

9.1.3 Dermal Toxicity 1 (Rittenhouse & Narcisse, 1975)

Species/strain:	Rabbit/New Zealand White
Number/sex of animals:	Intact skin group: 3 males; Abraded skin group: 3 males.
Observation period:	14 days.
Method of administration:	A single dermal dose of 5 g/kg was applied under an occlusive dressing on the back area for 24 hours.
Test method:	Similar to OECD TG 402
Mortality:	None.
Clinical observations:	Three days after dosing, skin in most treated areas was erythematous. At day 14, the fur was matted with OLOA 216Q and the skin appeared normal.
Morphological findings:	None.
Comment:	None.
LD_{50}:	> 5 000 mg/kg.
Result:	OLOA 216Q was of low dermal toxicity in rabbits.

9.1.4 Dermal Toxicity 2 (Glaza, 1997b)

Species/strain:	Rat/Sprague-Dawley
Number/sex of animals:	5/sex.
Observation period:	14 days.
Method of administration:	A single dermal dose of 2 000 mg/kg applied to the back area under a semi-occlusive dressing for 24 hours.
Test method:	OECD TG 402
Mortality:	None.
Clinical observations:	See Draize scores.

Morphological findings: None.

Draize scores:

Draize scores for oedema were zero for all animals during the study.

Time after treatment (days)	*Animal #*									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Erythema										
1	[i]1	0	0	0	0	0	0	0	0	1
3	1	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0

[i] see Attachment 1 for Draize scales

Comment: Slight erythema was seen in 2 animals on day 1 and 1 animal on day 3.

LD_{50}: > 2 000 mg/kg.

Result: OLOA 216Q was of low dermal toxicity in rats.

9.1.5 Inhalation Toxicity

No inhalation toxicity study report was provided for assessment.

9.1.6 Skin Irritation 1 (Rittenhouse & Narcisse, 1975)

Species/strain: Rabbit/New Zealand White

Number/sex of animals: 6 females.

Observation period: 7 days.

Method of administration: A dermal dose of 0.5 mL was applied under an occlusive dressing to an intact area and an abraded area on the back of each rabbit for 24 hours.

Test method: OECD TG 404

Draize scores (intact skin):

Time after treatment (days)	*Animal #*					
	1	*2*	*3*	*4*	*5*	*6*
Erythema						
1	[a]1	2	2	2	2	2
2	1	2	2	2	2	2

3	1	2	2	2	2	2
7	0	2	0	0	1	2
Oedema						
1	0	2	1	2	2	2
2	0	0	2	1	1	2
3	0	0	1	0	2	1
7	0	0	0	0	0	0

Draize scores (abraded skin):

Time after treatment (days)	***Animal #***					
	1	***2***	***3***	***4***	***5***	***6***
Erythema						
1	[a]2	2	2	2	2	2
2	1	2	2	1	2	2
3	0	2	2	1	2	2
7	0	2	0	0	0	2
Oedema						
1	0	2	1	1	2	2
2	0	1	1	0	2	2
3	0	0	2	0	2	1
7	0	0	0	0	0	1

[a] see Attachment 1 for Draize scales

Comment: The results in this study were observed after a 24-hour dermal treatment.

Most animals had moderate erythema and oedema in both intact and abraded skin areas for 3 days. The irritation effects persisted in 3 rabbits at day 7.

Mean score for all animals (intact skin): erythema, 1.94; and oedema, 1.05.

Result: OLOA 216Q was a moderate irritant to the skin of rabbits under the experimental conditions.

9.1.7 Skin Irritation 2 (Glaza, 1997c)

Species/strain: Rabbit/New Zealand White

Number/sex of animals: 3/sex.

Observation period: 7 days.

Method of administration: A dermal dose of 0.5 mL was applied under a semi-occlusive dressing to the back area of each rabbit for 4 hours.

Test method: OECD TG 404

Draize scores:

Time after treatment	*Animal #*					
	1	*2*	*3*	*4*	*5*	*6*
Erythema						
4 hours	[a]2	1	2	2	2	2
24 hours	1	1	2	2	1	2
48 hours	1	1	1	1	1	1
72 hours	1	1	1	1	1	1
96 hours	1	1	1	1	1	1
7 days	0	0	0	0	0	0
Oedema						
4 hours	2	1	2	3	2	2
24 hours	2	2	2	2	1	2
48 hours	1	2	1	1	1	1
72 hours	1	1	1	1	1	0
96 hours	1	1	1	1	0	0
7 days	0	0	0	0	0	0

[a] see Attachment 1 for Draize scales

Comment: Slight to moderate irritation was noted in animals after treatment. All irritation cleared by day 7.

Mean scores: erythema, 1.2; and oedema, 1.3.

Result: OLOA 216Q was a slight to moderate irritant to the skin of rabbits.

9.1.8 Eye Irritation 1 (Rittenhouse & Narcisse, 1975)

Species/strain: Rabbit/New Zealand White

Number/sex of animals: 6 females.

Observation period: 7 days.

Method of administration: An ocular dose of 0.1 mL was applied to conjunctival sac of one eye. The untreated eye served as the control.

Test method: OECD TG 405

Draize scores:

Draize scores for cornea (opacity and area) and iris were zero for all animals during the study.

	Time after instillation														
Animal	***1 hour***			***24 hours***			***48 hours***			***72 hours***			***7 days***		
Conjunctiva	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***
1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

2	2	0	0	2	0	0	1	0	0	0	0	0	0	0	0
3	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
4	1	0	0	2	0	0	1	0	0	1	0	0	0	0	0
5	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0
6	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0

[1] see Attachment 1 for Draize scales
r = redness c = chemosis d = discharge

Comment: One hour after treatment, all animals had conjunctival redness which resolved by day 7.

Mean scores: erythema, 0.6; and oedema, 0.0.

Result: OLOA 216Q was a slight to moderate irritant to the eyes of rabbits.

9.1.9 Eye Irritation 2 (Glaza, 1997d)

Species/strain: Rabbit/New Zealand White

Number/sex of animals: Unwashed group: 6 (sex not provided);
Washed group: 3 (sex not provided).

Observation period: 96 hours.

Method of administration: An ocular dose of 0.1 mL was applied to conjunctival sac of the right eye. The untreated left eye served as control.

Animals in the washed group had their eyes washed for 1 minute starting 30 seconds after installation.

Test method: OECD TG 405

Draize scores of unwashed eyes:

Draize scores for iris were zero for all animals during the study.

	Time after instillation									
Animal	***1 hour***		***1 day***		***2 days***		***3 days***		***4 days***	
Cornea	***o***	***a***	***o***	***a***	***o***	***a***	***o***	***a***	***o***	***a***
1	[1]0	0	0	0	0	0	0	0	0	0
2	1	1	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0

Conjunctiva	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***
1	2	0	1	2	0	0	1	0	0	0	0	0	0	0	0
2	1	1	2	2	0	0	1	0	0	0	0	0	0	0	0
3	1	0	2	1	0	0	1	0	0	0	0	0	0	0	0
4	1	0	2	2	0	1	1	0	0	0	0	0	0	0	0

5	1	0	2	2	1	1	0	0	0	0	0	0	0	0	0
6	1	0	1	2	1	1	1	0	0	1	0	0	0	0	0

Draize scores of washed eyes:

Draize scores for cornea (opacity and area) and iris were zero for all animals during the study.

	Time after instillation											
Animal	***1 hour***			***1 day***			***2 days***			***3 days***		
Conjunctiva	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***
1	1	0	2	2	0	0	0	0	0	0	0	0
2	1	0	2	2	0	0	1	0	0	0	0	0
3	1	0	2	1	0	0	0	0	0	0	0	0

[1] see At[1] see Atachment 1 for Draize scales
o = opacity a = area r = redness c = chemosis d = discharge

Comment: The results of sodium fluorescein examinations at 24 hours were negative to all the animals.

Rinsing the eyes after treatment showed no substantial effects on the severity and persistence of ocular irritation due to OLOA 216Q.

Mean scores (unirrigated eyes): corneal effects, 0.0; iris effects, 0.0; conjunctival irritation, 0.94; and conjunctival oedema, 0.1.

Result: OLOA 216Q was a slight to moderate irritant to the eyes of rabbits.

9.1.10 Skin Sensitisation (Buehler method) (Morris, 1997)

Species/strain: Guinea pig/Hartley

Number of animals: Test group: 10/sex;
Naive control group: 5/sex.

Induction procedure:

test group: Topical Inductions:
day 0-14 Dermal inductions of 25% OLOA 216Q in mineral oil (0.3 mL) were given by an occluded Hill Top Chamber at left shoulder site of each animal for 6 hours. Three inductions were applied on weekly basis at the same site.

control group: No treatment.

Challenge procedure:

day 28 Test and naive control groups:
A 6 hour occluded dermal application of 5% OLOA 216Q in mineral oil (0.3 mL) was given by a Hill Top Chamber to a skin site that had not been exposed to OLOA 216Q previously.

Test method: OECD TG 406

Challenge outcome:

	Test animals		*Naive Control animals*	
Challenge concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
5%	**6/20 (30%)	2/20 (10%)	3/10 (30%)	2/10 (20%)

* time after patch removal

** number of animals exhibiting positive response (Draize scores≥1)

Comment: A screening pilot study was carried out to select the concentrations for the main study.

The positive rates from the naive control group were greater than 15% after challenge. The incidence and severity of responses of the test group were similar to that of the naive control group.

The naive control group did not receive inductions of the vehicle in this study. No positive control group was included in the study.

Result: Inconclusive, due to the high positive responses in the naive control group.

9.2 Repeated Dose Toxicity of OLOA 216Q

9.2.1 Combined Four-Week Repeated-Dose Oral Toxicity, Reproduction and Neurotoxicity Screen in Rats (Schroeder, 1998)

Species/strain: Rat/Sprague-Dawley CD.

Number/sex of animals: Subchronic study: 4 dose groups of 6/sex per group, the control and high dose groups had additional 6/sex each as the recovery groups.

Neurotoxicity study: 2 dose groups (control and high dose) of 6 males per group, those 2 groups had additional 6 males per group as the recovery groups.

Reproduction study: 4 dose groups of 12/sex per group.

Method of administration: Oral by gavage.

Dose/Study duration: Control group: 0 mg/kg/day;
Low dose group: 50 mg/kg/day;
Mid dose group: 300 mg/kg/day;
High dose group: 1 000 mg/kg/day
(vehicle: corn oil).

Subchronic study
Animals were treated once daily for 29 consecutive days. The animals in the recovery groups had 2-week treatment free period after treatment.

Neurotoxicity study
Animals were treated once daily for 28 consecutive days. The animals in the recovery groups had 2-week treatment free period after treatment.

Reproduction study
Parental animals (F_0) were treated for 31 days prior to mating and the treatment continued during the ensuring mating, gestation and lactation period to lactation Day 4. The males were treated through postmating

until sacrifice (total of 70 days of treatment).

Test method: OECD TG 407

Clinical observations:

SUBCHRONIC/NEUROTOXICITY GROUPS: ALL ANIMALS SURVIVED DURING THE STUDY. CLINICAL EXAMINATIONS, BODYWEIGHTS, BODY WEIGHT GAINS AND FEED EFFICIENCY DATA IN THE TEST GROUPS INCLUDING THE RECOVERY GROUPS WERE COMPARABLE WITH CONTROL GROUPS.

Functional observational battery (FOB) data from neurotoxicological examinations were found normal compared with controls. However, in 1 of the 2 pretest trials, a decrease in forelimb and hindlimb grip strength and an increase in landing foot splay were found. The reasons were unclear.

Reproductive study: No mortality or abnormal clinical observations were seen during the study.

During the premating period, bodyweights and food consumption/feed efficiency in treated animals of both sexes were normal when compared with controls.

No adverse effect related to treatment were found in the reproduction examinations including mating indices (both sexes), female pregnancy rates and male fertility indices.

During the postmating period, lower food consumption, bodyweights and bodyweight gain in high-dose males were considered to the treatment-related. In females, maternal bodyweights and food consumption during gestation and lactation periods, gestation length and parturition data were comparable with controls. The litter size and pup data (bodyweight, sex distribution, surviving and dead pup observations) did not show any abnormalities in treated groups.

Clinical chemistry/Haematology:

SUBCHRONIC GROUP: BIOCHEMISTRY RESULTS IN TEST GROUPS INCLUDING THE RECOVERY GROUPS WERE COMPARABLE WITH CONTROL GROUP EXCEPT LOWER CHOLESTEROL LEVELS WERE FOUND IN MID AND HIGH-DOSE MALES. THE REASON WAS UNCLEAR.

Haematological data in the test groups including the recovery group were comparable to that of the control group except lower mean corpuscular volume in mid-dose females and lower mean corpuscular haemoglobin values in mid and high-dose females. However, no dose-response relationship established.

Urinalysis data in the test groups including the recovery group were comparable to that in the control group.

Necropsy:

SUBCHRONIC/NEUROTOXICITY GROUPS: ORGAN WEIGHT RESULTS IN THE LOW AND MID-DOSE GROUPS WERE COMPARABLE WITH THE CONTROL GROUP. HIGH-DOSE FEMALES HAD INCREASES IN ABSOLUTE ADRENAL WEIGHTS AND ADRENAL TO BRAIN WEIGHT RATIOS, WHICH WERE TREATMENT-RELATED. ADDITIONALLY, THE HIGH-DOSE FEMALES IN THE RECOVERY GROUP HAD SLIGHT HIGHER LUNG WEIGHTS, ABSOLUTE AND RELATIVE TO EITHER THE FINAL BODYWEIGHTS OR BRAIN WEIGHTS. AS NO DOSE-RESPONSE RELATIONSHIP WAS FOUND FOR THIS CHANGE, IT WAS NOT CONSIDERED TO BE TREATMENT-RELATED. HIGH-DOSE MALES IN THE RECOVERY GROUP HAD HIGHER ADRENAL WEIGHTS, BOTH ABSOLUTE AND RELATIVE TO EITHER FINAL BODYWEIGHTS OR BRAIN WEIGHTS. IN THE ABSENCE OF SIMILAR CHANGES IN HIGH DOSE MALES, IT IS NOT CONSIDERED TREATMENT RELATED.

All central nervous system and peripheral nervous tissues examined in the neurotoxicity study were within normal limits. The results of brain weight, length and width data did not show any abnormalities.

Microscopic examinations did not find any treatment-related abnormalities.

REPRODUCTIVE GROUP: ORGAN WEIGHT RESULTS IN ADULT MALES WERE COMPARABLE TO THOSE IN THE CONTROL GROUP EXCEPT SLIGHT HIGHER KIDNEY, LIVER AND TESTES TO BODYWEIGHT RATIOS IN HIGH-DOSE MALES DUE TO THE LOWER TERMINAL BODYWEIGHTS OF THESE ANIMALS. THE ORGAN WEIGHT DATA IN THE ADULT FEMALES WERE COMPARABLE TO THE CONTROL GROUP EXCEPT FOR A STATISTICALLY SIGNIFICANT INCREASE IN ADRENAL WEIGHTS.

FINE VACUOLAR CHANGES WERE SEEN IN THE CELLS OF THE ZONA FASCICULATA OF THE ADRENAL CORTEX IN SEVERAL FEMALES FROM ALL GROUPS. THE SEVERITY SEEN IN MID AND LOW DOSE GROUPS WAS COMPARABLE WITH CONTROLS. SEVERE CHANGES OCCURRED IN THE HIGH-DOSE FEMALES. THIS CHANGE IS PROBABLY INDICATIVE OF LIPIDOSIS.

Sperm analysis showed that the mean percent motility, caudal epididymal sperm count and sperm morphology were normal.

There were no macroscopic findings related to the treatment in the adult animals or pups.

Comment:

In the range finding study consisting of 3/sex/dose, treatment related decreases in bodyweights and bodyweight gains, and slight decrease in food consumption were found at 1 000 mg/kg/day level after 14 days treatment.

Several changes in the main study were considered to be treatment-related:

- In the males at 1 000 mg/kg/day, decreases in bodyweights and bodyweight gains were observed during the mating and postmating period (week 5-10). Efficiency of food utilization was reduced in this group during the postmating period (week 8-10).

- In the females at 1 000 mg/kg/day, an increase in adrenal weights was found in both subchronic and reproductive groups. This change was accompanied by an increased severity of fine vacuolar changes in the cells of the zona fasciculata in the adrenal cortex.

Examination results on neurotoxic and reproductive indices from the test groups were comparable to that from the control groups.

Result:

The NOEL for subchronic toxicity was 300 mg/kg/day based on the changes of bodyweights, bodyweight gains, food utilisation, and adrenal weights and structure at 1 000 mg/kg/day. The NOEL for reproductive toxicity and neurotoxicity was ≥ 1 000 mg/kg/day (the highest dose tested).

9.3 Genotoxicity of OLOA 216Q

9.3.1 *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay (Lawlor, 1997)

Strains:	*S. typhimurium* TA1535, TA1537, TA98 and TA100; *E. coli* WP2*uvr*A.
Metabolic activation:	Liver fraction (S9 mix) from rats pretreated with Aroclor 1254.
Concentration range:	Triplicate plates were prepared for each bacterial strain and dose level, in both the presence and the absence of S9-mix. 25% Pluronic F127 in ethanol was used as the vehicle. First and second tests: 0, 100, 250, 500, 1 000, 5 000 and 10 000 µg/plate in all strains. Third test: 0, 5, 10, 50, 100, 500 and 5 000 µg/plate in all strains. Additionally, 100, 250, 500, 1 000, 5 000 and 10 000 µg/plate were tested in TA98. Positive controls: (When with S9 mix)

benzo(a)pyrene for TA98; and
2-aminoanthracene for the remaining strains.

(When without S9 mix)
2-nitrofluorene (2-NF) for TA98;
sodium azide for TA100 and TA1535;
ICR-191 for TA1537; and
4-nitroquinoline-N-oxide for WP2*uvr*A.

Test method: OECD TG 471 (plate incorporation method)

Comment: Cytotoxicity of OLOA 216Q in the test system was determined in a dose range finding study.

Slight precipitation was observed at 5-1 000 µg/plate and moderate precipitation, at 500-10 000 µg/plate. There was no toxicity to the bacteria under the test conditions.

Under the conditions of the study, OLOA 216Q caused no substantial increases in revertant colony numbers over control counts at any concentration in either the presence or absence of the rat liver microsomal enzymes.

All positive controls responded appropriately.

Result: OLOA 216Q was non mutagenic under the conditions of the test.

9.3.2 Micronucleus Assay in the Bone Marrow Cells of the Mouse (Ivett, 1997)

Species/strain: Mouse/Cr1:CD-1(ICR)BR

Number and sex of animals: 5/sex/group.

Doses: 1250, 2 500 and 5 000 mg/kg (vehicle: peanut oil).
Samples were taken at 24, 48 and 72 hours.

Positive control: cyclophosphamide (CP).

Method of administration: Intraperitoneal injection.

Test method: OECD TG 474

Comment: A pilot study including dose limit test and dose selection test was carried out.

No animal deaths occurred and no adverse effects were observed following treatment. The test article did not induce statistically significant changes in PCE:NCE ratio.

No significant increase in micronucleated polychromatic erythrocytes compared to the controls.

Positive control responded appropriately.

Result: OLOA 216Q was non-clastogenic in bone marrow cells of the mouse *in vivo*, under the conditions of the test.

9.4 Overall Assessment of Toxicological Data of OLOA 216Q

OLOA 216Q was of very low acute oral toxicity (LD_{50} >5 000 mg/kg) in rats and low acute dermal toxicity in rats (LD_{50} >2 000 mg/kg) and rabbits (LD_{50}> 5 000 mg/kg). It was a slight to moderate skin irritant in rabbits with the degree of irritation increasing with the duration of exposure. OLOA 216Q was a slight to moderate eye irritant in rabbits. A skin sensitisation study in guinea pigs using Buehler method was inconclusive. However, the positive responses in the test animals after challenge were likely to be caused by irritant effects rather than the skin sensitisation.

A combined subchronic toxicity study and neurotoxicity and reproductive screen in rats was provided. The NOEL for subchronic toxicity was 300 mg/kg/day based on the decrease in food consumption and bodyweight gain in males and pathological changes in the adrenal glands of females at 1 000 mg/kg/day. The NOEL for reproductive toxicity and neurotoxicity was ≥ 1 000 mg/kg/day (highest dose tested).

In genotoxicity studies, OLOA 216Q was non-mutagenic in bacteria and non-clastogenic in an *in vivo* micronucleus assay in mice.

Results from acute dermal toxicity study 1 (summary only available) showed that most treated skin areas were erythematous for three days after a 24-hour dermal exposure in rabbits. OLOA 216Q induced moderate skin irritation that persisted up to 7 days. In a second rabbit study conducted according to OECD guidelines, slight to moderate irritation was observed. Irritant effects cleared by the end the observation period (day 7). Based on the available data, OLOA 216Q is not classified as a hazardous substance according to NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999b).

9.5 Acute Toxicity of OLOA 219

Summaries (only) were provided for assessment in some instances as indicated in the table below.

Summary of the acute toxicity of OLOA 219

Test	Species	Outcome	*Reference*
acute oral toxicity 1*	rat	LD_{50}>16.1 g/kg	Meyding, 1962
acute oral toxicity 2	rat	LD_{50}>5 g/kg	Cushman, 1986a
acute dermal toxicity 1*	rabbit	LD_{50}>15 g/kg	Meyding, 1962
acute dermal toxicity 2	rabbit	LD_{50}>5 g/kg	Cushman, 1986b
acute inhalation toxicity	rat	LC_{50}>1 670 mg/m^3	Griffis, 1985
skin irritation 1*	rabbit	Moderate to severe irritant	Meyding, 1962
skin irritation 2	rabbit	Slight to moderate irritant	Cushman, 1986c
eye irritation 1*	rabbit	Moderate irritant	Meyding, 1962
eye irritation 2	rabbit	Slight to severe irritant	Cushman, 1985a
skin sensitisation 1	guinea pig	Inconclusive	Cushman, 1985b
skin sensitisation 2	guinea pig	Inconclusive	Carey, 1993
skin sensitisation 3	guinea pig	Maybe a skin sensitiser	Morris, 1994
Insult patch test	human	Slight irritant and skin sensitiser	Carey, 1997

* Summary reports only provided for assessment.

9.5.1 Oral Toxicity 1 (Meyding, 1962)

Species/strain:	Rat/Sprague-Dawley.
Number/sex of animals:	7 males per dose group.
Observation period:	14 days.
Method of administration:	Oral by gavage at 1.07, 10.7 and 16.1 g/kg.
Test method:	OECD TG 401
Mortality:	All animals survived except one died at 16.1 g/kg level on day 2 due to an intubation into lungs.
Clinical observations:	None.
Morphological findings:	One animal died on day 2 due to an intubation error.
Comment:	The single death was not considered to be due to the treatment.
LD_{50}:	> 16.1 g/kg.
Result:	OLOA 219 was of very low acute oral toxicity in rats.

9.5.2 Oral Toxicity 2 (Cushman, 1986a)

Species/strain:	Rat/Sprague-Dawley
Number/sex of animals:	Test group: 5/sex; Control group: 5/sex.
Observation period:	14 days.
Method of administration:	A single oral dose of 5.0 g/kg (vehicle: peanut oil) was given by gavage. The control group received vehicle alone.
Test method:	OECD TG 401
Mortality:	None.
Clinical observations:	None.
Morphological findings:	No treatment-related findings.
Comment:	None.
LD_{50}:	> 5 g/kg.
Result:	OLOA 219 was of very low acute oral toxicity in rats.

9.5.3 Dermal Toxicity 1 (Meyding, 1962)

Species/strain:	Rabbit/albino.
Number/sex of animals:	2/sex.
Observation period:	14 days.
Method of administration:	A single dermal application at 15 g/kg (undiluted) under an occlusive

	dressing for 24 hours.
Test method:	OECD TG 402
Mortality:	None.
Clinical observations:	Severe erythema and oedema were seen at removal. The oedema subsided within 2-3 days, however slight oedema persisted in 3 animals until sacrifice. Erythema subsided in 3-4 days but persisted to a slight degree in 2 rabbits until sacrifice. Slight desquamation and dryness of skin was also observed.
Morphological findings:	None.
Comment:	Draize scores were not provided.
LD_{50}:	> 15 g/kg.
Result:	OLOA 219 was of low dermal toxicity in rabbits.

9.5.4 Dermal Toxicity 2 (Cushman, 1986b)

Species/strain:	Rabbit/New Zealand White.
Number/sex of animals:	Test group: 5/sex; Control group: 5/sex.
Observation period:	14 days.
Method of administration:	A single dermal dose of 5.0 g/kg (vehicle: mineral oil) was applied to trunk under an occlusive dressing for 24 hours. The control group received vehicle alone.
Test method:	OECD TG 402
Mortality:	None.
Clinical observations:	Reduced food intake was observed in 8/10 test animals after treatment. Dry and flaky skin and/or scabbed skin at the application site were seen in all test animals. Slight to moderate erythema with slight oedema was observed in both sexes after 24 hours, and recovered after 7-14 days. Eschar formation was observed in 6/10 animals.
Morphological findings:	Minimal acanthosis (diffuse hyperplasia and thickening of epidermis) and/or hyperkeratosis (hypertrophy of skin) were observed in 9/10 treated animals.
Comment:	No Draize scores were provided.
LD_{50}:	> 5 g/kg.
Result:	OLOA 219 was of low acute dermal toxicity in rabbits.

9.5.5 Inhalation Toxicity (Griffis, 1985)

Species/strain:	Rat/Sprague-Dawley.
Number/sex of animals:	Test group: 5/sex;

	Control group: 5/sex.
Observation period:	14 days.
Method of administration:	Vapours and condensation aerosol from heated OLOA 219 (1.67 mg/L) were administered by nose-only exposure to animals for 60 minutes.
Test method:	OECD TG 403
Mortality:	None.
Clinical observations:	None.
Morphological findings:	None.
Comment:	The average mass median aerodynamic diameter of the aerosol was 3.7 μm, and the average total aerosol concentration was 0.87 mg/L.
LC_{50}:	> 1.67 mg/L (1 670 mg/m^3).
Result:	OLOA 219 can be at most of moderate acute inhalation toxicity in rats.

9.5.6 Skin Irritation 1 (Meyding, 1962)

Species/strain:	Rabbit/albino
Number/sex of animals:	6 (sex not provide).
Observation period:	72 hours.
Method of administration:	A single dermal application (0.5 mL undiluted) under an occlusive dressing to both abraded and intact skin sites of each animal for 24 hours.
Test method:	OECD TG 404

Draize scores for intact skin:

Time after treatment (hour)	***Animal #***					
	1	***2***	***3***	***4***	***5***	***6***
Erythema						
24	[a]1	2	2	2	2	1
72	0	4	3	2	3	4
Oedema						
24	0	0	4	3	4	4
72	0	2	2	0	0	0

[a] see Attachment 1 for Draize scales

Draize scores for abraded skin:

Time after treatment (hour)	*Animal #*					
	1	*2*	*3*	*4*	*5*	*6*
Erythema						
24	[a]1	2	1	1	2	1
72	2	2	3	0	2	2
Oedema						
24	4	4	4	4	4	4
72	0	0	2	0	0	0

[a] see Attachment 1 for Draize scales

Comment: OLOA 219 induced moderate to severe irritation to both intact and abraded skin. The oedema subsided after 72 hours, but erythema became worse and substantial drying of the skin occurred.

Mean scores could not be calculated due to lack of 48 hour observations.

Result: OLOA 219 was a moderate to severe irritant to the skin of rabbits.

9.5.7 Skin Irritation 2 (Cushman, 1986c)

Species/strain: Rabbit/New Zealand White

Number/sex of animals: 12 (sex not provided).

Observation period: 14 days.

Method of administration: A single dermal dose (0.5 mL) was applied under a semi-occlusive dressing on the back of each rabbit for 4 hours.

Test method: OECD TG 404

Draize scores:

Time after treatment	*Animal #*											
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
Erythema												
1 hour	[a]2	2	2	0	3	2	0	2	2	2	2	2
24 hours	1	1	1	0	2	0	0	0	1	1	2	0
48 hours	0	1	0	0	0	0	0	0	0	0	0	0
72 hours	0	0	0	0	0	0	0	0	0	0	0	0
7 days	0	0	0	0	0	0	0	0	0	0	0	0
14 days	0	0	0	0	0	0	0	0	0	0	0	0
Oedema												
1 hour	[a]2	1	1	0	2	1	0	1	1	2	1	1

24 hours	0	0	0	0	1	0	0	0	0	0	1	0
48 hours	0	0	0	0	0	0	0	0	0	0	0	0
72 hours	0	0	0	0	0	0	0	0	0	0	0	0
7 days	0	0	0	0	0	0	0	0	0	0	0	0
14 days	0	0	0	0	0	0	0	0	0	0	0	0

[a] see Attachment 1 for Draize scales

Comment: OLOA 219 caused slight to moderate erythema and oedema after treatment. All irritation signs cleared by 72 hours.

Mean scores: erythema, 0.27; and oedema, 0.05.

Result: OLOA 219 was a slight to moderate irritant to the skin of rabbits.

9.5.8 Eye Irritation 1 (Meyding, 1962)

Species/strain: Rabbit/albino

Number/sex of animals: 6 (sex not provide).

Observation period: 72 hours.

Method of administration: A single ocular application (0.1 mL) to the conjunctival sac of the left eye. Untreated right eye served as the control.

Test method: OECD TG 405

Draize scores:

Draize scores for cornea and iris were zero for all animals at 24, 48 and 72 hours. The scores for "sclera & palpebrae" were provided in the report as combined scores for redness, chemosis and discharge of conjunctiva.

	Time after instillation		
Animal	***1 day***	***2 days***	***3 days***
Sclera & palpebrae			
1	10	4	6
2	8	2	6
3	14	6	6
4	12	6	6
5	14	8	10
6	12	6	6

Comment: Palpebral irritation occurred which was characterised by diffuse beefy redness and obvious swelling with partial eversion of the lids after 24 hours. These signs were partially reversed after 72 hours.

Result: OLOA 219 was a moderate to severe irritant to the eyes of rabbits.

9.5.9 Eye Irritation 2 (Cushman, 1985a)

Species/strain:	Rabbit/New Zealand White.
Number/sex of animals:	9 males.
Observation period:	21 days.
Method of administration:	A single ocular application (0.1 mL) to the conjunctival sac of one eye. The untreated eyes served as the control. After 30-second exposure, the eyes of 3 rabbits were rinsed for 1 minute with distilled water.
Test method:	OECD TG 405

Draize scores of eyes:

The Draize scores for cornea (opacity and area) and iris were zero for all animals during the study. The scores for conjunctiva were listed below.

Time after instillation	***Conjunctiva***	**Animal**								
		Unrinsed eyes						**Rinsed eyes**		
		1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***
1 hour	***r***	*3*	*3*	*2*	*2*	*3*	*2*	*3*	*2*	*3*
	c	*1*	*1*	*0*	*1*	*1*	*1*	*1*	*1*	*1*
	d	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
1 day	***r***	*2*	*3*	*3*	*2*	*3*	*2*	*2*	*3*	*2*
	c	*1*	*1*	*1*	*1*	*2*	*1*	*1*	*4*	*3*
	d	*0*	*0*	*0*	*0*	*1*	*0*	*0*	*0*	*0*
2 days	***r***	*2*	*2*	*2*	*1*	*1*	*1*	*1*	*2*	*3*
	c	*1*	*0*	*0*	*0*	*0*	*0*	*0*	*2*	*3*
	d	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
3 days	***r***	*1*	*1*	*2*	*0*	*1*	*0*	*1*	*2*	*3*
	c	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*2*
	d	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
4 day	***r***	*1*	*1*	*1*	*0*	*0*	*0*	*0*	*0*	*2*
	c	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*2*
	d	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
7 days	***r***	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
	c	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*
	d	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
10 days	***r***	*0*	*0*	*1*	*0*	*0*	*0*	*0*	*0*	*0*
	c	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*
	d	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
14 day	***r***	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
	c	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*

	d	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
17 days	***r***	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
	c	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*
	d	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
21 day	***r***	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
	c	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
	d	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*

r = redness c = chemosis d = discharge

Comment: No corneal opacity or iritis was observed. Slight to severe conjunctival redness and slight chemosis were seen after treatment and persisted. All the signs had disappeared by day 14 and 21 for unrinsed and rinsed eyes, respectively.

Mean scores: conjunctival irritation, 1.6; conjunctival oedema, 0.4; iris, 0.0 and cornea, 0.0.

Result: OLOA 219 was a moderate to severe irritant to the eyes of rabbits.

9.5.10 Skin Sensitisation 1 (Buehler method) (Cushman, 1985b)

Species/strain: Guinea pig/Hartley albino

Number of animals: Test group: 15 males;
Test vehicle group: 10 males;
Positive control group: 10 males;
Positive control vehicle group: 10 males.

Induction procedure:

test group: day 1-22: Topical Inductions:
10 occluded dermal inductions were applied on alternate days. The first application (0.3 mL of 0.1% OLOA 219 in mineral oil) was administered by a Hill Top Chamber to the right flank for 6 hours. The remaining 9 applications (0.4 mL of 0.1% OLOA 219 in mineral oil) were given under an occlusive dressing for 6 hours at the same site.

positive control group: Treated similarly to the test animals using 1-chloro-2,4-dinitrobenzene (DNCB, 0.1% in a vehicle of 80% ethanol) instead of OLOA 219 in the topical applications.

Challenge procedure:

day 36: Test and its vehicle groups:
A 6 hour occluded application of 1% of OLOA 219 in mineral oil (0.3 mL) was given by a Hill Top Chamber to the left flank of each animal.

Positive control and its vehicle groups:
These animals were challenged as describe above with 0.1% DNCB in acetone.

Test method: OECD TG 406

Challenge outcome:

Challenge concentration	*Test Group*			*Test Vehicle Group*		
	*24 hours**	*48 hours**	*72 hours*	*24 hours*	*48 hours*	*72 hours*
1% OLOA 219	**3/15 (20%) ***[0.2]	6/15 (40%) [0.4]	9/15 (60%) [0.9]	3/10 (30%) [0.3]	5/10 (50%) [0.5]	6/10 (60%) [1.0]
Challenge concentration	*Positive Control Group*			*Positive Control Vehicle Group*		
	*24 hours**	*48 hours**	*72 hours*	*24 hours*	*48 hours*	*72 hours*
0.1% DNCB	10/10 (100%) [3.7]	10/10 (100%) [3.9]	9/10 (90%) [3.5]	7/10 (70%) [1.1]	5/10 (50%) [0.6]	1/10 (10%) [0.3]

* time after patch removal
** number of animals exhibiting positive response
*** mean irritation score

Comment: The positive rates from the test vehicle group were greater than 15% after challenge. Slight to well-defined erythema in several animals and well-defined oedema in one animal were observed after challenge in the test group. The incidence and severity of responses of the test group were similar to that of the test vehicle group.

The positive control group responded appropriately.

Result: Inconclusive, due to the high positive responses in the test vehicle group.

9.5.11 Skin Sensitisation 2 (Buehler method) (Carey, 1993)

Species/strain: Guinea pig/Hartley albino

Number of animals: Test group: 20 females;
First challenge control group: 10 females;
Second challenge control group: 10 females.

Induction procedure:

test group:
day 1-15

Topical Inductions:
3 occluded dermal inductions were applied weekly over a 15 day period. The application (0.3 mL of 10% OLOA 219 in petrolatum) was administered by a Hill Top Chamber to flank site of each animal for 6 hours.

control group: The 2 challenge control groups had no topical induction.

Challenge procedure:

day 29

Test and first challenge control groups:
A 6 hour occluded application of 5% of OLOA 219 (0.3 mL) was given by a Hill Top Chamber to a naive site in the flank of each animal.

Test and second challenge control groups:
Same treatment as described in the first challenge.

day 36

Test method: OECD TG 406

Challenge outcome:

Challenge concentration	***Test Group***				***Challenge Control Group***			
	24 hours*		***48 hours****		***24 hours***		***48 hours***	
	erythema	oedema	erythema	oedema	erythema	oedema	erythema	oedema
Ist challenge 5% OLOA 219	**20/20 (100%)	20/20 (100%)	4/20 (25%)	1/20 (5%)	10/10 (100%)	9/10 (90%)	2/10 (20%)	0/10 (0%)
2nd challenge 5% OLOA 219	20/20 (100%)	20/20 (100%)	11/20 (55%)	8/20 (40%)	10/10 (100%)	10/10 (100%)	6/10 (60%)	0/10 (0%)
100% petrolatum					8/10 (80%)	7/10 (70%)	1/10 (10%)	0/10 (0%)

* time after patch removal
** number of animals exhibiting positive response

Comment: No positive control groups were included in this study. The laboratory provided historic positive control data in the report.

The percentage of positive responses in the challenge control group was >15%. The occurrence and severity levels of skin reaction after challenges in the test group were not greater than those in the challenge control groups.

Result: Inconclusive, due to the high positive responses in the challenge control group.

9.5.12 Skin Sensitisation 3 (Buehler method) (Morris, 1994)

Species/strain: Guinea pig/Hartley albino

Number of animals: Test group: 10 /sex;
Naive control group (first challenge): 5/sex;
Naive control group (second challenge): 5/sex.

Induction procedure:

test group:
day 1-15 — Topical Inductions:
3 occluded dermal inductions were applied weekly over 15 day period. The application (0.3 mL of 25% OLOA 219 in mineral oil light) was administered by a Hill Top Chamber to each animal for 6 hours.

control group: The control group had no topical induction.

Challenge procedure:

day 29 — Test and naive control groups:
A 6 hour occluded application of 1% of OLOA 219 in mineral oil light (0.3 mL) was given by a Hill Top Chamber to the flank of each animal.

Same treatment as described in first challenge with challenge doses at 0.5% and 1%.

day 35

Test method: OECD TG 406

Challenge outcome:

Challenge concentration	***Test Group***		***Naive Control Group***	
	24 hours*	***48 hours****	***24 hours***	***48 hours***
Ist challenge 1%				
Scoring scale 1:	13 (65%)	10 (50%)	6 (60%)	7 (70%)
Scoring scale 2:	7 (35%)	8 (40%)	2 (20%)	0
Total:	**20/20 (100%)	18/20 (90%)	8/10 (80%)	7/10 (70%)
2nd challenge 0.5%				
Scoring scale 1:	10 (50%)	8 (40%)	6 (60%)	8 (80%)
Scoring scale 2:	6 (30%)	7 (35%)	0	1 (10%)
Total:	16/20 (80%)	15/20 (75%)	6/10 (60%)	9/10 (90%)
2nd challenge 1%				
Scoring scale 1:	14 (70%)	12 (60%)	4 (40%)	8 (80%)
Scoring scale 2:	4 (20%)	7 (35%)	0	0
Total:	18/20 (90%)	19/20 (95%)	4/10 (40%)	8/10 (80%)

* time after patch removal
** number of animals exhibiting positive response
Scoring scale 1: slight but confluent or moderate patchy erythema
Scoring scale 2: moderate erythema

Comment: No positive control groups were included in this study. The test laboratory provided historic positive control data in the report.

The occurrence of skin reaction to the challenges in the test group was comparable to that in the challenge control groups. However, the severity of reaction in the test group were higher than that in the naive control group (see table above).

Result: OLOA 219 may be a skin sensitiser to the skin of guinea pigs.

9.5.13 Repeat Insult Patch Test in Humans (Buehler, 1997)

Group design:

Pilot study:
24 persons completed the pilot study: 11 received A and B, and 13 received A and C.
Group A: 100% mineral oil (vehicle);
Group B: 100% OLOA 219;
Group C: 50% OLOA 219 in vehicle.

Main study:
96 persons completed the main study, and received both A and B.
Group A: 100% mineral oil (negative control);
Group B: 100% OLOA 219.

Induction procedure:

week 1-3: Topical Inductions:
9 inductions were carried out 3 times per week over a 3-week period. The patch (0.1 mL) was administered by a Webril portion of Professional Medical Products to the same site on upper arm of each person for 24 hours.

Semi-occluded and occluded patches were used for pilot and main

studies, respectively.

Challenge procedure:

After a 10 to 17-day rest period: A 24-hour dermal application of 100% of OLOA 219 to a naive site of each person.

Test method: Similar to draft OECD TG

Challenge outcome:*

Pilot study

Challenge concentration	***Group A (vehicle)***		***Group B (100% OLOA 219)***		***Group C (50% OLOA 219)***	
	24 hours*	***48 hours****	***24 hours***	***48 hours***	***24 hours***	***48 hours***
100%	***0/24	0/24	0/11	1/11	0/13	1/13

Main study

Challenge concentration	***Group A (vehicle)***		***Group B (100% OLOA 219)***	
	24 hours*	***48 hours****	***24 hours***	***48 hours***
100%	4/96	0/96	9/96	1/96

* persons dropped from the study were not included;
** time after patch removal;
*** number of subjects exhibiting positive response

Comment: Mild erythema (grade 1) was observed in all test subjects during the induction phase of the pilot study and main study. These responses were sporadic and typically did not persist. After challenge, mild erythema was seen in some persons, the number of positive and the levels of severity were sufficient to be classified as a sensitiser.

Result: OLOA 219 was a slight skin irritant and a skin sensitiser to the skin of humans.

9.6 Repeat Dose Toxicity of OLOA 219

9.6.1 Combined Oral Subchronic Toxicity, Reproduction and Neurotoxicity Study of OLOA 219 in Rats (Lamb, 1993)

Species/strain: Rat/Sprague-Dawley

Number/sex of animals: Subchronic study: 4 dose groups of 5/sex per group, the control and high dose groups had additional 5/sex as the recovery groups.

Neurotoxicity study: 2 dose groups (control and high dose) of 5 males per group, and an additional 5 males per group as the recovery groups.

Reproduction study: 4 dose groups of 12/sex per group.

Method of administration: Oral by gavage.

Dose/Study duration: Control group: 0 mg/kg/day;
Low dose group: 50 mg/kg/day;
Mid dose group: 200 mg/kg/day;
High dose group: 1 000 mg/kg/day
(vehicle: peanut oil).

Subchronic and neurotoxicity groups
Animals were treated once daily for at least 28 consecutive days. The animals in the recovery groups had a 2-week treatment free period.

Reproduction group
Adult males were treated for at least 28 consecutive days prior to mating, through postmating until sacrifice for a total minimum of 70 days of treatment. Adult females were treated for at least 28 consecutive days prior to mating and the treatment continued during mating, gestation and lactation period to lactation Day 4.

Test method: OECD TG 407

Clinical observations:

Subchronic group: All animals survived the study. Both male and female animals at high-dose had salivation, clear material and red/yellow staining around the mouth 1 hour after dosing. A slight decrease of bodyweight during weeks 3-4 with partially recovery during weeks 5-6 in high-dose males was observed. Food consumption was unaffected by treatment.

Other clinical observation results from the test and recovery groups were comparable to the controls.

Neurotoxicity group: All animals survived the study. Similar to the animals in subchronic study, both male and female animals at high-dose had salivation, clear material and red/yellow staining around the mouth 1 hour after dosing. Decreases of bodyweight and bodyweight gain in high-dose males were considered treatment-related. Some improvement in bodyweight during the recovery period was observed.

Examination results of functional observational battery (FOB) including home cage observation, handling observation, open field observation, sensory observation, neuromuscular and physiological observations in the test groups were comparable to that in the control group.

Reproductive group: All animals survived except 1 male at low-dose died immediately after dosing due to intubation trauma in week 1. Both male and female animals at high-dose had salivation, clear material and red/yellow staining around the mouth 1 hour after dosing. These signs were seen in low- and mid-dose animals as well as in control animals, but less frequently.

Decreases of bodyweight and bodyweight gain in high-dose males were treatment-related in the subchronic and neurotoxicity studies. When evaluated as g/kg/day, food consumption in high-dose males was increased as a reflection of the reduction of bodyweight. In high-dose females, bodyweight gain was reduced during days 7-14, 14-20 and 0-20. The food consumption in high-dose females was reduced during lactation days 1-4 ascribed to reduced live litter size and not maternal toxicity. The mid-dose females had slightly lower bodyweight on gestation days 14 and 20.

Mating and fertility indices in both male and female animals of the test groups (F_0) were comparable to that in the control group.

Clinical chemistry/Haematology

Subchronic group: Glucose levels in high-dose males and low, mid and high-dose females were lower than the controls but were within the historic control range and the responses were not dose-related. Cholesterol levels in high-dose males and females were within the historic control range, but lower than the controls. The toxicological significance of these changes is unclear.

In the recovery groups, the haemoglobin and hematocrit values at week 6 in high-dose (recovery group) males were lower than the controls. This was not considered to be treatment-related because they were comparable to the week 4 results and within the historical control ranges.

Other clinical chemistry and haematological results from the test and recovery groups were comparable to the controls.

Necropsy:

Subchronic group: Increases in adrenal weights and ratios of adrenal to bodyweight or brain weight were noted in high-dose males and females from both test and recovery groups. In addition, higher ratios of kidney/bodyweight in high-dose males and liver/bodyweight in high-dose females were found in the test groups.

Other macro- and microscopic examination results from the test and recovery groups were comparable to the controls.

Neurotoxicity group: Macroscopic examinations results including brain weights and dimensions, and microscopic examinations from the test group were comparable to the controls.

Reproductive group:
F_0 DATA
The low-dose male, which died during the study, had reddened adrenal glands, haemorrhagic thymus and dark red lungs. One female each at low-, mid- and high-dose failed to deliver and were necropsied on post-mating day 25. These females were nongravid and internally normal. One female at high-dose had total litter loss on lactation day 2. No remarkable internal findings were seen at necropsy.

Adrenal gland weights and ratios of adrenal to bodyweight or brain weight were significantly higher in high-dose males, and the adrenal gland weights were slightly higher in mid- and high-dose females. In addition, pituitary gland weights and ratios of pituitary gland to bodyweight or brain weight were higher in high-dose males.

F_1 Litter data

Live litter size in the high-dose group was lower than controls and historic control data due to prenatal mortality. The group mean number of former implantation sites was significantly decreased at high dose level. The mean number of corpora lutea in the high-dose group was lower than the concurrent control group value and was also outside the observed range of historical control data for rats. These reductions were reflected by the significantly increased pre-implantation loss values. A secondary component of the prenatal mortality was post-implantation loss which was evident in the percentage data.

Live litter size in mid-dose group was slightly lower than control and historic data. The reduction was considered to be an equivocal effect of the treatment. Live litter size, and pre- and post-implantation loss in low-dose group were comparable to the controls.

Dead pups found in the control, low, mid and high dose groups were 7, 3, 2 and 2, respectively. The remainder were sacrificed on lactation day 4. No necropsy findings which related to the treatment were found in any pup. Hyoid bone ossification was present in all control and high-dose pups on lactation days 4.

Comment:

The laboratory provided historic control data.

The low-dose male which died during the study had histologically normal adrenal glands.

The following changes were considered to be treatment-related in this study.

- In both males and females at 1 000 mg/kg/day, salivation, clear material, and red/yellow staining around the mouth and/or red material around the nose were seen 1 hour after dosing.

- In males at 1 000 mg/kg/day, decreased bodyweight and bodyweight gain during weeks 3-4 in

subchronic study, weeks 2-4 in neurotoxicity study and weeks 2-10 in the reproduction study.

- Increased adrenal gland weights (absolute and relative) were noticed in both males and females at 1 000 mg/kg/day, and females at 200 mg/kg/day. An increase in pituitary gland weights was observed in high-dose males.

- In the females at dose levels of 200 and 1 000 mg/kg/day, decreased live litter size was found due to increased pre- and post-implantation losses. The prenatal mortality was also reflected in decreased gestation bodyweight and subsequent lactation food consumption at high-dose.

The examination results on neurotoxic and reproduction indices from the test groups were comparable to that from the control groups.

Result:

The NOEL was 50 mg/kg/day for systemic toxicity and reproductive toxicity based on the clinical signs, and changes in bodyweight, adrenal and pituitary gland weights, and live litter size. The NOAEL was ≥ 1000 mg/kg/day for neurotoxicity (highest dose tested).

9.6.2 Twenty-Eight Day Dermal Toxicity Study (Korenaga, 1986)

Species/strain: Rat/Sprague-Dawley CD.

Number/sex of animals: 12/sex/group.

Method of administration: A dermal dose of 1.0 mL/kg was applied under a semi-occlusive dressing 6 hours per day, 5 days per week for 4 weeks.

Dose/Study duration: Control group: 0%;
Low dose group: 2% (20 mg/kg/day);
Mid dose group: 10% (100 mg/kg/day);
High dose group: 25% (250 mg/kg/day)
(vehicle: mineral oil).

Test method: OECD TG 410

Clinical observations:

All animals survived the study. No treatment related clinical signs of toxicity were observed in the control and test groups except chromodacryorrhea, chromorhinorrhea, and sores/scabs on the skin over the throat areas (in most animals) due to the use of plastic collars.

Irritation was seen in control and test groups.

Mean of the Sum of Draize Scores for Erythema and Oedema

Time after treatment	*0% (0 mg/kg/day)*		*2% (20 mg/kg/day)*		*10% (100 mg/kg/day)*		*25% (250 mg/kg/day)*	
	male	*female*	*male*	*female*	*male*	*female*	*male*	*female*
0 day	0	0	0	0.1	0	0	0	0
2 days	0.1	0.2	0.2	0.3	0.1	1.7	0	0.8
9 days	0.2	0.8	0.8	1.2	1.4	0.8	2.3	1.7
16 days	0.3	1.2	0.4	1.5	0.8	1.2	1.8	1.4
23 days	0.2	1.0	0.3	0.7	0.2	0.6	0.8	0.7
27 days	0	0.5	0	0.6	0	0.4	0.4	0.7

Clinical chemistry/Haematology

Platelet counts in the low and mid-dose females and eosinophil count in low-dose females were higher than the controls, but within the range of historic control values and lacked a dose-related trend.

Compared to control animals, an increase in SGPT was observed in high-dose males, and a decrease in alkaline phosphatase, in low and high-dose females. However, these values were within the historic control ranges.

Pathology:

Gross skin observations at necropsy included dryness and flakiness in animals at all dose levels.

Acanthosis at the treatment sites were observed in the controls, and increased in incidence and severity in the high-dose animals.

Several sporadic observations were noticed but none of them was considered to be treatment-related.

Comment:

Several changes were noticed in the test animals in this study but none of them showed a dose-response relationship and were considered to be treatment-related.

Result:

The NOEL for dermal toxicity was ≥ 250 mg/kg/day (the highest dose tested) in this study.

9.7 Genotoxicity of OLOA 219

9.7.1 *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay (Carver, 1985a)

Strains:	*S. typhimurium* TA98, TA100, TA102; *Escherichia coli* WP2*urv*A
Metabolic activation:	Liver fraction (S9 mix) from rats pretreated with Aroclor 1254.
Concentration range:	Triplicate plates were prepared for each bacterial strain and dose level, in both the presence and the absence of S9-mix. 25% Pluronic F127 in ethanol was used as the vehicle. 0, 0.033, 0.1, 0.33, 1.0 and 3.33 mg/plate in all strains. Positive controls: (When with S9 mix) Danthron for TA102; 2-aminoanthracene for TA98, TA100 and WP2*uvr*A. (When without S9 mix) 2-nitrofluorene (2-NF) for TA98; sodium azide for TA100; mitomycin C for TA102; 1-ethyl 2-nitro 3-nitrosoguanidine for WP2*uvr*A.
Test method:	OECD TG 471

Comment: No cytotoxicity was observed at any concentration.

A slight higher number of revertant colonies in TA102 with S9-mix was not considered to be biological significant.

Result: OLOA 219 was non mutagenic under the conditions of the test.

9.7.2 Chromosomal Aberration Assay in Mouse Lymphoma Cells (Carver, 1985b)

Cells: Mouse lymphoma L5178Y-3.7.2C cells

Metabolic activation system: Liver fraction (S9 mix) from rats pretreated with Aroclor 1254.

Dosing schedule: Triplicate samples were prepared for each dose level, in the presence and the absence of S9-mix. 5% Pluronic F68 in water was used as the vehicle.

Metabolic Activation	*Experiment/ Study Number*	**Test concentration (µg/mL)**	**Controls**
-S9		treatment time = 48 hours 0, 60, 70, 80, 90, 100, and 110 µg/mL	Positive: none. Negative: vehicle
+S9		0, 75, 100, 150, 200, 250, and 275 µg/mL	Positive: DMBA Negative: vehicle

DMBA 7,12-dimethylbenzanthracene

Test method: OECD TG

Comment: Preliminary tests were performed for the determination of toxicity and selection of doses. Toxicity was observed >100 µg/mL with and without S9-mix.

No positive controls were included in the tests without S9-mix.

OLA 219 did not induce a significant increase in the mutant frequencies of cultures tested either with or without metabolic activation.

Result: OLOA 219 was non-clastogenic under the conditions of the test.

9.8 Other Studies of OLOA 219

9.8.1 Oral Teratogenicity and Development Toxicity Study in Rats (Roberts, 1990)

Species/strain: Rat/Sprague-Dawley CD.

Number/sex of animals: 15 mated female per dose group.

Method of administration: Oral by gavage.

Dose/Study duration: Control group: 0 mg/kg/day;
Low dose group: 50 mg/kg/day;

Mid dose group: 300 mg/kg/day;
High dose group: 1 000 mg/kg/day
(vehicle: peanut oil).

After mating, animals were treated once daily during days 6-15 of presumed gestation and were sacrificed on day 20.

Test method: OECD TG 414 & 415

Clinical observations:

Numbers of rats inseminated were 14, 14, 14 and 15 in the control, low, mid and high-dose groups, respectively.

On day 12 of gestation, 1 control female died due to a gavage injury, and 1 mid-dose female was found dead for unknown reasons.

No clinical signs that related to treatment were observed in the tested animals during the study.

Maternal observations:

Lower maternal bodyweight gains were noticed in high-dose females throughout the dosing period. Animals in the mid-dose group had a slight lower maternal bodyweight gain on days 6-7 and a higher average bodyweight gain during days 16-20. Mean gravid uterine weights were comparable between control and test groups.

There were lower absolute food consumption on days 7-8 and higher relative food consumption values on days 17-18, 19-20 in high-dose females.

No significant differences were found between the control and test groups in incidence of pregnancy, the average values for corpora lutea, implantations, and live litter sizes. The treatment at all dose levels did not affected foetal sex ratios, foetal bodyweight, or percentage of dead or resorbed conceptuses. There were no abortion, premature deliveries or dead conceptuses found.

Foetal observations:

External*:* Subcutaneous haemorrhages in foetuses, which were likely related to the sacrifice technique, were observed in each group. A decrease in the foetal incidence of paleness in the low-dose group, but this finding was not considered to be biological significant.

Skeletal*:* In the mid-dose group, there was an increase in foetal incidence of incomplete ossification of the sternebrae and pubis. However, this was not considered to be treatment-related due lack of a dose-response relationship. Foetuses and litters in the high-dose group had a higher incidence of rudimentary 14th ribs. The foetal incidences for hyoid, non-ossified and/or incompletely ossified were significantly higher in the mid and high-dose groups, and showed a possible dose-related response.

Visceral*:* There were no dose-related or dose dependent visceral observations found in any dose group as compared to controls.

Pathology:

Macroscopic and microscopic changes observed in the adult females did not appear to be treatment-related.

Changes attributable to gavage injury were observed in the female animal died on day 12.

Comment:

Two observations in this study were considered to be treatment-related:

- Lower bodyweight gain and food consumption in high-dose adults.
- Delayed ossification of the hyoid bone was noticed in the foetuses of mid and high-dose groups. Additionally, foetuses and litters in the high-dose group had an increase in rudimentary ribs.

Result:

The maternal NOEL was determined to be 300 mg/kg/day, and the developmental NOEL was 50 mg/kg/day in this study.

9.8.2 Developmental Toxicity Study (Nemec 1994)

Species/strain:	Rat/Sprague-Dawley CD.
Number/sex of animals:	25 mated females per dose group.
Method of administration:	Oral by gavage.
Dose/Study duration:	Control group: 0 mg/kg/day; Low dose group: 50 mg/kg/day; Mid dose group: 300 mg/kg/day; High dose group: 1 000 mg/kg/day (vehicle: peanut oil). After mating, animals were treated once daily during days 6-15 of presumed gestation and were sacrificed on day 20.
Test method:	OECD TG

Clinical observations:

All animals survived till day 20. High-dose females had staining/matting/material around the nose and mouth 1 hour following dosing. No other clinical signs that related to the treatment were observed in tested animals during the study.

Maternal observations:

Lower maternal bodyweights and cumulative bodyweight gains were observed in high-dose females. There was lower food consumption during days 6-16 in high-dose females. Animals in low and mid-dose groups had comparable maternal bodyweights and bodyweight gains to controls.

Mean gravid uterine weights were comparable in control and test groups.

No significant differences were found between the test and control groups in laparohysterectomy data including post-implantation loss, viable foetuses, foetal sex ratios, foetal bodyweights, and numbers of corpora lutea and implantation sites.

Foetal observations:

External: No external developmental variations were observed in foetuses at all dose levels except 1 mid-dose foetus with exencephaly (brain lying outside of the skull).

Visceral: Three visceral malformations were found. In the mid-dose group, 1 foetus had hydrocephaly (accumulation of cerebrospinal fluid within the skull) consisting of severely increased cavitation of the third ventricle with compression of the cerebral cortex, and one with malpositioned testes and a distended ureter. One foetus from the high-dose group had a meningocele (hernial protrusion of the meninges through a defect in the skull or vertebral column) with a large portion of the right cerebral cortex located between the

meninges and skull, with extensive haemorrhaging internal and external to the meninges, and another one from high-dose group had a right subclavian artery which coursed retroesophageal prior to joining the aortic arch at the level of the left subclavian artery.

Skeletal: The number of foetuses with bent ribs was higher in the high-dose group. One foetus in the mid-dose group had a costal cartilage anomaly at skeletal examination.

The numbers of foetuses (litters) with malformations were 0(0), 0(0), 2(2) and 1(1) in the control, low, mid and high dose groups, respectively. Foetal external, soft tissue and skeletal malformations were considered to be spontaneous in origin, except for the higher number of foetuses with bent ribs in the high-dose group.

Pathology:

One control female had dark red lung and 1 low-dose female had a unilateral dilated renal pelvis. In the mid-dose group, 1 female had red clotted material in the trachea and a dark red lung and another had fused placentas. One female in the high-dose group had multiple cysts on the spleen. These macroscopic and microscopic changes observed in the adult females did not appear to be treatment-related.

Comment:

Two observations in this study were considered to be treatment-related:

- Lower maternal bodyweights and food consumption in high-dose adults.
- Higher incidence of foetuses with bent ribs in the high-dose group.

Result:

The NOEL is determined to be 300 mg/kg/day for both parental and developmental toxicity in this study.

9.8.3 Two-Generation Reproductive Toxicity Study in Rats (Nemec, 1995a)

Species/strain: Rat/Sprague-Dawley CD.

Number/sex of animals:

	Group	*Male*	*Female*
F_0	Control	30	30
	Low-dose	30	30
	Mid-dose	30	30
	High-dose	30	30
F_1	Control	30	30
	Low-dose	30	30
	Mid-dose	30	30
	High-dose	30	30
	Satellite 1	28	30
	Satellite 2	30	29

F_1 satellite 1 consisted of 28 F_1 high-dose males and 30 F_1 control females, and F_1 satellite 2 had 30 F_1 control males and 29 F_1 high-dose females.

Method of administration: Oral by gavage.

Dose/Study duration:

Control group: 0 mg/kg/day;
Low dose group: 50 mg/kg/day;
Mid dose group: 300 mg/kg/day;
High dose group: 1 000 mg/kg/day
(vehicle: peanut oil).

F_0 generation
Parental animals (F_0) were treated from week 0 for at least 71 days prior to

mating (week 9). The treatment continued during mating, gestation and lactation period until F_1 weaning (lactation day 21, or week 20).

F_1 generation
F_1 animals were treated from postnatal day 22 (week 16) for at least 77 days prior to mating (week 29) for control, low, mid and high-dose groups, or at least 88 days prior to mating (week 30) for satellite groups. The treatment continued during the ensuing mating, gestation and lactation period until F_2 weaning (lactation day 21, or week 38).

F_2 generation
No treatment was given to F_2 animals. All F_2 animals were sacrificed on lactation day 21.

Test method: OECD TG 416

Clinical observations and mortality:

F_0 generation: All animals survived except 1 high-dose female that died in week 14. The cause of death was unknown (see *Necropsy examinations*).

High-dose male and female animals had salivation and yellow, red, brown, clear and/or tan staining/matting/material at dosing, or 1 hour after dosing. High-dose females also had red discharge from the vaginal opening 1 hour following dosing.

Mid-dose animals had and clear, yellow, tan and/or red staining/matting/material around the mouth, and the females also had salivation.

F_1 generation: Three females in the high-dose group were found dead during the study, one of them had dystocia (abnormal labour), coolness to touch, pale colour and apparent blockage of the vaginal opening. Another female in the high-dose group was sacrificed during the study due to an apparent vaginal blockage. All other animals survived to the schedule necropsy.

Animals in high-dose group had yellow, brown, tan, clear and/or red staining/matting/material on several body surfaces and salivation one hour after dosing. Two males in high-dose group showed unkempt appearance at the weekly physical examinations. In addition, males in the high-dose group had a higher incidence of ejaculatory plugs during pre-mating phase than other groups. The biological significance of the presence of ejaculatory plugs is unknown.

F_1 satellite groups: One female in satellite 1 group died during parturition with 1 pup still in the birth canal. Yellow staining around the moth was observed 1 hour following dosing on the day prior to death.

Animals from the high-dose groups had salivation and red, tan, clear and/or yellow staining/matting/material on several body surfaces 1 hour following dosing.

Males from the high-dose group had a greater incidence of ejaculatory plugs during the pre-mating phase than controls, and the incidence was reduced during the recovery period.

Reproductive performance:

F_0 generation: Reproductive performance was adversely affected by the treatment in high-dose animals. The mean number of days between pairing and coitus in the high-dose group was significantly higher than controls but within historical control range.

		Control	***Low-dose***	***Mid-dose***	***High-dose***
Fertility indices	F_0 males	96.7%	86.7%	93.3%	73.3%
	F_0 females	96.7%	93.3%	93.3%	73.3%

Mating indices	F_0 male	96.7%	90%	100%	86.7%
	F_0 female	96.7%	100%	100%	96.7%
F_0 males that did not sire a litter		1	4	2	8
Females mated without delivery		0	2	2	7

F_1 generation: The mean numbers of days between pairing and coitus in all treated group were similar to that in the control group. Some changes in reproductive performance that related to treatment were observed in the high-dose group.

		Control	***Low-dose***	***Mid-dose***	***High-dose***
Fertility indices	F_1 males	90%	83.3%	93.3%	76.7%
	F_1 females	93.3%	93.3%	100%	76.7%
Mating indices	F_1 male	93.3%	86.7%	93.3%	93.3%
	F_1 female	96.7%	96.7%	100%	96.7%
F_1 males that did not sire a litter		3	5	2	7
Females mated without delivery		2	2	0	12

F_1 satellite groups: Reproductive performance was affected by the treatment in the satellite group 2. The mean number of days between pairing and coitus was similar among the 2 satellite groups and control group.

		Control	***Satellite 1***	***Satellite 2***
Fertility indices	males	93.3%	89.3%	
	females	96.7%		100%
Mating indices	male	90.0%	89.3%	
	female	93.3%		55.2%
F_0 males that did not sire a litter			3	13
Females mated without delivery				15

Body weights and food consumption:

F_0 generation: Mean bodyweights and mean bodyweight gains in mid and high-dose males were reduced compared to controls. The mid and high-dose females had lower bodyweight gains during weeks 0-1 and 7-8.

Mean gestation bodyweight of high-dose females at gestation day 20 was lower than the controls. The mean bodyweight gains were reduced throughout gestation and lactation in high-dose females.

Weekly food consumption and food consumption during gestation and lactation were not affected at all dose levels, except during lactation when high-dose females had lower food consumption than controls.

F_1 generation: Decreases in mean weekly bodyweights and mean bodyweight gains were observed in both males and females of the high-dose group, became less persistent in the animals of mid-dose group and were occasional in the animals of low-dose group.

Mean bodyweights and bodyweight gains during gestation in high-dose females were lower than controls.

There were no differences in bodyweights and bodyweight gains during lactation in all treated groups and control group.

Weekly food consumption and food consumption during gestation and lactation were not affected at any dose level, except that high-dose females had lower food consumption during lactation than controls. When calculating the food consumption in g/kg/day basis, some significant differences were found due to the decreased bodyweights in high-dose group.

F_1 satellite groups: Mean bodyweights of satellite group 1 males and of satellite group 2 females were lower than the satellite group 2 males and satellite group 1 females, respectively.

Mean bodyweights of satellite group 2 females were reduced during gestation and lactation when compared to the satellite group 1 females.

Weekly food consumption and food consumption during gestation of the females were similar between 2 satellite groups. When calculating the food consumption in g/kg/day basis, some significantly differences were found due to the decreased bodyweights in high-dose group. Food consumption during lactation was reduced in females of satellite group 2 comparing with that in satellite group 1.

Gestation length and parturition:

F_0 generation: Mean gestation lengths were comparable between the treated groups and the controls.

F_1 generation: Mean gestation lengths were comparable between the treated groups and controls. Dystocia was observed in 1 mid-dose and 4 high-dose females.

F_1 satellite groups: Mean gestation lengths were comparable between the 2 satellite groups. No signs of dystocia were observed.

Litter data:

F_1 litters: The numbers of dead pups in mid and high-dose groups on lactation day 0 were higher than the controls and the historic data. The percentages of dead pups in mid and high-dose groups were dose-related. Pup viability indices in the treated groups for lactation days were comparable to the controls. F_1 pup sex ratios were not affected by treatment.

One dead pup each from the control, mid-dose and high-dose groups during the post-natal period had malformations. One control group pup had unilateral ablepharia (absence of eyelids), exencephaly, macroglossia (excessive size of tongue) and an omphalocele (protrusion of part of the intestine through a large defect in the abdominal wall at the umbilicus), and one at mid-dose level had mandibular micrognathia (unusual or undue smallness of the jaw). Anury was noted for a pup in the high-dose group. The physical condition of F_1 pups during lactation was similar in all groups, including the control group.

In high-dose group, the mean live litter size was reduced and mean pup bodyweights became lower post-natally.

No treat-related abnormalities were found at scheduled necropsy on the postnatal day 28.

F_2 litters: The numbers of dead pups on lactation day 0 were higher in high-dose group than the control group. Pup viability indices in the three treated groups during lactation days were comparable to the control group. F_2 pup sex ratios in the treated groups were comparable to those in the control group.

The physical condition of F_2 pups during lactation was similar in all the groups including the control group. One pup from the high-dose group, found dead during postnatal period, had red contents in the stomach and intestine.

Mean live litter sizes and pup weights were not affected by the treatment except that live litter size in the high-dose group was lower than in the controls.

No treat-related abnormalities were found at scheduled necropsy on postnatal day 21.

F_2 satellite litters: The number of dead pups and the proportion of dead pup to total pups on lactation day 0 in satellite group 2 was higher than in satellite group 1 or the controls. Pup viability index in satellite group 2 was slightly lower than that in satellite group 1 or the control group for lactation days 1 and 4 (before selection), with no difference at lactation days 4 (after selection) and 7.

F_2 pup sex ratios in the 2 satellite groups were comparable.

In satellite group 1 and 2, 6 and 12 pups, respectively were found dead during postnatal days. The physical condition of F_2 satellite pups during lactation was similar in the 2 satellite groups. Mean pup bodyweights in the 2 satellite groups were similar but the satellite group 2 had lower live litter size than the satellite group 1 or the control group.

No treat-related abnormalities were found at scheduled necropsy on lactation day 7.

Necropsy examinations:

F_0 generation: The female in the high-dose group that died in week 14 had reddened adrenal glands, dark red contents and areas in the stomach and a haemorrhagic thymus gland. Microscopic examination revealed hepatocellular necrosis in the liver. The animal retained 12 foetuses that had no apparent malformations *in utero*.

No treatment-related macroscopic findings were observed in F_0 parental animals.

Examinations of spermatogenesis in several males which failed to sire a litter, did not reveal any treatment related changes in gross sperm morphology, apparent relative numbers or motility. One low-dose male had nonmotile, abnormal sperm in the epididymides, 1 high-dose male had motile, abnormal sperm in the epididymides, and another high-dose male had nonmotile, abnormal sperm in the left epididymis and motile, abnormal sperm in the right epididymis.

In the high-dose females, there was an increase in mean absolute liver weight and a decrease in ovary weights, and in high dose males there was a decrease in mean absolute epididymides weight. Higher pituitary gland weights were seen in mid-dose males and high-dose males and females. The mean final bodyweights in mid and high-dose males were lower than the controls.

No microscopic lesions attributed to the treatment were found in F_0 animals.

F_1 generation: In the high-dose group, 3 females were found dead during the study and 1 female was euthanized. Necropsy revealed that at least 2 of them had mottled lungs and red fluid contents in the uterus. Microscopic examination showed hydrometra in the uterus in 2 animals and one had nonsuppurative inflammation of the liver. The sacrificed animal had medullary cysts in the kidneys and endometrial hyperplasia of the uterus.

At the scheduled necropsy, no treatment-related macroscopic findings were observed in F_1 parental animals.

Examinations of spermatogenesis in males which failed to sire a litter, did not reveal any treatment related changes in gross sperm morphology, apparent relative numbers or motility.

In high-dose females, there was an increase in mean absolute liver weight and a decrease in ovary weights, and in high-dose males there was a decrease in mean absolute epididymides and testes. Higher pituitary gland weights were seen in high-dose males and females. The mean final bodyweights in mid (males) and high-dose (males and females) animals were lower than the controls.

No microscopic lesions attributed to the treatment were found in F_1 animals.

F_1 satellite groups: One female of satellite group 2 died during parturition and had dark red stomach contents. She had 7 foetuses *in utero*, delivered 2 foetuses and had 1 foetus in breech position in the vagina. All the foetuses had no apparent malformation.

Satellite group 1 males had lower final bodyweights, mean absolute testes and epididymides weights, and higher absolute pituitary gland weights than the males in satellite group 2.

Comment:

Treatment related changes in this study are summarised below.

- Five females (1 F_0, 3 F_1 and 1 F_2) in the high-dose group died prior to the scheduled necropsies.
- Both males and females at high-dose level had yellow, red, brown tan and/or clear staining/matting/material on various body surfaces and salivation. These clinical signs became less frequent in animals at the mid-dose level.
- Both males and females in the high-dose group had lower fertility indices. Increased occurrence of not siring litters was seen in high-dose males, and higher numbers of mating without delivery and dystocia were observed in high-dose females. Female reproductive function appeared to be more affected by treatment than male reproductive function.
- Mean bodyweights and bodyweight gains were reduced in high-dose males and females, and occasionally reduced in mid-dose males and females.
- Increases of pituitary gland weights were seen in high-dose males and females. In addition, the high-dose animals had increases in liver weight in females, and decreases in epididymides and testes weight in males and ovary weight in females. Increases of pituitary gland weight were seen in mid-dose males.
- The mid and high-dose groups had lower survival pup on lactation day 0, and lower mean live litter sizes and mean pup bodyweight. Pup viability indices were reduced on lactation day 1 and 4 (before selection) in F_2 satellite group 2.

Result:

The NOAEL for systemic parental toxicity is determined to be 50 mg/kg/day based on clinical signs, reduced bodyweight gain, and increases in pituitary gland and liver weights and decreases in testes, epididymides and ovary weights at higher doses.

The NOAEL for reproductive toxicity is determined to be 300 mg/kg/day based on the lower fertility indices at 1 000 mg/kg/day.

The NOAEL for neonatal toxicity is determined to be 50 mg/kg/day based on the lower pup survival, live litter sizes and pup bodyweights at 1 000 mg/kg/day.

There was no evidence of cumulative toxic effects across generations in this study.

9.8.4 Oral Reproduction/Developmental Toxicity Screening Study with Finished Oil in Rats (Nemec, 1995b)

Test material:	C1234-24-10B: 5% OLOA 219 in Chevron 100 Neutral; C1234-24-10A: 25% OLOA 219 in Chevron 100 Neutral; C1234-24-10C: vehicle control.
Species/strain:	Rat/Sprague-Dawley CD.
Number/sex of animals:	12/sex per group.
Method of administration:	Oral by gavage.
Dose/Study duration:	Control group: 1.15 mL/kg/day of C1234-24-10C; Low dose group: 1 g/kg/day of C1234-24-10B (50 mg/kg/day); High dose group: 1 g/kg/day of C1234-24-10A (250 mg/kg/day). Parental (F_0) males were treated for at least 14 consecutive days prior to

mating and the treatment continued for a total 30 days.

Parental (F_0) females were treated for at least 14 consecutive days prior to mating and the treatment continued during the ensuring mating, gestation and lactation period to lactation day 4, for a total at least 39 days.

Test method: OECD TG 421

Clinical observations and mortality:

NO MORTALITY OR ABNORMAL CLINICAL OBSERVATIONS WERE SEEN IN F_0 MALES AND FEMALES AT BOTH DOSE LEVELS DURING THE STUDY.

Reproductive observations:

F_0 generation: Reproductive performance including fertility indices and mating indices was not affected by the treatment in both F_0 males and females from the low and high-dose groups when compared to controls. The mean numbers of days between pairing and coitus in the treated groups were similar to control group values.

Bodyweights and food consumption:

Mean bodyweights of low and high-dose males were comparable to the control group, but the mean bodyweight gains in those 2 groups were lower than the controls. The bodyweight data for the 2 female groups were comparable to the controls.

The mean gestation bodyweights and bodyweight gains, mean lactation bodyweights and bodyweight gains of the 2 female test groups were comparable to the control group.

Gestation length and parturition:

The mean lengths of gestation of the 2 female test groups were comparable to the control group.

No signs of dystocia at parturition were seen in the study.

Litter data:

Data of live birth and viability indices, sex ratios, general physical condition and mortalities, live litter size and pup body weights were comparable between the 2 test groups and the control group.

Necropsy examination:

F_0 generation: At the scheduled necropsy of F_0 males and females, sporadic findings were observed, but none of them were related to the treatment.

Brain, liver, kidney, testes, ovary, pituitary or epididymides weight on an absolute basis and relative to final bodyweight in F_0 males and females were comparable to those in the control group except that low-dose females had slightly lower kidney weights and liver/bodyweight ratios, and high-dose males had slightly higher kidney/bodyweight and testes/bodyweight ratios. However, these changes were not considered to be treatment-related.

There were no microscopic findings related to the treatment in the F_0 animals.

F_1 generation: One pup in the high-dose group had a major blood vessel variation including the right subclavian and right carotid arising independently from the aortic arch.

Comment:

No treatment-related clinical signs or reproductive performance were observed in F_0 animals. Reduction of bodyweight gain was noticed in F_0 males at both low and high-dose levels.

No neonatal toxicity was observed at low and high dose levels.

Result:

The NOEL for reproductive toxicity was ≥250 mg/kg/day (highest dose tested).

9.9 Overall Assessment of Toxicological Data of OLOA 219

OLOA 219 was of very low acute oral toxicity (LD_{50} > 5 g/kg) in rats, low acute dermal toxicity (LD_{50} > 5 g/kg) in rabbits, and would have at the most moderate acute inhalation toxicity (LC50 > 1 670 mg/m^3) in rats. It was a moderate to severe skin irritant after a 24 hour exposure and a slight to moderate skin irritant after a 4 hour exposure in rabbits, and a moderate to severe eye irritant in rabbits. Three skin sensitisation studies (Buehler method) were provided. However, high positive responses were observed in the control groups. Two studies were inconclusive and the third study showed higher positive rates in the test group than the controls. The notifier provided an additional report of repeat insult patch test in humans. OLOA 219 was found to be a slight skin irritant and a skin sensitiser in humans.

OLOA 219 was neither mutagenic in bacteria nor clastogenic in mouse lymphoma cells.

Six repeat dose studies in rats were provided in the submission covering subchronic toxicity, neurotoxicity, reproductive toxicity, and developmental toxicity. They are summarised in the table below. The lowest NOAEL or NOEL for subchronic (oral), reproductive and development toxicity is determined to be 50 mg/kg/day. The lowest NOAEL for neurotoxicity is ≥1 000 mg/kg/day and the lowest NOEL for dermal toxicity is ≥250 mg/kg/day. The overall NOEL for OLOA 219 is established as 50 mg/kg/day.

Author	Study	Dose (mg/kg/day)	NOAL/NOAEL (mg/kg/day)	Findings at higher dose.
Lamb, 1993	28 day, oral	0, 50, 200 and 1 000	NOEL=50 (subchronic)	Clinical signs, reduced bodyweight gains and changes in organ weights.
				Highest dose tested.
			NOAEL ≥1 000 (neurotoxicity)	
				Reduced bodyweights, and decrease in live litter size.
			NOEL=50 (reproductive)	
Korenaga, 1986	28 day, dermal	0, 20, 100 and 250	NOEL ≥250 (dermal)	Highest dose tested.
Roberts, 1990	Treated during gestation days 6-15, oral	0, 50, 300 and 1 000	NOEL=300 (parental)	Reduced bodyweight gains and food consumption.
			NOEL=50 (developmental)	Malformation.
Nemec, 1994	Treated during gestation days 6-15, oral	0, 50, 300 and 1 000	NOEL=300 (parental).	Reduced bodyweights and food consumption.
			NOEL=300 (developmental)	Malformation.
Nemec, 1995a	140 days per generation, oral	0, 50, 300 and 1 000	NOAEL=50 (parental)	Mortality, clinical signs, reduced bodyweight gains, and changes of organ weights.
			NOAEL=300 (reproductive)	Lower fertility indices.

			NOAEL=50 (developmental)	Lower pup survival rates, live litter sizes and pup bodyweights.
Nemec, 1995b	>30 days, oral	0, 50 and 250 (in finished oil)	NOEL ≥250 (reproductive & developmental)	Highest dose tested

OLOA 219 is classified as a hazardous substance based on its sensitisation effects according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999b). The risk phase assigned for OLOA 219 is R43 (May cause sensitisation by skin contact).

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

No ecotoxicity data for the notified chemical were provided. However, ecotoxicity data for the chemicals CMA #607 (Chemical Manufacturers Association), CMA #608, CMA #502, CMA #503 and "old" OLOA 216Q were provided as surrogates for the notified chemical produced in its various forms. The notifier states that these CMA chemicals are almost identical to the notified chemical in that they are propylene tetramer alkylated phenates. Further, CMA #607 and CMA #502 are like OLOA 219 type phenates, and CMA #608, CMA #503 and "old" OLOA 216Q are like OLOA 216 and OLOA 218 type phenates. The majority of the tests were conducted according to OECD protocols, and the data is summarised and discussed below.

Acute Toxicity Data for CMA #607 & CMA #503 (surrogates for OLOA 219 type phenates)

Test	*Species*	*Results (Nominal) mg/L*	*Reference*
Acute Toxicity [OECD 203] CMA #607 ≅ OLOA 219	Fathead Minnow *Pimephales promelas*	LC_{50} (96 h) > 1,000 NOEC (96 h) > 1,000 Dispersion	Kowalski, 1994
Acute Toxicity [OECD 203] CMA #607 ≅ OLOA 219	Fathead Minnow *Pimephales promelas*	LC_{50} (96 h) > 1,000 NOEC (96 h) > 1,000 WAF	Boeri, 1993a
Acute Toxicity [OECD 203] Preliminary Test CMA #503 ≅ OLOA 219	Sheepshead Minnow *Cyprinodon variegatus*	100% WSF (10 g/L) No mortality over 96 hours	Breteler, 1986a
Acute Immobilisation [OECD 202] CMA #607 ≅ OLOA 219	Water Flea *Daphnia magna*	EC_{50} (48 h) > 1,000 NOEC (48 h) < 100 WAF	Boeri, 1993b
Acute Toxicity (no method specified) CMA #503 ≅ OLOA 219	Brown Shrimp *Crangon crangon*	LC_{50} (96 h) > 100 NOEC (96 h) < 100 Dispersion	Douglas & Sewell, 1988a
Acute Toxicity [OECD Section 2, General Procedures] CMA #503 ≅ OLOA 219	Mysid Shrimp *Mysidopsis bahia*	LC_{50} (96 h) = 1,800 NOEC (96 h) < 500 WSF	Suprenant, 1986
Growth Inhibition [OECD 201] CMA #607 ≅ OLOA 219	Freshwater Alga *Selenastrum capricornutum*	E_bC_{50} (96 h) > 500 $NOEC_b$ (96 h) > 500 E_rC_{50} (96 h) > 500 $NOEC_r$ (96 h) > 500 WAF	Magazu, 1994
Respiration Inhibition [OECD 209] CMA #607 ≅ OLOA 219	Activated Sludge Bacterium	EC_{50} (3 h) > 1,000	Boeri, 1994a

EC_{50}: Daphnia test - The concentration estimated to immobilise 50% of the daphnia.
EC_{50}: Activated sludge test - The concentration at which the respiration rate of activated sludge bacteria is 50% of that shown by the control.
E_bC_{50}: The concentration of test substance that results in a 50% reduction in growth of alga relative to the control.

E_rC_{50}: The concentration of test substance that results in a 50% reduction in growth rate of alga relative to the control.
LC_{50}: Median lethal concentration.
NOEC: No Observed Effect Concentration.
$NOEC_r$: No Observed Effect Concentration (based on the average number of cells/mL).
$NOEC_b$: No Observed Effect Concentration (based on average specific growth rate).
WAF: Water Accomodated Fraction.
WSF: Water Soluble Fraction.

ACUTE TOXICITY AGAINST FISH

Fathead minnow (Dispersion method)

The tests on this freshwater species were conducted over 96 hours under static conditions at nominal concentrations of 0 (control), 100, 300 and 1,000 mg/L. Each test solution was equipped with a mechanical stirrer, thereby continuously mixing the test substance and dilution water. Each test was conducted in duplicate using 10 fish in each test vessel, pH levels of 7.9 to 8.4 and a dissolved oxygen range of 7.4 to 9.0 mg/L. The water used was dechlorinated tapwater adjusted to a hardness of 160 to 180 mg/L as Ca CO_3. It was observed that all non-control test vessels had test substance stuck to weigh boats and floating on the surface throughout the test. In addition test vessels containing 1,000 mg/L had test substance on the bottom of test vessels.

One hundred percent survival occurred in the control exposure and at all test concentrations. Exposure of fathead minnows to CMA #607 resulted in a 96 hour LC_{50} > 1,000 mg/L, which is the highest tested concentration, and a NOEC estimated to be 1,000 mg/L.

Fathead minnow (WAF)

The acute toxicity of the WAF of 100, 300 and 1,000 mg/L mixtures of CMA #607 was investigated over 96 hours under static renewal conditions. Preparation of the three WAFs involved stirring the mixtures of test substance in water for 24 hours, settling the mixtures for 1 hour and siphoning off the water phase containing the WAF and ensuring that no settled or surface floating test substance was transferred. A control sample containing none of the test substance was also tested. A duplicate for each level was tested, using 10 fish in each test vessel, pH levels of 7.0 to 8.0 and a dissolved oxygen range of 7.4 to 8.7 mg/L. The water used was filtered well water adjusted to a hardness of 176 mg/L as Ca CO_3.

In one of the controls one fish out of ten died, and in the duplicate control all fish survived. There was 100 per cent survival in all test concentrations. No sublethal effects were noted. Exposure of fathead minnows to CMA #607 resulted in a 96 hour LC_{50} > 1,000 mg/L (expressed as the nominal amount of test substance used to prepare the WAF) and a NOEC of 1,000 mg/L (nominal).

Sheepshead Minnow

A tiered approach was employed to determine the acute lethal effect of CMA #503 on sheepshead minnow, a salt-water fish species. The Tier I preliminary study used a 100% WSF for a maximum of 96 hours with daily renewal of the test and control solutions. Preparation of the 100% WSF test solution involved stirring (16 to 20 hours) 150 grams of CMA #503 in 15 liters of dilution water with a subsequent settling period of two hours. Duplicate WSFs and duplicate control samples, containing none of the test substance, were tested. Each test involved using 10 fish for each test vessel, pH levels of 7.8 to 8.1 and a dissolved oxygen range of 6.2 to 7.6 mg/L. The water used was filtered natural seawater.

There was 100 per cent survival in the controls and test levels. Based on these results the 100% WSF (10 g/L) of CMA 503 is not toxic to the test population of sheepshead minnows. A Tier II definitive study was not required as less than 50% mortality was observed during the exposure period. The testing laboratory noted that their test protocol followed OECD TG 203. However, the OECD guideline does not refer to tiered testing or a cut off of less than 50% mortality requiring no further testing. The OECD guideline refers to preliminary testing in the form of a range-finding study for a subsequent definitive test. Given the 100% survival in both test solutions, the requirement for no further testing appears reasonable.

ACUTE TOXICITY AGAINST INVERTEBRATES

Daphnia magna

The acute toxicity of the WAF of 100, 300 and 1,000 mg/L mixtures of CMA #607 to daphnia was investigated over 48 hours under static renewal conditions. A control sample containing none of the test substance was also tested. Preparation of the WAFs involved the same preparation as described for fathead minnow (WAF). A duplicate for each level was tested, using 10 daphnids in each test vessel, pH levels of 7.0 to 8.5 and a dissolved oxygen range of 8.4 to 8.7 mg/L. The water used was filtered well water adjusted to a hardness of 176 mg/L as $CaCO_3$.

In both of the controls there was 100 per cent survival. In the WAF of three test concentrations there was 75 to 95 per cent survival. Insoluble material was not noted during the test. No sublethal effects were observed during the test, although some of the daphnids exposed to each concentration were floating. Exposure of daphnids to CMA #607 resulted in a 48 hour EC_{50} > 1,000 mg/L (expressed as the nominal amount of test substance used to prepare the WAF) and a NOEC of < 100 mg/L, the lowest nominal concentration tested.

BROWN SHRIMP

The acute toxicity of CMA 503 to this salt-water invertebrate was determined over 96 hours under semi-static conditions at nominal concentrations of 0 (control), 100, 500 and 1,000 mg/L of CMA 503. On a daily basis surviving shrimps were temporarily removed while each aquarium was thoroughly cleaned and refilled with fresh seawater. The test substance was heated to 90 °C to aid dispensing, and introduced directly onto the surface of the water away from a stirrer that was installed in each test solution. This avoided seizure of the stirrer motor as the substance cooled and became highly viscous in the water. The study involved three test concentrations and a control, each in duplicate, with 10 fish tested for each replicate. The pH level and dissolved oxygen of the test solutions were measured as 8.3 and 9.9 mg/L, respectively and the water used was synthetic seawater. It was observed that there was very poor dispersion of CMA 503 with only a few small globules circulating throughout the water column. Most of the test substance adhered to the screens around the shielded propeller stirrers.

There was 90 percent survival in both of the control exposures after 96 hours. The test concentrations 500 and 1000 mg/L were terminated after 48 hours due to the viscous nature of the test material sticking the dead and live shrimps together. From the cumulative mortality data it is probable that the 48 to 96 hour LC_{50} values lie between 100 to 500 mg/L (ie > 100 mg/L, nominal), although it should be stressed that most of the adverse effects were probably due to physical rather than chemical properties. The NOEC is < 100 mg/L, the lowest nominal concentration tested.

Mysid Shrimp

To determine the acute toxicity of CMA #503 on this salt-water invertebrate, a Tier II definitive study was conducted under static renewal test conditions, with daily renewal of the test solution during a 96 hour exposure period. A Tier I preliminary study and a Tier II range finding study preceded the definitive test.

Test solutions were the water soluble fraction of CMA #503 made up in filtered natural sea water at nominal concentrations of 0 (control), 500, 1,000, 2,000, 4,000 and 8,000 mg/L. Test solutions of CMA #503 were prepared separately for each test concentration. Preparation involved addition of the requisite quantity of test substance to dilution water, stirring the mixture for 24 hours followed by a settling period of 24 hours, and the gentle siphoning of the WSF away from each mixture so as to avoid transferring settled or surface floating test substance. Each test was conducted in duplicate using 10 mysids for each test vessel, and pH and dissolved oxygen levels were 7.9 to 8.1 and 4.7 to 7.1 mg/L, respectively.

Despite the first mortalities being observed at 72 hours for the 500 mg/L treatment, where the number of mortalities for one of the duplicates was 30% and the other duplicate 0%, the number of mortalities tended to increase for treatment levels greater than 1,000 mg/L. For example, for duplicates of each treatment level, mortalities increased from 10% at 1,000 mg/L (48 hours) to 70% at 8,000 mg/L (48 hours). At 8,000 mg/L, the highest nominal concentration tested, mortalities for the duplicates were 100% and 90%. It was noted that for exposure to the 4,000 and 8,000 mg/L treatments, all surviving mysids at each 24 hour observation interval were lethargic.

Probit analysis of the results provided a 96 hour LC_{50} and 95% confidence interval of 1,800 (1,400-2,400, nominal) mg/L and a corresponding 96 hour NOEC of < 500 mg/L (nominal). It can be concluded that CMA #503 displays some toxicity to mysids, at concentrations below the level of its water solubility.

ACUTE TOXICITY AGAINST ALGAE

Algae

A test on algal growth inhibition was performed under static conditions at 22 to 25 °C over 96 hours on the freshwater alga *Selenastrum capricornutum* with the WAF of five concentrations of CMA 607 and a dilution water control. Preparation of the WAF involved mixing the solutions of CMA 607 for 24 hours, settling for 1 hour and siphoning off the WAF. The test substance was not heated prior to preparation of the WAF and the nominal concentrations of WAF were 0 (control), 63, 130, 250, 350 and 500 mg/L. Each test, including the control was conducted in triplicate with the cell density determined visually by means of direct microscopic examination with a haemocytometer. The water used for testing was sterile enriched media adjusted to a pH of 7.5.

It was noted that the test vessels containing WAF at 500 mg/L were cloudy at test initiation and insoluble material adhered to the inside of the test vessel was observed from 24 hours. The EC_{50} values could not be calculated because cell growth by algae exposed to all five concentrations of WAF was greater than 50% of the control at 24, 48, 72 and 96 hours, resulting in a 96 hour E_bC_{50} and E_rC_{50} of > 500 mg/L (highest tested nominal concentrations). No effects (size differences, unusual cell shapes, colours, flocculations) were noted in any of the treatments. Analysis of the results using acceptable statistical methods (eg. Kruskal and Wallis' test and Dunnett's test) provided a 96 hour $NOEC_b$ of > 500 mg/L (based on the average number of cells/mL at each concentration) and a 96 hour $NOEC_r$ of > 500 mg/L (based on the average specific growth rate).

An aliquot of test media taken from each 500 mg/L WAF at 96 hours, when cultured in fresh media for an additional 72 hours, revealed that the WAF at this nominal concentration was algistatic rather than algicidal.

ACTIVATED SLUDGE RESPIRATION INHIBITION

A test on the inhibition of activated sewage sludge respiration by CMA 607 was conducted under static conditions at 19.8 to 20.1 °C with three concentrations of CMA 607 and controls. Nominal concentrations were 0 (control), 100, 300 and 1,000 mg/L, with each test level in duplicate and insoluble test material observed on the bottom of non-control test vessels. Preparation of the test solutions involved direct addition of CMA #607 to sterilised, filtered, dechlorinated tap water, without the use of a solvent. At time 0 for each test vessel, an aliquot of synthetic sewage was diluted to volume with water containing the appropriate concentration of CMA 607, followed by addition of an aliquot of microbial inoculum. After a three hour incubation period the dissolved oxygen content was measured for 10 minutes.

Inhibition of the activated sludge respiration was < 50% of the control rate at all tested concentrations, indicating that CMA 607 was not acutely toxic. Exposure of the activated sludge to CMA 607 resulted in an EC_{50} > 1,000 mg/L, the highest tested nominal concentration. The three hour EC_{50} determined during a reference toxicant test with this batch of activated sludge and 3,5-dichlorophenol was 12 mg/L, thereby confirming the validity of the test. It is noted that the remaining requirement for validation of the test i.e. that control respiration rates are within 15% of each other, was not met. However, the slightly greater difference was considered to have minimal impact and not effect the validity of the test.

Acute Toxicity Data for "old" OLOA 216Q, CMA #608 and CMA #502 (surrogates for OLOA 216 and OLOA 218 type phenates)

Test	*Species*	*Results (Nominal) mg/L*	*Reference*
Acute Toxicity [OECD 203] "old" OLOA 216Q ≅ OLOA 216 & 218	Rainbow Trout *Oncorhynchus mykiss*	LC_{50} (96 h) > 1,000 NOEC (96 h) > 1,000 WAF	Ward et al, 1997
Acute Toxicity [OECD 203] CMA #502 ≅ OLOA 216 & 218	Sheepshead Minnow *Cyprinodon variegatus*	100% WSF (10 g/L) See Comments	Breteler, 1986b
Acute Toxicity (no method specified) CMA #502 ≅ OLOA 216 and 218	Brown Shrimp *Crangon crangon*	LC_{50} (96 h) > 1,000 100 < NOEC (96 h) < 500 Dispersion	Douglas & Sewell, 1988b
Acute Immobilisation [OECD 202] "old" OLOA 216Q ≅ OLOA 216 & 218	Water Flea *Daphnia magna*	EC_{50} (48 h) > 1,000 NOEC (48 h) = 1,000 WAF	Kowalski, 1997a
Growth Inhibition [OECD 201] "old" OLOA 216Q ≅ OLOA 216 & 218	Freshwater Alga *Selenastrum capricornutum*	E_bC_{50} (96 h) > 1,000 $NOEC_b$ (96 h) > 360 E_rC_{50} (96 h) > 1,000 $NOEC_r$ (96 h) > 220 WAF	Kowalski, 1997b
Respiration Inhibition [OECD 209] CMA #608 ≅ OLOA 216 & 218	Activated Sludge Bacterium	EC_{50} (3 h) > 1,000	Boeri, 1994b

ACUTE TOXICITY AGAINST FISH

Rainbow trout (WAF)

The acute toxicity of the WAF of a 1,000 mg/L mixture of OLOA 216Q was investigated over 96 hours under static, renewal conditions at 12 ± 1°C. Preparation of the WAF involved stirring the mixture of test substance in water for 20 hours, settling the mixture for 4 hours and siphoning off the water phase containing the WAF, ensuring that no settled or surface floating test substance was transferred. A control sample containing none of the test substance was also tested. Ten fish were distributed to each of three replicates of the control and treatment, with pH levels of 7.0 to 7.6 and a dissolved oxygen range of 7.1 to 10.2 mg/L. The water used was carbon filtered deionised water adjusted to a hardness of 40-48 mg/L as $CaCO_3$.

There was 100% survival in each of the controls. There was 97% survival in the test concentrations, with a single mortality at 72 hours resulting from accidental removal of the fish during renewal of the test solution. No sublethal effects were noted. Exposure of rainbow trout to "old" OLOA 216Q resulted in a 96 hour LC_{50} > 1,000 mg/L (expressed as the nominal amount of test substance used to prepare the WAF) and a NOEC of 1,000 mg/L (nominal).

Sheepshead Minnow

A tiered approach was employed to determine the acute lethal effect of CMA #502 on sheepshead minnow, a salt-water fish species. The Tier I preliminary study used a 100% WSF for a maximum of 96 hours with daily renewal of the test and control solutions. Preparation of the 100% WSF test solution involved stirring (16 to 20 hours) 150 grams of CMA #502 in 15 litres of dilution water, a settling period of two hours and removal of the WSF, ensuring that no settled or surface floating test substance was transferred. Duplicate WSF solutions and duplicate control solutions, containing none of the test substance, were tested. Each test involved using 10 fish

for each test vessel, pH levels of 7.6 to 8.3 and a dissolved oxygen range of 6.1 to 7.9 mg/L. The water used was filtered natural seawater.

There was 100 per cent survival for each of the controls. For one of the 100% WSF solutions there was 100% survival over 96 hours. However, for the duplicate 100% WSF solution, 100% mortality was observed at 48 hours and the testing laboratory calculated a mean mortality for the duplicates of 50%. The testing laboratory did not perform a Tier II definitive study and concluded that based on these results, the 100% WSF (10 g/L) of CMA 502 was not toxic to $\geq$ 50% of the test population of sheepshead minnow. The testing laboratory noted that their test protocol followed OECD TG 203. However, the OECD guideline does not refer to tiered testing or a cut off of less than 50% mortality requiring no further testing. The OECD guideline refers to preliminary testing in the form of a range-finding study for a subsequent definitive test. Given the massive difference between results for the test solutions and the lack of explanation as to the cause of the difference, the findings of this test do not appear sound. The test should have been repeated or a definitive test conducted.

ACUTE TOXICITY AGAINST INVERTEBRATES

Brown Shrimp

The acute toxicity of CMA 502 to this salt-water invertebrate was determined over 96 hours under semi-static conditions at nominal concentrations of 0 (control), 100, 500 and 1,000 mg/L of CMA 502, employing direct dispersion of the test substance in water. The study involved duplicates of each test level, with 10 fish tested for each replicate. The pH level and dissolved oxygen of the test solutions were measured as 8.1 and 10.0-10.1 mg/L, respectively. Refer to brown shrimp tested against CMA 503 for further details.

After 96 hours, there was 100% survival in one of the controls and 90% survival in the other control, with the single mortality observed at 48 hours. From the cumulative mortality data there were insufficient mortalities in 20 shrimp exposed to concentrations of up to 1000 mg/L (nominal) for 96 hours to calculate LC_{50} values. Consequently, the LC_{50} value is estimated to be > 1000 mg/L (nominal), the highest concentration tested. Mortalities (maximum of 50% and 20% for the 1000 mg/L duplicates at 96 hours) may have been due to physical rather than chemical properties of the test substance, as it was observed that there was very poor dispersion of CMA 502 and most of the test substance adhered to the screens around the shielded propeller stirrers. Although no statistical analysis was performed to determine the NOEC, it appears from the tabulated results that the NOEC is between 100 and 500 mg/L (nominal).

Daphnia magna

The acute toxicity of the WAF of 0 (control), 130, 220, 360, 600 and 1,000 mg/L mixtures of "old" OLOA 216Q to daphnia was investigated over 48 hours under static conditions. Preparation of the WAF involved mixing an appropriate amount of test substance with dilution water for 20 hours, a settling period of 4 hours and siphoning away the water phase containing the WAF. A duplicate for each level was tested, using 10 daphnids for each replicate, pH levels of 7.4 to 8.4 and a dissolved oxygen range of 7.4 to 8.5 mg/L. The water used was filtered, deionised tap water adjusted to a hardness of 160-164 mg/L as $CaCO_3$.

In both of the controls there was 100 per cent survival with no sublethal effects. There was greater than 90% survival in all of the WAF test solutions with no mortalities observed in the duplicates of the highest test level, 1,000 mg/L (nominal). Insoluble material was not noted during the test. Exposure of daphnids to OLOA 216Q resulted in a 48 hour EC_{50} > 1,000 mg/L (expressed as the nominal amount of test substance used to prepare the WAF) and a NOEC of 1,000 mg/L.

Algae

A test on algal growth inhibition was performed under static conditions at 23 to 25°C over 96 hours on the freshwater alga *Selenastrum capricornutum* with the WAF of five concentrations of "old" OLOA 216Q and a dilution water control. Preparation of the WAF involved mixing the solutions of OLOA 216Q for 20 hours, settling for 4 hours and siphoning away the WAF. The test substance was not heated prior to preparation of the WAF and the nominal concentrations of WAF were 0 (control), 130, 220, 360, 600 and 1,000 mg/L. Each test, including the control was conducted in triplicate with the cell density determined visually by means of direct microscopic examination with a haemocytometer. The water used for testing was sterile enriched media adjusted to a pH of 7.5.

The 96 hour values for E_bC_{50} and E_rC_{50} were determined to be greater than the highest tested nominal concentration of 1,000 mg/L (calculated using the number of cells per mL and average specific growth rate, respectively). No effects (size differences, unusual cell shapes, colours, flocculations) were noted during the test. The probit method was used to calculate the EC_{50} values despite cautionary statements, because the EC_{50} values compared well with values calculated by other methods. Analysis of the results using acceptable statistical methods (eg. Shapiro Wilks test and Bartletts test) provided a 96 hour $NOEC_b$ of 360 mg/L (calculated using the number of cells per mL) and a 96 hour $NOEC_r$ of 220 mg/L (calculated using the average specific growth rate).

An aliquot of test media taken from each 1,000 mg/L WAF at 96 hours, when cultured in fresh media for an additional 72 hours, revealed that the WAF at this nominal concentration was algistatic rather than algicidal.

ACTIVATED SLUDGE RESPIRATION INHIBITION

A test on the inhibition of activated sewage sludge respiration by CMA 608 was conducted under static conditions at 20.0 to 20.5°C with three concentrations of CMA 608 and controls. Nominal concentrations were 0 (control), 100, 300 and 1,000 mg/L, with each test level in duplicate and insoluble test material observed on the bottom of non-control test vessels. Preparation of the test solutions involved direct addition of CMA 608 to sterilised, filtered, dechlorinated tap water, without the use of a solvent. At time 0 for each test vessel, an aliquot of synthetic sewage was diluted to volume with water containing the appropriate concentration of CMA 608, followed by addition of an aliquot of microbial inoculum. After a three hour incubation period, the dissolved oxygen content was measured for 10 minutes.

Inhibition of the activated sludge respiration was less than 50% of the control rate at all tested concentrations, indicating that CMA 608 was not acutely toxic. Exposure of the activated sludge to CMA 608 resulted in an EC_{50} > 1,000 mg/L, the highest tested nominal concentration. The three hour EC_{50} determined during a reference toxicant test with this batch of activated sludge and 3,5-dichlorophenol was 16 mg/L and the control respiration rates were within 15% of each other, thereby confirming the validity of the test.

CONCLUSION

Ecotoxicity data for five chemicals, provided as surrogates for the low and high calcium versions of the notified chemical, indicate that based on the conditions of the individual tests, most of these chemicals are not toxic to the species of fish, micro-invertebrates and alga tested up to the level of their solubility in water. The exceptions were that one of the surrogates (CMA 503) exhibited some toxicity to a micro-invertebrate (mysid shrimp), at concentrations below the level of its water solubility and results from preliminary testing of another surrogate (CMA 502) against a salt-water species of fish (sheepshead minnow) were inconclusive. In addition, effects observed for two surrogates (CMA 502 and CMA 503) tested against a micro-invertebrate (brown shrimp), were likely due to physical effects. Further, the two surrogates (CMA 607 and "old" OLOA 216Q) tested against alga were shown to be algastatic. Treatment of activated sludge bacterium with two of the surrogates (CMA 607 and CMA 608) caused some inhibition in respiration, but they were not toxic to the bacterium.

Based on the claimed similarity of the surrogate chemicals to the different versions of the notified chemical, the ecotoxicity data for the surrogate chemicals indicate that the the notified chemical should not be toxic to the organisms tested except for mysid shrimps, up to its level of solubility in water.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The environmental hazard from the notified chemical is considered to be small provided that the material is used as indicated, and that disposal of waste oil follows approved practices. Apart from transport accidents or accidental spills or leaks, minimal release of the notified chemical is expected as a result of lubricant formulation and transfer to engine crankcases, and the waste generated would either be incinerated or placed into landfill. However, as a component of passenger vehicle engine lubricants, the notified material may be inappropriately released to the environment through disposal by DIY automobile enthusiasts of the waste engine oil in weed control, to landfill, stormwater drains, or in fence painting. No information was provided on the market share of the notified chemical in the lubricants market and the total amount of notified chemical used as

an additive in passenger vehicle lubricants. However, in comparison with the worst case scenario where all of the notified chemical would be sold to the automotive market and approximately 7% disposed of inappropriately by DIY enthusiasts, much less notified chemical would be disposed of inappropriately in the described range of uses, and effects would be mitigated by the diffuse pattern of release.

If deposited on soil or into landfill, the notified chemical will be immobilised through adsorption onto soil particles. If released into waterways it would associate with organic matter and sediments. The notified chemical is not readily biodegradable, but in landfill it would be expected to slowly degrade through biological and abiotic processes. Incineration of waste oil would destroy the notified chemical with evolution of water vapour and oxides of carbon and sulphur and produce calcium compounds that would be assimilated with the ash. Sludges from waste treatment plants or oil recycling facilities could also be incinerated.

Direct exposure to the water compartment is considered to be unlikely, thereby limiting the potential for bioaccumulation.

Based on a variety of ecotoxicity tests for surrogate chemicals conducted against a number of freshwater and marine organisms (fish, invertebrates and algae), the notified chemical is not expected to be toxic to the aquatic species against which the surrogates have been tested, up to its level of solubility in water. However, the notified chemical may exhibit some level of toxicity to mysid shrimps, below its level of water solubility and results for a surrogate tested against sheepshead minnow were inconclusive. It may be concluded that despite some inappropriate disposal, levels in water are unlikely to reach those presenting a hazard to aquatic organisms.

After the additional import volume of the notified chemical by the extension applicant, the fate of the notified chemical and the environmental impact are expected to be very similar to those for the original submission due to the same use, and therefore the potential risk due to the additional import volume remains low.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

No toxicological data were available on the notified chemical to assess it against the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999b). The notifier selected New OLOA 216Q and New OLOA 219 to represent the low calcium versions (New OLOA 216C, 216Q and 218A) and the high calcium versions (New OLOA 219, 219M and 219C), respectively. No toxicological data have been supplied for New OLOA 216Q or New OLOA 219. Toxicological studies provided were performed on two closely related analogues, OLOA 216Q and OLOA 219.

OLOA 216Q was of very low acute oral toxicity and low acute dermal toxicity. It caused slight to moderate skin and eye irritation in animals. Evidence for skin sensitisation in the guinea pig (Buehler) was inconclusive. The NOEL for subchronic toxicity was 300 mg/kg/day based on reduced body weight gain, food utilization and an increased adrenal weight which was accompanied by histopathological changes at the higher dose level. The NOEL for neurotoxicity and reproductive toxicity was ≥1 000 mg/kg/day (highest dose tested). In genotoxicity testing, OLOA 216Q was non-mutagenic in bacteria and non-clastogenic in a micronucleus assay in mice. Based on the results of OLOA 216Q, the New OLOA 216Q would not be classified as a hazardous substance according to NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999b).

OLOA 219 has very low acute oral toxicity, low acute dermal toxicity and at the most moderate acute inhalation toxicity. It caused slight to moderate skin irritation and moderate to severe eye irritation. It was a skin sensitiser in one animal study. A repeat insult patch test performed in humans was considered to be positive for skin sensitisation. Repeat dose studies in rats assessed subchronic toxicity, neurotoxicity, reproductive toxicity, and developmental toxicity of OLOA 219. The lowest NOEL or NOAEL when orally administered was 50 mg/kg/day, whereas the NOEL for dermal application was ≥250 mg/kg/day. Effects observed at higher doses (up to 1 000 mg/kg/day) in these studies were reduced food consumption, body weight gain, and changed organ weights. Reduced fertility indices and live litter size were apparent in the reproduction studies, while foetal malformations were observed in the developmental studies. No neurotoxicity was observed at doses up to 1 000 mg/kg/day. OLOA 219 was neither mutagenic in bacteria nor clastogenic in mouse lymphoma cells. Based on the results of OLOA 219, the New OLOA 219 would be classified as a hazardous substance based on the skin sensitisation effects according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999b) with Risk phase R43 (May cause sensitisation by skin contact).

The paraffinic petroleum distillates used as adjuvants in New OLOA products are Category 2 carcinogens, with concentration cut-offs of 0.1 %, unless the petroleum distillate is shown to satisfy the condition that it contains less than 3 % DMSO extract as measured by IP 346. The notifier has indicated that this is the case for the petroleum distillates used in New OLOA products.

Occupational Health and Safety

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 except in the event of spills. Skin and eye contact with the notified chemical in high concentrations 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. Cleaning operations are automated and exposure will not occur. All tasks are of short duration and infrequent and 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 health effects is considered to be low. However, exposure must be prevented to protect against skin sensitisation particularly for the high calcium products.

The system for reformulating New OLOA products to produce finished lubricants is enclosed and automated. The possibility of exposure is therefore limited and typically of short duration. Workers involved in transferring the imported oil additive containing the notified chemical, and blending the additive into finished oil may be exposed to drips and spills. In addition, occupational exposure to the drips and spills of the final lubricating oil containing the notified chemical is possible for workers filling and labelling the finished oil products. Workers involved in cleaning and maintenance of tanks and blending equipment may also have general dermal exposure to oil residues. It is reported that workers will wear coveralls, gloves and eye protection in all sections of the blending plant. Overall, exposure to the notified chemical is likely to be negligible and the risk of adverse health effects is considered to be low. However, exposure to the liquid and mist must be prevented to protect against skin sensitisation.

Workers handling marine vessels, railway diesel engines and heavy-duty vehicle diesel engines will contact lubricating oil with up to 12.5% notified chemical. Skin and eye contact with the notified chemical may occur via splashes, drips or spills when transferring the formulated lubricant into engines and during drum cleaning operations. Duration and frequency of tasks is likely to be comparable to those at marine terminals and blending plants. Overall exposure is likely to be negligible and the risk of adverse health effects is considered to be low. As it is possible that individuals may become sensitised to the notified chemical, employers will need to ensure that mechanics handling the notified chemical are informed about the potential for skin sensitisation.

At the petrol car manufacturing and repairing sites, dermal exposure is likely during addition or changing of engine oils, and while handling equipment which has been in contact with the lubricating oils. The finished lubricant contains 0.5-3.5% notified chemical. Exposure during each top up of reservoirs will be of short duration. However, it is recommended that the workers wear protective clothing and gloves to minimise the risk of skin sensitisation from the high calcium products containing the notified chemical.

Workers who become sensitised to lubricant oil containing the notified chemical should not continue to handle it in the workplace.

Public health

The populations who are potentially exposed to this material include consumers who may periodically either add or change their own automotive engine oil. The potential for public exposure to the notified chemical during transport, storage, or disposal is considered to be low. The amounts to which the public is likely to be exposed is expected to be small and exposure is expected to be brief and intermittent. The most likely routes of exposure to the notified chemical are skin and eye contact and wearing personal protective equipment can eliminate exposure. The notified chemical will not pose a significant hazard to public health when used in the proposed manner.

13. RECOMMENDATIONS

Occupational Health and Safety Matters

To minimise occupational exposure to Chemical in New OLOA 216C, 216Q, 218A, 219, 219C and 219M the following guidelines and precautions should be observed:

- The high calcium OLOA products are determined to be hazardous substances because they cause skin sensitisation. The label and MSDS for high calcium OLOA products should include R43 (May cause sensitisation by skin contact) and disclose the chemical name(s) of component(s) which caused skin sensitisation.

- 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 with high calcium OLOA products.

- 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 high calcium OLOA products. 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 high calcium OLOA products 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 high calcium OLOA products occurs. The notifier recommends Nitrile, Viton or silver shield gloves. Chemical impervious clothing is also 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);

- If products containing the notified chemical are hazardous to health in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999b), 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.

- OLOA products are identified as a C2 combustible liquid and should be stored, handled and used in accordance with AS 1940 (SAA 1993);

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

14. MATERIAL SAFETY DATA SHEET

Material Safety Data Sheet

The MSDSs for the notified chemical and products containing the notified chemical provided by the notifier and by the extension applicant were in accordance with the NOHSC *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC 2003). They are published here as a matter of public record. The accuracy of the information on the MSDS remains the responsibility of the applicants.

Label

The labels for the notified chemical and products containing the notified chemical provided by the notifier and by the extension applicant were in accordance with the NOHSC *National Code of Practice for the Labelling of Workplace Substances* (NOHSC 1994). The accuracy of the information on the labels remains the responsibility of the applicants.

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:

Erythema Formation	*Rating*	*Oedema Formation*	*Rating*
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

Opacity	*Rating*	*Area of Cornea involved*	*Rating*
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

Redness	*Rating*	*Chemosis*	*Rating*	*Discharge*	*Rating*
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

Values	*Rating*
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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