File No: STD/1468

December 2013

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Benzoic acid, 2-hydroxy-, mono-C14-18-alkyl derivs., calcium salts (2:1)

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

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

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1468	Cintox Australia Pty Ltd	Benzoic acid, 2-hydroxy-, mono-C14-18-alkyl derivs., calcium salts (2:1)	Yes	≤ 2000 tonnes per annum	Lubricating oil additive

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the table below.

Hazard classification	*Hazard statement*
Skin irritation (Category 3)	H316: Causes mild skin irritation
Skin sensitizer (Category 1)	H317: May cause allergic skin reaction

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:
R38: Irritating to skin
R43: May cause sensitisation by skin contact

Human health risk assessment
Provided that the recommended controls are being adhered to, under the conditions of the occupational setting, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

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

Environmental risk assessment
On the basis of the PEC/PNEC ratio and the assessed use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - H316: Causes mild skin irritation
  - H317: May cause allergic skin reaction

Health Surveillance

- As the notified chemical is a skin sensitiser, employers should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of skin sensitisation.

CONTROL MEASURES
Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical during reformulation:
  - Enclosed, automated processes, where possible

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical:
  - Avoid contact with skin and eyes

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical:
  - coveralls
  - impervious gloves
  - goggles

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

- A copy of the (M)SDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Disposal

- The notified chemical should be disposed of in accordance with local regulations for recycling, re-use or recovery of calorific content.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by physical containment, collection and subsequent safe disposal.

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if
  - the concentration of the notified chemical in the finished lubricating oils exceeds 2%.

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from a lubricating oil additive, or is likely to change significantly;
  - the amount of chemical being introduced has increased, or is likely to increase, significantly;

- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

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

(Material) Safety Data Sheet
The (M)SDS of the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Cintox Australia Pty Ltd (ABN: 63 122 874 613)
Suite 1, Level 2, 38-40 George Street
PARRAMATTA NSW 2150

NOTIFICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: analytical data, degree of purity, impurities, use details, import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: vapour pressure, hydrolysis as a function of pH, adsorption/desorption, dissociation constant, explosive properties, oxidising properties and all toxicological endpoints.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

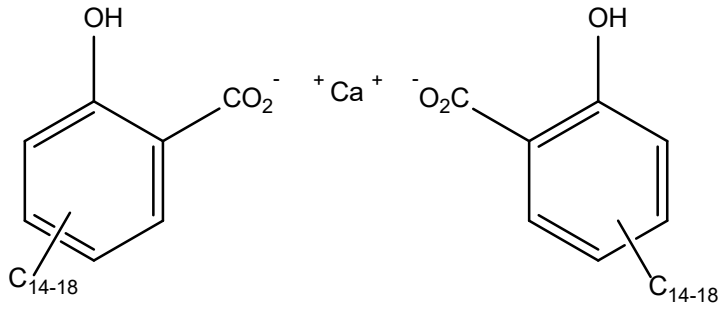
MARKETING NAME(S)
SP 8107 (contains < 50% notified chemical)

CAS NUMBER
114959-46-5

CHEMICAL NAME
Benzoic acid, 2-hydroxy-, mono-C14-18-alkyl derivs., calcium salts (2:1)

MOLECULAR FORMULA
Unspecified

STRUCTURAL FORMULA



MOLECULAR WEIGHT
772-885 Da

ANALYTICAL DATA
Reference NMR, IR, and UV spectra were provided.

3. COMPOSITION

Degree of Purity > 85%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 ºC AND 101.3 kPa: Brown liquid

Property	Value	Data Source/Justification
Pour Point	-38 °C	Measured
Boiling Point	Decomposes from 150 °C	Measured
Density	1007.7 kg/m^3 at 20 °C	Measured
Vapour Pressure	1.68 x 10^{-9} kPa at 25 °C	Analogue data
Water Solubility	< 0.103 g/L	Measured
Hydrolysis as a Function of pH	Not determined	Does not contain any readily hydrolysable functional groups and is expected to be stable at the environmental pH range (4-9)
Partition Coefficient (n-octanol/water)	log Pow > 3.9 at 20 °C	Measured
Adsorption/Desorption	Not determined	Expected to partition to phase boundaries based on its surface activity
Dissociation Constant	Not determined	Significant dissociation is not expected based on the low water solubility of the notified chemical
Flash Point	188 °C	Measured
Flammability	Not determined	Not expected to be highly flammable based on measured flash point
Autoignition Temperature	372 °C	Measured
Explosive Properties	Not determined	Contains no functional groups that imply explosive properties
Oxidising Properties	Not determined	Contains no functional groups that imply oxidative properties

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

Reactivity
The notified chemical is expected to be stable under normal conditions of use.

Physical hazard classification
Based on the submitted physico-chemical data depicted in the above table, the notified chemical is not recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported into Australia as part of finished lubricant oil products at < 2% concentration. The notified chemical may also be imported into Australia as part of an additive package at < 30% concentration for reformulation into lubricant oils.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤ 100	≤ 100	≤ 100	≤ 1000	≤ 2000

PORT OF ENTRY
Sydney, Melbourne, Fremantle and Brisbane

TRANSPORTATION AND PACKAGING
The additive packages (containing the notified chemical at < 30% concentration) and the finished oil products (containing the notified chemical at < 2% concentration) will be imported in either 500-26,000 L isotanks or 205 L steel drums and will be shipped directly to customers. It is estimated that 50% of the finished lubricant oil products will be delivered by tank truck or rail car, 30% in 205 L drums and 20% in 1-4 L containers to end-use customers.

USE
The notified chemical will be used as a lubricating oil additive in automotive engines at up to 2% concentration in finished products.

OPERATION DESCRIPTION
The notified chemical will be imported into Australia as part of finished lubricating oil for automotive engines at up to 2% concentration.

The notified chemical will also be imported in solution at < 30% concentration for reformulation into lubricant oils.

Formulation of lubricant oils
In the case where the notified chemical is to be reformulated, it will be distributed to oil lubricant manufacturers. At the blending sites, the notified chemical will be transferred using pumps into storage tanks. From these tanks the notified chemical will be transferred into blending tanks through computer-controlled and fully automated valves. The notified chemical will be blended at up to 2% concentration with other components in a fully closed and automated system. The finished oil product will then be returned to the storage tanks via an automated process. Samples may also be taken by laboratory staff prior and after reformulation for quality testing. The finished oils will then be packed off into drums or small containers, or transferred through hard piping to tank trucks or rail cars for distribution to end-use customers (service stations or retail outlets).

End-use
At end-use sites, the finished lubricant oils containing the notified chemical at < 2% concentration will be transferred (by automated or manual means) to automobile engines.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Unloading isotanks and drums	0.5	30
Sampling and analysing additive packages	0.2	30
Sampling and analysing finished oils	0.2	220
Packaging	0.5	220
Distribution	0.5	220

EXPOSURE DETAILS

Transport and storage

Transport and storage workers may come into contact with the notified chemical at < 30% concentration or as a component of finished lubricant oil products (< 2% concentration) only in the unlikely event of accidental rupture of containers.

Formulation of products

Dermal and ocular exposure of workers to the notified chemical at < 30% concentration may occur during formulation when connecting and disconnecting hoses and during sample testing. Inhalation exposure is not expected due to the low vapour pressure of the notified chemical. Exposure is expected to be minimised through the use of enclosed systems and through the use of personal protective equipment (PPE) such as coveralls, safety glasses and impervious gloves.

End-use

Workers may be exposed to engine oils containing the notified chemical at < 2% concentration during use, for example, at automobile manufacturing sites, car dealerships or automotive service centres during transfer, charging or top-up activities.

At car manufacturing sites, the finished engine oil containing the notified chemical (< 2% concentration) will likely be added to engines using automated systems and exposure is unlikely. However, dermal and ocular exposure from drips, spills and splashes as well as from handling equipment contaminated with engine oil is possible. The potential for dermal and ocular exposure is expected to be reduced by the wearing of PPE, e.g. gloves, protective clothing and goggles.

At automotive service centres, professional users such as mechanics may experience dermal or ocular exposure to the engine oil products containing the notified chemical (< 2% concentration) when transferring engine oil to cars. The potential for dermal and ocular exposure may be mitigated through the use of PPE (e.g. gloves, protective clothing and goggles). Overall, workers exposure to the notified chemical (< 2% concentration in finished engine oils) is not expected to be significant.

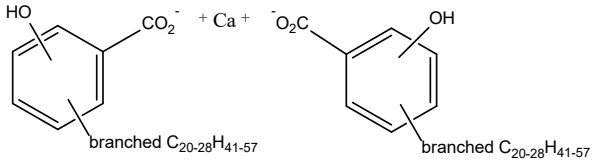
6.1.2. Public Exposure

The notified chemical will be used as a component of engine oils at < 2% concentration. Once engine oil containing the notified chemical is added to the engine, the general public is not expected to be exposed to the notified chemical during its use in the engine. DIY users may experience limited dermal and accidental ocular exposure to engine oils containing < 2% of the notified chemical when changing/topping-up the engine oil in their vehicles. However, such activities are expected to occur infrequently.

Overall, public exposure to the notified chemical is expected to be low, and further limited by the infrequent use of the finished engine oils (containing < 2% notified chemical).

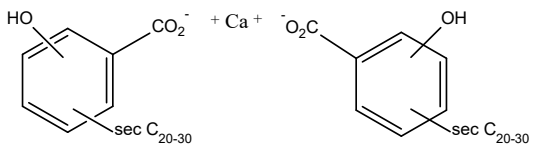
6.2. Human Health Effects Assessment

Information on the expected health effects of the notified chemical are based on the notified chemical as well as two acceptable analogues of the notified chemical (Analogues 1-2).



Analogue 1
Benzoic acid, hydroxy-, mono-C20-28-branched alkyl derivs., calcium salts (2:1) CAS No. 900185-23-1

Other names: SP8055; OLOA 16305



Analogue 2
Phenol, 2(or 4)-C20-30-sec-alkyl derivs., reaction products with carbon dioxide, distn. residues from manuf. of phenol (tetrapropenyl) derivs. and phenol (tetrapropenyl) derivs., calcium salts (2:1) CAS No. 220795-13-1

Other names: SP 7077

The analogues and the notified chemical are members of the carboxylate/salicylate class. They share the same chemical features and functionality and only differ in the alkyl chain length and branching. This is not expected to significantly alter their physical-chemical properties. Overall, the toxicological properties of the notified chemical are considered to be well represented by the analogue data.

Key results from toxicological investigations conducted on the notified chemical and the analogue chemicals are summarised below. For full details of the available studies, refer to Appendix B.

*Endpoint**	*Result and Assessment Conclusion*
Rat, acute oral toxicity[1]	LD50 > 5000 mg/kg bw; low toxicity
Rat, acute dermal toxicity[1]	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation[2]	irritating
Rabbit, eye irritation[2]	slightly irritating
Guinea pig, skin sensitisation – non-adjuvant test[3]	evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days[2]	NOAEL = 150 mg/kg bw/day
Mutagenicity – bacterial reverse mutation[4]	non mutagenic
Genotoxicity – in vitro chromosomal aberration test	non genotoxic
Genotoxicity – in vivo micronucleus test[2]	non genotoxic
Rat, reproductive and developmental toxicity	NOAEL = 500 mg/kg bw/day

[1] Test substance: 28% notified chemical, 51% highly refined mineral oil, 21% inorganic calcium salts
[2] Results of studies conducted on analogues
[3] Test substance: notified chemical in mineral oil (concentration unknown)
[4] Test substance: 43% notified chemical, 48% highly refined mineral oil, 9% inorganic calcium salts

Toxicokinetics
The notified chemical has a relatively high molecular weight, is a salt and is of low water solubility; hence dermal absorption is expected to be limited. Absorption across the GI tract may occur. This is supported by evidence of systemic toxicity in a 28-day repeated dose oral toxicity study.

Acute toxicity.
A test substance containing the notified chemical at 28% concentration was found to be of low acute oral and dermal toxicity in studies conducted in rats. These results are consistent with rat studies conducted on Analogue 1 (acute oral toxicity LD50 > 2000 mg/kg bw) and Analogue 2 (acute dermal toxicity LD50 > 2000 mg/kg bw). Based on the result of these studies, the notified chemical is not expected to be acutely toxic by the oral and dermal routes.

Irritation and sensitisation.
In studies conducted in rabbits, Analogue 1 was found to be irritating to the skin and Analogue 2 was slightly irritating to the eyes. Based on the result of these studies, the notified chemical is expected to be a skin irritant and a slight eye irritant.

The notified chemical was found to be a sensitiser in a Modified Buehler test conducted in guinea pigs. This result is consistent with the positive skin sensitisation results found for Analogue 1 in three independent guinea pig non-adjuvant skin sensitisation studies.

Repeated Dose Toxicity.
In a 28-day repeated dose oral toxicity study on the notified chemical, the NO(A)EL was established as 150 mg/kg bw/day based on slight changes in clinical chemistry with minimal corresponding changes in liver and kidney weights, and slightly longer prothrombin time in the high dose group. A 28-day repeated dose oral toxicity study was also conducted on Analogue 1. The NOAEL was established as 400 mg/kg bw/day based upon body weight and coagulation changes in the high dose group. All effects were resolved at the end of the 14-day recovery period.

Mutagenicity/Genotoxicity.
The notified chemical was found to be negative in a bacterial reverse mutation assay with and without metabolic activation. The notified chemical was also negative in a chromosome aberration assay. In addition, Analogue 1 was non mutagenic in a bacterial reverse mutation assay and was non genotoxic in an *in vitro* mammalian chromosome aberration test. Analogues 1 and 2 were also found to be non genotoxic in an *in vivo* mouse micronucleus test. Based on the results of these studies, the notified chemical is not expected to be genotoxic.

Reproductive/Developmental toxicity
The notified chemical was tested in a developmental/reproductive screening study in rats. It was found that there were no adverse effects nor signs of systemic toxicity at the maximum dose tested (500 mg/kg bw/day). Thus, the NOAEL for parental reproductive toxicity and neonatal toxicity was determined to be 500 mg/kg bw/day.

Health hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

Hazard classification	***Hazard statement***
Skin irritation (Category 3)	H316: Causes mild skin irritation
Skin sensitizer (Category 1)	H317: May cause allergic skin reaction

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase(s):

R38: Irritating to skin
R43: May cause sensitisation by skin contact

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on analogue studies, the notified chemical is a skin irritant. Although not classifiable, it is slightly irritating to the eyes. Furthermore, the notified chemical is a skin sensitiser. The notified chemical may cause systemic toxicity (NOAEL 150 mg/kg bw/day). However, given the limited potential for dermal absorption, systemic toxicity by the dermal route is not expected. Toxicity by the inhalation route is not known. However, based on the low vapour pressure, and the high viscosity of the formulation, inhalation exposure is not expected.

Reformulation
Reformulation workers may be at risk of irritating and sensitising effects when handling the imported additive packages containing the notified chemical at < 30% concentration. However, the risk is expected to be minimised by the use of largely enclosed and automated processes, and use of appropriate PPE including coveralls, impervious gloves, and eye protection. Therefore, the risk to the health of reformulation workers is not considered to be unreasonable.

End-use
End users (e.g. mechanics in automotive centres) will be exposed to the notified chemical at < 2% concentration when transferring engine oil to cars. Given the low concentration and provided that the recommended controls are used, exposure should be low. Therefore, the risk to professional end users from use of the notified chemical at < 2% concentration in engine oil products is not considered unreasonable.

6.3.2. Public Health
The public will be exposed to the notified chemical at < 2% concentration during DIY engine oil change/top-

up activities. Given the low concentration and expected infrequent use of engine oils by the public, exposure should be low. Therefore, the risk to the public from use of the notified chemical at < 2% concentration in engine oil products is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported into Australia as a component of finished lubricating oil at < 2% concentration or as solution at < 30% concentration for reformulation into lubricant oils for automotive engines. Significant release of the notified chemical to the environment is not expected during transport and storage except in the unlikely event of an accidental spillage or leakage.

Any notified chemical spilled during reformulation is expected to be contained with inert material and either sent to on-site waste treatment facilities or be reused in blending processes. At the on-site waste treatment facilities, residues of the notified chemical will be separated from the aqueous stream. The aqueous waste undergoes further treatment involving pond aeration and biological treatment before being released to the sewage system. The remaining non-aqueous waste will be incinerated. As a result of these treatments, greater than 90% removal of the notified chemical is estimated by the notifier. Therefore, the accidental release from reformulation of the notified chemical and finished oils is unlikely to be significant.

RELEASE OF CHEMICAL FROM USE

Environmental exposure from use of the finished oil would be from spills during addition of the finished oil to the engine, or as leaks from the engines. It is not possible to estimate these losses, though they are expected to be minimal since the notified chemical is present in the finished oil at less than 2%.

RELEASE OF CHEMICAL FROM DISPOSAL

After reformulation, empty import drums containing residual notified chemical are expected to be steam cleaned, with the residual waste sent to on-site wastewater treatment facilities. Assuming 0.1% of the notified chemical remains in the container after use, a worst case estimate of 2000 kg/yr (2000 tonnes/year × 0.1%) of the notified chemical will be sent to the waste treatment. It is estimated by the notifier that up to 98% of the notified chemical may be removed during waste treatment processes. Therefore, the amount of the notified chemical released to sewer from the cleaning of empty drums is estimated to be 40 kg/yr (= 2000 kg/year × 2%).

The major release of the notified chemical to the environment will come from inappropriate disposal of waste or used oils. Oil products containing the notified chemical will be poured into automotive engines by service station workers or by do-it-yourself (DIY) consumers. A survey by the Australian Institute of Petroleum (AIP, 1995) indicates that of the annual sales of engine oils in Australia, 60% of oils are potentially recoverable (i.e. not burnt in the engines during use). This report also indicates that around 86% of oil changes take place in specialised automotive service centres, where old oil drained from crankcases is disposed of responsibly (e.g. oil recycling or incineration). Assuming this is the case, negligible release of the notified chemical should result from these professional activities. The remaining 14% of oil is removed by DIY consumers. In these cases, some of the used oil would be either incinerated, left at transfer stations where it is again likely to be recycled, or deposited into landfill. It was estimated that DIY activities account for 7-10% of the unaccounted used oil (Meinhardt, 2002).

According to a survey tracing the fate of used lubricating oil in Australia (Snow 1997), only approximately 20% of used oil removed by DIY consumers is collected for recycling, approximately 25% is buried or disposed of in landfill, 5% is disposed of into stormwater drains and the remaining 50% is used in treating fence posts, killing grass and weeds or disposed of in other ways. In a worst case scenario involving the 14% of used oil removed by DIY consumers, up to 0.7% of the total import volume of the notified chemical may enter the aquatic environment via disposal to stormwater drains (= 14% × 5%). Therefore, the amount of the notified chemical released to the aquatic environment from disposal of used oil due to DIY consumers is expected to be 14,000 kg/yr (= 2000 tonnes/year × 0.7%). In addition to this, considering the unknown fate of some of the oil used by DIY consumers, a small proportion may also be disposed of to sewers. Since the use of the lubricating oils will occur throughout Australia, all releases resulting from use or disposal of used oil will be very diffuse,

and release of the notified chemical in neat concentrations is unlikely except as a result of transport accidents.

7.1.2. Environmental Fate

Biodegradation studies conducted on Analogue 1 indicate the notified chemical is not expected to be readily biodegradable. However it is expected to be inherently biodegradable based on a study on the notified chemical. The analogues and the notified chemical are members of the carboxylate/salicylate class. They are synthesised following the same processes. There is a difference in alkyl chain length and branching between them, however this is not expected to significantly alter their physical-chemical properties. Therefore, the environmental fate for the notified chemical is considered to be well represented by the analogue data.

It is estimated that 40 kg/year of notified chemical may be released to sewer from the waste water for the cleaning of empty drums. Notified chemical released to sewers is likely to be partially removed by biodegradation or adsorption to sludge during the wastewater treatment plants (STP) processes. During STP processes, 52% of notified chemical is estimated to be removed from the STP effluent by either partitioning to sludge (19%) or biodegradation (33%) using SimpleTreat (EC, 2003). Notified chemical released to surface waters is expected to degrade and disperse in the aqueous environment. Sludge from STP containing the notified chemical is expected to be disposed of to landfill or applied to agricultural soils.

The measured partition coefficient (n-octanol/water) was determined to be > 3.9, indicating that the notified chemical may be bioaccumulative. However, bioaccumulation of the notified chemical in aquatic life is expected to be low given that bioconcentration factors (BCFs) for surfactants in the aqueous phase are generally below the level for concern (EOSCA, 2000). The majority of the notified chemical will be either sent to landfill or thermally decomposed to recover the calorific value. In landfill, the notified chemical is not expected to leach due to the low water solubility, and will undergo degradation processes via biotic and abiotic pathways. Either way, the notified chemical will finally be decomposed into small molecules of water, oxides of carbon and inorganic salts.

7.1.3. Predicted Environmental Concentration (PEC)

The release of the notified chemical to surface waters may be up to 14,000 kg/year (= 2000 tonnes/year × 0.7%) due to inappropriate disposal of used oils. In this worst case scenario, it is assumed that the release goes into stormwater drains in a single metropolitan area with a geographical footprint of 500 km^2 and an average annual rainfall of 500 mm, all of which drains to stormwater. With a maximum annual release into this localised stormwater system of 14,000 kg and the annual volume of water drained from this region estimated to be 250 × 10^6 m^3, the calculated predicted environmental concentration (PEC) will be up to 56 µg/L. This result reflects a worst-case scenario upper limit, as in reality releases of the notified chemical will be distributed over multiple regional/farming areas. Moreover, the notified chemical will be further diluted if it reaches the ocean.

Based on the release scenario in section 7.1, the amount of the notified chemical released to sewer from the waste water generated during cleaning of drums is estimated to be 40 kg/yr. Under the worst case scenario, it is assumed that the waste water will be treated at a STP with a daily flow of 40 ML and there is no removal of the notified chemical during STP processes. Assuming the release occurs 260 days per year, corresponding to working days, the calculated PEC in river is estimated as follows:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Release Volume to Sewer from waste water for drums cleaning	40	kg/year
Days per year where release occurs	260	days/year
Daily chemical release:	0.154	kg/day
Individual Sewage Treatment Plant Average Daily Flow:	40	ML/day
Removal within STP	0%	
PEC-River	3.85	µg

Based on the above calculations, the worst-case PEC in river for the notified chemical due to the combined releases from drum cleaning and the disposal of the used oil by DIY users is up to 59.85 µg/L (= 56 + 3.85). Therefore, the PEC for the aquatic compartments are calculated as follows:

Predicted Environmental Conc□ntration (PEC) for release to the aquatic compartment		
Combined PEC	≤ 59.85	µg/L
Dilution Factor – River	1	

Dilution Factor – Ocean	10	
PEC – River	≤ 59.85	µg/L
PEC – Ocean	≤ 5.98	µg/L

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on water accommodated fractions (WAF) of the notified chemical and Analogue 1 are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity		
Notified chemical (28% concentration))	LL50 (96 h) > 1000 mg/L WAF	Not harmful to fish
Analogue 1	LL50 (96 h) > 1000 mg/LWAF	
Daphnia Toxicity		
Notified chemical (28% concentration)	EL50 (48 h) > 1000 mg/L WAF	Potentially harmful to aquatic invertebrates
Notified chemical (43% concentration)	NOEL (48 h) = 10 mg/L WAF	
Analogue 1	EL50 (48 h) > 1000 mg/LWAF	
Algal Toxicity		
Notified chemical (43% concentration)	EL50 (96 h) > 1000 mg/L WAF	Not harmful to algae
Notified chemical (28% concentration)	EL50 (72 h) > 1000 mg/L WAF	
Analogue 1	EL50 (96 h) > 1000 mg/LWAF	

The daphnia toxicity for a test substance containing the notified chemical at 43% concentration indicates the notified chemical is potentially harmful to aquatic organisms. However, the test substance in this study was a complex mixture with unknown concentration of components in the test solution. The actual concentration of the notified chemical in the test media was not determined and the concentration cannot be calculated as the water solubilities of the components of the test substance are unknown. Therefore, the resultant ratio of components in the test media is unknown. The classification for the chemicals should only be based on toxic responses observed in the soluble range for the notified chemical. In addition, the 48 hour EL50 for daphnia was not defined in the test and conflicting results were obtained for products containing different concentrations of notified chemical (as above). Therefore, the notified chemical is not formally classified under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009).

7.2.1. Predicted No-Effect Concentration

The notified chemical is unlikely to be imported into Australia at concentrations where toxic effects were observed toward daphnia. Therefore, the Predicted No-Effect Concentration (PNEC) has been calculated based on the EL50 for the notified chemical at 28% concentration. An assessment factor of 1000 was used as although three acute toxicity endpoints are available from three trophic levels, the test results were obtained for the test substance which only contains less than 30% notified chemical.

EL50	> 1000	mg/L
Assessment Factor	1000	
PNEC:	> 1000	µg/L

7.3. Environmental Risk Assessment

Risk Asesssment	PEC µg/L	PNEC µg/L	Q
Q - River	< 59.85	> 1000	< 0.06
Q - Ocean	< 5.98	> 1000	< 0.006

The Risk Quotients (Q = PEC/PNEC) for the worst case scenario have been calculated to be < 1 for the river

and ocean compartments. Although the notified chemical may be released into waterways, it is not expected to pose a risk to the aquatic environment as ecotoxicologically significant concentrations are unlikely to be reached under the assessed scenario. The notified chemical is expected to inherently degrade in the environment and bioaccumulation is not expected based on its surface activity. Therefore, on the basis of the PEC/PNEC ratio and the assessed use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Pour Point -38 °C

Method	OECD TG 102 Melting Point/Melting Range.
Remarks	ASTM D 5950 with an automatic apparatus was used rather than ASTM D 97 as it was reported to offer better repeatability and reproducibility.
Test Facility	Chevron (2012a)

Boiling Point Decomposition from 150 °C

Method	Similar to OECD TG 103 Boiling Point.
Remarks	Determined by thermogravimetric analysis. Decomposition began at 150 °C and the distillation of decomposition products was complete by 860 °C.
Test Facility	Chevron (2012a)

Density 1,007.7 kg/m^3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids.
Remarks	Tested using an oscillating densitometer
Test Facility	Chevron (2012b)

Water Solubility < 0.103 g/L

Method	EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks	Flask Method. Only study summary is available. The test substance contains 28% notified chemical, 51% mineral oil and 21% inorganic calcium salt. As the test substance is a mixture and the various components have different limits of solubility, the solubility of the mixture is dependent on loading rate. The concentrations of components of the test material in the aqueous phase were 30 ± 16 mg/L and 103 ± 6 mg/L at 1 and 100 g/L loading rates, respectively. The solubility of the test substance determined by UV analysis was in broad agreement. Inorganic carbon and calcium analysis indicated that the test substance was physically stable in water.
Test Facility	ACC (2006)

Water Solubility 0.9 - 90 × 10^{-3} g/L at 20 °C

Method	UK Health And Safety Executive, the UK Competent Authority for the notification of new substances.
Remarks	Only study summary is available. The test substance is a mixture containing 43% notified chemical, 48% mineral oil and 9% inorganic calcium salt. OECD shake flask method was determined to be not appropriate for this test substance. The test substance contains aromatic rings, which absorb strongly in the ultraviolet region. The UV absorptions of aqueous solutions of the test substance were, therefore, used as an approximate indicator of the concentration of those components in water.
Test Facility	ACC (2006)

Partition Coefficient (n-octanol/water) log Pow > 3.9 at 20 °C

Method	UK Health And Safety Executive, the UK Competent Authority for the notification of new substances.
Remarks	The test substance is a mixture containing 43% notified chemical, 48% mineral oil and 9% inorganic calcium salt. Water solubility and partition coefficient (n-octanol/water) were estimated based on ultraviolet absorption of aqueous solutions.

	The results indicated that the solubility of the test substance in octanol was > 0.64 mg/L. The estimated water solubility of the test substance was in the range of 0.9-90 mg/L. Based on the highest water solubility values of 90 mg/L, the log Pow was calculated to be > 3.9. The lowest water solubility value of 0.9 mg/L gave an estimated log Pow of > 5.9.
Test Facility	ACC (2006)

Flash Point 188 °C (pressure not reported)

Method	EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks	Tested using the Pensky-Martens closed cup method. The pressure was not noted though it is presumed to be atmospheric.
Test Facility	Chevron (2012b)

Autoignition Temperature 372 °C

Method	ASTM E659 Standard test method for Autoignition Temperature of Liquid Chemicals
Remarks	The sample was placed into a heated 500 mL glass flask at a predetermined temperature. The contents of the flask were observed in a dark room for 10 minutes following either insertion of the sample or until autoignition occurs. Autoignition was evidenced by a sharp rise in temperature and a Chromel-Alumel thermocouple was used for measuring the temperature inside the flask.
Test Facility	Southwest Research Institute (2013)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Product containing notified chemical (28%)

METHOD: OECD TG 401 Acute Oral Toxicity.
- Species/Strain: Rat/Fisher 344
- Vehicle: None
- Remarks - Method: Only a study summary was available

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5M/5F	5000	0/10

- LD50: > 5000 mg/kg bw
- Signs of Toxicity: All animals displayed a hunched posture, diarrhoea and an 'unkempt' appearance within 2.5 hours of dosing. Anogenital staining was observed in all animals by day 2. All symptoms had abated by the 4th day.
- Effects in Organs: None described
- Remarks - Results: There were no unscheduled deaths in the study. Some clinical signs were observed and were resolved within 4 days.

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

TEST FACILITY: ACC (2006)

B.2. Acute toxicity – oral

TEST SUBSTANCE: Analogue 1

METHOD: OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
- Species/Strain: Rat/Sprague Dawley
- Vehicle: None
- Remarks - Method: 1 female died of a gavage error, so a second group of 4 females were also dosed with the test article.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	3 F	2000	1/3
II	4 F	2000	0/4

- LD50: > 2000 mg/kg bw
- Signs of Toxicity: There were no signs of toxicity recorded.
- Effects in Organs: No effects on the organs were noted.
- Remarks - Results: One female died due to a dosing error. There were no other unscheduled deaths.

CONCLUSION: The test substance, and by inference, the notified chemical are of low toxicity via the oral route.

TEST FACILITY: Charles River (2006a)

B.3. Acute toxicity – dermal

TEST SUBSTANCE	Product containing notified chemical (28%)
METHOD	OECD TG 402 Acute Dermal Toxicity.
Species/Strain	Rat/Fisher 344
Vehicle	None
Type of dressing	Occlusive
Remarks - Method	Only a study summary was available

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5M/5F	2000	0/10

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Dose sites were stained brown and in male rats, erythema was observed from day 2. Treated skin returned to normal by day 4.
Signs of Toxicity - Systemic	Anogenital staining was observed in female rats from day 2.
Effects in Organs	No effects on the organs were noted
Remarks - Results	There were no unscheduled deaths in the study. Some clinical signs were observed and were resolved within 4 days.
CONCLUSION	The test substance is of low toxicity via the dermal route.
TEST FACILITY	ACC (2006)

B.4. Acute toxicity – dermal

TEST SUBSTANCE	Analogue 2
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test.
Species/Strain	Rat/Sprague Dawley
Vehicle	Sesame oil DAB 10
Type of dressing	Occlusive
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5M/5F	2000	0/10
II	5M/5F	2500	0/10

LD50	> 2500 mg/kg bw
Signs of Toxicity - Local	No signs of local irritation or toxicity were observed
Signs of Toxicity - Systemic	No signs of systemic toxicity were observed
Effects in Organs	No effects were observed in organs
Remarks - Results	There were no signs of toxicity observed after application of the test article.
CONCLUSION	The test substance, and by inference, the notified chemical are of low toxicity via the dermal route.
TEST FACILITY	LPT (1992)

B.5. Irritation – skin

TEST SUBSTANCE	Analogue 1
METHOD	Similar to OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	None
Observation Period	14 days
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations. Observations were made at 1, 24, 48 and 72 hours, and 4, 7, 10 and 14 days following treatment.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	2	2	2	2	< 14 days	0
Oedema	1	1	1	1	< 14 days	0

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

Remarks - Results	All animals displayed well-defined erythema and very slight oedema by the 24-hour observation time. Well-defined erythema persisted in all animals to the 7-day observation period. The irritation was completely resolved in 1/3 animals by day 10 and in all animals by day 14.
CONCLUSION	The test substance, and by inference, the notified chemical are irritating to the skin.
TEST FACILITY	Charles River (2006b)

B.6. Irritation – eye

TEST SUBSTANCE	Analogue 2
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	Group I: 6 animals; Group II: 3 animals
Observation Period	14 days
Remarks - Method	No significant protocol deviations. Observations were made at 1, 24, 48 and 72 hours, and 4, 7 and 14 days following treatment. The test was performed with two groups of animals. For Group I, six animals were treated with 0.1 mL of the test substance and the eyes were not washed after treatment. For Group II, three animals were treated in a similar manner to Group I, except that 30 seconds following introduction of the test substance, the treated eye was gently irrigated with 100 mL of lukewarm tap water for one minute.

RESULTS

Group I: 6 animals (unwashed)

Lesion	*Mean Score* Unwashed*	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Conjunctiva: redness	1.7	2	< 14 days	0
Conjunctiva: chemosis	1.8	3	< 7 days	0
Conjunctiva: discharge	0.8	3	< 4 days	0
Corneal opacity	0.6	2	< 7 days	0
Iridial inflammation	0.2	1	< 72 h	0

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

Group II (washed)

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

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

Remarks - Results

Group I (unwashed):
Corneal opacity was noted in 3/6 animals with all effects clearing in less than 7 days. Iridial inflammation (grade 1) was noted in two animals at the 1, 24 and 48-hour observation. Conjunctivae irritation (redness grade 2, chemosis grade 2 and discharge grade 2 or 3) was noted in all treated eyes at the 1-hour observation. These effects had resolved in all animals in 7 days. Alopecia was observed around the treated eyes in one animal at day 7 and in three animals at the day 14 observation.

Group II (washed):
Conjunctival irritation (redness grade 1 or 2, chemosis grade 1 or 2 and discharge grade 2) was observed in all treated animals one hour after treatment. Redness (grade 1) and chemosis (grade 1) was noted in one animal at the 24 and 48-hour observation. All effects were resolved at the 72-hour observation. No corneal or iridial effects were noted.

CONCLUSION

The test substance, and by inference, the notified chemical are slightly irritating to the eyes.

TEST FACILITY

Safepharm (1998a)

B.7. Skin sensitisation

TEST SUBSTANCE

Product containing notified chemical (concentration not stated)

METHOD

Similar to OECD TG 406 Skin Sensitisation - Modified Buehler Method.

Species/Strain

Guinea pig/Hartley [Crl:HA]

PRELIMINARY STUDY

Maximum Non-irritating Concentration:
topical: 10%

MAIN STUDY

Number of Animals

Test Group: 20 Control Group: 10

INDUCTION PHASE

Induction Concentration:
topical: 6%

Signs of Irritation

Signs of irritation in the induction phase were not reported.

CHALLENGE PHASE

1st challenge

topical: 10%

Remarks - Method

Test animals received three induction doses spaced 1 week apart over a three week period. Induction exposures were 6 hours long, after which the bandages were removed and the sites wiped clean. The challenge phase consisted of a single application of the test substance two weeks after the final induction dose. All challenge applications were 6 hours long and the bandages were removed and application sites wiped after exposure.

The induction and challenge concentrations were based on a preliminary

irritation study. Slight to moderate dermal reactions were noted at the highest concentrations tested (100% and 75%). The 25% concentration resulted in 1/4 with a slight dermal reaction (grade 1) and the remainder with a very slight dermal reaction. Very slight dermal irritation was noted at lower concentrations (10%, 5%, 2.5% and 1%). An induction concentration of 6% was selected for dosing since the recommended carboxylate skin threshold is 6%. The 10% concentration was chosen for the challenge phase as it is essentially the highest non-irritating concentration. The vehicle used was mineral oil.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: 1st challenge*	
		24 h	*48 h*
Test Group	10%	1/20	5/20
Control Group	10%	0/10	0/10

Remarks - Results

There were no substance related clinical findings notes during the study. In addition, there were no remarkable body weight changes during the study.

In the test group 1/20 animals at 24 hours and 5/20 animals at 48 hours displayed grade 1 moderate patchy erythema. In the control group there were no signs of significant irritation (i.e. grade 1 or above).

CONCLUSION

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

TEST FACILITY

WIL (2013)

B.8. Skin sensitisation

TEST SUBSTANCE

Analogue 1 (6% concentration in mineral oil)

METHOD

Similar to OECD TG 406 Skin Sensitisation - Buehler.

Species/Strain

Guinea pig/Hartley-derived albino

PRELIMINARY STUDY

Maximum Non-irritating Concentration:
topical: 10%

MAIN STUDY

Number of Animals

Test Group: 20 Control Group: 10

INDUCTION PHASE

Induction Concentration:
topical: 100%

Signs of Irritation

Slight erythema was observed in the majority of animals after each induction.

CHALLENGE PHASE

1st challenge topical: 10%
2nd challenge topical: 10%
3rd challenge topical: 10%

Remarks - Method

A 6% solution of Analogue 1 was prepared in mineral oil and administered at the concentrations indicated above. Initial range finding studies indicated that the same level of irritation was observed following administration of 100%, 75%, 50% and 25%. The 100% concentration was deemed acceptable for dosing the induction phase of the study as this was the highest concentration that resulted in irritation. There were three control groups in the study, each with 5 males and 5 females. The first group was dosed with the test article at the first challenge, the 2nd group at

the 2nd challenge, and the third group at the 3rd challenge. The test animals were dosed at all of the challenges.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*					
		1st challenge		*2nd challenge*		*3rd Challenge*	
		24 h	*48 h*	*24 h*	*48 h*	*24 h*	*48 h*
Test Group	10%	1/20	3/20	3/20	3/20	0/20	0/20
Control Group	10%	0/10	0/10	0/10	0/10	0/10	0/10

Remarks - Results

Skin irritation scores of 1 were noted in 1/20 animals at 24 hours, and 3/20 at the 48 hour interval. On the second challenge, dermal scores of 1 were noted in 3/20 animals at both the 24 and 48 hour intervals. No reactions were noted in any animals on the 3rd challenge, at any time point. There were no signs of irritation observed in the control animals.

Given the lack of response in the 3rd challenge, the study authors suggest that the dermal scores noted in the 1st and 2nd challenge may be due to an irritation response rather than a sensitisation response. However, as a conservative approach, the study authors have concluded the test substance to be a skin sensitiser.

CONCLUSION

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

TEST FACILITY

Charles River (2011a)

B.9. Skin sensitisation

TEST SUBSTANCE

Analogue 1 (6% concentration in mineral oil)

METHOD

OECD TG 406 Skin Sensitisation - Buehler.

Species/Strain

Guinea pig/Hartley-derived albino guinea pigs

PRELIMINARY STUDY

Maximum Non-irritating Concentration:
topical: 10%

MAIN STUDY

Number of Animals

Test Group: 20 Control Group: 10

INDUCTION PHASE

Induction Concentration:
topical: 100%

Signs of Irritation

Slight erythema was observed in the majority of animals after each induction.

CHALLENGE PHASE

1st challenge topical: 10%

2nd challenge topical: 10%

Remarks - Method

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st challenge		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	10%	13/20	14/20	17/20	17/20
Control Group	10%	0/10	0/10	0/10	0/10

Remarks - Results	Dermal irritation scores of 1 were noted in 13/20 test animals at 24 hours, and 14/20 at 48 hours. Following the 2nd challenge, dermal scores of 1 were noted in 17/20 at 24 hours and scores of 1-2 were noted in 17/20 animals after 48 hours. Dermal scores in the control animals were limited to 0 or ±.
CONCLUSION	There was evidence of reactions indicative of skin sensitisation to the test substance under the conditions of the test.
TEST FACILITY	Charles River (2011b)

B.10. Skin sensitisation

TEST SUBSTANCE	Analogue 1 (6% concentration in mineral oil)
METHOD	OECD TG 406 Skin Sensitisation - Buehler.
Species/Strain	Guinea pig/Hartley-derived albino guinea pigs
PRELIMINARY STUDY	Maximum Non-irritating Concentration: topical: 10%
MAIN STUDY	
Number of Animals	Test Group: 20 Control Group: 10
INDUCTION PHASE	Induction Concentration: topical: 100%
Signs of Irritation	Slight erythema was observed in the majority of animals after each induction.
CHALLENGE PHASE	
1st challenge	topical: 10%
2nd challenge	topical: 10%
Remarks - Method	

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st challenge		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	10%	8/20	7/20	13/20	12/20
Control Group	10%	0/10	0/10	0/10	0/10

Remarks - Results	Dermal irritation scores of 1 were noted in 8/20 test animals at 24 hours, and 7/20 at 48 hours. Group mean dermal irritation scores were higher in the test animals that the control group. Following the 2nd challenge, dermal scores of 1 were noted in 13/20 at 24 hours and 12/20 animals after 48 hours. Dermal scores in the control animals were limited to 0 or ±.
CONCLUSION	There was evidence of reactions indicative of skin sensitisation to the test substance under the conditions of the test.
TEST FACILITY	Charles River (2011c)

B.11. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain	Rat/Sprague-Dawley Crl:CD

Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days for control and high dose group only
Vehicle	Corn oil
Remarks - Method	Only a study summary was available.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	21M/14F	0	0/35
low dose	7M/7F	50	0/14
mid dose	7M/7F	150	0/14
high dose	21M/14F	500	0/35

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

Clinical Observations
Excessing salivation was noted in the 150 mg/kg bw/day and 500 mg/kg bw/day dose groups throughout the study. Near the end of the study yellow material was occasionally observed one hour after dosing in 500 mg/kg/day animals of both sexes. The body weight gains of the males in the 500 mg/kg/day group were consistently lower than those for control animals. This finding was, however, determined not be statistically significant. The additional 500 mg/kg/day group males showed significantly reduced mean body weight gain from week 3 to 4. During the recovery period mean body weights for the high dose group were similar to the control.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Statistically prolonged prothrombin time was present in one high dose male at the 4 week evaluation. Elevated alkaline phosphatase and alanine aminotransferase levels were noted in the 500 mg/kg/day group males and females. These effects were linked to increased liver weights. These levels were found to be similar to control animals after the recovery period.

Effects in Organs
Increased relative and absolute liver and thyroid weights were noted in males and females in the high dose group. These levels returned to control organ weights following the 2 week recovery period. There were no associated histological changes observed in these organs at necropsy.

Remarks – Results
The findings of excessive salivation and yellow material post dosing were not considered adverse effects. Hence, the NOAEL was determined to be 150 mg/kg bw/ day.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 150 mg/kg bw/day in this study, based on no adverse effects at this level.

TEST FACILITY ACC (2006)

B.12. Repeat dose toxicity

TEST SUBSTANCE Analogue 1

METHOD OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.

Species/Strain	Rat/ Crl:CD(SD)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days

Vehicle	Corn oil
Remarks - Method	No significant protocol deviations. Following the 28 days dose administration, 5 rats/sex/group were sacrificed. The remaining 5/rats/sex in the control and high dose groups were sacrificed following a 14-day recovery period.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 M/ 5 F	0	0/5
low dose	5 M/ 5 F	160	0/5
mid dose	5 M/ 5 F	400	0/5
high dose	5 M/ 5 F	1000	0/5
control recovery	5 M/ 5 F	0	0/5
high dose recovery	5 M/ 5 F	1000	0/5

Mortality and Time to Death

There were no unscheduled deaths in this study.

Clinical Observations

In the 1000 mg/kg/day group males and females displayed salivation, clear material around the mouth, and also some incidences of red material around the mouth. These findings were usually observed at 1 hour post-dosing. These findings were also noted in the 400 mg/kg/day group at a lower incidence. These finding were not observed during the recovery period.

Decreased body weight gain and body weight was noted in males in the 1000 mg/kg/day group.

There were no test substance related effects on food consumption or functional observational battery evaluations.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

At the end of the dosing period, increases in mean prothrombin time was observed in males and females in the 1000 mg/kg/day group, relative to controls. Increased mean activated thromboblastin time was also observed in males in the 1000 mg/kg/day group. These parameters were similar to the control groups after the recovery period.

Statistically significant increases in levels of alanine aminotransferase and aspartate aminotransferase were noted in males and females in the 400 and 1000 mg/kg/day and 1000 mg/kg/day groups, respectively. Statistically significant lowered levels of globulin and total protein were observed in the 1000 mg/kg/day males. This was also observed in the 1000 mg/kg/day females; however, these effects were not statistically significant. Since no correlating macroscopic or microscopic changes were observed in the liver, the changes observed were considered non-adverse.

Effects in Organs

There were no test article related effects on organ weights.

Remarks – Results

The clinical observations and the effects upon serum chemistry were not considered to be adverse based on the absence of microscopic changes in the 400 and 1000 mg/kg/bw group. Effects at 1000 mg/kg/bw were fully resolved after the recovery period.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 400 mg/kg bw/day in this study, based on body weight and coagulation changes observed at the highest dose.

TEST FACILITY WIL (2006)

B.13. Genotoxicity – bacteria

TEST SUBSTANCE	Product containing notified chemical (43%)
METHOD	Similar to OECD TG 471 Bacterial Reverse Mutation Test. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1538, TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA, WP2
Metabolic Activation System	S9 mix
Concentration Range in Main Test	a) With metabolic activation: 31.25-2000 µg/plate b) Without metabolic activation: 31.25-2000 µg/plate
Vehicle	Water containing 20% w/v Tween 80
Remarks - Method	Only a study summary was available.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 4000	> 2000	> 2000	Negative
Present				
Test 1		> 2000	> 2000	Negative

Remarks - Results	No toxicologically significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test substance, either with or without metabolic activation. All the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.
CONCLUSION	The test substance was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	ACC (2006)

B.14. Genotoxicity – bacteria

TEST SUBSTANCE	Product containing notified chemical (28%)
METHOD	Similar to OECD TG 471 Bacterial Reverse Mutation Test.. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1538, TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA, pKM101
Metabolic Activation System	S9 mix from Aroclor 1254 induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 31.25-5000 µg/plate b) Without metabolic activation: 31.25-5000 µg/plate
Vehicle	5% Tween in 1:1 heptane: acetone
Remarks - Method	Only a study summary was available.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	Unknown	> 5000	≥ 1000	Negative
Present				
Test 1		> 5000	≥ 1000	Negative

Remarks - Results	No toxicologically significant increases in the frequency of revertant

colonies were recorded for any of the bacterial strains, with any dose of the test substance, either with or without metabolic activation.

All the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

CONCLUSION The test substance was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY ACC (2006)

B.15. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.

Cell Type/Cell Line	Human peripheral blood lymphocytes
Metabolic Activation System	S9 mix
Vehicle	Water
Remarks - Method	Only a study summary was available.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	18.75, 37.5*, 75*, 150*, 300, 400, 500, 600	4	20
Test 2	6.25, 12.5*, 25, 50*, 75, 100*, 150	20	0
Present			
Test 1	12.5*, 25*, 50, 75*, 150, 200, 300	4	20

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 900	≥ 150	≥ 75	Negative
Test 2	≥ 270	≥ 75	≥ 75	
Present				
Test 1	≥ 90	≥ 100	≥ 75	Negative

Remarks - Results

CONCLUSION The notified chemical was not clastogenic to human peripheral blood lymphocytes treated in vitro under the conditions of the test.

TEST FACILITY ACC (2006)

B.16. Genotoxicity – bacteria

TEST SUBSTANCE Analogue 1

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
Plate incorporation procedure

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA$^-$
Metabolic Activation System	Liver preparations (S9 mix) from rats treated with phenobarbital/ß-naphthoflavone

Concentration Range in Main Test	a) With metabolic activation: 15-5000 µg/plate b) Without metabolic activation: 15 5000 µg/plate
Vehicle	Tetrahydrofuran (THF)
Remarks - Method	The negative control was THF and positive controls were N-ethyl-N'-nitro-N-nitrosoguanidine (2 µg/plate for WP2urvA$^-$, 3 µg/plate for TA100 and 5 µg/plate for TA1535), 9-aminoacridine (80 µg/plate for TA1537) and 4-nitroquinoline-1-oxide (0.2 µg/plate for TA98), in the absence of S9 mix and 2-aminoanthracene (1 µg/plate for TA100, 2 µg/plate for TA1535 and TA1537, 10 µg/plate for WP2urvA$^-$) and benzo[a]pyrene (5 µg/plate for TA98) in the presence of S9 mix.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5000	> 5000	≥ 500	Negative
Test 2		> 5000	≥ 500	Negative
Present				
Test 1	> 5000	> 5000	≥ 500	Negative
Test 2		> 5000	≥ 500	Negative

Remarks - Results	The test substance was tested up to the maximum recommended dose level of 5000 µg/plate. No toxicologically significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test substance, either with or without metabolic activation. All the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.
CONCLUSION	The test substance, and by inference, the notified chemical are not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	SafePharm (2006a)

B.17. Genotoxicity – in vitro

TEST SUBSTANCE	Analogue 1
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Cell Type/Cell Line	Human lymphocytes
Metabolic Activation System	S9 fraction from rat liver induced with Aroclor 1254
Vehicle	50% Pluronic F127 in ethanol
Remarks - Method	The positive controls used in the study were mitocycin C (without metabolic activation) and cyclophosphamide (with metabolic activation).

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	5.76, 8.24, 11.8, 16.8*, 24.0*, 34.3*, 49.0*, 70.0, 100, 143, 204, 292, 417, 595, 850	3 h	22 h
Test 2	1.56, 3.13, 6.25, 12.5, 25.0, 37.5*, 50.0*, 75.0, 100*, 150, 200*	22 h	22 h
Present			
Test 1	5.76, 8.24, 11.8, 16.8, 24.0*, 34.3*, 49.0*, 70.0*, 100, 143, 204, 292, 417, 595, 850	3 h	22 h
Test 2	12.5, 25.0, 37.5*, 50.0*, 75.0, 100*, 150, 200*	3 h	22 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*		
	Cytotoxicity	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	292*	≥ 49	Negative
Test 2	> 200	≥ 75	Negative
Present			
Test 1	292*	≥ 70	Negative
Test 2	> 200	≥ 75	Negative

*No significant cytotoxicity in plates at higher concentrations, except at 850 µg/mL.

Remarks - Results

Test 1:
Only dead cells were observed in the cultures treated with 850 µg/mL. In the assay without metabolic activation, a slight precipitate was observed after dosing at 292 µg/ml, at wash in cultures treated with ≥ 417 µg/mL and at harvest of the cultures treated with ≥ 49.0 µg/mL.

In the assay with metabolic activation, a precipitate was observed after dosing at ≥ 292 µg/mL, at wash in the cultures treated with 143, 204, 292, 417 and 850 µg/mL and at harvest of the cultures treated with ≥ 70 µg/mL.

Test 2:
In the assay without metabolic activation, a precipitate was observed after dosing at 200 µg/mL and prior to harvest of cultures treated with ≥ 75 µg/mL.

In the assay with metabolic activation, a precipitate was observed after dosing at 200 µg/mL, at wash of the cultures treated with ≥ 150 µg/mL, and at harvest of the cultures treated with ≥ 75 µg/mL.

The test substance did not induce any statistically significant increases in the mutant frequency at any tested concentration in each exposure group with and without metabolic activation.

The positive and vehicle controls gave satisfactory responses, confirming the validity of the test system.

CONCLUSION

The test substance, and by inference, the notified chemical are not clastogenic to human lymphocytes treated in vitro under the conditions of the test.

TEST FACILITY

Covance (2006)

B.18. Genotoxicity – in vivo

TEST SUBSTANCE

Analogue 2

METHOD

OECD TG 474 Mammalian Erythrocyte Micronucleus Test.

Species/Strain: Mouse/ Crl:CD^{tm}(ICR)BR, albino
Route of Administration: Intraperitoneal
Vehicle: Arachis oil
Remarks - Method: No significant protocol deviations

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time Hours*
I (vehicle control)	7 M	0	24
II (vehicle control)	7 M	0	48

III (low dose)	7 M	187.5	24
IV (mid dose)	7 M	375	24
V (high dose)	7 M	750	24
VI (high dose)	7 M	750	48
VII (positive control, CP)	5 M	50	24

CP=cyclophosphamide.

RESULTS

Doses Producing Toxicity: There were no unscheduled deaths in this study. Clinical signs were observed in animals treated with 750 mg/kg in both the 24 and 48 hour groups. The effects observed were hunched posture and lethargy.

Genotoxic Effects: There were statistically significant increases in the frequency of micronucleated polychromatic erythrocytes in the low and mid-dose 24-hour exposure groups. The response was inversely dose-related and did not exceed the upper limit of the historical background range for vehicle control values. Therefore, it was considered by the study authors that the increases had no toxicological significance.

Remarks - Results: There were no statistically significant increases in the frequency of micronucleated polychromatic erythrocytes in any of the test material groups when compared to the vehicle controls.

The observation of clinical signs in the high dose group suggests that systemic absorption had occurred.

The positive control produced a marked increase in the number of micronucleated polychromatic erythrocytes, confirming the validity of the test system.

CONCLUSION: Analogue 2 was not clastogenic under the conditions of this in vivo micronucleus test.

TEST FACILITY: SafePharm (1998b)

B.19. Genotoxicity – in vivo

TEST SUBSTANCE: Analogue 1

METHOD: OECD TG 474 Mammalian Erythrocyte Micronucleus Test.

Species/Strain: Mouse/ Crl:CD^{tm}(ICR)BR, albino

Route of Administration: Intraperitoneal

Vehicle: Arachis oil

Remarks - Method: No significant protocol deviations

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time Hours*
I (vehicle control)	7 M	0	24
II (vehicle control)	7 M	0	48
III (low dose)	7 M	300	24
IV (mid dose)	7 M	600	24
V (high dose)	7 M	1200	24
VI (high dose)	7 M	1200	48
VII (positive control, CP)	5 M	50	24

CP=cyclophosphamide.

RESULTS

Doses Producing Toxicity: There were no unscheduled deaths in this study. Clinical signs were observed in animals treated with 1200 mg/kg in both the 24 and 48 hour groups. The effects observed were hunched posture and ptosis.

Genotoxic Effects	There were no statistically significant increases in the frequency of micronucleated polychromatic erythrocytes in any of the test material groups when compared to the vehicle controls.
Remarks - Results	In the 24-hour 300 mg/kg dose group, the mean polychromatic erythrocyte/non-polychromatic erythrocyte value was high with a large standard deviation. This was due to two animals with low numbers of non-polychromatic erythrocytes. The reason for this was not determined but may be an artefact. The observation of clinical signs in the high dose group suggests that systemic absorption had occurred. The positive control produced a marked increase in the number of micronucleated polychromatic erythrocytes, confirming the validity of the test system.
CONCLUSION	The test substance, and by inference, the notified chemical are not clastogenic under the conditions of this in vivo micronucleus test.
TEST FACILITY	SafePharm (2005)

B.20. Reproductive/Developmental toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 421: Reproduction/Developmental Toxicity Screening Test
Species/Strain	Rat/ Crl:CD(SD)
Route of Administration	Oral – gavage
Exposure Information	Exposure days: 14 days premating period through lactation day 3. Post-exposure observation period:
Vehicle	Corn oil
Remarks - Method	Only a study summary was available.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	12 M/12 F	0	0/24
Low dose	12 M/12 F	50	0/24
Mid dose	12 M/12 F	150	0/24
High dose	12 M/12 F	500	0/24

Mortality and Time to Death

There were no unscheduled deaths throughout the study.

Effects on Dams

All females in the mid and high dose groups displayed excessive pawing of the cage floor and/or walls and wiping of the mouth of the floor and/or walls following dosing. These effects were noted to a lesser extent in high dose males. Salivation and presence of clear material on various surfaces were also noted in the mid and high dose females up to 2 hours following administration. Salivation was also noted in high dose males at up to 2 hours following administration. These effects were considered to be due to an aversion to the taste of the test article and not adverse.

Higher than mean liver weights were noted for males and females in the high dose group. This effect, however, was not considered adverse since these are consistent with hepatic enzyme induction which was adaptive in nature.

Effects on Foetus

Pups were unaffected by maternal test article administration. The number of live pups, litter size, physical condition, weights and postnatal survival were unaffected at any dose level.

Remarks - Results

Neither male nor female reproductive parameters were affected by the test substance. In addition, there were no effects observed on pups survival and growth.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 500 mg/kg bw/day for parental and neonatal systemic toxicity in this study, based on no adverse effects at this dose level.

TEST FACILITY ACC (2006)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE: Analogue 1

METHOD
- Inoculum: Activated sludge
- Exposure Period: 28 days
- Auxiliary Solvent: None
- Analytical Monitoring: TOC analyser for CO_2 analysis
- Remarks - Method: The test substance was determined to be insoluble in water. In order to increase the dispersion of the test substance in the test medium and the surface area of the test substance exposed to the test organisms, the test substance was adsorbed onto silica gel prior to dispersion in test culture medium. The sample biodegradability is calculated from the released CO_2 compared to blank and the reference.

  The test was conducted according with the test guideline above. No significant deviation from the protocol was reported.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
6	2	6	75
14	3	14	80
22	2	22	76
28	6	28	79

- Remarks - Results: All validity criteria are satisfied.

  The toxicity control attained 29% degradation after 14 days and 44% degradation after 28 days, implying that the test substance was not toxic to the sewage treatment micro-organisms.

CONCLUSION: The test substance and, by inference, the notified chemical are not readily biodegradable but have potential to biodegrade in the environment.

TEST FACILITY: SafePharm (2006b)

C.1.2. Inherent biodegradability

TEST SUBSTANCE: Product containing notified chemical (43%)

METHOD: Similar to ISO (1997) Headspace CO_2 biodegradation test with modifications recommended by CONCAWE.
- Inoculum: Activated sludge
- Exposure Period: 56 days
- Auxiliary Solvent: Not specific
- Analytical Monitoring: Inorganic carbon production
- Remarks – Method: Only a study summary is available.

  Biodegradation was calculated as net inorganic carbon (IC) production and expressed as a percentage of the theoretical maximum inorganic production (ThIC), based on the quantity of test substance (as carbon) added initially.

RESULTS

Test substance		*Hexadecane*	
Day	*% Degradation*	*Day*	*% Degradation*
56	63	56	94

Remarks – Results: The test substance contains 43% notified chemical and 48% mineral oil.

The study was reviewed by Petroleum Additives Panel Health & Environmental Research Task Group, American Chemistry Council. All validity criteria were reported to be met.

CONCLUSION: The test substance is considered to have inherent, ultimate biodegradability.

TEST FACILITY: ACC (2006)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Product containing notified chemical (28%)

METHOD: OECD TG 203 Fish, Acute Toxicity Test – Semi-static.

Species	*Oncorhynchus mykiss*
Exposure Period	96 hours
Auxiliary Solvent	Not specified
Water Hardness	266-278 $CaCO_3$/L
Analytical Monitoring	Not available
Remarks – Method	Only a study summary is available.

The test substance was prepared as a Water Accommodated Fraction (WAF). Seven fish were exposed to the control and each of WAF preparations at the loading rate of 220, 460 and 1000 mg/L. The test media were renewed daily.

RESULTS

LL50	> 1000 mg/L WAF at 96 hours.
NOEL	1000 mg/L WAF at 96 hours.
Remarks – Results	The test substance contains 28% notified chemical, 51% mineral oil and 21% inorganic calcium salt. No toxicity was observed at dose level up to and including 1000 mg/L WAF at 24, 48, 72 and 96 hours exposure. The test substance contains 28% notified chemical, 51% mineral oil and 21% inorganic calcium. The actual concentration of notified chemical in the test solution was not determined and the results were based on the nominal loading rate.

The study was reviewed by Petroleoum Additives Panel Health & Environmental Research Task Group, American Chemistry Council. The results were considered to be reliable with restriction. Restriction was due to the lack of analytical confirmation of the test concentrations.

CONCLUSION: The test substance is not harmful to fish.

TEST FACILITY: ACC (2006)

C.2.2. Acute toxicity to fish

TEST SUBSTANCE Analogue 1

METHOD OECD TG 203 Fish, Acute Toxicity Test – Semi-static.

Species	Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	Not specified
Water Hardness	120 mg $CaCO_3$/L
Analytical Monitoring	Total organic carbon (TOC) analysis
Remarks – Method	The test substance was prepared as a Water Accommodated Fraction (WAF) due to its expected low water solubility. The test substance (21.0 g) was added via a syringe to the surface of 21 litres of dechlorinated water to give the 1000 mg/L loading rate. After addition of the test substance, the dechlorinated tap water was stirred for 24 hours and allowed to stand for 4 hours afterward. A wide bore glass tube, covered at one end with Nescofilm was submerged into the vessel, sealed end down, to a depth of approximately 5 cm from the bottom of the vessel. A length of Tygon tubing was inserted into the glass tube and pushed through the Nescofilm seal. The aqueous phase or Water Accommodated Fraction (WAF) was removed by mid-depth siphoning (the first 75-100 mL discarded) to give the 1000 mg/L loading rate WAF. Microscopic inspection of the WAF showed no micro-dispersion or undissolved test substance to be present. The test medium was renewed daily. The test was conducted according to the test guideline above without significant deviation from the protocol.

RESULTS

Concentration		*Number of Fish*	*Mortality*				
Nominal (mg/LWAF)	*Actual (mg carbon/L)*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	1.86	20	0	0	0	0	0
1000	1.89	31	0	0	0	0	0

LL50	> 1000 mg/L WAF at 96 hours.
NOEL	1000 mg/L WAF at 96 hours.
Remarks – Results	Sample of the control and 1000 mg/L loading rate WAF were taken at 0 hour and 24 hours for TOC analysis. Considering the background level of carbon in the control vessels and also the low level of carbon in the test vessels, all the results were around the limit of quantitation (LOQ) of the analytical method and hence did not provide definitive evidence of stability of the test preparations. Therefore, the results were only based on nominal loading rate of WAF. All fish of the control and the treatment at 1000 mg/L survived the 96 h WAF toxicity test. No sub-lethal effects were observed for 31 fish exposed to a 1000 mg/L loading rate WAF for a time period of 96 hours. All validity criteria were met.

CONCLUSION The test substance, and by inference, the notified chemical are not harmful to fish.

TEST FACILITY SafePharm (2006c)

C.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Product containing the notified chemical (28%)

METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Not available
Water Hardness	186 mg $CaCO_3$/L
Analytical Monitoring	Not available
Remarks - Method	Only a study summary is available. The test substance was prepared as a Water Accommodated Fraction (WAF). Twenty daphnids (10 daphnids/replicate) were exposed to the control and each of WAF preparations at the loading rate of 100, 220, 460 and 1000 mg/L.
RESULTS	
EL50	> 1000 mg/L WAF at 48 hours
NOEL	1000 mg/L WAF at 48 hours
Remarks - Results	The test substance contains 28% notified chemical, 51% mineral oil and 21% inorganic calcium salt. No immobilization was observed at dose level up to and including 1000 mg/L WAF at both 24 and 48 hours exposure. The actual concentration of the notified chemical in the test media was not determined and the results were based on a nominal loading rate. The study was reviewed by Petroleoum Additives Panel Health & Environmental Research Task Group, American Chemistry Council. The results were considered to be reliable with restriction. Restriction was due to the lack of analytical confirmation of the test concentrations.
CONCLUSION	The test substance is not harmful to aquatic invertebrates.
TEST FACILITY	ACC (2006)

C.2.4. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Product containing the notified chemical (43%)
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Not available
Water Hardness	170 mg $CaCO_3$/L
Analytical Monitoring	Not available
Remarks - Method	Only a study summary is available. The test substance was prepared as a Water Accommodated Fraction (WAF). Thirty daphnids (10 daphnids/replicate) were exposed to the control and each WAF preparations at the loading rate of 10, 100 and 1000 mg/L.
RESULTS	
EL50	Not reported
NOEL	10 mg/L WAF at 48 hours
Remarks - Results	Immobilization of all *daphnia magna* was observed at 1000 mg/L WAF at both 24 and 48 hours. Immobilization of all *daphnia magna* was observed at 100 mg/L WAF at 48 hours. The no observed effect level was 10 mg/L WAF.

The test substance contains 43% notified chemical, 48% mineral oil and 9% inorganic calcium salt. The actual concentration of the notified chemical in the test media was not determined and the results were based on a nominal loading rate.

The study was reviewed by Petroleoum Additives Panel Health & Environmental Research Task Group, American Chemistry Council. The results were considered to be reliable with restriction. Restriction was due to the lack of analytical confirmation of the test concentrations.

The endpoints in this study indicate the test substance is potentially toxic to daphnia based on the nominal loading rate of WAF. However, no toxic effects were observed for daphnia in the other test conducted on a product containing different percentages of notified chemical (see above). Regarding these conflicting results, it is important to recognise that duration of mixing and energy input can have a marked influence on the composition, particle size and proportion of dispersed and non-dispersed test material in the WAF preparations. It is not clear whether the toxicity observed here was due to the soluble parts of the notified chemical, toxicity of other components of the test substance, or due to other physical/biological effects. The actual concentration of the notified chemical in the test media was not measured during the test. For these reasons, the daphnia toxicity reported in this study was not used in the risk assessment report as its validity cannot be verified

CONCLUSION The test substance is potentially harmful to aquatic invertebrates.

TEST FACILITY ACC (2006)

C.2.5. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Analogue 1

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - static.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Not specified
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	TOC analysis
Remarks - Method	The test substance (2.5 g) was added via a syringe to the surface of 2.5 litres of reconstituted water to give the 1000 mg/L loading rate. After addition of the test substance, the reconstituted water was stirred for 24 hours and allowed to stand for 4 hours afterward. A wide bore glass tube, covered at one end with Nescofilm was submerged into the vessel, sealed end down, to a depth of approximately 5 cm from the bottom of the vessel. A length of Tygon tubing was inserted into the glass tube and pushed through the Nescofilm seal. The aqueous phase or Water Accommodated Fraction (WAF) was removed by mid-depth siphoning (the first 75-100 mL discarded) to give the 1000 mg/L loading rate WAF. Microscopic inspection of the WAF showed no micro-dispersion or undissolved test substance to be present.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal (mg/L WAF)	*Actual (mg carbon/L)*		*24 h*	*48*
Control	< LOQ	20	0	0
1000	< LOQ	40	0	0

EL50	> 1000 mg/L WAF at 48 hours.
NOEL	1000 mg/L WAF at 48 hours
Remarks - Results	Sample of the control and 1000 mg/L loading rate WAF were taken at 0 hour (fresh media) and 48 hours (old media) for TOC analysis. Considering the background level of carbon in the control vessels and also the low level of carbon in the test vessels, all the results were around the limit of quantitation (LOQ) of the analytical method and hence did not provide definitive evidence of stability of the test preparations. Therefore, the results were only based on nominal loading rate of WAF. All validity criteria were satisfied.
CONCLUSION	The test substance and, by inference, the notified chemical are not harmful to aquatic invertebrates.
TEST FACILITY	SafePharm (2006d)

C.2.6. Algal growth inhibition test

TEST SUBSTANCE	Product containing notified chemical (43%)
METHOD	Not available
Species	*Pseudokirchneriella subcapitata*
Exposure Period	96 hours
Concentration Range	Nominal: Control, 0.1, 0.5, 2, 10, 50, 200 and 1000 mg/L Actual: Not determined
Auxiliary Solvent	Not specified
Water Hardness	Not specified
Analytical Monitoring	Not performed
Remarks - Method	Only a study summary is available. The test substance was prepared as a Water Accommodated Fraction (WAF) due to its expected low water solubility. A static test was carried out.

RESULTS

Biomass		*Growth*	
E_bL50	*NOE_bL*	*E_rL50*	*NOE_rL*
mg/L WAF at 96 h	*mg/L WAF*	*mg/L WAF at 96 hours*	*mg/LWAF at 96 hours*
Not determined	Not determined	> 1000	Not reported

Remarks - Results	The test substance contains 43% notified chemical, 48% mineral oil and 9% inorganic calcium salt. The actual concentration of the notified chemical in the test media was not determined and the results were based on a nominal loading rate. The study was reviewed by Petroleoum Additives Panel Health & Environmental Research Task Group, American Chemistry Council. The results were considered to be reliable with restriction. Restriction was due to the lack of analytical characterization of the WAF and the limited methodology contained in the report.
CONCLUSION	The test substance is not harmful to algae.
TEST FACILITY	ACC (2006)

C.2.7. Algal growth inhibition test

TEST SUBSTANCE	Product containing notified chemical (28%)
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Raphidocelis subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: Control, 10, 22, 46, 100, 220, 460 and 1000 mg/L Actual: Not determined
Auxiliary Solvent	Not specified
Water Hardness	Not specified
Analytical Monitoring	Not specified
Remarks - Method	Only a study summary is available. The test substance was prepared as a Water Accommodated Fraction (WAF) due to its expected low water solubility. A static test was carried out.

RESULTS

Biomass		*Growth*	
E_bL50	*NOE_bL*	*E_rL50*	*NOE_rL*
mg/L WAF at 72 h	*mg/L WAF at 72 h*	*mg/L WAF at 72 h*	*mg/LWAF at 72 h*
Not reported	Not reported	> 1000	< 10

Remarks - Results	The test substance contains 28% notified chemical, 51% mineral oil and 21% inorganic calcium salt. The actual concentration of the notified chemical in the test media was not determined and the results were based on a nominal loading rate. The study was reviewed by Petroleoum Additives Panel Health & Environmental Research Task Group, American Chemistry Council. The results were considered to be reliable with restriction. Restriction was due to the lack of analytical characterization of the WAF
CONCLUSION	The test substance is not harmful to algae.
TEST FACILITY	ACC (2006)

C.2.8. Algal growth inhibition test

TEST SUBSTANCE	Analogue 1
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Pseudokirchneriella subcapitata*
Exposure Period	96 hours
Concentration Range	Nominal: 1000 mg/L Actual: < LOQ mg carbon/L
Auxiliary Solvent	Not specified
Water Hardness	~3 mg $CaCO_3$/L
Analytical Monitoring	TOC analysis
Remarks - Method	The test substance (2.5 g) was added to the surface of 2.5 litres of culture medium via a syringe give the 1000 mg/L loading rate. After addition of the test substance, the culture medium was stirred for 24 hours and allowed to stand for 4 hours afterward. A wide bore glass tube, covered at one end with Nescofilm was submerged into the vessel, sealed end down, to a depth of approximately 5 cm from the bottom of the vessel. A length of Tygon tubing was inserted into the glass tube and pushed through the Nescofilm seal. The aqueous phase or Water Accommodated Fraction (WAF) was removed by mid-depth siphoning (the first 75-100 mL discarded) to give the 1000 mg/L loading rate WAF. Microscopic inspection of the WAF showed no micro-dispersion or undissolved test

substance to be present.

RESULTS

Biomass		*Growth*	
E_bL50	*NOE_bL*	*E_rL50*	*NOE_rL*
mg/L WAF at 96 h	*mg/L WAF*	*mg/L WAF at 96 h*	*mg/LWAF*
> 1000	1000	> 1000	1000

Remarks - Results

Sample of the control and 1000 mg/L loading rate WAF were taken at 0 hour (fresh media) and 96 hours (old media) for TOC analysis. Considering the background level of carbon in the control vessels and also the low level of carbon in the test vessels, all the results were around the limit of quantitation (LOQ) of the analytical method and hence did not provide definitive evidence of stability of the test preparations. Therefore, the results were only based on nominal loading rate of WAF.

All validity criteria were satisfied.

CONCLUSION

The test substance and, by inference, the notified chemical are not harmful to algae.

TEST FACILITY

Safepharm (2006e)

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