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September 2012

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

NExBTL Renewable Diesel

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

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:

Street Address:	Level 7, 260 Elizabeth Street, SURRY HILLS NSW 2010, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX	+ 61 2 8577 8888
Website:	www.nicnas.gov.au

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/1420	Cintox Australia Pty Ltd	NExBTL Renewable Diesel	Yes	≤600000 tonnes per annum	Component of fuel

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*
Aspiration hazard (Category 1)	H304 – May be fatal if swallowed and enters airways

In Australia, additional non-GHS hazard statements apply (see *Guidance on the Classification of Hazardous Chemicals Under the WHS Regulations* for further information; Safe Work Australia, 2012). Based on the available information, the following additional (non-GHS) hazard statement is also recommended:

AUH066 – Repeated exposure may cause skin dryness and cracking

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:

R65 Harmful: may cause lung damage if swallowed
R66 Repeated exposure may cause skin dryness or cracking

Based on the submitted physico-chemical data, 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*
Flammable Liquids (Category 4)	H227 – Combustible liquid

The environmental hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS)* is presented below. Environmental classification under the GHS is not mandated in Australia and carries no legal status but is presented for information purposes.

Hazard classification	*Hazard statement*
Chronic (Category 3)	H412 – Harmful to aquatic life with long lasting effects

Human health risk assessment
Under the conditions of the occupational settings described, 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 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:
  - H304 – May be fatal if swallowed and enters airways
  - AUH066 – Repeated exposure may cause skin dryness and cracking

- The following should be used for products containing the notified chemical:
  - Conc. ≥10%: H304
  - AUH066 (if applicable)

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 (as imported):
  - Enclosed, automated processes, where possible
  - Exhaust ventilation, if appropriate

- 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 (as imported):
  - Avoid contact with skin
  - Avoid inhalation
  - Use in ventilated areas

- 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 (as imported):
  - Impervious gloves
  - Coveralls
  - Respiratory protection (if ventilation is inadequate)

  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.

Public Health

- As liquid hydrocarbons are included in Schedule 5 of the SUSMP, any labelling and/or packaging requirements for products containing the notified chemical, which are available to the public, should be adhered to.

Disposal

- The notified chemical should be disposed of by recovery of calorific content in accordance with local regulations, or to landfill after containment with absorbent material.

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component of fuels, or is likely to change significantly;
- the amount of chemical being introduced has increased from 600 tonnes per annum, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

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

No additional secondary notification conditions are stipulated.

(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: chemical name, CAS number, molecular and structural formulae, molecular weight, analytical data and import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: flammability.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
NExBTL Renewable Diesel

OTHER NAME(S)
Renewable hydrocarbons (diesel type fraction)
NExBTL Biodiesel

MOLECULAR WEIGHT
MW <500 Da

ANALYTICAL DATA
Reference IR spectrum was provided.

3. COMPOSITION

DEGREE OF PURITY >95%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Clear liquid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	<-20 °C	Measured
Boiling Point	127 to 286 °C at 100.44 to 101.15 kPa	Measured
Density	772 kg/m^3 at 20 °C	Measured
Vapour Pressure	0.087 kPa at 25 °C	Measured
Water Solubility	9.36×10^{-8} to 3.59×10^{-3} g/L at 25 °C	Calculated
Hydrolysis as a Function of pH	Not determined	No hydrolysable groups are present in the notified chemical
Partition Coefficient (n-octanol/water)	log Pow >6.5 at 25 °C	Measured
Adsorption/Desorption	log Koc >5.63 at 25°C	Measured
Dissociation Constant	Not determined	No dissociable groups present
Flash Point	64 ± 2 °C at 101.325 kPa	Measured
Flammability	Upper: 7.5% Lower: 0.6%	Analogue data. The expected flammability limits for the notified chemical are expected to be similar to regular diesel
Autoignition Temperature	204±5 °C	Measured
Explosive Properties	Not explosive	Measured
Oxidising Properties	Not oxidising	Estimated based on chemical structure
Viscosity	3.97×10^{-6} m^2/s at 20 °C 2.6×10^{-6} m^2/s at 40 °C	Measured
Stability in Organic Solvents	Stable in polar and non-polar solvents.	Measured

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

The viscosity of the notified chemical (2.6 mm^2/s at 40 °C) indicates that the notified chemical is an aspiration hazard according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS)* and the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004). See Section 6.2 for further

details regarding the health hazard classification.

Reactivity
The notified chemical is not expected to react with air or water.

Physical hazard classification
Based on the submitted physico-chemical data depicted in the above table, 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***
Flammable Liquids (Category 4)	H227 – Combustible liquid

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported neat as a liquid.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤600000	≤600000	≤600000	≤600000	≤600000

PORT OF ENTRY
Any major Australian port where a petroleum refinery or oil product terminal is located.

IDENTITY OF MANUFACTURER/RECIPIENTS
Australian refineries or oil product terminals.

TRANSPORTATION AND PACKAGING
The notified chemical will be imported into Australia in bulk by ship and transported in bulk through pipelines. Barge or truck transport will also be used. It will be first transported from the ports of entry to the refinery, and then to customers.

USE
The notified chemical will be used as a fuel for diesel powered cars, trucks, off road equipment, agriculture, power plants and marine applications.

OPERATION DESCRIPTION
The notified chemical will be imported by ship and transferred by pipeline directly into a storage tank at a refinery, using an ISO 14001 procedure that allows for virtually no losses. A vacuum back flush will remove fuel from the unloading hoses, which will then be further capped to prevent any fuel spillage. Only a few grams that reside on the surface of the hoses will be allowed to dry.

From its storage tank, the notified chemical will be managed in two possible ways through pipelines:

- Blended into regular refinery produced diesel (at 1-10% notified chemical).
- Supplied to specific markets that require low emissions diesel fuel.

The notified chemical (either as a component of blended fuels or in its pure form) will be distributed from the refinery by marine barge or by truck. There will be no distribution by rail or drum.

Approximately 50% of the imported volume will go to commercial end users such as trucking fleets, marine, agriculture and construction companies, <20% to retail truck stops and service stations, approximately 10% to government and military, and <20% to railroads.

The loading of trucks will occur at the distribution center, where the diesel will be loaded with no spills or leaks expected. Trucks will be drained but not cleaned. Marine barges will not be cleaned. The trucks will deliver

fuel to tanks located at commercial trucking fleets, marine tugs or small ships, agriculture users, railroads, service stations, truck stops and construction companies. Workers will also be involved in fuelling vehicles and in the maintenance and cleaning of equipment and pipelines.

Blended diesel fuel will be analysed to ensure that the physical and chemical specifications are met. This analysis will be performed in laboratories. Remaining diesel fuel left over from the analysis will be disposed of into a waste drum, which will be recycled back to the refinery.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration*	*Exposure Frequency*
Unloading	1-8 hours/shipment	1-2 times per year
Sampling and Analysis	10 minutes/shipment	1-2 times/week
Truck & Barge Distribution	1 hour	240 days/week
End-use fuelling	10-30 minutes	200 days/year

EXPOSURE DETAILS

Occupational exposure to the notified chemical is possible via the dermal, ocular and inhalation routes and may occur during the import, loading/unloading, transport, and handling of the fuel containing the notified chemical.

During importation and unloading, worker exposure is expected to be low as the diesel is transferred via pipelines using a standard procedure that allows for virtually no losses. A vacuum back flush will remove fuel from the unloading hoses, which will then be further capped to prevent any fuel spillage. Only a small quantity (few grams) that is left on hoses will be allowed to dry.

Worker exposure is also expected to be low during initial transfer of notified chemical from the storage tank to the refinery, as transfer will occur by pipeline.

At refineries, the notified chemical is either blended at 1-10% into regular refinery produced diesel or sold directly to specific markets. While detailed information regarding the handling of the notified chemical at the refinery during blending has not been provided, exposure is expected to be low during blending, as it will be carried out mainly through pipelines.

Exposure to the notified chemical is expected to be low during sampling and analysis of blended fuel at the refinery, as the workers will be wearing appropriate personal protective equipment (PPE) when performing this in the chemical laboratory.

Worker exposure during transportation by marine barge is expected to be low, as loading and unloading will consist of attaching hoses to the truck and storage vessels for fuel transfer. A special air back flush system will be used to prevent spillage during transfer. Dermal exposure as a result of drips and spills is possible during the connection and disconnection of transfer hoses. Marine barge will not be cleaned. Similarly, exposure is also expected to be limited during transportation by trucks, as loading and unloading is expected to be completed with minimal spills or leaks. The drivers will likely wear gloves and long sleeve shirts when unloading the fuel. Trucks will be drain-dried but not cleaned.

6.1.2. Public Exposure

The notified chemical is intended for use as a fuel for diesel power cars, trucks, off road equipment, agriculture, power plants and marine applications. Therefore, the general public may only be directly exposed to the notified chemical via the dermal and inhalation routes, accidentally when vehicle and equipment users fuel their vehicles at service stations and truck stops. Overall, direct exposure to the notified chemical in blended diesel is expected to be low, less than that described above, and similar to that for currently used diesel fuels.

General population exposure may occur primarily through contaminated air as many components of diesel are commonly found in urban air. Secondary exposure to soil, water and the food chain, via environmental transport of residual diesel emissions to the atmosphere from vehicle exhaust, is also possible.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 >2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 >2000 mg/kg bw; low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation – adjuvant test	limited evidence of sensitisation
Mouse, skin sensitisation – local lymph node assay	evidence of sensitisation
Reproductive/repeat dose toxicity	NOAEL ≥1000 mg/kg bw/day (reproductive, foetal and systemic)
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* gene mutation	non genotoxic
Genotoxicity – *in vitro* chromosome aberration	non genotoxic

Toxicokinetics, metabolism and distribution.
Absorption across biological membranes (gastrointestinal tract and skin) is likely based on the low molecular weight (<500 Da) and high partition coefficient (log Kow >6.5) of the notified chemical (and noting that the dermal absorption may be enhanced due to the expected irritant/defatting effects of the notified chemical). The notified chemical may also be absorbed across the respiratory tract.

Acute toxicity.
The notified chemical was of low acute oral and dermal toxicity in rats (LD50 >2000 mg/kg bw). Effects consistent with drying and defatting were observed in females in the acute dermal toxicity study. No acute inhalation toxicity data were provided for the notified chemical. Therefore, harmful effects following inhalation exposure to the notified chemical cannot be ruled out. The (M)SDS of the notified chemical indicates an exposure limit of 5 mg/m^3 (8 hours; oil mist) and based on the viscosity of the notified chemical, it is classified as an aspiration hazard.

Irritation and Sensitisation.
The notified chemical was a slight skin and eye irritant in rabbits. While the effects that were observed in these studies would not warrant classification of the chemical as a skin or eye irritant, the notifier has indicated that the chemical should be classified on the basis that repeated exposure may cause skin dryness and cracking (R66 risk phrase; AUH066 non-GHS hazard statement). This effect is consistent with the effects noted in the acute dermal toxicity study.

An LLNA study on the notified chemical indicated the potential for skin sensitisation (stimulation indices of 2.09, 3.23 and 5.97 at 25, 50 and 100% concentration, respectively). However, borderline positive results (stimulation indices of 3.01-3.03) were also obtained in the study for substances that were intended to be negative controls. Limited evidence of sensitisation was noted in a guinea pig maximisation test (100% induction concentration; 25% challenge concentration; 15% response, whereas a response of ≥30% is expected for mild/moderate sensitisers in an adjuvant test). The notified chemical does not contain structural alerts for skin sensitisation. Therefore, based on the available information, the notified chemical is not considered to be a skin sensitiser.

Reproductive and repeat dose toxicity.
In a two-generation reproductive study, rats were administered the notified chemical by gavage at 0, 50, 250 or 1000 mg/kg bw/day and mated to produce subsequent generations. There were no treatment related effects on reproductive performance or on pups. Effects in the kidneys were attributed to α2-microglobulin accumulation and, in the absence of associated effects, were not considered by the study authors to be adverse. Increased liver weights and hepatocellular hypertrophy were observed and were considered to be an adaptive effect. The NOAEL for reproductive, foetal and systemic toxicity was established as ≥1000 mg/kg bw/day in this study, based on the lack of adverse effects.

Mutagenicity.
Bacterial reverse mutation, *in vitro* gene mutation and *in vitro* chromosomal aberration studies on the notified chemical were negative.

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 table below.

Hazard classification	***Hazard statement***
Aspiration hazard (Category 1)	H304 – May be fatal if swallowed and enters airways

In Australia, additional non-GHS hazard statements apply (see *Guidance on the Classification of Hazardous Chemicals Under the WHS Regulations* for further information; Safe Work Australia, 2012). Based on the available information, the following additional (non-GHS) hazard statement is also recommended:

AUH066 – Repeated exposure may cause skin dryness and cracking

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:

R65 Harmful: may cause lung damage if swallowed
R66 Repeated exposure may cause skin dryness or cracking

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Dermal, inhalation and ocular exposure to the notified chemical may occur during handling of the notified chemical itself (as imported) or during handling of formulated fuels containing the notified chemical, with the most frequent exposure of workers expected to occur when connecting/disconnecting hoses. The primary risks associated with use of the notified chemical are related to the aspiration hazard and the risk of dermal effects following repeated exposure. The notified chemical is also classified as a flammable liquid (category 4).

The workers of most concern are those that will handle the notified chemical itself, both in large volume and on a frequent basis. When handling large volumes of the notified chemical (e.g. following import and at refinery sites), containment measures are expected to be in place. In addition, appropriate PPE (including impervious gloves and coveralls) is expected to be worn by workers to minimise exposure to the notified chemical. Operations are expected to occur in adequately ventilated areas [noting that the (M)SDS of the notified chemical indicates an exposure limit of 5 mg/m^3 (8 hours; oil mist)]. In addition, respiratory protection should be used by workers if significant inhalation exposure is expected. Similar control measures are expected to be in place for workers conducting sampling and analysis tasks.

Therefore, the risk to the health of workers is not considered to be unreasonable provided that control measures (e.g. containment and/or adequate ventilation and appropriate PPE, such as coveralls, impervious gloves and respiratory protection, as applicable) are in place to minimise exposure of workers to the notified chemical (as imported).

6.3.2. Public Health

The public may be exposed to the notified chemical (in general, at ≤10% concentration) during refuelling operations. Liquid hydrocarbons are included in Schedule 5 of the *Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP), with packaging/labelling requirements for products available to the public.

During refuelling operations, exposure is expected to be brief and infrequent. Refuelling will occur outdoor and vapours will quickly dissipate into the ambient air. Therefore, the risk to public health is not considered to be unreasonable.

Although most of the notified chemical will be combusted as a fuel, the general population may be exposed at low levels mainly through exposure to contaminated air, as many components of diesel are commonly found in urban air. Secondary exposure to soil, water and the food chain, via environmental transport of residual diesel emissions to the atmosphere from vehicle exhaust, is also possible. However, the risk of secondary exposure of the general public to the notified chemical is expected to be low, also considering the low water solubility, ready biodegradability and high log Koc and log Kow of the notified chemical.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be transferred to diesel storage tanks at refineries using a pipeline from the ship in which it is imported. From its storage tank, the notified chemical may be either blended into regular refinery produced diesel or be supplied to specific markets that require low emissions diesel fuel. Releases may occur as a result of accidental spills from the processes of unloading, transportation and blending/reformulation. These releases are not expected to be significant.

RELEASE OF CHEMICAL FROM USE

Less than 1% of the notified chemical is expected to be released during use. Small amounts (expected to be less than a gram per operation) will be spilt on the ground during vehicle refuelling, and will largely evaporate into the atmosphere and disperse. Most of the notified chemical will be combusted as fuel to generate water and oxides of carbon.

RELEASE OF CHEMICAL FROM DISPOSAL
Any wastes (small releases) of the fuel containing the notified chemical are expected to be absorbed by absorbent material for disposal to landfill. For releases of large amounts, the preferred disposal option is thermal decomposition at an approved facility.

7.1.2. Environmental Fate
The notified chemical is readily biodegradable. For the details of the environmental fate studies please refer to Appendix C. The individual components of the notified chemical are lipophilic and have the potential to bioaccumulate, which can be significantly reduced due to ready biodegradability of the notified chemical in the environment. Furthermore, significant bioaccumulation in aquatic organisms is not expected because of the low aquatic exposure from the use pattern.

Most of the notified chemical is expected to be combusted as fuel. A small amount of the notified chemical may be sent to landfill as collected wastes to undergo slow biotic or abiotic degradation processes. Very small amounts of the notified chemical may be spilt on the ground and will most likely evaporate into the atmosphere. Some of the notified chemical may be thermally decomposed at an approved facility. In the above routes, the notified chemical is expected to be finally decomposed into water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)
The calculation of predicted environmental concentration (PEC) was not necessary due to the limited release of the notified chemical expected from the proposed use pattern.

7.2. Environmental Effects Assessment
The results from ecotoxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h EL50 >1000 mg/L (filtered WAF)	Not acutely harmful up to its limit of water solubility
Daphnia Toxicity	48 h EL50 >100 mg/L (filtered WAF)	Not acutely harmful up to its limit of water solubility
	21 day NOEL = 1 mg/L (WAF)	Harmful on a chronic basis
Algal Toxicity	72 h EL50 >100 mg/L (WAF)	Not acutely harmful up to its limit of water solubility
	72 h NOEL = 100 mg/L (WAF)	
Inhibition of Bacterial Respiration	3 h IC50 >1000 mg/L	Not harmful to microbial respiration
Mud Shrimp Toxicity	10 day acute LC50 = 1200 mg/kg dry weight sediment	Very slightly toxic*

* Based on Mensink *et al*., 1995 for the acute toxicity classification for earthworms.

The notified chemical is not acutely harmful to aquatic organisms, and is very slightly toxic to the sediment species mud shrimp. It is chronically harmful to daphnids.

Under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009) the notified chemical is classified as acutely not harmful to aquatic organisms. The notified chemical is chronically harmful to daphnids based on the 21 day NOEL of 1 mg/L. Based on the acute toxicity to fish, daphnids and alga, the notified chemical is not formally classified under the GHS; and is not considered to be harmful to aquatic life. However, based on the chronic toxicity to daphnids, the notified chemical is formally classified as Chronic Category 3 under the GHS - Harmful to aquatic life with long lasting effects.

7.2.1. Predicted No-Effect Concentration
The calculation of predicted no-effect concentration was not considered necessary because limited release of the notified chemical to the aquatic environment is expected based on the proposed use pattern.

7.3. Environmental Risk Assessment
The Risk Quotient (PEC/PNEC) was not calculated since no significant release to the aquatic environment is expected. The notified chemical is not expected to pose an unreasonable risk to the aquatic environment based

on the assessed use pattern.

For PBT consideration, the notified chemical is not persistent. It is not considered to meet the criteria for bioaccumulation given its rapid biodegradability, despite its low water solubility and low molecular weight (<1000 Da). It is not considered to meet the criterion for toxicity based on the provided chronic study on daphnids indicating a 21 day NOEL of 1 mg/L.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point <-20 ºC

Method	EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks	The appearance of the test substance changed from clear liquid to white, translucent liquid at -17 ºC.
Test Facility	Harlan (2009a)

Boiling Point 127 to 286 ºC at 100.44 to 101.15 kPa

Method	EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks	Distillation method at atmospheric pressure was used.
Test Facility	Harlan (2009a)

Density 772 kg/m^3 at 20 ºC

Method	EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks	Pycnometer method.
Test Facility	Harlan (2009a)

Vapour Pressure 0.087 kPa at 25 °C

Method	EC Directive 92/69/EEC A.4 Vapour Pressure
Remarks	The vapour pressure was determined using an isoteniscope system with measurements being made at several temperatures and linear regression analysis used to calculate the vapour pressure at 25 °C.
Test Facility	Harlan (2009b)

Water Solubility Overall solubility range 9.36×10^{-8} to 3.59×10^{-3} g/L

Method	EC Directive 92/69/EEC A.6 Water Solubility WSKOWWIN version 1.41
Remarks	It was not possible to determine the water solubility using the flask method. Due to the presence of a suspension of excess undissolved test substance it was not possible to isolate a saturated solution for analysis without filtration. Filtration of the sample solution had previously been demonstrated to be detrimental to validation recoveries, possibly due to the losses through lipophilic adsorption during the procedure. The water solubilities of the individual components present in the notified chemical were estimated by fragment constant methodology using compositional information and WSKOWWIN version 1.41. The accuracy of the software was assessed by comparing the obtained data to available literature values. The water solubility of the individual test substance components was estimated to be in the range 9.36 x 10^{-8} to 3.59 x10^{-3} g/L at 25 ºC.
Test Facility	Harlan (2009a)

Partition Coefficient (n-octanol/water) log Pow >6.5

Method	EC Council Regulation No 440/2008 A.8 Partition Coefficient
Remarks	HPLC method was used. The sample was monitored in the UV region and the elution of the notified chemical was confirmed by fraction collection and subsequent GC analysis. In the absence of any dissociating group, no manipulation of the mobile phase pH was necessary to ensure the molecular form of each component. Therefore, the determination was performed at approximately neutral pH. The gradient method was used. All components of the test substance were demonstrated to elute after the retention time of the highest calibration standard (log Pow = 6.5) and therefore have a partition coefficient value greater than the highest calibration standard.

Test Facility Harlan (2009a)

Adsorption/Desorption log K_{oc} >5.63
– screening test

Method EC Directive No 440/2008 C.19 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography
Remarks HPLC method was used. The sample was monitored in the UV region and the elution of the notified chemical was confirmed by fraction collection and subsequent GC analysis. The gradient method was used, and the determination was performed at approximately neutral pH. All components eluated shared a common adsorption coefficient factor, that was higher than the highest calibration standard ($\log K_{oc}$>5.63). Therefore, the absorption coefficient (K_{oc}) of the notified chemical was determined to be greater than 4.27 x 10^5, log K_{oc} >5.63.
Test Facility Harlan (2009a)

Adsorption/Desorption log K_{oc} >5.63
– screening test

Method EC Directive 2001/59/EC C.19 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography
Remarks The test was carried out at approximately neutral pH. The HPLC conditions followed did not demonstrate a peak for the test substance. To determine the retention characteristics of the test substance, fractions of the eluate were collected and analysed by gas chromatography. It was confirmed that the test substance was present in the second fraction (from 60.1 to 73.6 minutes when the mobile phase was adjusted to 100% methanol), whereas no test substance was detected in the first fraction (from 0 to 60 minutes with the mobile phase composition of methanol:water (55:45v/v)). The log K_{oc} was determined to be >5.63.
Test Facility SafePharm (2008a)

Flash Point 64 ± 2 °C at 101.325 kPa

Method EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks The flash point was determined using a closed cup equilibrium method.
Test Facility Harlan (2009b)

Autoignition Temperature 204±5 °C

Method EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks The auto-ignition temperature was determined by heating aliquots of the test substance in a flask and observed for any ignition. The procedure was repeated, varying the sample size as required until the lowest temperature at which ignition, if any, occurred within 5 minutes of insertion, was determined.
Test Facility Harlan (2009b)

Explosive Properties Not explosive

Method EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks The test substance was subjected to the BAM fall hammer test and Koenen steel tube test. The BAM friction test, which is not applicable to liquids, was not performed. The test substance was determined not to have explosive properties.
Test Facility Harlan (2009)

Oxidizing Properties Not oxidising

Method EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids).
Remarks The structure of the test substance was assessed for chemical groups that would imply oxidising properties. Based on the chemical structure of the test substance, its oxidising

properties were predicted to be negative.
Test Facility Harlan (2009b)

Viscosity 3.97×10^{-6} m^2/s at 20 °C
2.60×10^{-6} m^2/s at 40 °C

Method OECD TG 114 Viscosity of Liquids.
Remarks The determinations were performed in duplicate at 20±0.5 °C and 40±0.5 °C using a U-tube viscometer.
Test Facility Harlan (2009a)

Stability in Organic Solvents Stable in polar and non-polar solvents.

Method In house method.
Remarks The stability of the notified chemical was investigated in a polar organic solvent (methanol) and a non-polar organic solvent (hexane) over a 30 day period at 25± 2 °C. Solutions of the notified chemical were prepared in the two organic solvents. After preparation of the solutions, aliquots were transferred into a number of separate glass stoppered vessels which were then placed into storage at 25± 2 °C in the dark.

An aliquot of each of the remaining solutions was analysed at the initial time point. Individual vessels of each solution were removed for analysis after storage periods of 8 and 20 days. Triplicate vessels for each solution were removed for analysis after storage period of 30 days. The concentration of the notified chemical in the sample solutions was determined by gas chromatography.
Test Facility Harlan (2009a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE Notified chemical

METHOD OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method
Species/Strain Rat/Sprague-Dawley CD
Vehicle None
Remarks - Method No significant protocol deviations.

RESULTS

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

LD50 >2000 mg/kg bw
Signs of Toxicity No clinical signs of toxicity were observed. All animals gained weight over the 14 day observation period.
Effects in Organs None

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

TEST FACILITY SafePharm (2005a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE Notified chemical

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

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5M + 5F	2000	0/10

LD50 >2000 mg/kg bw
Signs of Toxicity - Local No irritation was noted in males. Hyperkeratinisation or crust formation was observed in females throughout the observation period, with scabs observed in some females. The study authors note that the scabs may have been attributed to the animals scratching the treatment site. The authors further note the observations in females may be due to a drying/defatting effect of the test substance.
Signs of Toxicity - Systemic None
Effects in Organs None
Remarks - Results The use of semi-occlusive dressing may have resulted in loss of the applied test substance through volatilisation.

CONCLUSION The notified chemical was of low toxicity via the dermal route, under the conditions of the study.

TEST FACILITY SafePharm (2006a)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion
Species/Strain Rabbit/New Zealand White
Number of Animals 3 males
Vehicle None
Observation Period 72 hours
Type of Dressing Semi-occlusive
Remarks - Method No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0.7	0.3	0.3	2	<72 hours	0
Oedema	0	0	0	1	<24 hours	0

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

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY SafePharm (2007a)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion
Species/Strain Rabbit/New Zealand White
Number of Animals 3 male
Observation Period 72 hours
Remarks - Method No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0	0.3	0	1	<48 hours	0
Conjunctiva: chemosis	0	0	0	1	<24 hours	0
Conjunctiva: discharge	0	0	0	1	<24 hours	0
Corneal opacity	0	0	0	0	no effects	0
Iridial inflammation	0	0	0	0	no effects	0

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

Remarks - Results Slight conjunctival redness, chemosis and discharge were observed at one hour in all animals. The only observation at 24 hours was slight conjunctival redness in one animal.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY SafePharm (2007b)

B.5. Skin sensitisation

TEST SUBSTANCE Notified chemical

METHOD	OECD TG 406 Skin Sensitisation – Guinea Pig Maximisation Test
Species/Strain	Guinea pig/Dunkin-Hartley
PRELIMINARY STUDY	Intradermal: 25% (maximum non-necrotising concentration) Topical: 25% (maximum non-irritating concentration)
MAIN STUDY	
Number of Animals	Test Group: 20 females Control Group: 10 female
INDUCTION PHASE	Induction Concentration: intradermal: 25% topical: 100%
Signs of Irritation	Dryness was observed in 4 animals and a scab was observed in 9 animals following topical induction.
CHALLENGE PHASE	
1st challenge	topical: 12.5% and 25%
Remarks - Method	No significant protocol deviations. Olive oil was used as the vehicle. The test sites were treated with 10% sodium lauryl sulphate 24 hours before topical induction to induce irritation.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*		
		24 h	*48 h*	*72 h*
Test Group				
	12.5%	0/20	0/20	0/20
	25%	3/20	1/20	0/20
Control Group				
	12.5%	0/10	0/10	0/10
	25%	0/10	0/10	0/10

Remarks - Results	Slight to moderate erythema was observed in 3/20 (15%) animals at 24 hours and 1/20 (5%) animals at 48 hours. There were no observations in controls. A response of ≥30% is expected for mild to moderate sensitisers in an adjuvant test. A concurrent positive control study was not conducted. However, the positive control studies with α-hexylcinnamaldehyde (HCA) had been previously conducted in the laboratory.
CONCLUSION	There was limited evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Phycher (2008)

B.6. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain	Mouse/CBA/Ca (female)
Vehicle	Acetone:olive oil (4:1)
Remarks - Method	In an irritation screening study, single mice were administered three consecutive daily 25 µL doses to the dorsal surface of each ear of either undiluted test substance, kerosene or n-octadecane, then observed for 3 days. No systemic toxicity or notable weight changes were observed. The main study was conducted using 5 mice/group at 0, 25, 50 or 100% concentration. A concurrent positive control was conducted using 15% HCA in the vehicle. Additional groups of 5 mice were administered undiluted kerosene or n-octadecane.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node*)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	1204	-
25	2513	2.09
50	3886	3.23
100	7189	5.97
100 (n-octadecane)	3650	3.03
100 (kerosene)	3623	3.01
Positive Control (HCA)		
15	5053	4.20

*Scintillation measurements taken 3 times for each sample, data presented as mean of all measurements.

Remarks - Results	No systemic toxicity or notable weight changes were observed. Local toxicity effects were not reported. There was a clear (dose-related) increase in the stimulation index (SI) in the groups treated with the test substance and a positive response (SI >3) was noted in the groups treated at 50% and 100%. A positive response was noted in the positive control group. Borderline positive responses were also noted in the groups treated with kerosene and n-octadecane. As these groups were intended as negative controls (no reference data provided), the study authors note that the positive responses observed in these groups suggest the possibility of confounding.
CONCLUSION	Under the conditions of the study, there was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	SafePharm (2008b)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test – Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, TA102
Metabolic Activation System	S9 fraction from phenobartbitone/β-naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 50-5000 µg/plate b) Without metabolic activation: 50-5000 µg/plate
Vehicle	Acetone
Remarks - Method	No significant protocol deviations. A range-finding study was conducted in the TA100 strain in the presence and absence of metabolic activation between 0.15-5000 µg/plate. Vehicle and positive controls were used in parallel with the test substance.

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	≥1500	negative
Test 2	-	>5000	≥1500	negative

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Present				
Test 1	> 5000	> 5000	≥ 1500	negative
Test 2	-	> 5000	≥ 1500	negative

Remarks - Results: There were no statistically significant increases in the frequencies of revertant colonies in treated plates. The positive controls demonstrated a positive response, confirming the validity of the test system.

CONCLUSION: The notified chemical was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY: SafePharm (2005b)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 476 In vitro Mammalian Cell Gene Mutation Test
Cell Type/Cell Line: Mouse lymphoma/L5178Y
Metabolic Activation System: S9 fraction from phenobartbitone/β-naphthoflavone induced rat liver
Vehicle: Acetone
Remarks - Method: No significant protocol deviations.

A preliminary toxicity test was conducted (4 hour exposure period with and without metabolic activation, and 24 hour exposure period without activation) at concentrations ≤5000 µg/mL. In the preliminary cytotoxicity tests, a cloudy precipitate was observed at ≥78.13 µg/mL and an oily precipitate was formed at ≥ 625 µg/mL in the 4 hour exposure groups, and at ≥312.5 µg/mL in the 24 hour exposure group.

Vehicle and positive controls were used in parallel with the test substance.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	0, 40, 80, 160, 320, 480, 640	4 hours	2 days	10-14 days
Test 2	0, 40, 80, 160, 320, 480, 640	24 hours	2 days	10-14 days
Present				
Test 1	0, 40, 80, 160, 320, 480, 640	4 hours	2 days	10-14 days
Test 2	0, 40, 80, 160, 320, 480, 640	4 hours	2 days	10-14 days

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	>5000	>640	≥40	negative
Test 2	>5000	>640	≥80	negative
Present				
Test 1	>5000	>640	≥40	negative
Test 2	-	>640	≥40	negative

*Observed in preliminary study.

Remarks - Results: There were no statistically significant increases in mutant frequency in treated plates. The positive controls demonstrated a positive response,

confirming the validity of the test system.

CONCLUSION The notified chemical was not mutagenic to mouse lymphoma L5178Y cells treated *in vitro* under the conditions of the test.

TEST FACILITY SafePharm (2008c)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test

Cell Type/Cell Line	human lymphocytes
Metabolic Activation System	S9 fraction from phenobartbitone/β-naphthoflavone induced rat liver
Vehicle	acetone
Remarks - Method	No significant protocol deviations.

Vehicle and positive controls (mitomycin C and cyclophosphamine) were used in parallel with the test substance.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	78.13, 156.25, 312.5*, 625*, 1250*, 2500*	4 hours	24 hours
Test 2	78.13, 156.25, 312.5, 625*, 1250*, 2500*	24 hours	24 hours
Present			
Test 1	78.13, 156.25, 312.5*, 625*, 1250*, 2500*	4 hours	24 hours
Test 2	78.13, 156.25, 312.5, 625*, 1250*, 2500*	4 hours	24 hours

*Cultures selected for metaphase analysis. MMC, mitomycin C; CP, cyclophosphamine.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	>5000	>2500	≥1250	negative
Test 2	>5000	>2500	≥78.13	negative
Present				
Test 1	>5000	>2500	≥1250	negative
Test 2	-	>2500	≥1250	negative

Remarks - Results An oily precipitate was observed at ≥1250 μg/mL for the 4 hour exposures, and at ≥78.13 μg/mL for the 24 hour exposure.

There were no statistically significant increases in the frequencies of chromosomal aberrations at any tested concentration. The positive controls demonstrated a positive response, confirming the validity of the test system.

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

TEST FACILITY SafePharm (2007c)

B.10. Reproductive toxicity – two generation study

TEST SUBSTANCE Notified chemical

METHOD OECD TG 416 Two-Generation Reproduction Toxicity

Species/Strain	Rat/Wistar
Route of Administration	Oral – gavage
Exposure Information	Exposure period: approximately 11-18 weeks
Vehicle	Arachis Oil BP
Remarks – Method	In a preliminary range-finding study, rats (3/sex/dose) were administered the test substance by gavage for 21-days at 0, 200, 600 or 1000 mg/kg bw/day. There were no treatment-related clinical signs of toxicity or effects on body weight gain. There were no treatment-related findings reported at necropsy. In the main study, parental (P) generation rats (28/sex/dose) were administered the test substance by gavage at 0, 50, 250 or 1000 mg/kg bw/day for 18 weeks. A subset of the P generation (10/sex/dose) was subject to examinations, which included functional observations (behavioural, functional performance tests and sensory reactivity), body weight and feed consumption. Ophthalmological examination was conducted pre-treatment and again at week 10, and haematology and clinical chemistry analyses, and histopathological analyses, were conducted during week 11 of the study. Animals were paired and mated at week 11 until pregnancy was detected. The litters were maintained until weaning on day 21 postpartum, followed by culling to the F1 generation (24/sex/dose). Remaining F1 pups and the P generation females were sacrificed and subject to necropsy at this time. All P generation males were terminated during week 18. Selected tissues were subject to histopathological examination and epididymal spermatozoa were analysed for performance. Following weaning, the F1 pups were administered appropriate doses of the test substance for 11 weeks before mating. Subsequent F2 litters were maintained before study completion on day 21 postpartum. Histopathological analyses were conducted on P and F1 parental animals.

RESULTS

Mortality and Time to Death

There were no treatment related mortalities during the study. Incidental deaths (that were considered by the study authors to be unrelated to treatment) included one P generation male treated at 250 mg/kg bw/day which was found dead on day 76 and one P generation female treated at 25 mg/kg bw/day which was killed *in extremis* on day 106 following complete litter loss and signs of poor health.

Subchronic study

There were no mortalities in the subchronic groups. Clinical observations included increased salivation post-dosing in animals treated at 250 and 1000 mg/kg bw/day and red/brown staining around the mouth in the animals treated at 1000 mg/kg bw/day. These effects were attributed by the study authors to gavage administration of an unpleasant tasting and/or irritant test material. There were no treatment related observations in behavioural, functional or sensory reactivity assessments.

There were no treatment related effects on bodyweight gains. Mean food consumption was statistically significantly increased during weeks 7, 8 and 10 in males treated at 1000 mg/kg bw/day. Additionally, there were some statistically significant increases in mean water consumption in males treated at 250 and 1000 mg/kg bw/day.

There were statistically significant increases in neutrophil counts in males treated at 250 and 1000 mg/kg bw/day (↑81% and ↑71%, respectively). There was a statistically significant decrease in alkaline phosphatase levels in females treated at 1000 mg/kg bw/day (↓47%). The relevance of these effects is unclear, but these are unlikely to be treatment related in the absence of associated pathological findings.

There was a statistically significant increase in the relative liver weights in males treated at 1000 mg/kg

bw/day (↑13%). Histopathological findings in the liver (females only) included minimal to slight generalised hepatocyte enlargement (hypertrophy), particularly in animals treated at 250 and 1000 mg/kg bw/day. These changes were not considered to be of toxicological concern based on the lack of associated effects.

There were globular accumulations of eosinophilic material in the tubular epithelium in all males treated at 1000 mg/kg bw/day, with minimal occurrences at 50 and 250 mg/kg bw/day. The study authors note that this effect is consistent with hydrocarbon nephropathy, resulting from excessive accumulation of α2-microglobulin and that humans do not synthesise this protein. The presence of α2-microglobulin was confirmed by Mallory Heidenhain staining. In the absence of degenerative changes, this finding was not considered by the study authors to represent an adverse effect of treatment.

Effects on Parental (P) and F1 Generation Animals

Effects were similar in P and F1 animals. Clinical observations were similar to the subchronic groups (salivation and red/brown staining around the mouth). There were no treatment-related effects on bodyweight gains. Feed consumption increases were observed in both generations and in both sexes treated at 1000 mg/kg bw/day with statistical significance at some observation points. Mean water intake was also statistically significantly increased, mostly in the 1000 mg/kg bw/day groups.

There were no treatment related changes to oestrous cycle. There was a slight non-statistically significant decrease in pregnancy rate in P generation animals treated at 1000 mg/kg bw/day, but this was not considered to be treatment related as there was no change in the F1 generation.

Pre-implantation losses were increased in the F1 generation treated at 250 and 1000 mg/kg bw/day, and post-implantation losses were increased in the F1 generation in the 1000 mg/kg bw/day group. These increases were not statistically significant and were of a similar rate to that of the controls in the P generation. Mating and pregnancy indices, gestation length, the number of litters per treatment level, total number of corpora lutea and implantation sites, litter size, live birth, and viability indices, and sex ratio were all similar to control groups.

There was an increase in the homogenisation resistant testicular spermatid counts in males treated at 1000 mg/kg bw/day in the F1 generation (↑49%), but this was not considered by the study authors to be toxicologically significant due to the lack of associated changes to reproductive performance. There were no treatment related effects in the proportion of pre-antral, antral and pre-ovulatory phases of follicular development.

Statistically significant increases in relative liver weights were observed in both generations in males treated at 1000 mg/kg bw/day. A statistically significant increase in absolute spleen weights in P generation males treated at 1000 mg/kg bw/day was not considered by the study authors to be treatment related due to the lack of an increase in the F1 generation and the lack of associated histopathological findings.

Statistically significant increases in the incidence of generalised hepatocyte enlargement (hypertrophy) was observed in P and F1 females treated at 1000 mg/kg bw/day, and in P generation females treated at 250 mg/kg bw/day. In the kidney, globular accumulation of eosinophilic material in the tubular epithelium was noted (α2-microglobulin presence confirmed by Mallory-Heidenhain staining). This effect primarily occurred in P and F1 generation males treated at 1000 mg/kg bw/day, with minimal observations at 50 and 250 mg/kg bw/day. The study authors note the absence of other, more severe effects in the kidney.

Effects on Pups (F1 and F2)

There were no treatment related clinical signs in pups, and pup body weights and body weight gains were not affected by treatment. Sexual development and ano-genital distance were not significantly affected by treatment. There were no treatment related organ weight changes.

Remarks – Results

Treatment related effects were observed in the liver and kidneys. The effects in the kidneys were not considered adverse due to the absence of associated degenerative changes. In the liver, increased liver weights and hypertrophy were observed. These effects may be adaptive in nature and were not considered to be adverse based on the lack of associated effects.

CONCLUSION

The NOAEL for reproductive and systemic toxicity was established as ≥1000 mg/kg bw/day, due to the lack of adverse effects at the tested doses.

TEST FACILITY Harlan (2009c)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test
EC Directive 92/69/EEC C.4-C Biodegradation: Determination of the Ready Biodegradability: Carbon Dioxide Evolution Test

Inoculum	Activated sewage sludge microorganism from local domestic sewage treatment plant
Exposure Period	29 days
Auxiliary Solvent	None reported
Analytical Monitoring	Total Organic Carbon (TOC) analyser. The degree of degradation was assessed by determination of CO_2 produced.
Remarks - Method	No significant deviation in protocol.

In view of the low water solubility a modification to the standard method of preparation of the test concentration was performed. An approach endorsed by the International Standards Organisation (ISO1995) and the published literature is to absorb the test substance onto an inert support prior to dispersion in the test vessels. Using this method, the test substance is evenly distributed throughout the test medium and the surface area of test substance exposed to the test organism is increased, thereby increasing the potential for degradation.

The test was performed at 10 mg carbon/L in the dark at about 21 ºC. A blank control, a reference control using sodium benzoate and a toxicity control containing both the test substance and the reference item were performed. Each group was conducted in duplicate, except a single replicate for the toxicity control.

RESULTS

Test substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
10	61	10	65
29	82	29	76

Remarks - Results: A degradation degree of 84% was achieved for the toxicity control, suggesting that the notified chemical was not toxic to the sludge bacteria.

The notified chemical is considered to be readily biodegradable based on the test results.

CONCLUSION: The notified chemical is readily biodegradable.

TEST FACILITY: SafePharm (2008d)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Notified chemical

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

Species	Juvenile rainbow trout (*Oncorhynchus mykiss)*
Exposure Period	96 hours

Auxiliary Solvent	None
Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	Total Organic Carbon (TOC) analyser.
Remarks – Method	The study was performed by exposing 20 fish (two groups of 10) to water accommodated fractions (WAFs) of the test substance at a loading rate of 1000 mg/L for 96 hours at 12.9 – 15.0 ºC. After 23 hours of stirring the test substance/test medium mixtures were allowed to stand for 1 hour. 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. Visual examination of the WAFs showed small oily globules, which were removed by filtering through a glass wool plug. The aqueous phase or WAFs were removed by mid-depth siphoning (the first 75-100 mL discarded) to give the target loading rate WAFs. During the test the WAFs 1000 mg/L loading rate was observed to be a clear, colourless solution.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	Not applicable	20	0	0	0	0	0
1000	Not applicable	20	0	0	0	0	0

LL50	>1000 mg/L (filtered WAF) at 96 hours
NOEL	1000 mg/L (filtered WAF) at 96 hours
Remarks – Results	The dissolved oxygen levels during the test were not provided, which is considered not a concern considering no mortality was observed for both the control and treatment groups. All other test guideline criteria were met. TOC analysis of the test preparation at 0 (fresh media), 24 (old media), 72 (fresh media), and 96 hours (old media) showed no significant differences in the amount of carbon present in the 1000 mg/L loading rate WAF test vessels when compared to the control vessels. Therefore, given the background level of carbon in the control vessels and also low levels of carbon in the test vessels, it was considered that all the results were around the limit of quantification of the analytical method and hence could not provide definitive evidence of the stability of the test preparation. Based on the test results, the notified chemical is considered not harmful to fish up to the limit of its water solubility.

CONCLUSION The notified chemical is not harmful to fish up to the limit of its water solubility.

TEST FACILITY SafePharm (2006b)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test - Static.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	Total Organic Carbon (TOC) analysis
Remarks - Method	Daphnids of 20 (4 replicates of 5) were exposed to a WAF of the test

substance at a loading rate of 100 mg/L and 20 ºC. The preparation of WAFs was the same as described in the previous study for fish. At the start of the mixing period the WAFs 100 mg/L loading rate was observed to be clear, colourless water column with oily globules of test substance at the water surface. The aqueous phase or WAFs were removed by mid-depth siphoning (the first 75-100 mL discarded) to give the target concentration WAFs. After siphoning and for the duration of the test, the WAF 100 mg/L loading rate was observed to be a clear, colourless solution. Microscopic inspection of the WAF showed no micro-dispersion or undissolved test substance to be present.

A positive control using potassium dichromate was performed every 6 months at concentrations ranging 0.32 – 3.2 mg/L and 20 ºC for 48 hours.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*
Nominal	*Actual*		*48 h*
0	Not applicable	20	0
100	Not applicable	20	0

EL50 >100 mg/L at 48 hours (filtered WAF)
NOEL 100 mg/L at 48 hours (filtered WAF)
Remarks - Results All the test guideline criteria were met.

TOC analysis of the test preparation at 0 and 48 hours showed no significant differences in the amount of carbon present within the 100 mg/L loading rate WAF test vessels when compared to the control vessels. Therefore, given the background level of carbon in the control vessels and also the low level carbon in the test vessels, it was considered that all the results were around the limit of quantification of the analytical method and could not provide definitive evidence of the stability of the test preparation.

Based on the test results, the notified chemical is considered not harmful to daphnids up to the limit of its water solubility.

CONCLUSION The notified chemical is not harmful to daphnids up to the limit of its water solubility.

TEST FACILITY SafePharm (2005c)

C.2.3. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 211 *Daphnia magna*, Reproduction Test – semi static
Species *Daphnia magna*
Exposure Period 21days
Auxiliary Solvent None
Water Hardness 140 mg $CaCO_3$/L
Analytical Monitoring Total organic carbon (TOC) analysis
Remarks - Method Test daphnids were exposed (10 replicates of single daphnid per group) to a WAF of the notified chemical over a range of nominal loading rates of 1.0, 3.2, 10, 32 and 100 mg/L at 20 – 22 ºC. After 23 hours of stirring the notified chemical/test medium mixtures for loading rates were allowed to stand for 1 hour. 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 WAFs were removed by mid-depth siphoning (the first 75-100 mL discarded) to give the target loading rate WAFs. After 23 hours stirring and a 1 hour standing period the WAFs were observed to be clear, colourless solutions. Therefore, the WAFs were not siphoned through a glass wool plug. WAFs were renewed 3 times per week.

The old or expired test media preparations were observed green tinged due to the presence of algal cells used as feed for the daphnids.

Statistical analysis methods for the data (SAS computer software package):
- EL50 for immobilization: corrected chi-squared statistic for analysis of mortality data;
- Analysis of the daphnids' length data, lowest-observed-effect loading rate (LOELR) and no-observed-effect loading rate (NOELR): one way analysis of variance incorporating Bartlett's test for homogeneity of variance and Dunnett's multiple comparison procedure for comparing several treatments with a control.

Results

Nominal loading tested, cumulative mean number of offspring released, number of offspring released per female daphnid (*Daphnia magna*), mean length and standard deviations and survival of parental daphnids.

	Nominal loading Rate (mg/L)					
Test Day 21	*Control*	*1.0*	*3.2*	*10*	*32*	*100*
Total no. of offspring released by survived *Daphnia*	573	530	392	281	373	276
Total no. of offspring released per survived *Daphnid*	63	66	43	35	53	39
Mean length (standard deviation) of survival parent daphnids (mm)	4.4 (0.2)	4.5 (0.1)	4.4 (0.2)	4.4 (0.2)	4.3 (0.1)	4.3 (0.2)
No. of adult *Daphnids* Immobilised	1	2	1	2	3	3
% Survival	90	80	90	80	70	70

21 day EL50 (Immobilization)	>100 mg/L (WAF)
21 day EL50 (Reproduction)	>100 mg/L (WAF)
21 day LOELR	3.2 mg/L (WAF)
21 day NOELR	1.0 mg/L (WAF)
Remarks - Results	Only 10 animals were used for each test concentration which does not meet the test guideline criterion (> 20 per concentration). This is however not considered to be a concern considering <50% mortality was observed at all the test levels. All other test guideline criteria were met.

TOC analysis of the test preparation up to Day 7 showed that the levels in the test WAFs were lower than or approximately equal to the controls. Therefore, given the background level of carbon in the control vessels and also the low level carbon in the test vessels, it was considered that all the results were around the limit of quantification of the analytical method and could not provide definitive evidence of the stability of the test preparation.

No statistically significant differences were found between the control and all the test groups in terms of length of the surviving parental daphnids on Day 21 of the test.

The 21 day EL50s for immobilization and reproduction were determined to be >100 mg/L (WAF). The LOELR was determined to be 3.2 mg/L (WAF)

on the basis that significantly fewer live young per adult female (P <0.05) were produced at this loading rate when compared with the control. The NOELR was determined to be 1.0 mg/L (WAF) on the basis of parent immobilization and numbers of live young produced per adult surviving female. All the endpoints were determined by the study author and are considered acceptable.

The notified chemical is considered harmful to daphnids on a chronic basis based on the determined NOELR for WAF.

CONCLUSION The notified chemical is harmful to daphnids on a chronic basis.

TEST FACILITY SafePharm (2008e)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test
EC Directive 92/69/EEC C.3 Algal Inhibition Test

Species	*Scenedesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 100 mg/L (WAF)
Auxiliary Solvent	None
Water Hardness	Not provided
Analytical Monitoring	Total organic carbon (TOC) analysis; Coulter® Multisizer Particle Counter for cell density determination.
Remarks - Method	Following a range-finding test, a limited test was conducted at a single loading rate of 100 mg/L using water accommodation fractions (WAFs) of the notified chemical at 24±1 ºC under constant illumination (7000 lux) and pH 7.4 – 7.9. The initial cell density used was about 1×10^4 cells/L. The preparation of WAFs was the same as described in the previous chronic study for daphnids. Microscopic inspection of the WAFs found no micro-dispersion or undissolved test substance to be present. Six replicates for the test group and 3 replicates for the control were set up. Statistical analysis methods for the data (SAS computer software package): - Students t-test incorporating Bartlett's test for homogeneity of variance was carried out on the area under the growth curve data at 72 hours to determine any statistically significant differences between the test and control groups.

RESULTS

Biomass		*Growth*	
E_bL50	*NOEL*	*E_rL50*	*NOEL*
mg/L at 72h	*mg/L*	*mg/L at 72 h*	*mg/L*
>100	100	>100	100

Remarks - Results No reference control was reported in the study report. This is considered acceptable considering no statistically significant differences between the test and control groups were observed in the limited test. All other test guideline criteria were met.

The notified chemical is considered not harmful to alga up to its limit of water solubility.

CONCLUSION The notified chemical is not harmful to alga up to its limit of water solubility on an acute basis.

TEST FACILITY	SafePharm (2005d)

C.2.5. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 87/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test.
Inoculum	Activated sewage sludge microorganism from local domestic sewage treatment plant
Exposure Period	3 hours
Concentration Range	Nominal: 1000 mg/L
Remarks – Method	Following a range finding test, a limited test was performed at 1000 mg/L in triplicate at 21 ºC with the addition of a synthetic sewage as a respiratory substrate. Lab tap water was sued as the test water after being dechlorinated by passage through an activated carbon filter and partially softened, giving water a total hardness of 1401 mg/L as $CaCO_3$. The notified chemical was dispersed with the aid of ultrasonication in the test diluents for approximately 15 minutes prior to the addition of synthetic sewage, activated sewage sludge and water. Furthermore, each vessel was aerated to ensure that there was maximum contact between the test substance and activated sewage sludge. At the test concentration of 1000 mg/L a thick layer of test substance was visibly dispersed on the surface throughout the exposure period. This was considered to be due to the insoluble nature of the notified chemical in the test media. A blank control and a reference control using 3,5-dichlorophenol were also conducted.
RESULTS	
IC50	>1000 mg/L (nominal) for 3 hour
NOEC	1000 mg/L (nominal) for 3 hour
Remarks – Results	All the test guideline criteria were met. No significant inhibitory effects were observed for the test group comparing to the control. The notified chemical is not considered to be inhibitory harmful to sludge bacteria based on the test results.
CONCLUSION	The notified chemical is not inhibitory harmful to sludge bacteria.
TEST FACILITY	SafePharm (2006c)

C.2.6. Determination of acute toxicity to *Corophium volutator* in a sediment system

TEST SUBSTANCE	Notified chemical
METHOD	OPSAR Protocols on Methods for the Testing of Chemicals used in The Offshore Industry Part A: A Sediment Bioassay using an Amphoid *Corophium* sp Olso and Paris Commission 2005
Species	Adult *Corophium volutator* (mud shrimp), greater than 5 mm in body length at the start of the test, collected from wild population in Dalgety Bay on the north Shore of the Firth of Forth
Exposure Period	10 days
Auxiliary Solvent	None
Analytical Monitoring	The sample extracts were analysed by Gas Chromatography with Mass Spectrometric detection (GCMS).
Remarks - Method	Natural sediment were collected from the same site and stored in a temperature controlled room. Before use the sediment was sieved to

remove any infaunal organisms. The test water was seawater of salinity 39%, and was filtered to 1 µm before use.

The test was performed at nominal concentrations of 0, 1.2, 3.7, 12, 37, 117, 373 and 1165 mg/kg sediment dry weight at pH 7.5 – 8.1, 15±2 ºC in three replicates for the test and control groups. The measured concentrations for the 117, 373 and 1165 mg/kg sediment dry weight levels were 124, 329 and 981 mg/kg dry weight sediment, respectively. The dissolved oxygen ranged 7.0 – 9.0 mg/L (106% to 84% recovery). Sacrificial analytical replicates for Day 10 analysis for 117, 373 and 1165 mg/kg test concentrations were used. Food was not provided during the test. A total of 30 animals were used per treatment.

The mortality of the test animals during the holding period (in aerated static seawater of 36‰ salinity and at 15 ± 2 ºC) was estimated to be <10% and no symptoms of disease or abnormal behaviour were observed.

Analysis of the mortality data for determination of LC50: A computer programmed moving average angle method. A method of one way analysis of variance was used to determine any statistically significant differences between the test and control groups.

RESULTS

LC50	>1200 (95% CI 800 – 3700, nominal) mg/kg dry weight sediment at 10 day
NOEC	373 mg/kg dry weight at 10 days (nominal)
Remarks - Results	There was 3.33% mortality in the control treatment. Between 3.3% and 13.3% mortality occurred at nominal concentrations of 1.2 – 373 mg/kg dry weight sediment. Statistical analysis indicated no statistically significant difference compared to the control.

Based on the test results, the notified chemical is considered very slightly toxic to seawater sediment species according to Mensink (1995) for the acute toxicity classification for earthworms.

CONCLUSION The notified chemical is very slightly toxic to seawater sediment species.

TEST FACILITY AstraZeneca (2010)

BIBLIOGRAPHY

AstraZeneca (2010) NExBTL – Renewable Diesel: Determination of Acute Toxicity to *Corophium volutator* in a Sediment System (Study No. 09-0095/B, April, 2010). Brixham, UK, AstraZeneca UK Ltd (Unpublished report submitted by the notifier).

Harlan (2009a) NExBTL – Renewable Diesel: Determination of General Physico-Chemical Properties (Project No. 2106/0031, October, 2009). Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2009b) NExBTL – Renewable Diesel: Determination of Hazardous Physico-Chemical Properties (Project No. 2106/0032, November, 2009). Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2009c) NExBTL Renewable Diesel: Oral (Gavage) Two Generation Reproduction Study in the Rat with Evaluation of Subchronic Toxicity (Project No. 2106/0025, December, 2009). Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Mensink BJWG, Montforts M, Wijkhuizen-Maslankiewicz L, Tibosch H & Linders JBHJ (1995) Manual for summarising and evaluating the environmental aspects of pesticides. Bilthoven, The Netherlands, National Institute of Public Health and Environmental Protection, Report No. 679101022, Appendix 5, <http://www.rivm.nl/bibliotheek/rapporten/679101022.html>. Accessed on 11/07/2012.

NOHSC (2004) Approved Criteria for Classifying Hazardous Substances, 3rd edition [NOHSC:1008(2004)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

NTC (National Transport Commission) 2007 Australian Code for the Transport of Dangerous Goods by Road and Rail (ADG code), 7th Edition, Commonwealth of Australia.

Phycher (2008) NExBTL – Renewable Diesel: Skin Sensitisation in the Guinea Pig – Magnusson and Kilgman Maximisation Method (Study No. SMK-PH-07/0460-C1, May, 2008). Cestas, France, Phycher Bio Développement (Unpublished report submitted by the notifier).

Safe Work Australia (2012) Guidance on the Classification of Hazardous Chemicals Under the WHS Regulations.

SafePharm (2005a) Acute Oral Toxicity of NExBTL Biodiesel in the Rat – Acute Toxic Class Method (Project No. 2106/002, July, 2005). Derbyshire, UK, Safepharm Laboratories Ltd (Unpublished report submitted by the notifier).

SafePharm (2005b) NExBTL Biodiesel: Reverse Mutation Assay "Ames Test" Using *Salmonella typhimurium* (Project No. 2106/003, July, 2005). Derbyshire, UK, Safepharm Laboratories Ltd (Unpublished report submitted by the notifier).

Safepharm (2005c) NExBTL – Renewable Diesel: Acute Toxicity to *Daphnia magna* (Project No. 2106/004, July, 2005). Derbyshire, UK, Safepharm Laboratories Ltd (Unpublished report submitted by the notifier).

Safepharm (2005d) NExBTL – Renewable Diesel: Algal Inhibition Test (Project No. 2106/005, July, 2005). Derbyshire, UK, Safepharm Laboratories Ltd (Unpublished report submitted by the notifier).

SafePharm (2006a) NExBTL Biodiesel: Acute Dermal Toxicity (Limit Test) in the Rat (Project No. 2106/0007, October, 2006). Derbyshire, UK, SafePharm Laboratories Ltd (Unpublished report submitted by the notifier).

SafePharm (2006b) NExBTL – Renewable Diesel: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) (Project No. 2106/0009, October, 2006). Derbyshire, UK, SafePharm Laboratories Ltd (Unpublished report submitted by the notifier).

Safepharm (2006c) NExBTL – Renewable Diesel: Assessment of the Inhibitory Effect on the Respiration of Activated Sewage Sludge (Project No. 2106/0010, October, 2006). Derbyshire, UK, Safepharm Laboratories Ltd, (Unpublished report submitted by the notifier).

SafePharm (2007a) NExBTL Biodiesel: Acute Dermal Irritation in the Rabbit (Project No. 2106/0021, August, 2007). Derbyshire, UK, Safepharm Laboratories Ltd (Unpublished report submitted by the notifier).

SafePharm (2007b) NExBTL Biodiesel: Acute Eye Irritation in the Rabbit (Project No. 2106/0022, August, 2007). Derbyshire, UK, Safepharm Laboratories Ltd (Unpublished report submitted by the notifier).

SafePharm (2007c) NExBTL Biodiesel: Chromosome Aberration Test in Human Lymphocytes *in vitro* (Project No. 2106/0008, March, 2007). Derbyshire, UK, Safepharm Laboratories Ltd (Unpublished report submitted by the notifier).

SafePharm (2008a) NExBTL – Renewable Diesel: Determination of Adsorption/Coefficient (Project No. 2106/0029, May, 2008). Derbyshire, UK, SafePharm Laboratories Ltd (Unpublished report submitted by the notifier).

SafePharm (2008b) NExBTL – Renewable Diesel: Local Lymph Node Assay in the Mouse (Project No. 2106/0023, June, 2008). Derbyshire, UK, SafePharm Laboratories Ltd (Unpublished report submitted by the notifier).

SafePharm (2008c) NExBTL – Renewable Diesel: L5178Y TK +/- Mouse Lymphoma Assay (Project No. 2106/0026, August, 2008). Derbyshire, UK, SafePharm Laboratories Ltd (Unpublished report submitted by the notifier).

SafePharm (2008d) NExBTL – Renewable Diesel: Assessment of Ready Biodegradability; CO_2 Evolution Test (Project No. 2106/0028, May, 2008). Derbyshire, UK, SafePharm Laboratories Ltd (Unpublished report submitted by the notifier).

SafePharm (2008e) NExBTL – Renewable Diesel: *Daphnia magna* Reproduction Test (Project No. 2106/0027, August, 2008). Derbyshire, UK, SafePharm Laboratories Ltd (Unpublished report submitted by the notifier).

United Nations (2009) Globally Harmonised System of Classification and Labelling of Chemicals (GHS), 3rd revised edition. United Nations Economic Commission for Europe (UN/ECE), <http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html >.