File No: STD/1111

February 2005

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

ADK Sakuralube S-250

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

For the purposes of subsection 78(1) of the Act, this Full Public Report may be inspected at:

Library
National Occupational Health and Safety Commission
25 Constitution Avenue
CANBERRA ACT 2600
AUSTRALIA

To arrange an appointment contact the Librarian on TEL + 61 2 6279 1161 or + 61 2 6279 1163.

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

Director
Chemicals Notification and Assessment

TABLE OF CONTENTS

FULL PUBLIC REPORT

ADK Sakuralube S-250

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Nissho Iwai Australia Limited (ABN 16 000 213 132)
Level 28
459 Collins Street
Melbourne VIC 3000

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 Formula
Structural Formula
Molecular Weight
Spectral Data
Concentration in imported and finished products
Exact Use
Import Volume

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows:
Hydrolysis as a Function of pH
Partition Coefficient
Adsorption/Desorption
Dissociation Constant
Bioaccumulation

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None.

NOTIFICATION IN OTHER COUNTRIES
Germany – Annex VIIB Notification (2002).

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
ADK Sakuralube S-250

METHODS OF DETECTION AND DETERMINATION

METHOD	Ultraviolet/Visible (UV/Vis), Infrared (IR), Nuclear Magnetic Resonance (NMR) spectroscopy.
Remarks	Reference spectra were provided.
TEST FACILITY	Asahi Denka Kogyo K.K., Japan

3. COMPOSITION

DEGREE OF PURITY
>85%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
Two identified impurities formed a total of approximately 7% (w/w). These impurities may contribute to the observed toxicity profile of the notified chemical.

4. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as a solution at <50% in mineral oil.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 1	1-3	1-3	1-3	3-10

USE
The notified chemical will be imported as a solution in mineral oil, for blending with additives to produce engine oil. This engine oil will be sold for industrial, commercial and consumer use. It will also be re-blended with other oils and additives and repackaged for industrial, commercial and retail sale. The notified chemical will also be imported in blended solutions, for re-blending into final products for industrial, commercial and retail sale.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, transport and storage

PORT OF ENTRY
Sydney.

IDENTITY OF MANUFACTURER/RECIPIENTS
Not to be manufactured in Australia. Identity of recipients not yet finalised; expected to be in Sydney.

TRANSPORTATION AND PACKAGING
Solutions of the notified chemical will be imported and transported to blending facilities in 200 L steel drums. After blending, the oil product is discharged into steel drums, which will be transported to industrial customers and repackaging facilities. Reformulated products for commercial and retail sale will be repackaged into 4 L steel cans and plastic (HDPE) bottles.

5.2. Operation description

Blending Facilities
Workers in blending facilities open the import containers and transfer the contents (<50% solution of the notified chemical in mineral oil) to a mixer. These procedures are usually automated. Mineral or synthetic oil is then added automatically to the mixer tank. Blending occurs at 70˚C. After blending, the oil product (containing less than 5% notified chemical) is automatically discharged into steel drums. It is estimated that this process will take 12 hours, and will occur twice per year.

Re-blending Facilities
Workers in re-blending facilities will open drums containing the blended oil product (less than 5% notified chemical) and transfer the contents to a mixer for blending with other oils and additives such as viscosity modifiers and anti-oxidants. This blending is conducted at 70˚C. After blending, the final product (containing less than 1% notified chemical) is automatically discharged into 4 L steel cans or plastic (HDPE) bottles for commercial and retail sale.

End Use
Occupational end use of blended and re-blended/repackaged products containing less than 5% notified chemical is expected to include car manufacturers and maintenance service providers.

5.3. Occupational exposure

Number and Category of Workers

Category of Worker	*Number*	*Exposure Duration*	*Exposure Frequency*
Transport	1	8 hours/day	2 days/year
Warehouse	1	2 hours/day	6 days/years
Blending facility	2	12 hours/day	2 days/year
Repackaging	2	12 hours/day	2 days/year

Exposure Details

Transport & Warehousing

Exposure during transport or warehousing operations will only occur in the event of accidental spillage involving rupture of import containers.

Blending & Re-blending Facilities

During the blending process, workers should not come into direct contact with the notified chemical under normal operating conditions. Local exhaust systems will minimise inhalation exposure to mists or vapours. Blending is carried out in an automated, closed, non-dispersive system. Incidental exposure to the notified chemical could occur during connection and disconnection of hoses and pumps between the import containers and the mixing vessel. This will be minimised by use of chemical protective clothing, gloves and eye protection, and respiratory equipment where necessary.

Similar exposure patterns are likely during blending and re-blending for commercial and retail sale. Similar systems will be in place for exposure control, including use of closed automated processes, dedicated delivery lines and equipment, local exhaust ventilation, respiratory equipment and chemical protective clothing, gloves and eye protection.

End Use

Exposure to engine oils can be high during addition or replacement, however exposure will be limited by the low concentration (<1%) of notified chemical in blended products. End use workers will typically wear overalls but will not necessarily wear gloves or eye protection.

5.4. Release

RELEASE OF CHEMICAL AT SITE

Environmental release of the notified chemical is unlikely during importation, storage and transportation, and accidental spills and leaks. Catastrophic mechanical failure during a transport accident is the most likely reason for environmental release. Engineering controls (e.g. 200 L steel drum specifications), personnel training and emergency clean-up procedures (i.e. spill response instructions on the Material Safety Data Sheet and label) will limit the impact on the environment of such incidents.

Blending is conducted twice per year using automated, closed, non-dispersive systems. Drips and spills may potentially occur during manual handling (i.e. connection and disconnection of hose/pump lines); however, environmental release is not expected. After blending, the finished oil is automatically discharged into steel drums. Blending tanks are typically cleaned with lube oil, which will be recycled during subsequent blending or collected for incineration at authorised facilities. Less than 0.2% of the notified chemical is estimated to enter waste streams at the blending process. No aqueous wastes are generated during the blending process.

RELEASE OF CHEMICAL FROM USE

The finished lubricants for use in engine oils will be distributed to customers throughout Australia in 200 L drums. No information was available on whether the notified chemical is altered during use as a lubricant in internal combustion engines and therefore it is assumed to be unaffected. There may be some accidental losses (e.g. drips) when lubricant is added to vehicle engines, which may be about every 5,000-10,000 kilometres for passenger car petrol engines. These are expected to be minor spills that which would be mostly left on the ground or cleaned up and disposed of to landfill. The amount disposed of in this way should be less than 1% of the final lubricant. In the closed system of an

engine, there is no expected release of the notified chemical to the environment under normal conditions of use, except for unintended oil leaks, which would mostly drip to road and pavement surfaces. Spills/leaks from engines may potentially comprise 1% of the oil formulation. Since the use of the lubricating oils will occur throughout Australia, any releases from use of oils containing the notified chemical would be diffuse.

5.5. Disposal

Each year, about 581 million litres of lubricating oil is sold in Australia, and about 303 million litres of waste oil is generated. The remainder is consumed during engine operation, unrecoverable or unaccounted for (Meinhardt, 2002). The greatest potential for environmental release of the notified chemical is through disposal of oil product wastes. A survey by the Australian Institute of Petroleum (AIP 1995) indicates that of the annual sales of automotive engine oils in Australia, some 60% 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 (up to 1.4 tonnes of the estimated maximum 10 tonnes of notified chemical imported per annum) is removed by "do it yourself" (DIY) enthusiasts. 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. Meinhardt (2002) estimated that DIY activities account for 7-10% of the unaccounted used oil.

According to a survey tracing the fate of used lubricating oil in Australia (Snow, 1997), only approximately 20% of used oil removed by DIY enthusiasts 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 enthusiasts, the notified chemical could be collected for recycling (≤280 kg/y), buried or disposed of in landfill (≤350 kg/y), disposed of in stormwater drains (≤70 kg/y) and used in treating fence posts, to kill weeds or disposed of in other ways (≤700 kg/y). A proportion of the latter may potentially be disposed of to sewer. Therefore, about 0.7% (up to 70 kg/y) of the total import volume of the notified chemical could potentially enter the aquatic environment via disposal into the stormwater system. In addition to this, considering the unknown fate of some of the oil used by DIY operators, up to 7% (i.e. 50% of 14%; <700 kg/y) may also be sent to the sewer for disposal. 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 material in neat concentrations is very unlikely except as a result of transport accidents.

Spent packaging material and container residues are disposed of to landfill or incinerated. Emptied drums are likely to be cleaned with mineral oil and reconditioned, with oily waste potentially containing 2% of the formulation reused in subsequent batches or concentrated and incinerated. Emptied drums may also be collected for metal recycling. Assuming ~2.0% of the imported formulation remains in emptied drums, an estimated maximum quantity of ≤200 kg/y will be generated as waste by this route based on a total annual import volume of <10 t/y of the notified chemical.

5.6. Public exposure

There is potential for public exposure to the notified chemical from an accidental spillage during transportation. In the event of spillage, public exposure will be minimised if procedures outlined in the MSDS are followed. Specifically, spills should be soaked up with inert material (e.g. sand, silica gel, acid binder, universal binder, sawdust), and collected into tightly sealed and properly labelled containers for incineration or landfill. To further avoid public exposure via the environment, any spills should not be flushed into surface water, sewer or ground water systems.

Approximately 10% of blended and re-blended engine oil products are predicted to be sold to the public. Engine oils containing the notified chemical may be used to replace spent crankcase oil. Where the oil is changed by members of the public the potential for dermal exposure to the oil is high. However, the potential for exposure to the notified chemical is low given its low concentration (< 5%) in end use commercial and retail products, and the fact that engine oil is changed infrequently.

6. PHYSICAL AND CHEMICAL PROPERTIES

All physico-chemical studies were conducted using technical grade notified chemical, purity >90%.

Appearance at 20ºC and 101.3 kPa Dark brown paste.

Pour Point 55 ± 3ºC

METHOD EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks Method: inspection of test jar for signs of flow in the sample.
TEST FACILITY SafePharm Laboratories (2001b)

Boiling Point >360ºC at 101.3 kPa (estimate)

METHOD EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks Differential scanning calorimetry was conducted; however the test material decomposed (before any phase change) from approximately 308˚C, therefore no BP could be determined. BP estimated based on vapour pressure data.
TEST FACILITY SafePharm Laboratories (2001b)

Density 1090 kg/m^3 at 20ºC

METHOD EC Directive 92/69/EEC A.3 Relative Density.
Remarks Pycnometer method.

To enable the test material to be poured into the pycnometer, it was first warmed to approximately 105˚C, then the pycnometer and test material were equilibrated to 20˚C.
TEST FACILITY SafePharm Laboratories (2004)

Vapour Pressure 8.8 x 10^{-8} kPa at 25ºC

METHOD EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks Vapour Pressure balance measurements made at 90-112°C were extrapolated to 25°C. The test material changed to a green paste under the conditions of the test. The notified chemical is very slightly volatile (Mensink et al., 1995).
TEST FACILITY SafePharm Laboratories (2001c)

Water Solubility <2.3 x 10^{-3} g/L at 20ºC

METHOD EC Directive 92/69/EEC A.6 Water Solubility.
Remarks Modified Flask Method
No substance specific analytical method could be developed to quantify the test material dissolved in water. Additionally, it was thought that the test material dissociated in water. Therefore, the water solubility was estimated by visual observation of excess test material in each flask after shaking and standing periods. 2.3x10^{-3} g/L was the limit that could accurately be assessed using visual observation. However, using WSKOWIN software (Syracuse Research Corporation, 1998), water solubility was predicted to be 4.02x10^{-9} mg/L at 25˚C based on estimated chemical structure and the predicted partition co-efficient. The notified chemical is slightly soluble in water (Mensink et al., 1995).
TEST FACILITY SafePharm Laboratories (2001b)

Fat Solubility 210 g/kg HB307 solvent at 37±0.5°C

METHOD OECD TG 116 Fat Solubility of Solid and Liquid Substances.
Remarks Flask Method. Eight test material concentrations, in the range 1.9-8.1 g test material in 25 g fat, were tested. Test flasks were shaken at 37°C for ≤24 hours prior to filtration (0.45 µm) and analysis by Atomic Absorption Spectroscopy (AAS). Preliminary test results indicated that fat solubility was 137 g/L at 37±0.5

°C. Analytical Method: AAS at 313.3 nm after equilibration in standard fat.
TEST FACILITY SafePharm Laboratories (2004)

Hydrolysis as a Function of pH Not determined

Remarks Assessment of hydrolytic stability was not undertaken according to EC Method C7 of Commission Directive 92/69/EEC or OECD TG 111 predominantly due to the low water solubility of the notified chemical. In addition, no substance-specific method of analysis was available that would be capable of monitoring the test material and any possible degradation products separately. Based on chemical structure, hydrolysis is unlikely though the notified chemical may degrade.
TEST FACILITY SafePharm Laboratories (2004)

Partition Coefficient (n-octanol/water) log P_{ow} ≥5.34 at 20±0.5°C (estimated)

METHOD EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks The test material is not suitable for estimation of partition coefficient by HPLC. The shake flask method was also not suitable as the test material possibly dissociates in water and no substance-specific method of analysis could be developed. The partition co-efficient was estimated by visual assessment, using a method based on the shake flask method. Test material was visually assessed to have a solubility in water less than 2.30×10^{-4} % (w/w) at 20±0.5°C and a solubility in n-octanol greater than 50.2% w/w at 20±0.5°C. In support, based on an estimated chemical structure, a log K_{ow} of ~13 was derived using KOWWIN software, version 1.65 (Syracuse Research Corporation, 1999).
TEST FACILITY SafePharm Laboratories (2001b)

Adsorption/Desorption log K_{oc} >3.80 (estimated)

Remarks No testing was possible as the HPLC method was not considered valid for this type of compound. The adsorption coefficient was estimated using Quantitative Structure Activity Relationships (QSAR) for a non-hydrophobic chemical class and the partition co-efficient and using the equation: log K_{oc} = 0.52 log K_{ow} + 1.02.
TEST FACILITY SafePharm Laboratories (2004)

Dissociation Constant Not determined.

METHOD OECD TG 112 Dissociation Constants in Water.
Remarks Not determined using OECD TG 112 as the notified chemical has very low water solubility and, based on its structure, is unlikely to dissociate in water.

Particle Size Not applicable to a paste.

Flash Point 161 ±2ºC at 101.3 kPa

METHOD EC Directive 92/69/EEC A.9 Flash Point.
Remarks Closed cup equilibrium method.
TEST FACILITY SafePharm Laboratories (2001d)

Flammability Limits Not highly flammable.

METHOD EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks The test material did not propagate combustion in the preliminary test, obviating the need to perform the main test.
TEST FACILITY SafePharm Laboratories (2002)

Autoignition Temperature 396±5ºC

METHOD 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).

Remarks	Test conducted by heating the test substance in a flask and observing for any ignition.
TEST FACILITY	SafePharm Laboratories (2003a)

Explosive Properties Not determined.

METHOD	EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks	There are no chemical groups that would imply explosive properties. This is supported by experience in use.
TEST FACILITY	SafePharm Laboratories (2003a)

Oxidising Properties

METHOD	EC Directive 92/69/EEC A.17 Oxidising Properties (Solids).
Remarks	No chemical groups in the notified chemical indicate oxidising properties. This is supported by experience in use.

Reactivity Expected to be stable under normal environmental conditions.

Remarks	The chemical is combustible and will burn if involved in a fire, evolving noxious fumes (eg carbon and nitrogen oxides).

7. TOXICOLOGICAL INVESTIGATIONS

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral	Low toxicity LD50 >2500 mg/kg bw
Rat, acute dermal	Low toxicity LD50 >2000 mg/kg bw
Acute toxicity-inhalation	Data not provided
Rabbit, skin irritation	Severely irritating
Rabbit, eye irritation	Presumed to be severely irritating, based on severe skin irritation results
Guinea pig, skin sensitisation – adjuvant test	Limited evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOEL = 15 mg/kg bw/day
Genotoxicity – bacterial reverse mutation	Non mutagenic
Genotoxicity – in vitro chromosomal aberration	Non genotoxic
Genotoxicity – in vivo mouse micronucleus	Non genotoxic

7.1. Acute toxicity – oral

TEST SUBSTANCE ADK Sakuralube S-250

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

Species/Strain	Rat/Sprague-Dawley CD
Vehicle	Arachis oil BP
Remarks - Method	Test material was warmed at 70˚C in a water bath for suspension.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3/sex	2000	0/6

LD50	>2500 mg/kg bw
Signs of Toxicity	Hunched posture, lethargy, increased salivation and pilo-erection were observed in females up to 2 days after dosing.
Effects in Organs	No abnormalities observed at necropsy.
Remarks - Results	None.

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

TEST FACILITY SafePharm Laboratories (2001e)

7.2. Acute toxicity – dermal

TEST SUBSTANCE ADK Sakuralube S-250

METHOD OECD TG 402 Acute Dermal Toxicity – Limit Test.
EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal) – Limit Test.

Species/Strain	Rat/Sprague-Dawley CD
Vehicle	Arachis oil BP
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations.

RESULTS

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

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	Slight erythema was observed at all treatment sites up to 4 days after treatment. Crust formation was observed at all treatment sites 5 to 8 days after treatment.
Signs of Toxicity - Systemic	No signs of systemic toxicity were observed.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks - Results	None.

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

TEST FACILITY SafePharm Laboratories (2003b)

7.3. Acute toxicity – inhalation

Data not provided

7.4. Irritation – skin

TEST SUBSTANCE ADK Sakuralube S-250

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 males
Vehicle	None
Observation Period	14 days
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	2.7	2.7	2.7	3	7 days**	0
Oedema	2.7	3	2.7	3	7 days**	0

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

Remarks - Results	**7 day scores for erythema and oedema could not accurately be assessed due to adverse reaction (straw coloured scab extending up to 6 cm beyond the treatment sites).

CONCLUSION The notified chemical is severely irritating to the skin.

TEST FACILITY SafePharm Laboratories (2001f)

7.5. Irritation – eye

Remarks	The eye irritation study was not performed because the test material was found to be a severe irritant to rabbit skin. It was assumed that the test material would product severe effects in an eye irritation study. This is in accordance with OECD Testing Guideline 405.

CONCLUSION The notified chemical is presumed to be severely irritating to the eye.

7.6. Skin sensitisation

TEST SUBSTANCE	ADK Sakuralube S-250
METHOD	OECD TG 406 Skin Sensitisation – Magnusson and Kligman test. EC Directive 96/54/EC B.6 Skin Sensitisation – Magnusson and Kligman test.
Species/Strain	Guinea pig/Dunkin Hartley
Vehicle	6% acetone in arachis oil BP
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: Not determined. Lowest dose (1%) caused skin irritation (erythema graded 2 up to 72 hours) topical: 75% after 24 hour exposure. After 48 hour exposure, all doses (25-100%) caused skin irritation (erythema graded 2 up to 24 hours)
MAIN STUDY	
Number of Animals	Test Group: 10 males Control Group: 5 males
INDUCTION PHASE	Induction Concentration: intradermal: 1% topical: Neat
Signs of Irritation	After intradermal induction, discrete or patchy erythema was observed at the induction site in all test and control animals. In addition moderate and confluent erythema was observed in the test animals. After topical induction, moderate and confluent erythema, slight oedema, small superficial scattered scabs and dried blood were observed at topical induction sites of test animals. Discrete or patchy erythema was observed at topical induction sites of control animals.
CHALLENGE PHASE	
1st challenge	topical: 75% and 50%
2nd challenge	No second challenge was conducted.
Remarks - Method	No significant protocol deviations.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st challenge		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	50%	2/10	0/10		
	75%	7/10	0/10		
Control Group	50%	0/5	0/5		
	75%	1/5	0/5		

Remarks - Results	Discrete or patchy erythema was observed at challenge sites in 7/10 test animals and 1/5 control animals at the 75% challenge site; and in 2/10 test animals at the 50% challenge site, 24 hours after challenge. These reactions were not observed 48 hours after challenge. The study authors concluded that the observed effects were not due to sensitisation, based on the lack of persistence.
CONCLUSION	There was limited evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	SafePharm Laboratories (2001g)

7.7. Repeat dose toxicity

TEST SUBSTANCE	ADK Sakuralube S-250

METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/Sprague-Dawley CD
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: None
Vehicle	Arachis oil BP
Remarks - Method	Due to deterioration in physical condition, high dose females were not dosed on days 11 and 12. Due to deterioration in physical condition and concern for survival of animals in both female and male high dose groups, the high dose level was lowered to 500 mg/kg bw/day from day 13.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
I (control)	5/sex	0	0/10
II (low dose)	5/sex	15	0/10
III (mid dose)	5/sex	150	0/10
IV (high dose)	5/sex	750/500	2/10

Mortality and Time to Death

In the high dose group, one male was found dead on day 23, and one female was killed on day 24 due to excessive body weight loss. There were no other deaths during the study.

Clinical Observations

In the high dose groups, increased salivation immediately before and up to five hours after dosing was observed in animals of either sex. Hunched posture was observed from day 7, followed by tiptoe gait and pilo-erection from day 10. Despite a reduction in dose level to 500 mg/kg bw/day from day 13, there was no regression of these clinical symptoms, which continued to be observed in the high dose groups throughout the study period.

High dose animals of either sex also showed a significant drop in body weight gain during the first week. Following the dose reduction at day 13, female high dose animals gained weight comparably to controls; however lower body weight gain and body weight loss persisted in males throughout the study period. These effects were accompanied by reduced dietary intake and reduced food efficiency in the high dose groups, with effects more prominent for males.

During functional testing, a reduction in fore- and hind-limb grip strength was observed for high dose animals of either sex.
High dose males showed an increase in the startle response.

A significant reduction in overall motor activity was observed in high and mid dose males compared to controls, although this did not occur in a dose-dependent manner.

No adverse clinical observations were recorded for any other treatment group.

No treatment-related effects were observed in any other treatment group.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

High dose animals of either sex showed elevated plasma alanine aminotransferase, aspartate aminotransferase, bilirubin and alkaline phosphatase; as well as lowered glucose, total protein and albumin levels. High dose males also showed elevated creatinine and plasma chloride, and lowered potassium levels. Females treated with high dose or 150 mg/kg/day showed higher inorganic phosphate levels.

Lowered haemoglobin, haematocrit, erythrocyte count, mean corpuscular volume, mean corpuscular haemoglobin and lymphocyte count were observed in high dose animals of either sex, as well as elevated

prothrombin clotting time. Statistical significant was not achieved for erythrocyte count in females, mean corpuscular volume in males, mean corpuscular haemoglobin in males and lymphocyte count in males.

Effects in Organs

High dose animals of either sex had higher relative spleen, kidney and adrenal weight, and lower relative thymus weight, although this was statistically significant only for females. High dose females also had higher relative liver weight. Females treated with 150 mg/kg/day had higher relative spleen weight.

High dose males had significantly lower absolute brain and testes weights; however this was considered to be a function of lowered overall body weight, as relative brain weight was significantly higher compared to controls, while relative testes weight was not different to controls.

Macroscopic Findings

The high dose male found dead on day 23 showed normal autolytic post-mortem changes. The high dose female killed at day 24 had a dark pancreas, pale kidneys, raised limiting ridge of the stomach and thickening of the gastric epithelium.

Macroscopic abnormalities at terminal kill were only observed in high dose animals. The majority of high dose animals had pale kidneys; other observations included raised limiting ridge of the stomach, dark areas or discolouration of the stomach, pallor of the liver and reddened lungs. All high dose animals had cage tray lining material in the stomach.

Histopathology

The following treatment-related changes were observed:

Liver: Higher grades of severity of generalised hepatocyte vacuolation, generalised hepatocyte basophilia/degeneration, foamy vacuolation of hepatocytes and hepatocyte karyomegaly were observed in high dose animals of either sex.

Kidneys: Basophilia of cortical tubules was observed in all high dose animals, and in 2 females and 2 males from the 150 mg/kg/day groups.

Duodenum: Mucosal hypertrophy was observed in high dose females; it was also observed in treated males, but without a convincing relationship to treatment.

Jejunum: Foamy vacuolation of lamina propria cells was observed in high dose animals of either sex.

Lungs: Higher incidence and severity of alveolar macrophage accumulations and occasional granulomatous foci were observed in high dose animals of either sex.

Mesenteric lymph nodes: Accumulations of foamy macrophages were observed in all animals treated with the highest dose, or with 150 mg/kg/day.

Thymus: Lymphoid atrophy and/or foam cells were observed in high dose animals of both sexes. Isolated instances of foam cell accumulation was observed among the lower dose groups, but was considered to be unrelated to treatment.

Bone marrow: Adipose infiltration of the bone marrow, indicative of reduced marrow activity, was observed with higher severity in high dose animals of either sex.

Seminal vesicles: All high dose males showed reduced secretory content. This can be a direct effect of treatment but is usually associated with deteriorating general condition.

Remarks – Results

As there were indications of treatment-related changes in several tissues, histopathological examination was extended to include bone marrow, lungs, seminal vesicles, duodenum, jejunum, thymus, kidneys and mesenteric lymph nodes from all animals in the mid and low dose treatment groups.

In the high dose group, microscopic changes identified as adipose infiltration in the bone marrow indicated that haematological findings were likely to be associated with reduced bone marrow activity. Lymphoid atrophy and foam cell accumulation observed in thymus sections may represent a secondary response to deteriorating health, but a direct effect on immune function cannot be ruled out. Blood chemical results and histopathological examination of liver sections may be indicative of liver dysfunction. Microscopic

examination of kidneys, combined with the substantial increase in relative kidney weight, suggested an adverse effect on kidney function.

Elevated adrenal weight in high dose animals, and histopathological abnormalities in seminal vesicles of high dose males, were most probably due to a non-specific stress response associated with the decline in bodyweight and overall condition. Histopathological abnormalities in the lungs were consistent with small amounts of irritant material entering the lungs.

Two males and two females in the mid dose group also showed histopathological evidence of adverse effects on kidney function.

Accumulation of foamy macrophages in the mesenteric lymph nodes of high dose and mid dose animals was probably associated with normal clearance mechanisms effecting removal of test material.

CONCLUSION
The No Observed Effect Level (NOEL) was established as 15 mg/kg bw/day in this study, based on higher relative spleen weight, higher inorganic phosphate levels and adverse histopathological findings in the kidneys and mesenteric lymph nodes of animals treated with 150 mg/kg bw/day.

TEST FACILITY SafePharm Laboratories (2003c)

7.8. Genotoxicity – bacteria

TEST SUBSTANCE ADK Sakuralube S-250

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA$^-$
Metabolic Activation System	Phenobarbital/β-naphthoflavone-induced rat liver S9 fraction
Concentration Range in Main Test	a) With metabolic activation: 0.5-5000 µg/plate b) Without metabolic activation: 0.5-5000 µg/plate
Vehicle	Acetone
Remarks - Method	Plate incorporation method

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	150	150	5000	None
Test 2		150	5000	None
Present				
Test 1	150	150	5000	None
Test 2		150	5000	None

Remarks - Results No toxicity was observed to *E. coli* strain WP2uvrA- either with or without metabolic activation. The vehicle control plates gave counts of revertant colonies within the normal range. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with and without metabolic activation.

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

TEST FACILITY SafePharm Laboratories (2001h)

7.9. Genotoxicity – in vitro

TEST SUBSTANCE: ADK Sakuralube S-250

METHOD: OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.

Cell Type/Cell Line	Human lymphocytes from freshly drawn peripheral blood.
Metabolic Activation System	Phenobarbital/β-naphthoflavone-induced rat liver S9 fraction
Vehicle	Acetone
Remarks - Method	No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Expression Time*	*Harvest Time*
Absent				
Test 1	0*, 0.625, 1.25*, 2.5*, 5*, 10, 20	4 hours	18 hours	24 hours
Test 2	0*, 1.25, 2.5*, 5*, 7.5*, 10, 15	24 hours		24 hours
Present				
Test 1	0*, 0.625, 1.25*, 2.5*, 5*, 10, 20	4 hours	18 hours	24 hours
Test 2	0*, 2.5*, 5*, 10*, 15*, 20, 30	4 hours	18 hours	24 hours

*Cultures selected for metaphase analysis. The maximum dose level selected for metaphase analysis was that which induced approximately 50% mitotic inhibition in each case.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	All doses tested: 0.08-625 μg/mL	All doses tested	Not reported	None observed
Test 2	All doses tested: 0.08-625 μg/mL	All doses tested	Not reported	None observed
Present				
Test 1	All doses tested: 0.08-625 μg/mL	All doses tested	Not reported	None observed
Test 2		All doses tested	Not reported	None observed

Remarks - Results: All vehicle controls had frequencies of cells with aberrations within the range expected for normal human lymphocytes. All the positive control materials induced statistically significant increases in the frequency of cells with aberrations.

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

TEST FACILITY: SafePharm Laboratories (2003d)

7.10. Genotoxicity – in vivo

TEST SUBSTANCE: ADK Sakuralube S-250

METHOD: OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
EC Directive 2000/32/EC B.12 Mutagenicity - Mammalian Erythrocyte Micronucleus Test.

Species/Strain	Mouse/CD-1
Route of Administration	Intraperitoneal
Vehicle	Arachis oil BP

Remarks - Method	A preliminary range-finding test did not demonstrate any marked differences between the sexes; therefore the main test used only male mice. In the preliminary test, no evidence of toxicity was observed in animals dosed orally with up to 2000 mg/kg bw, therefore systemic absorption could not be confirmed using this route. The high dose for the main study was the maximum tolerated intraperitoneal dose, as determined in the preliminary study. Positive control (cyclophosphamide) was administered orally.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	14 males	0	24 hours (n=7) 48 hours (n=7)
II (low dose)	7 males	75	24 hours
III (mid dose)	7 males	150	24 hours
IV (high dose)	14 males	300	24 hours (n=7) 48 hours (n=7)
V (cyclophosphamide)	5 males	50	24 hours

RESULTS

Doses Producing Toxicity	150 and 300 mg/kg bw
Genotoxic Effects	None observed in any treatment group
Remarks - Results	Significantly lower polychromatic erythrocyte/normochromatic erythrocyte (PCE/NCE) ratios were observed in the 150 and 300 mg/kg groups (although this was not statistically significant at 48 hours), indicating bone marrow toxicity and hence systemic absorption. The positive control group showed a marked increase in the incidence of micronucleated PCEs compared to concurrent vehicle control groups.

CONCLUSION The notified chemical was not genotoxic under the conditions of the test.

TEST FACILITY SafePharm Laboratories (2003e)

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical.
METHOD	OECD Guideline 301B Ready Biodegradability; CO2 Evolution Test referenced as Method C.4-C of Commission Directive 92/69/EEC and USEPA Fate, Transport and Transformation Test Guidelines OPPT 835.3110 (m).
Inoculum	Mixed population of activated sewage sludge micro-organisms from Severn Trent Water plc sewage treatment plant, UK, which treats predominantly domestic sewage.
Exposure Period	29 days
Auxiliary Solvent	None
Analytical Monitoring	Inorganic carbon, CO_2
Remarks - Method	An initial test involving exposure of activated sewage sludge micro-organisms to a concentration of test material equivalent to 16.8 mg/L resulted in inhibition of CO_2 evolution in the test material vessels after 28 days. Therefore a lower concentration (8.4 mg/L or 5 mg C/L) was used in the definitive test. Test containers consisted of 5 L glass culture vessels with 3 L of test solution. Test solutions were tested in duplicate and consisted of a toxicity control with inoculated culture medium; a positive control (sodium benzoate; 17.1 mg/L) with inoculated culture medium (10 mg C/L); the test material (8.4 mg/L) with inoculated medium; and test material (8.4 mg/L) plus positive control (17.1 mg/L) with inoculated medium (15 mg C/L) to act as a toxicity control. To reduce solids content, sludge was washed 3 times by settlement and resuspension in culture medium to remove excessive DOC and a subsample was removed and the suspended solids (SS) concentration determined. Test containers were inoculated with 31 mL of inoculum, equivalent to 5 mg C/L or 30 mg SS/L. The test was conducted at 21°C in darkness. Test solution pH range was 7.9-7.9. Degradation of the test material was assessed by analysis of CO_2 production after collection of CO_2 in Dreschel bottles containing 0.05 M NaOH.

RESULTS

Test substance (8.4 mg/L)		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
1	0	1	0
3	8	3	39
8	8	8	70
14	14	14	78
20	21	20	86
27	23	27	89
28	26	28	96
29	31	29	97

Remarks - Results	The toxicity control (63% degradation after 28 days) and positive control (96% degradation after 28 days) satisfied validation criteria. The test material was not toxic to the microbes at the concentration tested (8.4 mg/L).
CONCLUSION	Only 26% biodegradation occurred after 28 days incubation indicating that the test substance is not readily biodegradable.
TEST FACILITY	SafePharm Laboratories (2001j)

8.1.2. Bioaccumulation

Not determined.

Remarks

The notified chemical has an estimated log K_{ow} of >5.34 at 20°C (potentially log K_{ow} ~13), and has a relatively high affinity to lipids. The moderate molecular weight of the notified chemical suggests it may be capable of crossing biological membranes. However, aquatic release of the notified chemical is likely to be limited based on its use pattern.

8.2. Ecotoxicological investigations

8.2.1. Acute toxicity to fish

TEST SUBSTANCE

Notified chemical.

METHOD

OECD TG 203 Fish Acute Toxicity Test referenced as Method C1 of Commission Directive 92/69/EEC – Semi-static conditions.

Species	Rainbow trout (*Oncorhynchus mykiss*); 1.33 g and 46 mm length.
Exposure Period	96 hours
Auxiliary Solvent	Tetrahydrofuran
Water Hardness	~100 mg/L (as $CaCO_3$)
Analytical Monitoring	Analysis was by voltametry after UV digestion and complexation using differential pulse mode and a dropping mercury electrode. Samples were analysed at 0, 24 and 96 hours.
Remarks – Method	Range-finding and definitive tests were performed. The notified chemical was dissolved in tetrahydrofuran at 10 mg/mL, diluted in dechlorinated tap water, filtered (0.2 µm) to remove undissolved test material and dispersed into a test vessel at 1.0 mg/mL. Based on preliminary studies, test concentrations >1.0 mg/L produced a precipitate. Test aquaria were monitored after 3 h, 6 h and daily for mortality and sublethal effects. 16 h light: 8 h dark. Test aquaria were aerated and test solution was renewed daily. Fish were not fed during the test. Test temperature 14.1-14.4°C. Dissolved oxygen >9.0 mg/L. pH range 7.8-8.1 (acceptable to protocol).

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality(%)*				
Nominal	*Actual**		*1h*	*24h*	*48h*	*72h*	*96h*
1.0	1.23	20 (2 replicates of 10)					0

*Limit of quantitation (LOQ) = 0.039 mg/L.

LC50	>1.0 mg/L at 96 hours
NOEC	1.0 mg/L at 96 hours.
Remarks – Results	No mortality or sublethal effects were noted in the solvent control. Analytical recoveries had acceptable levels of recoveries of test material in test media. Stability of the test material during the tests was evaluated; however, stability of the parent compound could not be determined and the results are reported as the nominal concentration. The test solution remained clear and colourless during the test. No mortality or sublethal effects were recorded at the highest test concentration, equivalent to the limit of solubility of the test substance in the test solution, including initial solvent dissolution above which marked precipitation occurred. Water solubility of <2.3 mg/L had previously been determined (SafePharm Laboratories, 2001b).

CONCLUSION

The notified chemical was not toxic to Rainbow Trout up to the limit of its water solubility.

TEST FACILITY SafePharm Laboratories (2003f)

8.2.2a. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical.

METHOD OECD TG 202: Daphnia sp. Acute Immobilisation Test - Static Test
Species *Daphnia magna* (<24 hours old, 1st instars)
Exposure Period 48 hours
Auxiliary Solvent Tetrahydrofuran
Water Hardness ~250 mg/L (as $CaCO_3$)
Analytical Monitoring Analysis by absorptive stripping voltametry (ASV) after UV digestion and complexation.
Remarks - Method Both range-finding and definitive tests were conducted. Aliquots of stock solutions of the notified chemical in tetrahydrofuran were separately dispersed in reconstituted water prior to adjusting the volume to 2 L to give the nominal test concentrations. Test aquaria consisted of 250 mL glass beakers containing ~250 mL of test solution. A control and solvent control (100 µL/L of tetrahydrofuran) were also tested. Immobilisation was recorded daily. Test temperature (21°C), DO (8.1-8.5 mg/L) and pH (7.9-8.0) were monitored daily and were acceptable. Illumination was for 16 hours/day. The 24 h EC50 value and confidence limits were calculated using the trimmed Spearman-Karber method and the 48 h EC50 value and confidence limits was calculated using the maximum-likelihood probit method using ToxCalc software.

RESULTS

Concentration mg/L			*Number of Daphnids*	*Percent (%)*	*Mortality*
Nominal	*Actual (unfiltered)*			*48 h*	
	t0	*t48 h*			
Control	<LOQ*		20 (2 replicates x 10 animals)		0
Solvent Control	<LOQ		"		0
0.010	0.00834	0.00761	"		0
0.018	ND		"		0
0.032	0.0283	0.0235	"		0
0.056	ND		"		0
0.10	0.0996	0.0899	"		15
0.18	ND		"		90
0.32	0.281	0.271	"		100
0.56	ND		"		100
1.0	0.970	0.898	"		100

*Limit of quantitation (LOQ) = 5 µg/L. ND = not determined.

LC50 0.33 mg/L (nominal) at 24 hours (95% CI 0.29-0.37).
0.13 mg/L (nominal) at 48 hours (95% CI 0.11-0.15).
NOEC 0.18 mg/L (nominal)
0.056 mg/L (nominal)
Remarks - Results As actual concentrations approximated nominal concentrations, the toxicity values are based on nominal concentrations. The test solutions were clear and colourless during the tests. Stability of the test material during the tests was evaluated; however, stability of the parent compound could not be determined.

CONCLUSION Very toxic (LC50 <1 mg/L) to Daphnia (United Nations, 2003).

TEST FACILITY SafePharm Laboratories (2001i)

8.2.2b. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 211 *Daphnia magna* Reproductive Test, referenced as Method C.20 of Commission Directive 2001/59/EC – Semi-static Test
Species	*Daphnia magna* (<24 h old 1st instar)
Exposure Period	21 days
Auxiliary Solvent	Tetrahydrofuran
Water Hardness	220-258 mg/L as $CaCO_3$
Analytical Monitoring	Analysis by absorptive stripping voltametry (ASV) after UV digestion and complexation. Test concentrations were analysed each 2-3 days during the test.
Remarks - Method	Stock solution was prepared by dissolving test material (250 mg) in tetrahydrofuran and adjusting the volume to 25 mL to give a concentration of 250 mg/25 mL. An aliquot (1.0 mL) was dispersed in 10 L reconstituted water and stirred (200 rpm for 10 mins), then filtered (0.2 µm) to give a nominal concentration of 1.0 mg/L. An aliquot (100 mL) was further diluted in a final volume of 1 L to give a final stock concentration of 0.1 mg/L. Aliquots (26 mL and 84 mL) of the 0.1 mg/L stock solution and aliquots (26, 84 and 260 mL) of the 1.0 mg/L stock solution were each separately dispersed in a final volume of 2 L to give the nominal test concentrations. No test material losses occurred during filtration. Water renewal was 3 times per week. Test chambers were not aerated during the test and were covered with plastic lids. Daphnids in test chambers were monitored daily for mortality. Daphnids were fed algal suspension during the test. Test temperature 21°C, pH 7.9-8.1, DO ≥8.0 mg/L. 16 h illumination (470-531 lux) (acceptable to protocol). The solvent control group was exposed to 26 µL/L of tetrahydrofuran. EC50 was determined using trimmed Spearman-Karber method using ToxCalc software version 5.0.23C. Initially test solutions were clear and colourless; however, 48-72 hour old test media was green tinged due to the addition of algal suspension as food for the daphnids.

Results

Concentration mg/L		*Number of D. magna*	*% Survival*	*Number of Live Young*
Nominal	*Actual**		*of adults*	*per female (cumulative)*
Control	-	10 (10 replicates of 1 daphnid)	100	115
Solvent control	-	"	100	121
0.0013	<LOQ	"	100	117
0.0042	Not determined	"	100	117
0.013	0.0166	"	100	118
0.042	Not determined	"	100	121
0.13	0.159	"	0	0

*Limit of quantitation (LOQ) 0.0039 mg/L.

EC50 (adult immobilisation)	0.074 mg/L at 21 days (95% CI 0.042-0.13 mg/L; nominal)
EC50 (reproduction)	0.074 mg/L at 21 days (95% CI 0.042-0.13 mg/L; nominal)
LOEC	0.13 mg/L at 21 days
NOEC	0.042 mg/L at 21 days
Remarks- Results	Stability of the test material during the tests was evaluated; however, stability of the parent compound could not be determined. Control and solvent results were acceptable. Adult mortality occurred mostly at the highest test concentration of 0.13 mg/L, with 50, 75 and 100% mortality at days 6, 7, and 13 indicating a prolonged toxic effect. In the highest test concentration only, surviving daphnids were markedly paler and smaller than control daphnids. No mortalities occurred in the 4 lowest test concentrations. Discarding the highest test concentration data, adult

	fecundity and daphnid length in other test concentrations was not significantly different from the solvent control.
CONCLUSION	Very toxic (LC50 <1 mg/L) to Daphnia (Mensink et al., 1995).
TEST FACILITY	SafePharm Laboratories (2003i).

8.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test referenced as Method C.3 of Commission Directive 92/69/EEC.
Species	*Scenedesmus subspicatus* Green Algae
Exposure Period	72 hours
Concentration Range	Nominal: 1.0 mg/L Actual: 0.948 mg/L
Auxiliary Solvent	Tetrahydrofuran
Water Hardness	Not determined
Analytical Monitoring	The concentration of notified chemical was tested at 0 and 72 h. Analysis was by ASV after UV digestion and complexation using differential pulse mode and a dropping mercury electrode.
Remarks - Method	Algae were exposed to a single test material concentration of 1.0 mg/L (6 replicates) in 250 mL flasks containing 100 mL under continuous illumination (7000 lux) and temperature 24±1°C. The test concentration was prepared by dissolving test material (100 mg) in tetrahydrofuran. The volume was adjusted to 10 mL to give a concentration of 100 mg/10 mL solvent stock solution. An aliquot (500 µL) of the solvent stock was dispersed in culture medium (5 L) to give the test concentration (1.0 mg/L). The test concentration was filtered (0.2 µm) to remove undissolved test material and dispersed into a test vessel inoculated with 10 mL of concentrated algal solution. The test solution and solvent control (tetrahydrofuran) contained 100 µL/L. Cell density was determined at 72 h using a Coulter counter. Initial cell density was ~104 cell/mL. Final cell density was >105 cell/mL. Test solution pH range 7.8-8.1.

RESULTS

Biomass		*Growth*
EbL50 at 96 h (mg/L)	*96 h NOEC*	*ErL50 at 96 h (mg/L)*
>1.0	*1.0 mg/L*	*>1.0 mg/L*

Remarks - Results	Cell cultures in the control and solvent control increased by factors of 37 and 42 respectively, meeting the test validation requirements. No effects on growth and biomass were recorded at the highest test concentration, equivalent to the limit of solubility of the test substance in the test solution, including initial solvent dissolution above which marked precipitation occurred. SafePharm Laboratories (2001b) had previously determined water solubility to be <2.3 mg/L. Actual test substance concentrations were 89 – 123 % of nominal and therefore toxicity values are reported using nominal concentrations.
CONCLUSION	The test substance was not toxic up to the limit of its water solubility.
TEST FACILITY	SafePharm Laboratories (2003g)

8.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical.

METHOD OECD TG 209 Activated Sludge, Respiration Inhibition Test and Commission Directive 87/302/EEC and US EPA Draft Ecological Effects Test Guidelines OPPTS 850.6800.

Inoculum A mixed population of activated sewage sludge micro-organisms from the aeration stage of the Severn Trent Water plc sewage treatment plant, which predominantly treats domestic sewage.

Exposure Period 3 hours

Auxiliary solvent Tetrahydrofuran

Concentration Range Nominal: 1000 mg/L

Remarks – Method Range-finding (10, 100 and 1000 mg/L) and definitive tests were performed. The definitive test included one test concentration of 1000 mg/L (3 replicate vessels). An amount of test material (500 mg) was added to water and subjected to ultrasonication (~30 mins), then synthetic sewage (16 mL), activated sewage sludge (200 mL) and water were added to a final volume of 500 mL and test concentration of 1000 mg/L. The solvent control contained 100 µL/L of tetrahydrofuran. A reference material (3,5-dichlorophenol) was also tested at 3.2 and 32 mg/L in the range-finding test to confirm the suitability of the inoculum.

RESULTS

	Nominal Concentration (mg/L)	*% Inhibition at 3 h*
Control	0	-
	0	-
Test substance	1000	6
	1000	4
	1000	4
3,5-dichlorophenol	3.2	19
	10	52
	32	87

EC50 >1000 mg/L in 3 hours

NOEC 1000 mg/L in 3 hours

Remarks – Results The test container had a slight oily slick and test material was stuck to the sides of the container at 0, 0.5 and 3 h as much of the test material was not dissolved at the concentration tested. Validation criteria for the control respiration rate and reference material EC50 (9.2 mg/L) were met.

CONCLUSION The test substance was only slightly inhibitory to microbial respiration at a concentration of 1000 mg/L after 3 hours. However, during a ready biodegradability test (SafePharm Laboratories, 2001d), the test material inhibited CO_2 evolution at a concentration equivalent to 16.8 mg/L after 28 days; however, no inhibition was detected at a test concentration of 8.4 mg/L. Reasons for the apparent discrepancy between the studies are not known but it may be due to acute versus chronic exposure conditions.

TEST FACILITY SafePharm Laboratories (2003h)

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

The notified chemical has a very low water solubility estimated at <2.3 x 10^{-3} g/L and potentially as low as 4.02 x 10^{-12} g/L at 25°C. With log K_{oc} >3.80, the notified chemical is expected to partition with organic matter, suspended particulates and accumulate in sediments and soils in the environment. In soils, the notified chemical is expected to be immobile. It has a low vapour pressure of 8.8 x 10^{-8} kPa and an estimated Henry's Law Constant of ~2.0 x 10^{-2} Pa m^3/mole, indicating that volatilisation to air is probably an insignificant migration pathway.

Although the formulation containing the notified chemical is not readily biodegradable over a 28 day test period, it is expected that it would biodegrade over time within a landfill environment. With low water solubility (0.001 g/L), moderate log K_{ow} of 4.64 and log K_{oc} of 4.44-4.56 (estimated), the notified chemical is likely to partition to soil particles and is likely to have low mobility in soil.

Predicted Environmental Concentrations (PEC)

Sewer

Based on recent information on waste oil disposal (Meinhardt, 2002; Snow, 1997), up to 7.7% (up to 700 kg/y) of the notified chemical may be inappropriately disposed of to the sewer by DIY activities. A PEC in the treated effluent, and downstream waterways, has been estimated with a sewage treatment plant (STP) model developed by the Department of the Environment and Heritage (Environment Australia, 2003). The model assumes that the notified chemical is discharged into the sewerage system and none is attenuated or biodegraded within this system. Australia has a population of ~20.1 million people, and an average value for water consumption of 200 L/person/day has been adopted for this national-level assessment (4020 ML/day for total population). Therefore the concentration of notified chemical in the Australian sewage network may be calculated on the basis of a maximum annual volume of ≤10 tpa. The approximate sewerage effluent concentration under these assumptions is 0.5 µg/L (700 x 10^9 µg per year ÷ 365 days/ year ÷ 4020 x 10^6 L/day). Based on dilution factors of 1 and 10 for inland river and ocean outfall discharges of STP-treated effluents, respectively, PECs of the notified chemical in freshwater and marine surface waters may, under these assumptions, approximate 0.5 µg/L ($PEC_{freshwater}$) and 0.05 µg/L (PEC_{marine}), respectively.

Stormwater

Spills/leaks from engines may comprise 1% of the oil formulation. These may enter the soil and stormwater compartments over a diffuse area based on the widespread use pattern. In addition, DIY users may potentially dispose of up to 70 kg/annum of waste oil to stormwater. A worst case may involve all of this estimated quantity (≤170 kg/annum of the notified chemical) being discharged into the stormwater drains in a single metropolitan area with a geographical footprint of 500 square kilometres, and an average annual rainfall of 500 mm. With a maximum annual release into this localised stormwater system of 1.7 x 10^{11} µg/y and the annual volume of water drained from this region estimated to be approximately 250 x 10^9 L, the resultant PEC in the stormwater is approximately ≤1.5 µg/L, with additional dilution, dispersion and sedimentation also likely to occur in the receiving environment.

9.1.2. Environment – effects assessment

Toxicity data were available for 4 taxonomic groups (freshwater fish, cladocerans, algae and sewage sludge microbes). The notified chemical was not toxic to fish or algae above the estimated water solubility (i.e. NOEC of 1.0 mg/L); however, the notified chemical was very toxic to daphnids in both acute and chronic exposures below the estimated water solubility level. A predicted no effect concentration (PNEC) for freshwater organisms for the notified chemical of 4.2 µg/L has been derived by dividing the lowest chronic NOEC value for daphnids (21 d NOEC of 0.042 mg/L) by an assessment factor of 10 to account for interspecies sensitivity. In the absence of marine toxicity data, the $PNEC_{freshwater}$ is tentatively extrapolated to the marine environment, an approach supported by a preliminary review of comparative data by ECETOC (2003).

The notified chemical did not inhibit the growth of sewage sludge microbes at a concentration of >1000 mg/L when exposed for 3 hours. Over a longer period of exposure (28 days), an exposure concentration of 8.4 mg/L was not inhibitory; however, exposure to 16.8 mg/L was inhibitory. Variation in the results is probably due to acute versus chronic exposure regimes.

Eventual degradation of the notified chemical to release simpler compounds is unlikely to pose an unacceptable risk to the environment.

9.1.3. Environment – risk characterisation

Risk quotient (RQ) values, where RQ = PEC/PNEC, for freshwater and marine receiving environments of 0.12 (i.e. 0.5 µg/L ÷ 4.2) and 0.012 (i.e. 0.05 ÷ 4.2), respectively, have been estimated based on a sewer disposal scenario described in 9.1.1 above. The probable degradation of the notified chemical in municipal STPs and the aquatic compartment is expected to reduce the risk to the environment from that estimated.

A risk quotient value (PEC/PNEC) for receiving environments of <0.36 (i.e. 1.5 µg/L ÷ 4.2) has been estimated based on a stormwater release scenario described in 9.1.1; however, the risk quotient is likely to be lower based on the widespread and diffuse use pattern, natural attenuation processes in the environment and release to the terrestrial environment. Therefore, the proposed use of the notified chemical is unlikely to pose an unacceptable risk to aquatic life.

9.2. Human health

9.2.1. Occupational health and safety – exposure assessment

Transport & Storage

Occupational exposure to the notified chemical during transport and storage of imported product containing less than 50% notified chemical is only likely in the event of accidental spills involving breach of import containers. Exposure in these circumstances is expected to be infrequent and acute, and can be limited by use of skin and eye protection, including gloves, goggles and protective clothing, during clean-up operations.

Blending and Re-blending Operations

During blending operations imported solutions of less than 50% notified chemical are diluted to less than 1% for use in oils. During re-blending operations, dilute oil products (less than 5% notified chemical) are further diluted into commercial and retail products. Exposure patterns will be similar for both processes. Intermittent dermal, eye and inhalation exposure is possible during connection and disconnection of transfer equipment, during sealing and labelling of drums, and during equipment cleaning and maintenance. Dermal and ocular exposure due to spills or splashes can be limited by the use of protective clothing, eyewear and gloves. Inhalation exposure to vapours and/or aerosols can be limited by the use of local exhaust ventilation. Exposure during the blending or re-blending process should be minimal as this is conducted in an automated, closed system.

Dermal exposure during blending operations was estimated using the EASE model (HSE, 1994). Assuming non-dispersive use and intermittent direct handling, the estimated dermal exposure during blending is 0-0.1 mg/cm^2/day of imported product containing less than 50% of the notified chemical. This equates to less than 0-0.05 mg/cm^2/day of the notified chemical. Adsorption of the notified chemical is suggested by its high fat solubility and predicted log P_{ow}, however with molecular weight >500 approximately 10% adsorption can be expected. Therefore, for a 70 kg worker with surface area for hands and forearms at 1960 cm^2, and assuming 10% adsorption, systemic exposure is estimated to be 0-0.14 mg/kg bw/day of the notified chemical. This level of exposure would be substantially reduced by the use of protective clothing and gloves.

The estimated atmospheric concentration of notified chemical during formulation, assuming non-dispersive use and direct handling in the absence of aerosol formation, is 0-2.2 mg/m^3 using the EASE model. However, the very low vapour pressure indicates that the notified chemical is essentially non-volatile. At equilibrium the notified chemical would have an atmospheric concentration of 2 x 10^{-7} g/L, therefore negligible inhalation exposure is expected.

Exposure to the notified chemical by all routes will also be limited by the frequency of handling, which is expected to be twice per year.

End Use
Industrial, commercial and retail end users will be exposed to the notified chemical during addition or replacement of engine oil products. Dermal exposure may be extensive, as end users may not wear gloves; there is also the possibility of ocular exposure as end users are unlikely to wear eye protection. However, overall exposure to the notified chemical will be limited by its low concentration (<5%) in end use products. Inhalation exposure is unlikely due to the low vapour pressure of the notified chemical and the unlikely production of mists or aerosols of end use products.

9.2.2. Public health – exposure assessment

Exposure of the public (mainly dermal) can occur during oil changes; however exposure is limited by the low frequency of use, limited contact time and low concentration of notified chemical in retail products.

9.2.3. Human health – effects assessment

The notified chemical is of low acute oral and dermal toxicity in rats. Acute inhalation toxicity data were not provided. The notified chemical has low volatility and is not expected to cause significant adverse effects by inhalation.

The notified chemical is not mutagenic in a bacteriological test, not genotoxic in a mouse micronucleus test, and not clastogenic to human lymphocytes in vitro.

The neat notified chemical is severely irritating to rabbit skin. Eye irritation was not tested because, in view of the results of the skin irritation test, the notified chemical is assumed to be severely irritating to eyes. There was limited evidence of skin sensitisation in an adjuvant study in guinea pigs.

In rats, a 4-week repeat dose oral toxicity study showed the NOEL to be 15 mg/kg bw/day. Higher doses of the notified chemical were shown to cause higher relative spleen weight and adverse histopathological findings in the kidneys and mesenteric lymph nodes (at 150 mg/kg/day), and extreme systemic adverse effects at doses of 500-750 mg/kg bw/day.

Based on the available data, the notified chemical is classified as a hazardous substance in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC 2002) and is assigned the following risk phrases:
R38 – Irritating to skin
R41 – Risk of serious damage to eyes

9.2.4. Occupational health and safety – risk characterisation

Blending & Re-blending
During blending operations, dermal exposure to the notified chemical was estimated to be 0-0.14 mg/kg bw/day of the notified chemical. The margin of exposure (MOE) for chronic toxicity is based on a NOEL of 15 mg/kg bw/day (although it should be noted that only slight effects were seen at a dose of 150 mg/kg bw/day). MOE greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. For dermal exposure, the MOE is calculated as >110. Therefore, the risk of chronic systemic toxicity using modelled worker data is acceptable for blending workers directly handling the notified chemical in up to 50% solution. PPE including protective clothing and gloves will further reduce dermal exposure.

Due to the non-volatility of the notified chemical, inhalation exposure is likely to be negligible, providing aerosols are not produced (unlikely, as blending does not involved high speed mixing). Use of local exhaust ventilation will further reduce exposure.

There may be a risk of serious eye damage and skin irritation to oil blending and packaging workers. There was also limited evidence of a risk of skin sensitisation. Exposure and hence the risk of irritation and sensitisation is most likely during the initial transfer of imported solution

containing up to 50% notified chemical. However, exposure is likely to be low and to occur intermittently only in the case where impervious gloves and goggles are not worn.

Exposure during the rest of the blending and re-blending processes is expected to be low, due to the low concentration of notified chemical in blended products. Therefore the overall occupational risk of skin or eye irritancy is low. There would be a low risk of skin sensitisation as contact should in all cases be intermittent.

End Use
Exposure to the notified chemical could occur during direct manual handling of engine oil products containing the notified chemical. Individuals showing signs of irritation should use gloves and suitable protective clothing.

9.2.5. Public health – risk characterisation

There should be a low risk of skin or eye irritancy or skin sensitisation to the public on exposure through changing engine oil products containing the notified chemical, or handling automotive parts which have been in contact with the oils, given the intermittent and limited nature of exposure and the low concentration (<5%) of the notified chemical in retail products.

10. CONCLUSIONS – ASSESSMENT LEVEL OF CONCERN FOR THE ENVIRONMENT AND HUMANS

10.1. Hazard classification

Based on the available data the notified chemical is classified as hazardous under the NOHSC *Approved Criteria for Classifying Hazardous Substances*. The classification and labelling details are:
R38 – Irritating to skin
R41 – Risk of serious damage to eyes

As a comparison only, the classification of the notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations 2003) is presented below. This system is not mandated in Australia and carries no legal status but is presented for information purposes.

	Hazard category	*Hazard statement*
Skin corrosion/irritation	2	Causes skin irritation
Serious eye damage/eye irritation	2A	Causes serious eye irritation
Chronic hazards to the aquatic environment	1	Very toxic to aquatic life with long lasting effects

10.2. Environmental risk assessment

On the basis of the PEC/PNEC ratio the notified chemical is not considered to pose an unacceptable risk to the environment based on its reported use pattern.

10.3. Human health risk assessment

10.3.1. Occupational health and safety

There is Moderate Concern to occupational health and safety under the conditions of the occupational settings described, based on the risk of skin and eye irritancy and possible skin sensitisation to oil blending workers.

10.3.2. Public health

There is No Significant Concern to public health when used as described.

11. MATERIAL SAFETY DATA SHEET

11.1. Material Safety Data Sheet

The MSDS of the notified chemical provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 2003). It is published here as a matter of public record. The accuracy of the information on the MSDS remains the responsibility of the applicant.

11.2. Label

The label for the notified chemical provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Labelling of Workplace Substances* (NOHSC, 1994). The accuracy of the information on the label remains the responsibility of the applicant.

12. RECOMMENDATIONS

REGULATORY CONTROLS
Hazard Classification and Labelling

- The NOHSC Chemicals Standards Sub-committee should consider the following health hazard classification for the notified chemical:
  - R38 Irritating to skin
  - R41 Risk of serious damage to eyes

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - ≥5% R36 Irritating to eyes
  - 10-20% R41 Risk of serious damage to eyes
  - ≥20% R38 Irritating to skin
    R41 Risk of serious damage to eyes

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical during blending and repackaging operations:
  - Local exhaust ventilation at all potential release sites

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical during blending and repackaging operations:
  - Impermeable gloves, protective clothing, chemical resistant goggles

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

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

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances*, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Emergency procedures

- Spills/release of the notified chemical should be handled as specified in the MSDS (wear eye protection, chemical resistant gloves, protective clothing and respiratory equipment as required; absorb spillages with inert material, transfer to labelled containers for disposal; avoid release to drains or ground water systems; clean spillage area with water and detergent).
- Spills/release of lubricants containing the notified chemical should not be released to waterways or sewer. Do not contaminate groundwater. Spills/leaks should be contained

by applying absorbent materials (e.g. sand, soil, diatomaceous earth) to the spill, or pumping of spilled material, into labelled containers. Clean the spillage area with water and detergent. Dispose of in a manner consistent with local jurisdiction waste management regulations by incineration.

Disposal

- Wastes containing the notified chemical should be disposed of in a manner consistent with local jurisdiction waste management regulations by incineration.

12.1. Secondary notification

The Director of Chemicals Notification and Assessment must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(2) of the Act:
- if any of the circumstances listed in the subsection arise.

The Director will then decide whether secondary notification is required.

No additional secondary notification conditions are stipulated.

13. BIBLIOGRAPHY

AIP (1995) AIP survey of used oil. Australian Institute of Petroleum Ltd.

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals). (2003). Aquatic Hazard Assessment II, Technical Report No. 91. Brussels, November 2003.

Environment Australia (2003) Model and Guidance for Estimating Predicted Environmental Concentrations to Surface Water and Soil From Chemicals Released to the Environment Through a Sewage Treatment Plant.

Meinhardt (2002) Used oil in Australia. Prepared by Meinhardt Infrastructure & Environment Group for the Australian Government Department of the Environment and Heritage, Canberra.

Mensink, B. J. W. G., Montforts, M., Wijkhuizen-Maslankiewicz, L., Tibosch, H. and Linders, J. B. H. J (1995). Manual for Summarising and Evaluating the Environmental Aspects of Pesticides. National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands. Report no. 679101022.

NOHSC (1994) National Code of Practice for the Labelling of Workplace Substances [NOHSC:2012(1994)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

NOHSC (2002) Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2002)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

NOHSC (2003) National Code of Practice for the Preparation of Material Safety Data Sheets, 2nd edn [NOHSC:2011(2003)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

SafePharm Laboratories (2001a) S-250: Statement of Non Performance of Rabbit Eye Irritation Test, Project Number 744/108. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2001b) S-250: Determination of General Physico-Chemical Properties, Project Number 744/103. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2001c) S-250: Determination of Vapour Pressure, Project Number 744/104. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2001d) S-250: Determination of Flash Point, Project Number 744/105. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2001e) S-250: Acute Oral Toxicity in the Rat – Acute Toxic Class Method, Project Number 744/106. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2001f) S-250: Acute Dermal Irritation in the Rabbit, Project Number 744/107. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2001g) S-250: Skin Sensitisation in the Guinea Pig – Magnusson and Kligman Maximisation Method, Project Number 744/109. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2001h) S-250: Reverse Mutation Assay "Ames Test" Using *Salmonella typhimurium* and *Escherichia coli*, Project Number 744/110. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2001i) S-250: Acute Toxicity to *Daphnia magna*, Project Number 744/111. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2001j) S-250: Assessment of Ready Biodegradability; CO2 Evolution Test, Project Number 744/112. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2002) S-250: Determination of Flammability (Solids), Project Number 744/132. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2003a) ADK Sakuralube S-250: Determination of Hazardous Physico-Chemical Properties, Project Number 744/153. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2003b) ADK Sakuralube S-250: Acute Dermal Toxicity (Limit Test) in the Rat, Project Number 744/154. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2003c) ADK Sakuralube S-250: Twenty-Eight Day Repeated Dose Oral (Gavage) Toxicity Study in the Rat, Project Number 744/155. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2003d) ADK Sakuralube S-250: Chromosome Aberration Test in Human Lymphocytes *In Vitro*, Project Number 744/156. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2003e) ADK Sakuralube S-250: Micronucleus Test in the Mouse, Project Number 744/157. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2003f) ADK Sakuralube S-250: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*), Project Number 744/158. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2003g) ADK Sakuralube S-250: Algal Inhibition Test, Project Number 744/159. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2003h) ADK Sakuralube S-250: Assessment of the Inhibitory Effect on the Respiration of Activated Sewage Sludge, Project Number 744/160. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2003i) ADK Sakuralube S-250: *Daphnia magna* Reproduction Test, Project Number 744/161. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

SafePharm Laboratories (2004) ADK Sakuralube S-250: Determination of General Physico-Chemical Properties, Project Number 744/152. SafePharm Laboratories Limited, Shardlow, Derbyshire, UK (Unpublished report provided by the notifier)

Snow R (1997) Used Oil Management. Paper presented at the Used Oil Management Conference, Brisbane, August 1997, Queensland Department of the Environment.

Syracuse Research Corporation. (1998) WSKOWIN software (version 1.33).

Syracuse Research Corporation (1999) KOWWIN software (version 1.65).

United Nations (2003) Globally Harmonised System of Classification and Labelling of Chemicals (GHS). United Nations Economic Commission for Europe (UN/ECE), New York and Geneva.