File No: EX/154 (STD/1281)

October 2010

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Z-76

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 Sustainability, Environment, Water, Population and Communities.

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

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

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NICNAS

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FULL PUBLIC REPORT

This assessment report is for an extension of the original assessment certificate for Z-76. Based on the submission of new information by the extension notifier, some sections of the original assessment report have been modified. These modifications have been made under the heading 'Extension Application' in the respective sections.

Z-76

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Lubrizol International, Inc. (ABN 52 073 495 603)
28 River Street
Silverwater, NSW 2128

Applicant for an Extension of the Original Assessment Certificate:
Southern Cross Oil Pty Ltd (ABN 43 121 686 253)
1/121 Fairbairn Road, Sunshine, VIC 3020

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, Other names, CAS number, Molecular formula, Structural formula, Molecular weight, Spectral data, Methods of detection and determination, Purity, Impurities, Additives/Adjuvants, Introduction volume, Details of use, and Identity of recipients.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows:
Dissociation constant, Particle size, and Flammability limits.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
The notified chemical is currently being notified globally by Lubrizol Inc.

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Z-76

Extension Application
Mobil 1 Synthetic ATF (product containing the notified chemical at <1% w/w)

MOLECULAR WEIGHT
> 400 Da

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

3. COMPOSITION

DEGREE OF PURITY
>80%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Clear amber liquid

Property	Value	Data Source/Justification
Pour Point	-8.15°C	Measured
Boiling Point	400 ± 0.5°C at 101.72 kPa	Measured
Density	882 kg/m^3 at 20°C	Measured
Vapour Pressure	5.7 x 10^{-10} kPa at 25°C	Measured
Water Solubility	1.19 x 10^{-4} -7.0 x 10^{-10} g/L at 25°C	Estimated
Hydrolysis as a Function of pH	Significant hydrolysis is unlikely to occur in the environmental pH range of 4-9.	Estimated based on its structure and the predicted low water solubility.
Partition Coefficient (n-octanol/water)	log Pow = 3.86-9.24 at pH 3.3	Measured
Adsorption/Desorption	log K_{oc} = 3.99-6.64	Estimated
Dissociation Constant	Expected to be ionised throughout the environmental pH range of 4-9.	Estimated based on its structure
Surface tension	Not determined	Based on its low water solubility
Particle Size	Not determined	The notified chemical is a liquid.
Flash Point	136 ± 2°C at 101.52 kPa	Measured
Flammability Linit	NA Not determined	The notified chemical is a liquid.
Autoignition Temperature	344 ± 5°C	Measured
Explosive Properties	Not explosive	Estimated based on chemical structure
Oxidising Properties	Not oxidising	Estimated based on chemical structure

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

Reactivity
Stable under normal conditions of use.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. It will initially be imported as a component of finished automotive transmission fluid (ATF) at concentrations of <1%. It is possible that additive concentrate containing 1-10% of the notified chemical will be imported for further formulation in Australia.

Extension Application
The notified chemical will not be manufactured or reformulated in Australia. It will be imported into Australia as a component of finished automotive transmission fluid (ATF) at concentrations of <1%.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	0-5	0-5	0-5	0-5	0-5
Extension Application					
Year	*1*	*2*	*3*	*4*	*5*
Tonnes	0-1	0-1	0-1	0-1	0-1

PORT OF ENTRY
Fremantle, Brisbane, Melbourne and Sydney

IDENTITY OF RECIPIENTS
Lubrizol International Inc. (Silverwater, NSW)

Extension Application
Southern Cross Oil Pty Ltd. (Sunshine, VIC)

TRANSPORTATION AND PACKAGING
The product containing the notified chemical will be imported in isotainers or 250 L drums. These will be then

transported by truck or rail to end users or repackaging/formulation facility. The packaging size of the finished ATF product will be 250 L drums and 1 L plastic bottles.

Extension Application
The finished ATF containing <1% of the notified chemical will be imported in 208 L drums and 20 L packs. No repackaging of the ATF containing <1% of the notified chemical will occur in Australia.

USE
The notified chemical is a friction modifier for use in the lubricant additive industry, primarily as automatic transmission fluids (ATF).

OPERATION DESCRIPTION
Formulation/blending
If blending occurs in the future, the additive concentrate containing 1 to 10% notified chemical will be transferred from storage to a blending area via forklifts and decanted from drums into blending tanks via either an automated pumping process or hand pumps. The blending process will be fully automated/enclosed and under local exhaust ventilation. The end products will be packaged automatically into containers ranging from drums for bulk shipment to large customers, such as original equipment manufacturers (OEMs), to smaller size plastic containers for aftermarket, garages or do-it-yourself (DIY) sales. Similar materials and products are blended in the same equipment, therefore, any residual material left in the blend tank or transfer lines remains for the next batch. Sampling will be conducted during the blending process. The equipment will be cleaned using mineral oil which will be recycled.

End use – bulk use
Initially, all ATF containing <1% notified chemical will be used by automobile manufacturers for factory fill operations (OEM use). The end product will be pumped from drums directly to the transmission through dedicated lines which will be an automated and enclosed process.

End use – non-bulk use
Initially, no ATF containing <1% notified chemical will be used for garages and do-it-yourself (DIY) use. During the possible future use (estimated to be minority), the end product will first be repackaged into smaller containers. The repackaging process will be automated, in which the end product will be pumped directly from the bulk container to the smaller bottles (plastic, 1 L).

Although some transmissions require the fluid to be replaced during servicing, many are now sealed units which are filled for the life of the transmission. These are expected to be serviced only by professional mechanics and often do not require replacement of ATF for the life of the transmission. When the replacement of ATF or 'topping off' the fluid level occurs, it is expected to be a manual process.

Extension application
The product containing the notified chemical will not be blended/repackaged in Australia and is intended for industrial and general consumer use. The end use will not differ from that of the original notifier.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number per site*	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	2-3	1-3	4-6
Blending	1-2	1-3	10-20
Packaging	2-3	2-5	10-20
Repacking	1	1-3	10-20
End use	1-3	2-4	Variable

EXPOSURE DETAILS

Transport and storage
Exposure to the notified chemical (at a concentration of up to 10%) will be unlikely to occur, except in the event of an accidental spillage and breach of packaging.

Formulation
During formulation of the notified chemical into finished ATF products, worker exposure to the additive concentrate (maximum concentration of 10%) will be limited during transfer and mixing due to the enclosed and automated process. Workers may be exposed to the notified chemical (concentration up 1%) by skin and inhalation or ocular contact during sampling and equipment cleaning. However, exposure should be minimised by use of mechanical ventilation systems, automated processes and personal protective equipment (PPE).

End uses
Worker exposure during bulk end use is expected to be limited due to the enclosed and automated processes and use of mechanical ventilation systems and PPE.

Workers may be exposed to the notified chemical during non-bulk use of ATF containing <1% of the notified chemical. The estimated dermal exposure is 1.8 mg/day, based on the EASE model using following inputs: direct handling, intermittent contact, non-dispersive use, and assuming an average exposed surface areas of 1800 cm^2 for forearms and hands. Therefore, for a 70 kg worker and a 100% dermal absorption factor, systemic dermal exposure is estimated to be 0.026 mg/kg bw/day for using ATF containing <1% of the notified chemical. Ocular and inhalation exposure may also occur, however, the extent is expected to be low due to short exposure duration, infrequent uses and low volatility of the notified chemical (vapour pressure of 5.7 x 10^{-10} kPa at 25^{o}C).

Extension application
No formulation and repackaging of the notified chemical will occur in Australia. Exposure to the finished product containing <1% of the notified chemical is only expected to occur during end use applications.

6.1.2. Public exposure
Public exposure to the finished ATF product containing <1% notified chemical via skin, optical, inhalation and potentially ingestion will be likely due to the manual application of the DIY products and the unlikely use of PPE. However, the public exposure is expected to be limited due to its infrequent use, assuming that most consumers do not change their own transmission fluid and this is an activity that would occur infrequently during the lifetime of the transmission.

Extension Application
Public exposure to the finished product containing <1% notified chemical will be similar to the original application.

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	severely irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – local lymph node assay	evidence of strong sensitisation
Rat, repeat dose oral toxicity – 28 days	NOAEL = 25 mg/kg bw/day
Bacterial reverse mutation assay	non mutagenic
Chromosome aberration test – in vitro	non clastogenic

Toxicokinetics
No data on toxicokinetics, metabolism and distribution were available. The notified chemical is not expected to be readily bioavailable, as it is hydrophobic (log Pow = 3.86-9.24) and has a relatively high molecular weight (>400 Da). It has the potential to be absorbed across biological membranes, but the absorption of significant amounts of notified chemical is unlikely.

No evidence of dermal absorption was observed in the acute dermal toxicity study, however, dermal absorption would be necessary for the strong sensitisation reactions seen in the LLNA study (see below).

Given the systemic toxicity of the notified chemical in the repeated oral toxicity study, it is expected to be absorbed after an oral dose. Due to its surface-active nature, absorption is most likely to occur from the intestine, through micellar uptake into the lymphatic system. This conclusion is supported by the findings of the repeat dose oral toxicity study, where mucosal hypertrophy and vacuolation of the intestine and sinus histiocytosis of mesenteric lymph nodes and vacuolation of histiocytes were observed. The effect on the lymph nodes could be caused by the strong sensitisation nature of the notified chemical acting on the lymphatic tissues of the mesentery.

Given the hydrophobic nature of the notified chemical, any absorbed chemical is not expected to distribute significantly throughout the body; rather it would be predisposed to be bound to cellular membranes and to distribute into adipose tissue (EC, 2003). If exposure were continuous, the potential for bioconcentration in these regions could result in more severe effects than would be expected for the given exposure level. Following an oral exposure to the notified chemical, a wider distribution may be possible, as micelles of the notified chemical may be carried to the general circulation through lacteals and the thoracic duct.

Acute toxicity

The notified chemical showed no lethality in acute oral and dermal toxicity studies. No signs of systemic toxicity were observed during the study. However, signs of dermal irritation similar to the dermal irritation study (see below) were noted in the acute dermal toxicity study.

Irritation and sensitisation

The skin irritation study after 4 hours exposure showed well-defined erythema and slight oedema in all animals, which was recovered by Day 14. In addition, light brown discolouration of the epidermis with loss of skin elasticity, crust formation, and slight desquamation were observed in test animals at the 72-hour, 7-day, and 14-day observation, respectively. Similar skin reactions were also found in this study after 1 hour exposure and in the acute dermal study after 24 hours exposure. However, these effects were not observed after 3-minute exposure. Whilst the crust formation observed may indicate full thickness destruction of skin tissue, the lack of corrosive effects in the eye irritation study and the repeated dose oral study (see below) does not support the classification of Corrosive. The severity of the effect meets the hazard classification criteria (the Criteria) under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) as a skin irritant.

Slight eye irritation (minimal to moderate conjunctival irritations) was observed in all test animals, which was recovered by Day 7. No effects were found in the cornea and iris. The severity of the effect does not meet the Criteria for classification.

A LLNA test yielded high Stimulation Index (SI, 12.36, 20.98, and 29.13 for test concentrations of 2.5, 5 and 10%, respectively). According to Schneider and Akkan (2004), an Effective Concentration inducing a SI of >3 (EC_3) was calculated to be 0.7%, indicating that the notified chemical is a strong skin sensitiser. Analysis of the notified chemical against known structural alerts for skin sensitisers (Barratt *et al*, 1994) showed that it was homologous to one of the known sensitisers.

There is no evidence to exclude the possibility that the notified chemical may induce respiratory sensitisation after repeated inhalation exposure.

Repeated dose toxicity

A 28-day repeated dose toxicity study displayed systemic effects at 1000 mg/kg bw/day. These effects include increased salvation, decreased body weight gain and increased water consumption, haematological (indication of inflammation and anaemia) and clinical chemistry disturbances, and histological changes (mainly mucosal hypertrophy and vacuolation of the mucosal lamina propria in the duodenum, jejunum and ileum and sinus histiocytosis of the mesenteric lymph nodes and vacuolation of histiocytes). The histopathological changes and changes in water consumption and some of the haematological and clinical chemistry disturbances persisted in the recovery group. Although these effects were described as less severe at the 150 mg/kg bw/day dose level, they were still observed at this dose level.

The notified chemical is a moderate/severe skin irritant and strong skin sensitiser, and therefore has the potential to cause local effects which may or may not be reversible. The fact that the irritation/sensitization effects seen with this chemical are severe/strong tends to suggest that the effects may not be readily reversible, and therefore

could lead to longer term adverse effects. It is not known as to whether the effects observed in the intestine and in the mesenteric lymph nodes, which are likely to be a consequence of the irritant/sensitization properties of the chemical, are serious enough to lead to longer term health effects on prolonged exposure or whether the effects are an adaptive physiological response which is reversible. The study examined animals at the high dose level for 14 days after treatment. In the intestine, the mucosal hypertrophy was not observed but the vacuolation of the lamina propria cells was still apparent, although reduced. In the mesenteric lymph nodes, both the sinus histiocytosis and the vacuolation of histiocytes were both still present but reduced in severity. The reversibility of these changes at 150 mg/kg bw/day was not examined, however, given their reduced incidence and severity at this dose level, it is reasonable to assume that improvements, but possibly not absence of the effects, would be observed.

The morphological changes which were observed in the intestine particularly are unlikely to resolve quickly given the nature of irritation/sensitivity reactions and the results of the reversibility study, particularly in situations of repeated or prolonged exposure. Whether the observed changes, if not given the opportunity to recover, will lead to further damage to the intestine is difficult to predict and a longer term study may be required to address this uncertainty. The nature and properties of this chemical as evidenced from the results of the toxicological studies indicate that prolonged oral exposure may cause serious damage to health and, as such, the hazard classification R48/22 is warranted. The No Observed Adverse Effect Level (NOAEL) was established as 25 mg/kg bw/day in this study, based on absence of adverse health effect at this dose level.

The notified chemical did not induce mutations in bacterial test and failed to induce significant chromosomal aberrations in mammalian cells in vitro. These results suggest that the notified chemical is not likely to be mutagenic to humans.

Classification

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 2004). The following risk and safety phrases apply to the chemical:
R38 Irritating to skin (cut-off for classification ≥ 20%)
R43 May cause sensitisation by skin contact (cut-off for classification ≥ 1%)
R48/22 Harmful: danger of serious damage to health by prolonged exposure if swallowed (cut-off for classification ≥ 10%)

6.3. Human health risk characterisation

6.3.1. Occupational health and safety

Considering the intended use of the notified chemical the main routes of exposure for all types of workers are dermal and ocular. The major health concerns are skin irritation and sensitisation as well as systemic effects.

Risk of skin sensitisation

During formulation of the finished ATF products and before mixing, workers handling the notified chemical may be exposed to maximum concentration of 10% in the additive concentrate. Considering the hazardous nature of the notified chemical there is a risk of skin sensitisation for these workers. However, the risk is not considered to be high as handling of the concentrate will be limited to enclosed transferring process into a mixing tank. Following the enclosed mixing process, workers involved in the formulation and bulk end use processes will only handle product containing <1% of the notified chemical. The risk of skin sensitisation is expected to be low due to the enclosed and automated system. In addition, the risk of skin sensitisation will be further reduced by employment of safe work practices and the appropriate use of personal protective equipment (PPE) including appropriate aprons and gloves.

Non-bulk end use of the formulated products, namely addition or changing of gear oils, may result in frequent exposure to a range of products containing <1% of notified chemical. Although the concentration of the notified chemical is low, considering the skin sensitising potency of the notified chemical and the likely dermal exposure, the risk for skin sensitisation cannot be excluded, especially for highly sensitive individuals. Employers should implement all necessary control measures to minimise dermal exposure including carrying a warning with regard to the skin sensitising potency on the MSDS for the additive concentrate.

Risk of skin irritation
The risk of skin irritation is expected to be low for workers involved in formulation of products containing the notified chemical and bulk- end uses due to the enclosed/automated processes, low concentration of the notified chemical and use of appropriate PPE, such as skin protection.
Although the non-bulk end use may result in frequent exposure to the ATF product containing the notified chemical, the risk of irritation is considered low, as the concentration of the notified chemical is <1%.

Risk of systemic effects
Although ingestion is not a likely route of exposure at the occupational settings and low dermal absorption of the notified chemical is expected, the systemic effects observed in the repeated oral animal study cannot be completely ruled out following repeated dermal exposure to the notified chemical. However, the risk of the systemic effects for workers involving in the formulation and bulk end uses is expected to be low due to limited dermal exposure from the enclosed/automated processes and use of appropriate PPE use.

Based on a NOAEL of 25 mg/kg bw/day, derived from a 28-day rat oral study and the reasonable worst-case worker exposure estimation during non-bulk end uses, the margin of exposure (MOE) is calculated as 833. MOEs greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. Therefore, the risk of systemic effects based on the modelled data is acceptable for non-bulk end use workers who handle ATF products containing <1% of the notified chemical.

Other potential risks
The risk of eye irritation will be low for workers due to the low concentration of the notified chemical and the enclosed/automated processes in the majority of the uses.

Risk of respiratory sensitisation cannot be excluded due to the strong potency of the skin sensitisation. However, based on the very low vapour pressure, worker exposure via inhalation will be limited. Therefore, the risk is not expected to be high.

Overall, the main risk for workers handling products containing the notified chemical is related to skin sensitisation and irritation. Appropriate control measures should be in place to minimise dermal exposure.

Extension Application
As there will not be any reformulation and repackaging, workers may only be exposed to the product containing the notified chemical at <1% during end use applications, as compared to the original application.

6.3.2. Public health
ATF products containing <1% may be available to the public for DIY manual application in the future. During this process dermal and ocular exposure is likely, especially considering that members of the public are likely not to use PPE. Although the concentration of the notified chemical is low (<1%), considering the skin sensitising potency of the notified chemical and the likely dermal exposure, the risk for skin sensitisation cannot be excluded, especially for highly sensitive individuals. Advice to consumers with regard to the skin sensitisation potential needs to be highlighted on the label. The risk of skin and eye irritation as well as systemic effects is expected to be low due to its infrequent use and low concentration of the notified chemical.

Extension Application
Considering the low concentration (<1%) of the notified chemical in the finished product and its infrequent use by public, the risk is not considered to be unacceptable, similar to the original application.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE
If reformulation of ATF occurs in the future, it is expected that this process will be highly automated with minimal release of the chemical. The residue in packing containers would be rinsed with oil for charging to the next batch. Similarly rinses from cleaning of equipment would be charged to the next batch.

RELEASE OF CHEMICAL FROM USE

Approximately 0.5% (<25 kg per annum) of the notified chemical is expected to remain in import containers as residue after repacking or filling of transmissions with the product.

Assuming that 25% (<1250 kg per annum) is used in top up applications and that 1% remains in these containers then a further maximum of 7.5 kg per annum will remain as residue in the repackaged product.

It is expected that only a small amount (<5%; <250 kg) of the ATF products would be released to the environment either from incorrect disposal from DIY enthusiasts and leaks from transmissions. This is likely to occur throughout Australia in a disperse manner.

Although some transmissions require the fluid to be replaced during servicing, many are now sealed units which are filled for the life of the transmission. These are expected to be serviced only by professional mechanics and often do not require replacement of the ATF for the life of the transmission. The ATF are expected to be collected either at the end of the useful life of the transmissions; or if required during servicing, and properly disposed of.

RELEASE OF CHEMICAL FROM DISPOSAL

Residues in import drums are expected to be cleaned out by licensed drum recyclers and properly disposed of (most likely by incineration).

Residue from the repackaged product is likely to be disposed of as domestic waste and deposited to authorised landfill.

Used ATF may be recycled, re-refined, burnt as low grade burner fuel or disposed of by incineration.

Transmissions which may not be fully drained of the product comprising the notified chemical are likely to be disposed of to landfill or undergo metal recycling at the end of their useful lives.

7.1.2 Environmental fate

For the details of the environmental fate studies please refer to Appendix C.

The notified chemical will share the same fate as the ATF in which it is blended. It is expected that 0.5% residue will remain in the "empty packaging" for import containers and it is estimated that 1% will remain in small packaging. Small packaging is likely to be sent to landfill, whilst larger operations are likely to send empty containers to licensed drum recyclers. It is expected that at most 25 kg of the notified chemical will be disposed of to landfill from small packaging. Although the notified chemical is not readily biodegradable, it is expected that it will degrade through a range of biotic and abiotic processes in landfill.

The ATF are expected to be collected either at the end of the useful life of the transmissions or if required during servicing, and properly disposed of. Used ATF may be recycled, re-refined, burnt as low grade burner fuel or disposed of by incineration. Automatic transmissions containing residual amounts of the notified chemical are expected to be disposed of to landfill or enter metal recycling.

The notified chemical is expected to be decomposed during re-refining to simpler organic molecules and completely combusted to oxides of carbon and nitrogen, and water vapour if burnt.

7.1.3 Predicted Environmental Concentration (PEC)

The calculation of a PEC has not been undertaken as the proposed use pattern of the notified chemical in ATF will lead to little aquatic exposure.

7.2. Environmental effects assessment

Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	LL50 >100 mg/L	The notified chemical is not toxic to Rainbow trout up to the limit of its water solubility.

Daphnia Toxicity	EL50 230 mg/L	The notified chemical exerts a toxic effect on aquatic invertebrates below the level of its water solubility.
Algal Toxicity	E_bL50 9.6 mg/L E_yL50 10 mg/L E_rL50 16 mg/L	WAFs of the notified chemical are toxic to algae. Noting that the analysis of the WAFs indicated that the components were present in extremely low concentrations (which were not quantifiable), the components of the WAFs are highly toxic to algae.
Inhibition of Bacterial Respiration	EC50 >1000 mg/L	The test substance is not considered inhibitory to sewage sludge up to the concentration tested.
Other		

7.2.1 Predicted No-Effect Concentration

A predicted no effect concentration (PNEC – aquatic ecosystems) of <160 µg/L has been derived by dividing the end point value of 16 mg/L by a worst-case scenario uncertainty (safety) factor of 100 (as usable toxicity data are available for three trophic levels).

7.3. Environmental risk assessment

Although a Predicted Environmental Concentration (PEC) and hence risk quotient (RQ) cannot be calculated, the RQ is expected to be low as the exposure of the notified chemical to the aquatic environment is expected to be minimal. Furthermore, due to the notified chemical's low water solubility, it is unlikely that the entire amount of chemical entering the aquatic environment will be available to aquatic species. The notified chemical therefore is unlikely to pose an unacceptable risk to the environment.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

Based on the available data the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)]. The classification and labelling details are:

R38 Irritating to the skin
R43 May cause sensitisation by skin contact
R48/22 Harmful: danger of serious damage to health by prolonged exposure if swallowed

and

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 irritation	2	Causes skin irritation
Skin sensitisation	1	May cause an allergic reaction
Specific target organ system toxicity – Repeated exposure	2	May cause damage to organs through prolonged or repeated exposure
Acute hazards to the aquatic environment	2	Toxic to aquatic life
Chronic hazards to the aquatic environment	2	Toxic 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 unacceptable risk to the health of workers, provided that the notified chemical is handled in such a way as to

minimise any potential exposure. Good working practices should be followed and appropriate personal protective equipment should be used where exposure might occur during handling.

When used in the proposed manner, the notified chemical is not considered to pose an unacceptable risk to the health of general public. However, the risk of a sensitisation response in exposed individuals cannot be ruled out.

Environmental risk assessment
The chemical is not considered to pose a risk to the environment based on its reported use pattern.

Risk assessment and recommendations relating to Extension Application
The use and the fate of the notified chemical will not change under the proposed extension. The increase in proposed introduction volume is not expected to significantly change the environment and health impacts. Therefore, there are no changes required in the existing risk assessment and recommendations.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- The Office of the ASCC, Department of Employment and Workplace Relations (DEWR), should consider the following health hazard classification for the notified chemical:
  - R38 Irritating to the skin
  - R43 May cause sensitisation by skin contact
  - R48/22 Harmful: danger of serious damage to health by prolonged exposure if swallowed

  - S24 Avoid contact with skin
  - S27 take off immediately all contaminated clothing
  - S28 After contact with skin, wash immediately with plenty of water
  - S36 Wear suitable protective clothing
  - S37 Wear suitable gloves

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - Conc ≥ 1% R43
  - Conc ≥ 10% R48/22
  - Conc ≥ 20% R38

- The National Drugs and Poisons Scheduling Committee (NDPSC) should consider the notified chemical for listing on the SUSDP.

Health Surveillance

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

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following isolation and engineering controls to minimise occupational exposure to the notified chemical during formulation for in finished products:
  - *Prevent leaks and spills*
  - *Wherever possible, direct handling of the notified chemical should be avoided; rather, some remote handling apparatus should be used.*

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical during formulation and use of finished products:
  - *Avoid contact with skin, eyes and clothing.*
  - *Avoid breathing mists.*
  - *A shower station should be available.*
  - *Avoid spills and splashing during use.*
  - *After exposure, any contaminated PPE should be thoroughly cleaned before re-use.*

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical during formulation and use of finished products:
  - *Chemical resistant gloves*
  - *Chemical resistant clothing which protects the body, arms, legs and feet*

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

- Only workers with sufficient education on the hazards of the notified chemical should handle it in any concentrated form, such as the imported product.

- 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.

Public Health

- The following measures should be taken by notifier to minimise public exposure to the notified chemical:
  - Products available to the public should contain the following warning statement:
    - Wear gloves when using
    - May cause allergic skin reaction

Environment

- The notified chemical should be disposed of by re-refining or authorised incineration.

- Spills or accidental release of the notified chemical should be handled by physical containment such as diking, whilst preventing entry into waterways and sewers. Collect free liquid for reuse to the extent practicable and dispose of the remainder. Residual liquid may be absorbed onto inert material (vermiculite, sand etc.) and collected for disposal.

Regulatory Obligations

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

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

(1) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from an additive in automatic transmission fluids, or is likely to change significantly;
  - the amount of chemical being introduced has increased from up to 5 tonnes, or is likely to increase, significantly;
  - if the chemical has begun to be manufactured in Australia;
  - additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

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

Material Safety Data Sheet
The MSDS of the notified chemical and products containing the notified chemical provided by the notifier were reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

Extension Application
The extension applicant has provided an MSDS for a product containing the notified chemical. The accuracy of the information on the MSDS remains the responsibility of the extension applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Pour Point -8.15 ºC

Method OECD TG 102 Melting Point/Melting Range.
EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Test Facility Safepharm Laboratories (2006a)

Boiling Point >400 ± 0.5 ºC at 101.72 kPa

Method OECD TG 103 Boiling Point.
EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks Differential scanning calorimetry (DSC) was used.
Test Facility Safepharm Laboratories (2006a)

Density 882 kg/m^3 at 20ºC

Method OECD TG 109 Density of Liquids and Solids.
EC Directive 92/69/EEC A.3 Relative Density.
Remarks Pycnometer method was used.
Test Facility Safepharm Laboratories (2006a)

Vapour Pressure 5.7 x 10^{-10} kPa at 25ºC

Method OECD TG 104 Vapour Pressure.
EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks A vapour pressure balance system was used, at 145-155°C.
Test Facility Safepharm Laboratories (2006b)

Water Solubility 1.19 x 10^{-4} -7.0 x 10^{-10} g/L at 25ºC

Method Estimated using Water NT™ Version 1.00/1.01, ©US EPA 2002
Remarks Water solubility trials with the notified chemical, according to OECD TG 105, were attempted. However, excess test material was inseparable from the test solutions even after filtration and centrifugation. Therefore, the water solubility was estimated using computer based estimation software. As the notified chemical consists of a mixture of compounds an estimate of the water solubility was made for the component with the smallest molecular weight and one toward the upper limit of the molecular weight range. The modelling was conducted for the neutral molecules, and protonation of the notified chemical's basic functionality would be expected to result in increased water solubility.
Test Facility Safepharm Laboratories (2006a)

Hydrolysis as a Function of pH Not Determined

Remarks Not determined due to the predicted low water solubility. The notified chemical does not contain functional groups which would be expected to undergo hydrolysis within the environmental pH range (4-9).

Partition Coefficient (n-octanol/water) log Pow = 3.86-9.24 at pH 3.3

Method OECD TG 117 Partition Coefficient (n-octanol/water).
EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks The partition coefficient was determined using the HPLC Method. The study was conducted under acidic pH (3.3) in order to ensure adequate ionisation for LCMS detection. It was considered that the basic conditions (pH 10-12) required to ensure all the notified chemical was present as the neutral base were outside the environmental pH range (and would have made LCMS analysis almost impossible). While pH of 3.3 is also outside the environmental pH range, it was chosen to allow LCMS detection and prevent interaction of the test material with the column. The range of results reflects the mixture

of components in the notified chemical.
Test Facility Safepharm Laboratories (2006a)

Adsorption/Desorption log K_{oc} = 3.99-6.64

Method Estimated using PCKOWIN version 1.66, ©US EPA 2002
Remarks Due to the predicted low solubility of the components in the notified chemical the adsorption behaviour was estimated using computer based estimation software. As the notified chemical consists of a mixture of compounds an estimate of the water solubility was made for the component with the smallest molecular weight and one toward the upper limit of the molecular weight range. The modelling was conducted for the neutral molecules, and protonation of the notified chemical's basic functionality would be expected to result in an increase in water solubility which would normally result in decreased adsorption of the notified chemical. However, protonation would increase the adsorption to clays and minerals.
Test Facility Safepharm Laboratories (2006a)

Dissociation Constant Not Determined

Remarks The notified chemical contains functional groups which are expected to display typical basicity with a pKa ~9-10. Thus, the notified chemical would be ionised throughout the environmental pH range of 4-9.

Flash Point 136 ± 2 °C at 101.52 kPa

Method EC Directive 92/69/EEC A.9 Flash Point.
Remarks Closed cup equilibrium method was used.
Test Facility Safepharm Laboratories (2006b)

Autoignition Temperature 344 ± 5°C

Method EC Directive 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).
Test Facility Safepharm Laboratories (2006b)

Explosive Properties Not explosive

Method EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks Estimated based on the chemical structure.
Test Facility Safepharm Laboratories (2006b)

Oxidizing Properties Not oxidising

Method EC Directive 92/69/EEC A.21 Oxidizing Properties (Liquids).
Remarks Estimated based on the chemical structure.
Test Facility Safepharm Laboratories (2006b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method. EC Directive 2004/73/EC B.1 *tris* Acute Toxicity (Oral).
Species/Strain	Rat/ Sprague Dawley CD
Vehicle	None
Remarks - Method	No significant deviation from the protocol.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity	No signs of systemic toxicity.
Effects in Organs	No abnormalities were noted following terminal necropsy on Day 14.
Remarks - Results	All animals survived the study and showed expected gain in body weight during the 14-day observation period.

CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Safepharm (2006c)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/ Sprague Dawley CD
Vehicle	None
Exposure duration	24 hours
Type of dressing	Semi-occlusive
Remarks - Method	No significant deviation from the protocol.

RESULTS

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

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	No erythema or oedema was observed at all treatment sites. Desquamation was noted at all treatment sites on the third day after dosing. Crust formation was observed at the treatment sites of 2 males and all female animals from Day 4, which lasted untill Day 6 to Day 10 after dosing. Small superficial scattered scabs were also noted in 3 females from Day 6 to Day 9.
Signs of Toxicity - Systemic	No signs of systemic toxicity were observed during the study.
Effects in Organs	No abnormalities were noted following terminal necropsy on Day 14.
Remarks - Results	All animals survived the study and showed expected gain in body weight during the 14-day observation period.

CONCLUSION	The notified chemical of low toxicity via the dermal route.
TEST FACILITY	Safepharm (2006d)

B.3. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 2004/73/EC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 Males for 4-hour exposure, 1 male each for 1-hour and 3-minutes exposure
Vehicle	None
Exposure Duration	4 hours, 1 hour, and 3 minutes
Observation Period	14 days (for 1-hour and 4-hour exposure)
Type of Dressing	Semi-occlusive
Remarks - Method	No significant deviation from the protocol.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	2	2	2	2	>14 days	0
Oedema	1.33	1.33	1	2	<7 days	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal (after 4 hours exposure).

Remarks - Results

4-hour exposure
Well-defined erythema was noted in 2 test animals 1 hour after patch removal and in all animals at the 24, 48 and 72-hour observations. Slight erythema was noted in one animal on Day 7. No erythema was observed by Day 14.

Slight oedema was noted in 2 animals at 1 and 24 hours after patch removal. Very slight oedema was observed in one animal 24 hours after patch removal and in all animals up to 72-hour observation. No oedema was observed by Day 7.

Light brown discolouration of the epidermis and loss of skin elasticity were each observed in 2 animals at the 72-hour observation. Crust formation and slight desquamation were noted in all animals at the 7-day and 14-day observation, respectively.

1-hour exposure (in one animal)
Well-defined erythema and very slight oedema were noted at the 24, 48 and 72-hour observation. Light brown discolouration of the epidermis was noted at the 72-hour observation and moderate desquamation noted at the 7-day observation.

3-minute exposure (in one animal)
No evidence of skin irritation or corrosion was noted.

CONCLUSION	The notified chemical is severely irritating to the skin.
TEST FACILITY	Safepharm (2006e)

B.4. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Directive 2004/73/EC B.5 Acute Toxicity (Eye Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 Males
Observation Period	72 hours (7 days for one animal)
Remarks - Method	No significant deviation from the protocol. A Rabbit Enucleated Eye Test (REET) was conducted prior to the eye irritation study, which indicated that the notified chemical was unlikely to cause severe ocular irritancy.

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	1.67	1	0.67	2	<7 d	0
Conjunctiva: chemosis	0.67	0.33	0.33	1	<48 h	0
Conjunctiva: discharge	0	0	0.67	1	<72 h	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

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

Remarks - Results	Minimal to moderate conjunctival irritation was noted in all treated eyes one hour after treatment and at the 24 and 48-hour observations, except in one treated eye at the 72-hour observation. No effects to the cornea or iris were observed in the study.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	Safepharm (2006f)

B.5 Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 429 Skin Sensitisation - Local lymph node assay EC Directive 2004/73/EC B.42 Skin Sensitisation - Local lymph node assay.
Species/Strain	Mouse CBA/CaBkl (Females)
Vehicle	Butanone
Remarks - Method	No significant deviation from the protocol. A preliminary screening test was conducted using the test material at concentrations of 10%, 25% and 50% in butanone. Based on the result, the test material at concentrations of 2.5%, 5% and 10% in butanone was selected for the main test.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	737.97 (± 275.87)	n/a
2.5	9122.17 (± 6080.77)	12.36
5	15482.17 (± 3697.67)	20.98
10	21497.93 (± 5086.54)	29.13
Positive Control (α-Hexylcinnamaldehyde, Tech 85%)		
5	Not documented	3.08
10	Not documented	4.54
25	Not documented	8.06

Remarks - Results

Preliminary screening test
The animal treated with 50% test material was killed on Day 3 due to the approach of the moderate severity limit (hunched posture and moderate redness to ears, head and neck). Bodyweight loss of 2 g was noted in the animal treated with 25% of the test material. No signs of systemic toxicity were observed in the animal treated with 10% of the test material.

Main test
No deaths or signs of systemic toxicity were observed in the main study.

An Effective Concentration inducing a SI of >3 (EC_3) was then calculated to be 0.7% indicating a strong sensitising property (Schneider and Akkan, 2004).

CONCLUSION

There was evidence of induction of a lymphocyte proliferative response indicative of strong skin sensitisation to the notified chemical.

TEST FACILITY

Safepharm (2006g)

B.6. Repeat dose toxicity

TEST SUBSTANCE

Notified chemical

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
Sprague-Dawley Crl:CD (SD) IGS BR

Route of Administration
Oral – gavage

Exposure Information
Total exposure days: 28 days
Dose regimen: 7 days per week
Post-exposure observation period: 14 days

Vehicle
Arachis oil BP

Remarks - Method
No significant deviation from the protocol. A dose range-finding study was initially performed with 6 animals (3/sex) using control and two test doses, 500 and 1000 mg/kg bw/day for 14 consecutive days.

In the main study, two recovery groups were included for control and high dose groups, using 5 male and 5 female animals per group. Recovery treatment-free period was 14 days after which all animals were subject to gross necropsy examination and histopathological evaluation similar to the non-recovery treatment groups.

RESULTS

Dose range-finding study

Increased salivation and staining around the mouth were observed during the study. No adverse effects on body weight or findings upon necropsy were reported in the test report.

Main study

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5/sex	0	0
low dose	5/sex	25	0
mid dose	5/sex	150	0
high dose	5/sex	1000	0
control recovery	5/sex	0	0
high dose recovery	5/sex	1000	0

Mortality and Time to Death

No mortality occurred during the study.

Clinical Observations

Increased salivation with associated red/brown staining around mouth was observed up to 1 hour after dosing in either sex treated with 1000 mg/kg bw/day from Day 3. Isolated incidents of this observation were also found at 150 mg/kg bw/day during the study. These clinical signs disappeared following cessation of the treatment.

No treatment related observations were found in behavioural and sensory reactivity assessments, and functional performance tests. Reduced bodyweight gain was found only in 1000 mg/kg bw/day males during Week 1 and Week 4. Slight reductions in dietary intake with or without corresponding disruptions in food efficiency were found in animals of either sex treated with 1000 mg/kg bw/day. Increased water consumption was found in animals of either sex treated with 1000 mg/kg bw/day from Week 3 onwards, with the effect still evident in female recovery group after the treatment-free period.

Laboratory Findings

Haematology

Elevated leucocyte counts, specifically in the neutrophil fraction, were found in animals of either sex treated with 1000 mg/kg bw/day, together with reductions in mean cell haemoglobin concentration. Males treated with 1000 mg/kg bw/day also displayed reduced mean cell volume, with increase in haematocrit counts and red blood cell counts. Furthermore, increases in platelet counts, activated partial thromboplastin time, and clotting times were found in females. Increased platelet counts were also evident in the female recovery group.

Similar effects were found at 150 mg/kg bw/day. An increase in haemotocrit counts and red blood cells was observed in males. Reductions in mean corpuscular haemoglobin concentration and increase in platelet and neutrophil count were seen in females.

Clinical Chemistry

Animals of either sex treated with 1000 mg/kg bw/day showed increases in aspartate aminotransferase and alanine aminotransferase, with reductions in alkaline phosphatase. Reductions in plasma cholesterol were also evident in animals of either sex treated with 1000 mg/kg bw/day and 150 mg/kg bw/day. In addition, males at 1000 mg/kg bw/day and 150 mg/kg bw/day showed statistically significant decreases in triglyceride and bilirubin levels. The decreased triglyceride levels persisted in the recovery group. Other sex-specific effects at 1000 mg/kg bw/day include increased urea, potassium, and decreased glucose in males and decreased potassium, phosphate and chloride in females. Males receiving 150 mg/kg bw/day also showed an increase in aspartate aminotransferase and alanine aminotransferase, with a reduction in plasma glucose.

Urinalysis

Increased (but not statistically significant) urine volume of reduced specific gravity was found at the end of the treatment period in animals of either sex treated with 1000 mg/kg bw/day.

Effects in Organs

Organ weight
No treatment-related effects were found in organ weight.

Necropsy findings
A fluid-filled duodenum, jejunum and ileum, with dark patches on the ileum was evident in one female treated with 1000 mg/kg bw/day. Small seminal vesicles were found in two males treated with 150 and 1000 mg/kg bw/day and small prostates were found in two males treated with 1000 mg/kg bw/day only.

Histopathology
Mucosal hypertrophy and vacuolation of the mucosal lamina propria were seen in the duodenum, jejunum and ileum in animals of either sex treated with 1000 mg/kg bw/day. The histopathological effects observed in the intestine at 150 mg/kg bw/day occurred at a lower incidence (mucosal hypertrophy was present in 2/5 duodenum, 0/5 ileum, 0/5 jujenum; vacuolation of the lamina propria cells was present in 0/5 duodenum, 0/5 ileum, 2/5 jejunum) and reported to be less severe than those observed at 1000 mg/kg bw/day. Vacuolation of lamina propria cells remained prevalent among the recovery group.

Slight to marked sinus histiocytosis of the mesenteric lymph nodes and vacuolation of histiocytes were observed in animals treated with 1000 mg/kg bw/day. The histopathological effects observed in the mesenteric lymph nodes at 150 mg/kg bw/day occurred at a lower incidence (sinus histocytosis 3 slight, 1 moderate/5; vacuolation of histiocytes 1 slight, 4 moderate /5). Both conditions persisted among the recovery group. Similar findings but with minimal grade of severity were also found at 25 mg/kg bw/day, however, this finding was not dose-related.

Remarks – Results
This study resulted in treatment-related changes at all dose levels, therefore, a no observed effect level (NOEL) cannot be established. However, the effect at 25 mg/kg bw/day was considered not to represent an adverse health effect due to isolated morphological changes at both lower incidence and grades of severity.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 25 mg/kg bw/day in this study, based on absence of adverse health effect at this dose level.

TEST FACILITY Safepharm (2007a)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. (Direct plate incorporation method)
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA$^-$
Metabolic Activation System	Phenobarbitone/β-naphthoflavone induced rat liver microsome preparations (S9 mix)
Concentration Range in Main Test	a) With metabolic activation: 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate b) Without metabolic activation: 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate
Vehicle	Acetone
Remarks - Method	A preliminary cytotoxicity test was performed using a vehicle control and a range of 10 concentrations of the notified chemical from 0.15 to 5000 µg/plate using *S. typhimurium*: TA100 and *E. coli*: WP2uvrA$^-$ strains. No significant deviation from the protocol. Appropriate known mutagens were tested in parallel to the notified chemical to validate the sensitivity of the assay.

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	≥50	≥1500	Negative
Test 2	≥1500	≥50	≥1500	Negative
Present				
Test 1	≥150	≥150	≥1500	Negative
Test 2	≥1500	≥150	≥1500	Negative

Remarks - Results

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains at any dose levels either with or without S9.

The test material caused a visible reduction in the growth of the bacterial background lawn to all the *S. typhimurium* strains, initially at 50 and 150 µg/plate in the absence and presence of S9, respectively. No toxicity was observed to *E. coli*: WP2uvrA$^-$ strains at any dose levels tested either with or without S9. An oily precipitate was noted at and above 1500 µg/plate which did not prevent the scoring of revertant colonies.

All of the positive control chemicals used induced marked increases in the frequency of revertant colonies.

CONCLUSION

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

TEST FACILITY

Safepharm Laboratories (2006h)

B.8 Chromosome aberration test – in vitro

TEST SUBSTANCE

Notified chemical

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: Chinese Hamster Lung (CHL) cells

Metabolic Activation System: Phenobarbitone/β-naphthoflavone induced rat liver microsome preparations (S9 mix) at 2% and 5%.

Vehicle: Acetone

Remarks - Method

A preliminary toxicity test was performed in the dose range between 0.12 to 60 µg/ml. Growth inhibition and mitotic index were examined to evaluate toxicity after 24h continuous treatment without metabolic activation and after 6h treatment in the presence and absence of metabolic activation followed with 18h of incubation.

No significant deviation from the protocol. Appropriate known mutagens were tested in parallel to the notified chemical.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period (h)*	*Harvest Time(h)*
Absent			
Test 1	0*; 0.94, 1.88*, 3.75*, 5.63, 7.5*, 11.25	6	24
Test 2	0*; 0.235, 0.47*, 0.94*, 1.88*, 3.75, 7.5	24	24
Present at %			
Test 1 @ 5%	0*; 3.75, 7.5, 15*, 30*, 45*, 60	6	24
Test 2 @ 2%	0*; 1.88, 3.75*, 7.5*, 15*, 30, 60	6	24

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 7.5*	≥ 7.5	> 11.25	Negative
Test 2	≥ 3.75*	> 1.88	> 7.5	Negative
Present				
Test 1	≥ 30**	≥ 30	> 60	Negative
Test 2	-	≥ 30	> 60	Negative

* Based on inhibition of Cell Growth Index; ** Based on inhibition of Mitotic index

Remarks - Results

The test material did not induce any statistically significant increases in the frequency of cells with aberrations, or in the number of polyploid cells at any dose level, either in the presence or absence of metabolic activation.

The positive controls showed significant increases in mutagenic colonies, confirming the effectiveness of the test conditions.

CONCLUSION

The notified chemical was not clastogenic to Chinese Hamster Lung (CHL) cells treated in vitro under the conditions of the test.

TEST FACILITY

Safepharm (2007b)

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.

Inoculum	Activated Sewage sludge obtained from a sewage treatment plant
Exposure Period	28 days
Auxiliary Solvent	Dispersed on Silica gel
Analytical Monitoring	CO_2 evolution
Remarks - Method	Solubility testing indicated that the test material had poor solubility. As the test substance was a liquid and of poor solubility, the test material was suspended in the inoculum adsorbed to silica gel.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
0	0	0	0
1	1	1	31
6	5	6	77
14	1	14	93
22	11	22	91
28	11	28	86

Remarks - Results: The notified chemical was not found to be inhibitory to activated sewage sludge bacteria under the conditions of this test. The validation criteria for the control were met.

CONCLUSION: The notified chemical cannot be classed as ready biodegradable.

TEST FACILITY: Safepharm Laboratories (2006i)

C.1.2. Bioaccumulation

Remarks: Not determined. Bioaccumulation of the notified chemical is not anticipated as the release to the aquatic environment will be very low and the notified chemical will be protonated throughout the environmental pH range.

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.
EC Directive 92/69/EEC C.1 Acute Toxicity for Fish -Semi-static.

Species	*Rainbow trout* (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	~ 140 mg $CaCO_3$/L
Analytical Monitoring	HPLC/MS
Remarks – Method	A range finding test was performed by preparing water accomodated

fractions (WAFs) of test substance of nominal concentrations of 10, 100 and 1000 mg/L by stirring the mixture of test substance and purified water for 23 hours and allowing the mixture to settle for 1 hour before siphoning the WAF. Three fish were subjected to the WAFs for 96 hours.

The main test was conducted by subjecting groups of ten fish to duplicate WAFs prepared at a loading rate of 100 mg/L of the test substance (prepared in the same manner as described previously). The test solutions were replaced every 24 hours. No micro-dispersions or undissolved material was present. The concentrations were determined by HPLC/MS on fresh an old samples at 0, 24, 72 and 96 hours. Analysis of the samples showed extremely low measured concentrations for each of the ion masses analysed. However, reference standards for each of the components detected in standard solutions of the test material or the WAFs do not exist, hence, it was not possible to reliable quantify the concentrations of each of the components in the WAFs. Examination of the total ion chromatograms generated from the HPLC/MS analysis of the WAFs showed there were significant differences in the peak profiles when compared to the standard solutions. The most significant peak in the mass spectrums of the WAFs was ascribed to an impurity which was solubilised in the WAFs.
pH 7.6-7.9
Temperature 13.7-14.5°C
Dissolved Oxygen 9.6 – 10.4 mg O_2/L
Light: 16 hours light and 8 hours dark with 20 minute transition.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	0	10	0	0	0	0	0
100	-	20	0	0	0	0	0

LL50	>100 mg/L at 96 hours
NOEL	100 mg/L (WAF) at 96 hours
Remarks – Results	No sub-lethal effects were observed throughout the definitive study.

CONCLUSION The notified chemical is not toxic to Rainbow trout up to the limit of its water solubility.

TEST FACILITY Safepharm Laboratories (2007c)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test - Static.
EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - Static.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	Approximately 250 mg $CaCO_3$/L
Analytical Monitoring	HPLC/MS
Remarks - Method	A range finding test was performed by preparing three WAFs of test substance of nominal concentrations of 10, 100 and 1000 mg/L by stirring the mixture of test substance and purified water for 23 hours and allowing the mixture to settle for 1 hour before siphoning the WAF. Ten daphnids were subjected to the WAFs for 96 hours.

The main test was conducted by subjecting duplicate test sample of ten daphnia to WAFs (concentrations detailed below) of the test substance (prepared in the same manner as described previously). As microscopic examination of the WAFs with the highest loading rates showed micro-dispersions all of the test substances were filtered. A reference substance (0.32, 0.56, 1.0, 1.8 and 3.2 mg/L of potassium dichromate) was also run. Analysis of the samples showed extremely low measured concentrations for each of the ion masses analysed. However, reference standards for each of the components detected in standard solutions of the test material or the WAFs do not exist, hence, it was not possible to reliable quantify the concentrations of each of the components in the WAFs. Examination of the total ion chromatograms generated from the HPLC/MS analysis of the WAFs showed there were significant differences in the peak profiles when compared to the standard solutions. The most significant peak in the mass spectrums of the WAFs was ascribed to an impurity which was solubilised in the WAFs.
pH 8.0
Temperature 19.9-20.3°C
Dissolved Oxygen 8.3-8.5 mg O_2/L
Light: 16 hours light and 8 hours dark with 20 minute transition.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	-	20	0	0
10	-	20	0	0
18	-	20	0	0
32	-	20	0	0
56	-	20	0	2
100	-	20	0	2
180	-	20	1	2
320	-	20	11	14
560	-	20	16	20
1000	-	20	19	20

EL50 350 (95% CI; 290-430) mg/L at 24 hours
230 (95% CI; 150-360) mg/L at 48 hours

NOEC 32 mg/L (WAF) at 48 hours

Remarks - Results The observed immobilisation data clearly show that the addition of the test material to the water column induced a toxic effect on the Daphnids. However, results of the chemical analyses for the test material in the WAFs were unreliable due to the low water solubility (predicted to be <1 mg/L for the major component) and the variability of the chromatographic profiles for the WAFs compared to the standard solutions.

CONCLUSION The notified chemical exerts a toxic effect on aquatic invertebrates below the level of its water solubility.

TEST FACILITY Safepharm Laboratories (2007d)

C.2.3. 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 *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*)

Exposure Period	72 hours
Concentration Range	Nominal: 0.1, 1.0, 10 and 100 mg/L Actual: -
Auxiliary Solvent	None
Water Hardness	Not specified
Analytical Monitoring	HPLC/MS
Remarks - Method	A range finding test was performed by preparing WAFs of test substance of nominal concentrations between 0.1 and 1000 mg/L by stirring the mixture of test substance and purified water for 23 hours and allowing the mixture to settle for 1 hour before siphoning the WAF. Algae were exposed to the WAFs for 72 hours. The main test was conducted by subjecting triplicate test samples of algae of cell density of approximately 10^4 cells per mL to the WAFs (concentrations detailed above) of the test substance (prepared in the same manner as described previously). Analysis of the samples showed extremely low measured concentrations for each of the ion masses analysed. However, reference standards for each of the components detected in standard solutions of the test material or the WAFs do not exist, hence, it was not possible to reliable quantify the concentrations of each of the components in the WAFs. Examination of the total ion chromatograms generated from the HPLC/MS analysis of the WAFs showed there were significant differences in the peak profiles when compared to the standard solutions. The most significant peak in the mass spectrums of the WAFs was ascribed to an impurity which was solubilised in the WAFs.

RESULTS

Biomass		*Yield*		*Growth*	
*NOEL**	*E_bL50*	*NOEL**	*E_yL50*	*NOEL**	*E_rL50*
(mg/L at 0-72 h	*mg/L at 0-72 h*	*mg/L at 0-72 h*	*mg/L at 0-72 h*	*mg/L at 0-72 h*	*mg/L at 0-72 h*
3.2	9.6	3.2	10	3.2	16

*No observed effect loading

Remarks - Results	Three endpoints were determined; the biomass integral, the Yield (cells/mL) and the growth rate (cell/mL/hour). The observed toxicity endpoints clearly show that the addition of the test material to the water column induced a toxic effect on the algae. However, results of the chemical analyses for the test material in the WAFs were unreliable due to the low water solubility (predicted to be < 1mg/L for the major component) and the variability of the chromatographic profiles for the WAFs compared to the standard solutions.
CONCLUSION	WAFs of the notified chemical are toxic to algae. Noting that the analysis of the WAFs indicated that the components were present in extremely low concentrations (which were not quantifiable), the soluble components of the WAFs are highly toxic to algae.
TEST FACILITY	Safepharm Laboratories (2007e)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	In accordance with OECD TG 209 Activated Sludge, Respiration Inhibition Test, EC Directive 88/302/EEC C.11 Biodegradation and US EPA Draft Ecological Effects Test Guidelines OPPTS 850.6800.

	Activated Sludge Respiration Inhibition Test
Inoculum	Activated Sewage Sludge
Exposure Period	3 hours
Concentration Range	Nominal: 1000 mg/L Actual: Not Determined
Remarks – Method	Activated sludge organisms from the Severn Trent Water Plc sewage treatment plant at Loughborough, Leicestershire, UK, which treats predominantly domestic sewage sludge were used. A range finding test was conducted using duplicate samples of a control and single samples 100 mg/L and 1000 mg/L of test substance. A reference substance (3,5-dichlorophenol) was also run at 3.2 mg/L and 32 mg/L. Synthetic sewage was added to the test substances and the O_2 consumption rates were measured and compared with the control. The main test was conducted by subjecting triplicate samples of 1000 mg/L of test substance to the inoculum and synthetic sewage sludge and measuring the O_2 consumption rate. A comparison was then made to the control which was run in duplicate. A reference substance (3,5-dichlorophenol) was also at concentrations of 3.2, 10, and 32 mg/L. pH 7.8-8.3 Total Hardness 100 mg $CaCO_3$/L

RESULTS

IC50	> 1000 mg/L
NOEC	1000 mg/L
Remarks – Results	The range finding test showed that 1000 mg/L of test substance had 0% inhibition of respiration of activated sewage sludge. The test substances were observed as dark brown dispersions with globules of test material throughout and some adhered to the side of the flask. The average % inhibition of the main test at 3 hours was 1.7%. The reference substance had an IC50 of 6.1 mg/L, which was within the accepted value of 5-30 mg/L. Some of the initial and final dissolved oxygen concentrations were below the test guidelines (6.5 mg/L and 2.5 mg/L, respectively). This was not considered to have an adverse effect as the oxygen consumption rate was determined over the linear portion of the oxygen consumption trace.

CONCLUSION	The test substance is not considered inhibitory to sewage sludge up to the concentration tested.
TEST FACILITY	Safepharm Laboratories (2006j)

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