File No: STD/1308

February 2009

**NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)**

# FULL PUBLIC REPORT

## Z-89

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, Water, Heritage and the Arts.

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 334-336 Illawarra Road, Marrickville NSW 2204.

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:


| | |
|---|---|
| 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**
**NICNAS**

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### 1. APPLICANT AND NOTIFICATION DETAILS

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

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, means of identification, purity, identity of impurities, details of use and import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: Dissociation constant, bioaccumulation, flammability limits, acute inhalation toxicity and induction of germ cell damage.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

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

### 2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Z-89

OTHER NAME(S)
OS219479 (Used for testing and trials)

MOLECULAR WEIGHT
< 500 Da

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

### 3. COMPOSITION

DEGREE OF PURITY > 92%

ADDITIVES/ADJUVANTS None

## 4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Pale yellow, slightly viscous liquid

| Property | Value | Data Source/Justification |
|---|---|---|
| Pour point | -20°C | Measured |
| Boiling Point | Boiled with decomposition at 371°C at 101.3 kPa | Measured |
| Density | 959 kg/m$^3$ at 20°C | Measured |
| Vapour Pressure | $2.0 \times 10^{-8}$ kPa at 25°C | Measured |
| Water Solubility | $\leq 1.21 \times 10^{-4}$ g/L at 20°C | Measured |
| Hydrolysis as a Function of pH | Stable at pH 4 and 7 (half-life more than a year). Hydrolysed at pH 9 (half-life 15.2 days). | Measured |
| Partition Coefficient (n-octanol/water) | log Pow = 7.18 at 20°C | Measured |
| Adsorption/Desorption | log $K_{oc}$ > 5.63 | Measured |
| Dissociation Constant | pKa = 12.3 | Estimated |
| Flash Point | 129°C at 101 kPa (closed cup) | Measured |
| Flammability | Not expected to be highly flammable | Estimated from measured flash point. |
| Autoignition Temperature | 356°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.
The notified chemical has a low vapour pressure, is highly lipophilic and sparingly soluble in water.
Based on the flash point, the notified chemical is not classified as flammable but would be considered to be a C1 combustible liquid [NOHSC:1015(2001)]

*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. The notified chemical will be imported as a component of finished lubricant at a concentration of ≤ 2%. The notified chemical may also be imported in a more concentrated product (≤ 10%) for reformulation within Australia.

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

| *Year* | *1* | *2* | *3* | *4* | *5* |
|---|---|---|---|---|---|
| *Tonnes* | 100-200 | 300-400 | 300-400 | 300-400 | 400-500 |

PORT OF ENTRY
Throughout Australia.

TRANSPORTATION AND PACKAGING
The products containing the notified chemical will be imported in isotainers and 55 and 330 gallon drums and will be transported throughout Australia by road or rail.

USE
The notified chemical will be used as an engine oil additive (concentration ≤ 2%).

OPERATION DESCRIPTION
The notified chemical will not be manufactured in Australia, but may be reformulated after importation.

*Reformulation*
At customer blending sites, the concentrate product containing the notified chemical (at ≤ 10%) will be formulated into engine oil products by mixing with oil and other additives. It will be either decanted from drums or isotainers into a trough from which it will be pumped into a blend tank, or pumped directly into the blend tank. Blend facilities are expected to be fully automated, well ventilated and closed systems.

After blending, the engine oil products containing the notified chemical (at ≤ 2%) will be packaged into containers. The packaging facility will usually be located near the blending operation area and the transfer of product to the packaging is expected to be fully automated.

Samples may be taken during the blending process for quality control testing. The samples will be collected by opening a valve in the blending vessel and filling a small container.

*End use*
Engine oil products containing ≤ 2% of the notified chemical will primarily be used in factories where cars are manufactured. The engine oil products containing ≤ 2% of the notified chemical may also be used in mechanical repair garages and sold to the public.

## 6. HUMAN HEALTH IMPLICATIONS

### 6.1 Exposure assessment

#### 6.1.1 Occupational exposure

EXPOSURE DETAILS
*Storage and transportation*
It is anticipated that waterside workers, transport drivers and warehouse workers would only be exposed to the material in the event of an accident

*Reformulation*
Dermal and ocular exposure to the notified chemical (≤ 10%) is possible when plant operators are connecting and disconnecting pump lines to storage tanks or blending vessels. It is expected that negligible exposure will occur during the fully automatic and closed blending process. The opportunity also exists for dermal exposure (≤ 2%) when cleaning up spills or leaks and during maintenance of the blend vessel. However, maintenance is not expected to occur frequently as residue in the blending vessel will generally be incorporated into the next blend. Workers involved in the blending process are expected to wear impermeable gloves, goggles or face shield and protective clothing to further minimise exposure.

Negligible exposure is expected during transfer of the concentrate product containing the notified chemical (≤ 2%) to packaging as this will be carried out using automated processes. Inhalation exposure is expected to be negligible given the very low vapour pressure of the notified chemical ($2.0 \times 10^{-8}$ kPa at 25°C). In addition, blending and packaging facilities are expected to be well ventilated.

*Sampling*
At blending facilities samples will be taken from blend vessels during the blending process. Dermal exposure to the notified chemical (at ≤ 2%) may occur during sampling. To minimise exposure the plant operator is expected to wear gloves, goggles and protective clothing.

*End use*
There is potential for dermal exposure during the connection and disconnection of the dip-pipe and pump as well as from handling automotive components contaminated with the engine oil. At workshops, mechanics may experience dermal and ocular exposure to final products containing < 2% of the notified chemical when adding the products to automobiles and other machinery. Exposure is expected to be minimised by the use of gloves, goggles and protective clothing.

### 6.1.2. Public exposure

Products containing the notified polymer are primarily intended for use by industry and therefore public exposure to the notified polymer is expected to be low. However, exposure to the notified polymer (≤ 2%) may occur during the changing of engine oil in automobiles by the public, although this will only be on an infrequent basis. Exposure will primarily be dermal, although ocular exposure is also possible. PPE is not expected to be worn by the public.

## 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 | oral LD50 > 2000 mg/kg bw<br>low toxicity |
| Rat, acute dermal toxicity | LD50 > 2000 mg/kg bw<br>low toxicity |
| Rat, acute inhalation toxicity | Not determined |
| Rabbit, skin irritation | slightly irritating |
| Rabbit, eye irritation | slightly irritating |
| Mouse, skin sensitisation – Local lymph node assay | no evidence of sensitisation |
| Rat, repeat dose oral toxicity – 28 days. | NOAEL = 750 mg/kg bw/day |
| Mutagenicity – bacterial reverse mutation | non mutagenic |
| Genotoxicity – in vitro Mammalian Chromosome Aberration Test | non genotoxic |
| Genotoxicity – in vivo | Not determined |

*Toxicokinetics, metabolism and distribution.*

Based on the low molecular weight (< 500 Da) and the lipophilicity of the notified chemical (water solubility ≤ $1.21 \times 10^{-4}$ g/L at 20ºC; log Pow = 7.18 at 20ºC) dermal absorption may occur, but the transfer from the stratum corneum into the epidermis is expected to be slow.

*Acute toxicity.*

The notified chemical is considered to be of low acute toxicity via the oral and dermal routes based on tests conducted in rats.

*Irritation and Sensitisation.*

Based on a test conducted in rabbits the notified chemical is considered to be slightly irritating to the skin and eye. The notified chemical was found to be a non-sensitiser in a local lymph node assay in mice.

*Repeated Dose Toxicity (sub acute, sub chronic, chronic).*

Oral administration of the test material to rats for a period of 28 consecutive days at dose levels of 750, 150 and 25 mg/kg/day resulted in treatment related effects at all dose levels and therefore a No Observed Effect Level (NOEL) could not be set. The treatment related effects were limited to microscopic liver and thyroid changes, with associated liver weight gains and a slightly altered metabolism. There were no associated inflammatory or degenerative changes in the organs affected, and so the effects are considered to be an adaptive response to the high doses of xenobiotics.

Therefore, the No Observed (Adverse) Effect Level (NO(A)EL) was established as 750 mg/kg bw/day in this study, based on the lack of toxicologically relevant effects seen in the study.

*Mutagenicity and carcinogenicity.*

The notified chemical was found to not be mutagenic using a bacterial reverse mutation test, and is not clastogenic to Chinese hamster lung cells *in vitro*.

***Health hazard classification***

Based on the available data the notified chemical is not classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

### 6.3. Human health risk characterisation

#### 6.3.1. Occupational health and safety

Occupational dermal and ocular exposure to the notified chemical for reformulation workers may occur during handling of the drums or isotainers, transfer of the concentrate product (≤ 10%) into the blend tank, cleaning and maintenance of the equipment. Dermal and ocular exposure may also occur in the end use where engine oil (≤ 2% notified chemical) is changed by automotive mechanics.

Local effects

Although the notified chemical is a slight eye and skin irritant, the severity of these effects will be reduced due to the relatively low concentrations in the products. In addition the exposure is expected to be minimised due to the use of personal protective equipment in the case of reformulation workers. Therefore the risk of irritation is not expected.

Systemic effects

The NOAEL for the notified chemical was found to be 750 mg/kg bw/day. The chemical is not acutely toxic via the oral or dermal routes and was not mutagenic or genotoxic.

Dermal exposure to the notified chemical during reformulation activities can be estimated using the EASE (1997) model assuming reasonable worst case defaults and based on non-dispersive use with intermittent direct handling. This gives an estimated daily exposure of 0.12 mg/kg bw/day for a 70 kg worker, assuming a 10% dermal absorption factor and an average surface area of 860 $cm^2$ for the hands (RVIM, 2006). Based on a 750 mg/kg bw/day NOAEL for the notified chemical derived from a 28-day rat oral repeat dose study the margin of exposure (MOE) for the proposed use is 6250. A MOE greater than or equal to 100 is considered acceptable to account for intra- and inter-species differences. Therefore, the risk of systemic effects is acceptable for workers involved in reformulation of products containing the notified chemical. For end-users the risk of repeated exposure to the notified chemical is reduced due to the lower concentrations ≤ 2%).

The risk of repeated exposure is not considered unacceptable, considering the estimated MOE and the toxicological profile of the notified chemical.

#### 6.3.2. Public health

The risk to the public is low assuming that most consumers do not change their own engine oil. For DIY users changing their own engine oil the risk is not considered unacceptable, given that draining of engine oil is an infrequent event and the concentration of the notified chemical in the engine oil is low.

## 7. ENVIRONMENTAL IMPLICATIONS

### 7.1. Environmental Exposure & Fate Assessment

#### 7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notification dossier states that the notified chemical will initially be imported as part of a finished engine oil. Should local blending commence, releases would be expected to be low as blending facilities use highly automated processes. Spills would be recycled or collected for incineration, together with residues rinsed from import containers, estimated at 1% of the import volume.

RELEASE OF CHEMICAL FROM USE

Some minor, diffuse exposure will result from spills during addition to and removal of oil from vehicles. Around 86% of oil changes take place in specialised automotive service centres, where release of the notified chemical from professional activities should be disposed of appropriately. The remaining 14% are removed by DIY enthusiasts. The DIY proportion of oil changes could potentially lead to improper disposal of used oil (55%) to soils or sediments and stormwater drains.

RELEASE OF CHEMICAL FROM DISPOSAL

Iso-containers and drums should be sent for cleaning and reconditioning by a licensed company. The resultant washings from such companies are typically passed to an on site waste treatment facility, with any waste sludge typically incinerated.

Old oil drained from crankcases at specialised automotive service centres is expected to be disposed of responsibly, either to oil recycling facilities or incineration.

Oils disposed of by DIY enthusiasts would be either incinerated, left at transfer stations where it is again likely to be recycled, or deposited into landfill. However, a survey tracing the fate of used lubricating oil in Australia found that only around 20% of used oil removed by 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.

### 7.1.2 Environmental fate

The notified chemical is likely to mainly be disposed of by incineration. Smaller amounts may be consigned to landfill, or disposed of inappropriately to land or stormwater. Incineration would destroy the notified chemical, while disposal to land or landfill would result in its immobilisation because of the strong sorption to soil organic carbon. If disposed of to water, the notified chemical is likely to spread across the surface of the water and sorb to suspended solids and sediment. The notified chemical is not readily biodegradable, but can be expected to slowly degrade in the environment. Bioaccumulation is not expected to be a significant pathway because the notified chemical has a very low water solubility and high octanol-water partition coefficient (> $10^7$) and is therefore not expected to be bioavailable to aquatic organisms.

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

### 7.1.3 Predicted Environmental Concentration (PEC)

A worst case estimated PEC might be calculated if it is assumed that 0.7% of the notified chemical (maximum 3.5 tonne) is released into stormwater drains in a single metropolitan area with a geographical footprint of 500 $km^2$ and an average annual rainfall of 500 mm, all of which drains to stormwater. With a maximum annual release into this localised stormwater system of 3500 kg and the annual volume of water drained from this region estimated to be approximately 250 x $10^6$ $m^3$, the resultant PEC is approximately 14 µg/L. It should be stressed that this result is very much a worst case scenario, and that in reality releases of the notified chemical would be very much more diffuse than indicated here, and also at significantly reduced levels.

## 7.2. Environmental effects assessment

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

| *Endpoint* | *Result* | *Assessment Conclusion* |
|---|---|---|
| Fish Toxicity | EC50 > 0.0011 mg/L | Nontoxic to the limit of water solubility, as modulated by the instability of the notified chemical. |
| Daphnia Toxicity | EC50 > 0.0082 mg/L | |
| Algal Toxicity | EC50 > 0.00018 mg/L | |
| Inhibition of Bacterial Respiration | EC50 > 1000 mg/L | |

The fish, daphnid and algal tests were conducted with water accommodated fractions obtained by mid-depth siphon from stirred suspensions. Results are expressed as mean measured concentrations. Their variability reflects the hydrophobicity of the notified chemical and its instability under the test conditions as seen in preliminary stability analyses. The result for the bacterial inhibition test is expressed as the nominal concentration.

### 7.2.1 Predicted No-Effect Concentration

Where acute toxicity data are available for three trophic levels, the predicted no effect concentration is normally derived by application of an assessment factor of 100 to the median effect concentration in the most sensitive species. This approach is impractical for the notified chemical as the median effect concentrations are not known. The notified chemical had no harmful acute effects in fish, daphnids or algae at concentrations up to the solubility limit, as modulated by its degradation. The mean measured no effect concentrations varied from 0.18 µg/L to 8.2 µg/L.

### 7.3. Environmental risk assessment

Comparison of the PEC of 14 µg/L with the mean measured no-effect concentrations of 0.18-8.2 µg/L suggests the possibility of toxic effects in aquatic life exposed to residues of the notified chemical that have been inappropriately disposed of to stormwater. However, in practical terms the notified chemical is not expected to pose a risk to the environment. This is because the PEC overestimates the likely level of exposure, as it reflects a worst case scenario with no consideration of the strong hydrophobicity of the notified chemical, which would favour sorption to sediment rather than dissolution in the water column. The actual no-effect concentrations are likely to be higher than the values obtained from testing, which are limited by the very low water solubility and instability of the notified chemical. Accidental spills of the notified chemical to water can be expected to be harmful to aquatic life, as it would be likely to coat biological membranes in such situations, but the notified chemical is not expected to pose a risk to the environment when it is used as proposed in engine oils.

## 8. CONCLUSIONS AND REGULATORY OBLIGATIONS

**Hazard classification**

Based on the available data the notified chemical is not classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)].

**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.

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

**Environmental risk assessment**

On the basis of the very low water solubility and the reported use pattern, the notified chemical is not considered to pose a risk to the environment.

**Recommendations**

CONTROL MEASURES

Occupational Health and Safety

- Employers should implement the following safe work practices to minimise occupational exposure during handling of products containing the notified chemical:
  - Avoid skin and eye contact

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to products containing the notified chemical:
  - Protective eyewear
  - Impervious gloves
  - Protective clothing

  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 *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Disposal

- The notified chemical should be disposed of by landfill.

**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 engine oil additive, or is likely to change significantly;
- the amount of chemical being introduced has increased from 500 tonnes, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

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

No additional secondary notification conditions are stipulated.

*Material Safety Data Sheet*
The MSDS of the 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.

## APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

**Melting Point/Freezing Point** -20°C

| | |
|---|---|
| Method | OECD TG 102 Melting Point/Melting Range. |
| Remarks | No significant protocol deviations. |
| Test Facility | Safepharm Laboratories (2007a) |

**Boiling Point** 371°C at 101.3 kPa

| | |
|---|---|
| Method | OECD TG 103 Boiling Point. |
| Remarks | The boiling point was determined using differential scanning calorimetry.<br>Decomposition was noted along with boiling. The decomposition occurred under both air and nitrogen atmospheres indicating that decomposition was not oxidative.<br>No significant protocol deviations. |
| Test Facility | Safepharm Laboratories (2007a) |

**Density** 959 kg/m$^3$ at 20°C

| | |
|---|---|
| Method | OECD TG 109 Density of Liquids and Solids. |
| Remarks | The relative density was determined using the pycnometer method.<br>No significant protocol deviations. |
| Test Facility | Safepharm Laboratories (2007a) |

**Vapour Pressure** $2.0 \times 10^{-8}$ kPa at 25°C

| | |
|---|---|
| Method | OECD TG 104 Vapour Pressure. |
| Remarks | The vapour pressure was determined using a vapour pressure balance at several temperatures, with linear regression analysis used to determine the vapour pressure at 25°C. No significant protocol deviations. |
| Test Facility | Safepharm Laboratories (2007b) |

**Water Solubility** $\leq 1.21 \times 10^{-4}$ g/L at 20°C

| | |
|---|---|
| Method | OECD TG 105 Water Solubility. |
| Remarks | Flask Method. The notified chemical was determined by HPLC analysis of dichloromethane extracts of the saturated aqueous solutions obtained by centrifugation and filtration of dispersions prepared at a nominal 0.2 g/L. The value cited has been corrected for the low recovery (25%) from filtered solutions prepared at a nominal 0.25 mg/L.<br>The water solubility has also been estimated (EPIWIN version 3.12) as 8.5 x $10^{-7}$ g/L. |
| Test Facility | SafePharm Laboratories (2007a) |

**Hydrolysis as a Function of pH**

| | |
|---|---|
| Method | OECD TG 111 Hydrolysis as a Function of pH.<br>EC Directive 92/69/EEC C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH. |

| *pH* | *T (°C)* | *t½* |
|---|---|---|
| 4 | 25 | > 1 year |
| 7 | 25 | > 1 year |
| 9 | 25 | 15.2 days |

| | |
|---|---|
| Remarks | The half-life at pH 9 was extrapolated from measurements at 30°C, 40°C and 50°C. The nominal concentration was 5 x $10^{-5}$ g/L, below the solubility limit. Acetonitrile (1%) was used to assist in dissolution. Hydrolysis was expected to occur at pH 9, based on the structure, and was confirmed by identification of the expected hydrolysis products. |
| Test Facility | SafePharm Laboratories (2007a) |

**Partition Coefficient (n-octanol/water)** log $P_{ow}$ = 7.18 at 20°C

Method OECD TG 117 Partition Coefficient (n-octanol/water).
Remarks HPLC Method. The test was conducted at neutral pH. The mobile phase was modified to allow higher calibration reference standards (nonylbenzene and tridecylbenzene) to elute. The result from HPLC is consistent with the preliminary estimate (log $P_{ow}$ > 5.19) obtained from the approximate solubilities in octanol (415 g/L) and water (< 2.7 mg/L).
Test Facility SafePharm Laboratories (2007a)

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

Method OECD TG 121 Adsorption - Desorption HPLC Screening Method.
Remarks The notified chemical eluted from the HPLC column after the reference substance DDT.
Test Facility SafePharm Laboratories (2007a)

**Dissociation Constant** pKa = 12.3

Method Computer based estimation software.
Remarks Measurement could not be carried out because of the very low water solubility of the notified chemical.
Test Facility SafePharm Laboratories (2007a)

**Flash Point** 129°C at 101 kPa

Method EC Directive 92/69/EEC A.9 Flash Point.
Remarks The flash point was determined using a closed cup equilibrium method. No significant protocol deviations.
Test Facility Safepharm Laboratories (2007b)

**Autoignition Temperature** 356°C

Method EC Directive 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks No significant protocol deviations.
Test Facility Safepharm Laboratories (2007b)

**Explosive Properties** Not explosive

Method EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks The structure of the notified chemical was assessed for chemical groups that imply explosive properties. No significant protocol deviations.
Test Facility Safepharm Laboratories (2007b)

**Oxidizing Properties** Not oxidising

Method EC Directive 92/69/EEC A.21 Oxidizing Properties (Liquids).
Remarks The structure of the notified chemical was assessed for chemical groups that imply oxidising properties.
No significant protocol deviations.
Test Facility Safepharm Laboratories (2007b)

# APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

## B.1. Acute toxicity – oral

| | |
|---|---|
| TEST SUBSTANCE | Notified chemical |
| METHOD | OECD TG 420 Acute Oral Toxicity - Fixed Dose Method.<br>EC Directive2004/73/EC B.1 bis Acute Toxicity (Oral) Fixed Dose Method. |
| Species/Strain | Rat/Sprague-Dawley CD (Crl : CD (SD) IGS BR) Female |
| Vehicle | Test substance administered as supplied |
| Remarks - Method | No significant protocol deviations.<br>All animals were dosed by gavage.<br>The sighting study was conducted using 1 animal dosed at 2000 mg/kg. As there was no mortality an additional four animals were dosed at 2000 mg/kg. |
| RESULTS | |
| Discriminating Dose | > 2000 mg/kg bw |
| Signs of Toxicity | There were no deaths.<br>No signs of systemic toxicity were noted. |
| Effects in Organs | No abnormalities were noted at necroscopy |
| Remarks - Results | Body weight gains were as expected. |
| CONCLUSION | The notified chemical is of low toxicity via the oral route. |
| TEST FACILITY | Safepharm Laboratories (2007c) |

## B.2. Acute toxicity – dermal

| | |
|---|---|
| TEST SUBSTANCE | Notified chemical |
| METHOD | OECD TG 402 Acute Dermal Toxicity – Limit Test.<br>EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal) – Limit Test. |
| Species/Strain | Rat/Sprague-Dawley CD (Crl : CD (SD) IGS BR) |
| Vehicle | Test substance administered as supplied |
| Type of dressing | Semi-occlusive. |
| Remarks - Method | No significant protocol deviations |

RESULTS

| *Group* | *Number and Sex of Animals* | *Dose mg/kg bw* | *Mortality* |
|---|---|---|---|
| I | 5 per sex | 2000 | 0 |

| | |
|---|---|
| LD50 | > 2000 mg/kg bw |
| Signs of Toxicity - Local | There were no test substance-related dermal reactions. |
| Signs of Toxicity - Systemic | There were no deaths or test-substance related clinical signs. There were no signs of systemic toxicity. |
| Effects in Organs | No abnormalities were noted at necroscopy |
| Remarks - Results | Body weight gains were as expected. |
| CONCLUSION | The notified chemical is of low toxicity via the dermal route. |
| TEST FACILITY | Safepharm Laboratories (2007d) |

### 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 |
| Vehicle | Test substance administered as supplied |
| Observation Period | 7 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* | 1 | 1 | 1.3 | 2 | < 7 days | 0 |
| *Oedema* | 0.3 | 0 | 0.7 | 1 | < 72 hours | 0 |

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

Remarks - Results: A single 4-hour, semi-occluded application of the test material to the intact skin of the three rabbits produced very slight to well-defined erythema and very slight oedema at the 24 hour observation. Slight erythema was noted at all treated skin sites at 48 hours. One treated skin site appeared normal at the 72-hour observation and the remaining two treated skin sites appeared normal at the 7-day observation.
No corrosive effects were noted.

CONCLUSION: The notified chemical is slightly irritating to the skin.

TEST FACILITY: Safepharm Laboratories (2007e)

### 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 |
| Remarks - Method | No significant protocol deviations |

RESULTS

| *Lesion* | *Mean Score* Animal No.* | | | *Maximum Value* | *Maximum Duration of Any Effect* | *Maximum Value at End of Observation Period* |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | | | |
| *Conjunctiva: redness* | 0.7 | 0.7 | 1 | 2 | < 72 hours | 0 |
| *Conjunctiva: chemosis* | 0.3 | 0.3 | 0.3 | 1 | < 48 hours | 0 |
| *Conjunctiva: discharge* | 0.3 | 0.3 | 0.3 | 1 | < 48 hours | 0 |
| *Corneal opacity* | 0 | 0 | 0 | 0 | 0 | 0 |
| *Iridial inflammation* | 0 | 0.3 | 0.3 | | < 48 hours | 0 |

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

Remarks - Results: A single application of the test material to the non-irrigated eye of three rabbits produced iridial inflammation and moderate conjunctival irritation. One treated eye appeared normal at the 48 hour observation

with the remaining two eyes appearing normal at the 72 hour observation.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY Safepharm Laboratories (2007f)

### 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/Ca (CBA/CaBkl) Female

Vehicle Acetone/olive oil 4:1

Remarks - Method A preliminary screening test was conducted on 1 mouse using 25 µL of undiluted test substance. No signs of systemic toxicity were noted. Mild redness to the ears was noted post dose on Day 3.
α-Hexylcinnamaldehyde was used as the positive control. The positive control assay was performed more than 6 months prior to the test substance assay. However, an assay using a different positive control in propylene glycol was conducted within 6 months of the test substance assay.

RESULTS

| *Concentration (% w/w)* | *Proliferative response (Mean DPM/animal (SD))* | *Stimulation Index (Test/Control Ratio)* |
|---|---|---|
| *Test Substance* | | |
| 0 (vehicle control) | 2329 (±2713) | |
| 25 | 2170 (±696) | 0.93 |
| 50 | 2244 (±366) | 0.96 |
| 100 | 3556 (±1600) | 1.53 |
| *Positive Control* | | |
| 5 | | 2.50 |
| 10 | | 4.03 |
| 25 | | 9.13 |

Recalculation of the stimulation index using the median rather than the mean.

| *Concentration (% w/w)* | *Proliferative response (Median DPM/animal)* | *Stimulation Index (Test/Control Ratio)* |
|---|---|---|
| *Test Substance* | | |
| 0 (vehicle control) | 1328 | |
| 25 | 2172 | 1.64 |
| 50 | 2302 | 1.73 |
| 100 | 3563 | 2.68 |

Remarks - Results There were no deaths and no signs of systemic toxicity were noted in the test or control animals.
Body weight changes of the test animals were comparable to those seen in the control animals.
There was a large outlier present in the vehicle control, which had a disintegrations per minute (dpm) value of 7158. The other 4 animals in the vehicle control had dpm values of 853, 1328, 1454 and 851. The data was therefore recalculated using the medians rather than the means in order to minimise the effect of the single outlier.
A stimulation index of less than 3 was observed for all concentrations of the test material.

| | |
|---|---|
| CONCLUSION | There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical. |
| TEST FACILITY | Safepharm Laboratories (2007g) |

**B.6. Repeat dose toxicity**

| | |
|---|---|
| TEST SUBSTANCE | Notified chemical |
| METHOD | OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.<br>EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral). |
| Species/Strain | Sprague-Dawley Crl:CD® (SD) IGS BR strain rats |
| Route of Administration | Oral – gavage |
| Exposure Information | Total exposure days: 28 days |
| Vehicle | Arachis oil BP |
| Remarks - Method | Two recovery groups, each of five males and five females, were treated with the high dose (750 mg/kg/day) or the vehicle alone for 28 consecutive days and then maintained without treatment for a further 14 days. |

RESULTS

| *Group* | *Number and Sex of Animals* | *Dose mg/kg bw/day* | *Mortality* |
|---|---|---|---|
| control | 5 per sex | 0 | 0 |
| low dose | 5 per sex | 25 | 0 |
| mid dose | 5 per sex | 150 | 0 |
| high dose | 5 per sex | 750 | 0 |
| control recovery | 5 per sex | 0 | 0 |
| high dose recovery | 5 per sex | 750 | 0 |

*Mortality and Time to Death*

There were no unscheduled deaths during the study.

*Clinical Observations*

Incidental findings of increased salvation, generalised red/brown fur staining, fur loss and hunched posture were noted during the study. These isolated, incidental external changes were considered of no toxicological importance.

*Behavioural Assessment* – One female in the non-recovery high dose group showed hunched during weeks 3 and 4. In the absence of any supporting clinical observations to suggest an effect of neurotoxicity the finding is considered to be of no toxicological importance.

*Functional Performance Tests* - There were no toxicologically significant changes in the functional performance parameters measured.

*Sensory Reactivity Assessments* - There were no treatment-related changes in sensory reactivity.

*Bodyweight* – Significant increases in bodyweight were seen in male animals in the low dose group and female animals in the non-recovery high dose group. These increases in bodyweight are of no toxicological significance.

*Food Consumption* – There was no effect on food consumption or food efficiency throughout the course of the study in comparison to the controls.

*Water Consumption* - An increase in water consumption was detected for non-recovery males in the high dose group from day 15 onwards when compared to controls.

*Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis*

*Haematology* – Non-recovery males treated in the high dose group showed an increase in clotting time, platelet count and activated partial thromboplastin time. Male animals in the high dose recovery group showed a decrease in the clotting time in comparison to the clotting time for the recovery control group. Male animals in the mid dose group showed a decreased total leucocyte count and differential leucocyte count (lymphocytes).

*Blood Chemistry* – Non-recovery animals of either sex in the high dose group showed an increase in plasma cholesterol levels. This effect was also seen in the mid dose males and high dose recovery females.
An increase in the albumin/globulin ratio was seen in all the male non-recovery dose groups and a decrease was seen in all the female non-recovery dose groups.
Non-recovery males in the mid and high dose groups showed a decrease in triglycerides and glucose levels and an increase in alanine aminotransferase. A decrease in creatine levels was also seen in non-recovery high dose males only.
In non-recovery high dose females an increase in the total protein was seen as well as decreases in the glucose and chloride levels. A decrease in glucose levels was also seen in mid dose females.
An increase in inorganic phosphorus was seen in the male high dose recovery group. In the female high dose recovery group a decrease in chloride levels and an increase in triglycerides levels was also seen.

*Urinalysis*
Females from all treatment groups showed an increase in reducing agents present in the urine. Males in the high dose groups had an increase in urine volume together with a reduction in the specific gravity. No effects were detected in the recovery animals following 14 days without treatment.

*Effects in Organs*
Non-recovery animals of either sex in the mid and high dose groups showed an absolute and relative increase in liver weights. High dose females in the recovery group also showed an absolute and relative increase in liver weights.
In non-recovery male animals in the high dose group an increase in the weight of the epididymides, kidneys and testes was seen. In the high dose recovery groups the males showed a decrease in the weight of the adrenals and the female animals an increase in the weight of the liver and spleen.
There were no adverse findings noted at necroscopy.

*Liver* – Centrilobular hepatocyte enlargement was observed in the low, mid and high dose groups for both sexes, with greater severity seen in the high dose groups. There was also evidence of regression of the condition among the high dose recovery group.

*Thyroid* - Follicular cell hypertrophy was observed in relation to treatment for animals of either sex in the mid and high dose groups and male animals in the low dose group. There was evidence of regression of the condition among the high dose recovery group.

Remarks – Results
The treatment related effects were limited to microscopic liver and thyroid changes, with associated liver weight gains and a slightly altered metabolism. There were no associated inflammatory or degenerative changes in the organs affected, and so the effects are considered to be an adaptive response to the high doses of xenobiotics.

CONCLUSION
Oral administration of the test material to rats for a period of 28 consecutive days at dose levels of 750, 150 and 25 mg/kg/day resulted in treatment related effects at all dose levels and therefore a No Observed Effect Level (NOEL) could not be set.

The treatment related effects observed were primarily related to adaptive changes in the liver or were of no toxicological significance. Therefore, the No Observed (Adverse) Effect Level (NO(A)EL) was established as 750 mg/kg bw/day in this study.

TEST FACILITY Safepharm Laboratories (2007h)

### B.7. Genotoxicity – bacteria

| | |
|---|---|
| TEST SUBSTANCE | Notified chemical |
| METHOD | OECD TG 471 Bacterial Reverse Mutation Test.<br>EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.<br>Plate incorporation procedure |
| Species/Strain | *S. typhimurium*: TA1535, TA1537, TA98, TA100<br>*E. coli*: WP2uvrA$^-$ |
| Metabolic Activation System | Rat S9 fraction from phenobarbitone/β-napthoflavone induced rat liver |
| Concentration Range in Main Test | a) With metabolic activation: 50 – 5000 µg/plate<br>b) Without metabolic activation: 50 – 5000 µg/plate |
| Vehicle | Dimethyl sulphoxide, test substance added as solution |
| Remarks - Method | No signs of toxicity were recorded in the preliminary test. |

RESULTS

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

| | |
|---|---|
| Remarks - Results | The test material was tested up to the maximum recommended dose level of 5000 µg/plate. No toxicologically significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation.<br><br>All the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains. |
| CONCLUSION | The notified chemical was not mutagenic to bacteria under the conditions of the test. |
| TEST FACILITY | Safepharm Laboratories (2007i) |

### B.8. Genotoxicity – in vitro

| | |
|---|---|
| TEST SUBSTANCE | Notified chemical |
| METHOD | OECD TG 473 In vitro Mammalian Chromosome Aberration Test.<br>EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test. |
| Cell Type/Cell Line | Chinese Hamster Lung |
| Metabolic Activation System | Rat S9 fraction from phenobarbitone/β-napthoflavone induced rat liver |
| Vehicle | Dimethyl sulphoxide |
| Remarks - Method | No significant protocol deviations. GLP compliant.<br>No adjustment for the purity of the notified chemical was made.<br>In test 1 the S9 mix was present at 5% of the final concentration, while in test 2 it was present at 2% of the final concentration. |

| *Metabolic Activation* | *Test Substance Concentration (µg/mL)* | *Exposure Period* | *Harvest Time* |
|---|---|---|---|
| *Absent* | | | |
| Test 1 | 0*, 3.75, 7.5*, 15*, 22.5*, 30, 45 | 6 hours | 24 hours |
| Test 2 | 0*, 1.88, 3.75, 7.5, 15*, 22.5*, 30* | 24 hours | 24 hours |
| *Present* | | | |
| Test 1 | 0*, 60.94, 121.88, 243.75, 487.5*, 975*, 1950* | 6 hours | 24 hours |
| Test 2 | 0*, 30, 60*, 120*, 240*, 360, 480 | 6 hours | 24 hours |

*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 | 30.47 | > 22.5 | > 22.5 | negative |
| Test 2 | 32 | 22.5 | > 30 | negative |
| *Present* | | | | |
| Test 1 | 1950 | 975 | > 1950 | negative |
| Test 2 | | 240 | 120 | negative |

Remarks - Results

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

The precipitate was seen to aggregate at 3900 µg/mL.

The test material did not induce any statistically significant increases in the frequency of cells with aberrations, or in the numbers of polyploid cells.

CONCLUSION

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

TEST FACILITY

Safepharm Laboratories (2007j)

# APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

## C.1. Environmental Fate

### C.1.1. Ready biodegradability

| | |
|---|---|
| TEST SUBSTANCE | Notified chemical |
| METHOD | Japanese Guideline: Biodegradability Test of Chemical Substances by Microorganisms |
| Inoculum | Activated sludge (mixed liquor suspended solid 3450 mg/L). |
| Exposure Period | 28 days |
| Auxiliary Solvent | None |
| Analytical Monitoring | BOD, DOC, analysis of residual test substance. |
| Remarks - Method | DOC measurements did not allow estimation of degradability as the test substance was insoluble in water. |
| RESULTS | There was no degradation of the test substance based on BOD. Measurement of residual test material achieved 97% recovery. |
| Remarks - Results | Primary degradation was confirmed by identification of the expected transformation product. |
| CONCLUSION | The notified chemical is not readily biodegradable. |
| TEST FACILITY | Mitsubishi (2008). |

### C.1.2. Bioaccumulation

Bioaccumulation was not tested as the strong hydrophobicity and high partition coefficient of the notified chemical are considered to preclude significant bioaccumulation by minimising bioavailability.

## 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. |
| Species | Rainbow trout |
| Exposure Period | 96 hours |
| Auxiliary Solvent | None |
| Water Hardness | 140 mg $CaCO_3$/L |
| Analytical Monitoring | HPLC (LOQ 0.000067 mg/L) |
| Remarks – Method | Fish were exposed to the WAF obtained by mid-depth siphon from stirred aqueous suspensions. The WAF remained clear and colourless throughout the exposure period. Analysis of test concentrations returned variable results, probably reflecting varying proportions of undissolved test material. Measured concentrations declined in the 24 hours between renewal of the test media, consistent with preliminary observations that the notified chemical was unstable in the light and dark at ambient temperatures. |
| RESULTS | All fish survived. |

| *Concentration mg/L* | *Number of Fish* | *Mortality* |
|---|---|---|

| *Nominal* | *Actual* | | *1 h* | *24 h* | *48 h* | *72 h* | *96 h* |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 |
| WAF | 0.0011 | 10 | 0 | 0 | 0 | 0 | 0 |
| WAF | 0.0011 | 10 | 0 | 0 | 0 | 0 | 0 |

LC50 > 0.0011 mg/L at 96 hours (time weighted mean measured concentration).
NOEC (or LOEC) 0.0011 mg/L at 96 hours.

CONCLUSION Nontoxic to fish to the limit of water solubility, as modulated by the instability of the notified chemical.

TEST FACILITY SafePharm Laboratories (2008a).

### C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical.

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - static.
Species *Daphnia magna*
Exposure Period 48 hours
Auxiliary Solvent None
Water Hardness 250 mg $CaCO_3$/L
Analytical Monitoring HPLC (LOQ 0.000067 mg/L)
Remarks – Method Daphnids were exposed to the WAF obtained by mid-depth siphon from stirred aqueous suspensions. The WAF remained clear and colourless throughout the exposure period. Analysis of test concentrations returned variable results, probably reflecting varying proportions of undissolved test material. Measured concentrations declined over the test period, consistent with preliminary observations that the notified chemical was unstable in the light and dark at ambient temperatures, and with its strongly hydrophobic nature.

RESULTS None of the twenty exposed daphnids were immobilised.

| *Concentration mg/L* | | *Number of D. magna* | *Number Immobilised* | |
|---|---|---|---|---|
| *Nominal* | *Actual* | | *24 h* | *48 h* |
| 0 | 0 | 20 | 0 | 0 |
| WAF | 0.0082 | 20 | 0 | 0 |

LC50 > 0.0082 mg/L at 48 hours (geometric mean measured concentration)
NOEC (or LOEC) 0.0082 mg/L at 48 hours
Remarks – Results Results with the reference substance potassium dichromate were within the normal range.

CONCLUSION Nontoxic to daphnids to the limit of water solubility, as modulated by the instability of the notified chemical.

TEST FACILITY SafePharm Laboratories (2008b)

### C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical.

METHOD OECD TG 201 Alga, Growth Inhibition Test.
Species *Desmodesmus subspicatus*
Exposure Period 72 hours

| | |
|---|---|
| Concentration Range | Nominal: 6.25-100 mg/L<br>Actual: 0.000087-0.00018 mg/L (geometric mean measured concentrations) |
| Auxiliary Solvent | None |
| Analytical Monitoring | HPLC (LOQ 0.00011 mg/L) |
| Remarks - Method | Algae were exposed to the WAF obtained by mid-depth siphon from stirred aqueous suspensions followed by centrifugation. The WAF was clear and colourless. Measured concentrations declined below the LOQ over the test period, consistent with preliminary observations that the notified chemical was unstable in the culture medium. |
| RESULTS | The algal response at the highest test concentration (0.00018 mg/L) was significantly different from that at lower concentrations. Growth was inhibited by 13%, and biomass by 19%. The NOECs for growth and biomass were 0.00014 mg/L. |

| *Growth* | | *Biomass* | |
|---|---|---|---|
| *EC50* | *NOEC* | *EC50* | *NOEC* |
| *> 0.0018 mg/L at 72 h* | *0.0014 mg/L* | *> 0.0018 mg/L at 72 h* | *0.0014 mg/L* |

| | |
|---|---|
| Remarks - Results | Cell concentrations in controls increased by a factor of 46, satisfying the validity criterion of a 16 fold increase. Results with the reference substance potassium dichromate were within the normal range. |
| CONCLUSION | Nontoxic to green algae to the limit of water solubility, as modulated by the instability of the notified chemical. |
| TEST FACILITY | SafePharm Laboratories (2008c) |

**C.2.4. Inhibition of microbial activity**

| | |
|---|---|
| TEST SUBSTANCE | Notified chemical |
| METHOD | OECD TG 209 Activated Sludge, Respiration Inhibition Test. |
| Inoculum | Activated sludge from UK sewage treatment plant handling predominantly domestic sewage. |
| Exposure Period | 3 hours |
| Concentration Range | Nominal: 1000 mg/L<br>Actual: not measured. |
| Remarks – Method | Oily globules of the test substance were observed on the surface and throughout the test medium. |
| RESULTS | |
| IC50 | > 1000 mg/L |
| NOEC | 1000 mg/L |
| Remarks – Results | Oxygen consumption at 3 hours varied by only 1% between test flasks and controls. Results with the reference substance potassium dichromate were within the normal range. |
| CONCLUSION | The notified chemical does not inhibit the respiration of activated sludge. |
| TEST FACILITY | SafePharm Laboratories (2007k) |

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