File No STD/1096

28 May 2004

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Two Eyed Musk

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Director
Chemicals Notification and Assessment

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
International Flavours and Fragrances Australia Ltd (ABN: 77 004 269 658)
301 Frankston-Dandenong Rd
DANDENONG SOUTH VIC 3175

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
No details are claimed exempt from publication.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
LVC Permit No 562

NOTIFICATION IN OTHER COUNTRIES
USA, EC- Spain (2003 & 2004), Canada (2003), Philippines (2003).

2. IDENTITY OF CHEMICAL

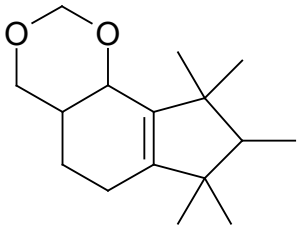
CHEMICAL NAME
Indeno[4,5,D]-1,3-dioxin, 4,4a,5,6,7,8,9,9b-octahydro-7,7,8,9,9-pentamethyl-

MARKETING NAME(S)
Two Eyed Musk

CAS NUMBER
365411-50-3

MOLECULAR FORMULA
$C_{16}H_{26}O_2$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
250.38

SPECTRAL DATA

ANALYTICAL METHOD	NMR, UV, IR Spectroscopy
Remarks	^{1}H NMR Peaks 0.83-1.03 ppm, 1.34-1.37 ppm, 1.52 ppm, 1.60-1.65 ppm, 1.91-1.98 ppm, 2.07-2.30 ppm, 3.95 ppm, 4.00 ppm, 4.12 ppm, 4.76 ppm, 5.02 ppm. UV Neutral pH (pH 7) λ_{max} = 203 nm ε_{max}=12,121 mol/L/cm Acid pH (pH ~2-3) λ_{max} = 203 nm ε_{max}=13,129 mol/L/cm Basic pH (pH~9-10) λ_{max} = 204 nm ε_{max}=7941 mol/L/cm IR Peaks at: 2953, 2872, 2837, 2742, 1470, 1455, 1388, 1362, 1172, 1142, 1090, 1078, 1039, 1021, 987, 915 cm^{-1} 2953, 2872 cm^{-1} (C-H stretching, alkane) 1172, 1142, 1039 cm^{-1} (C-O stretching, ether, aliphatic) 1388, 1362 cm^{-1} (C-H bending, alkane, gem-dimethyl)

METHODS OF DETECTION AND DETERMINATION

ANALYTICAL METHOD	IR spectroscopy and Gas Chromatography

3. COMPOSITION

DEGREE OF PURITY
98%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (>1% by weight)
None

ADDITIVES/ADJUVANTS
None

4. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as part of a finished fragrance oil (10% maximum, typically 4%) in sealed, polypropylene lined steel drums (205 L).

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

USE
Two-Eyed Musk will be used as an odourant in alcoholic perfumery, cosmetics, toiletries, household products, soaps, and detergents. The maximum concentration of Two-Eyed Musk in the imported fragrance oil will be 10%. The resulting concentration of Two-Eyed Musk in end-user consumer products is 0.01-0.8%.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, transport and storage

PORT OF ENTRY
Melbourne

IDENTITY OF MANUFACTURER/RECIPIENTS
International Flavours and Fragrances (Australia), Pty Ltd. (IFF)

TRANSPORTATION AND PACKAGING
Two-Eyed Musk will be imported into Australia as a component of finished fragrance oils in sealed, polypropylene lined steel drums (205 L) or as a component of a finished consumer products in standard consumer packagings. Two-Eyed Musk will be transported from the docks by road to the notifiers warehouse. The finished fragrance oil will then be transported to customers, typically by road, when needed. The finished consumer product will be transported to retails stores for distribution.

5.2. Operation description
The drummed fragrance oil will be used in the cosmetic industry for production of toiletries, shampoos, soap and household cleaning agents and detergents (containing < 0.8% Two-Eyed Musk) following mixing with other ingredients. The production process mainly involving a blending operation will be highly automated and will occur in a fully enclosed environment. Plant operators will only be involved in opening and closing drums, weighing and charging the mixing vessel, and cleaning and maintenance tasks. Waste will generally be disposed of by incineration or through a wastewater treatment plant prior to release to the environment.

5.3. Occupational exposure
Number and Category of Workers

Category of Worker	*Number*	*Exposure Duration*	*Exposure Frequency*
Transport workers	5	Not provided	Not provided
Mixer	5	4 hr/day	2 days/year
Drum handling	5	4 hr/day	2 days/year
Drum cleaning/washing	10	4 hr/day	2 days/year
Maintenance	5	4 hr/day	2 days/year
Quality control worker	2	0.5 hr/day	2 days/year
Packager	10	4 hr/day	2 days/year

Exposure Details

The number and category of workers will vary depending on the nature of the customers' business. However, it is anticipated that the notified chemical will be handled according to typical practices by cosmetic and consumer product manufacturers, including the use of adequate local ventilation, appropriate PPE, enclosed mixing vessel and filling areas as well as a high degree of process automation to protect workers.

Transport and warehouse

At the IFF facility, transport and warehouse workers will be exposed to the 10% fragrance oil only in the event of a spill due to an accident or leaking drum. Workers will wear protective overalls, hard hats, chemical resistant gloves and safety glasses.

Formulation workers

Most customer facilities (cosmetic and consumer product manufacturers) are expected to have fully automated systems with a few facilities not being fully automated. Exposure is possible during handling of the drums, pumping the formulation into mixing tanks, quality control testing, packaging and cleaning and maintenance of the equipment. Skin, inhalation, and eye contact (due to splashing) are likely to be the main routes of exposure. Workers will observe good personal hygiene practices and use industrial standard PPE such as coveralls, gloves, and safety glasses. The plant will have adequate ventilation and self-contained breathing apparatus if required. The production process will be in compliance with good manufacturing practices, including the availability of eyewash fountains and/or safety showers in the vicinity of the blending areas. Only workers qualified and trained in the safety of working with chemicals and chemical mixtures will be permitted to handle the Two-Eyed Musk mixtures.

5.4. Release

RELEASE OF CHEMICAL AT SITE

Since the notified polymer will not be manufactured locally, there will be no environmental exposure associated with this process in Australia. Environmental release of the notified substance is unlikely during importation, storage and transportation, and an accidental spill/leak is the most likely reason for environmental release. The size of the containers (205 L steel drums) and the concentration of the notified chemical (<10%) will limit the impact on the environment of such incidents.

Little material is expected to be lost during the formulation into the consumer products since the processes are mainly automated. Cleaning the blending equipment (automated mixing tank and filling machines) following each batch may generate wastewaters containing the notified chemical. The quantity of notified chemical remaining in wash water may approximate 1% of the import volume (5 kg/year). The disposal route for these wastewaters may include disposal to on-site wastewater treatment plants and/or sewer. Up to 5 kg of the notified chemical is expected to be left as residue in emptied import containers. The disposal route for container rinsate may include disposal to on-site wastewater treatment plants and/or sewer.

RELEASE OF CHEMICAL FROM USE

Since the notified chemical will be used in household, laundry and personal cleaning products perfumery and cosmetics, almost all (~97%) of Two-Eyed Musk will end up in the sewer. Approximately 5 kg of the notified chemical imported is expected to be lost as residues in consumer product containers, which are primarily sent to landfill.

5.5. Disposal

Spilled or leaked material should be collected using absorbent material into sealed container and dispose of in accordance with current applicable laws and regulations. Emptied imported drums containing residual quantities of the notified chemical mixture may be rinsed and re-used, sent to a metal recycler, or sent to a landfill for disposal. Drum rinsate will be discharged to on-site wastewater treatment plant and/or sewer. Following use, emptied consumer product containers are expected to be disposed of through domestic garbage disposal and then to landfill or a recycling program.

5.6. Public exposure

The notified chemical will be formulated into cosmetics and household products with concentration ranging from 0.01% to 0.32%. These products include body lotions, creams, suncreams and lotions;

hairsprays, shampoos, dishwashing liquid, fabric washing liquids, surface cleaners, deodorants sprays, air fresheners, bath products, shower gels, and toilet waters. Therefore general public may be repeatedly exposed to low-levels of Two-Eyed Musk via a number of different consumer products.

6. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20ºC and 101.3 kPa Colourless solid with a musky odour.

Melting Point/Freezing Point 38-46ºC

METHOD EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks Capillary Method
TEST FACILITY SafePharm Laboratories (2003a)

Boiling Point 293ºC at 101.92 kPa

METHOD EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks Differential Scanning Calorimetry
TEST FACILITY SafePharm Laboratories (2003a)

Density 1070 kg/m^3 at 22ºC

METHOD EC Directive 92/69/EEC A.3 Relative Density.
Remarks Gas Comparison Pycnometer
TEST FACILITY SafePharm Laboratories (2003a)

Vapour Pressure 3.6 X 10^{-4} kPa at 25ºC

METHOD EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks The vapour pressure was determined using a vapour pressure balance system with the temperature of the sample was controlled electronically. A sequence of runs was started after a sample of notified chemical had been under vacuum for approximately 19 minutes. Temperature and pressure readings were taken between 15ºC and 30ºC. Linear regression was used to calculate the vapour pressure at 25ºC. The result indicates that the notified chemical is moderately volatile (Mensink e*t al*. 1995).

The Henry's Law constant (H) was calculated from the molecular weight, the measured water solubility, and the measured vapour pressure according to the following equation: H = MW (g/mol) X Vapour Pressure (Pa)/Water Solubility (mg/L). H = 6.78 Pa m^3/mol, indicates that the substance is moderately volatile from water or moist soil (Mensink e*t al*. 1995).
TEST FACILITY SafePharm Laboratories (2003b)

Water Solubility 1.33 X 10^{-2}g/L at 20±0.5 °C

METHOD EC Directive 92/69/EEC A.6 Water Solubility (Flask method).
Remarks A preliminary test result indicated a water solubility of 9.79 X 10^{-3} g/L. The definitive test was conducted by diluting aliquots of notified chemical with glass double-distilled water; shaking (30°C) for 5 h, and allowing to stand (20°C) for not less than 24 hours. The solution was centrifuged (10,000 rpm for 10 mins) and the supernatant analysed by GC.

Based on Mensink e*t al*. (1995) the test results show that the notified chemical is moderately soluble (marginally above the slightly soluble class).
TEST FACILITY SafePharm Laboratories (2003a)

Fat Solubility Miscible in all proportions with standard fat at 37 ± 0.5°C

METHOD OECD TG 116 Fat Solubility of Solid and Liquid Substances (Modified).

Remarks The standard OECD method (TG 116) was not suitable for direct use with this notified chemical as it showed no upper limit for saturation in standard fat. Therefore, a modified procedure was used to illustrate the complete miscibility of the notified chemical with standard fat.

Mixtures of notified chemical (scraped from block as waxy flakes) and liquefied and mixed standard fat were prepared and shaken (at 37 ± 0.5°C for 3 hours) and phases observed for miscibility. Only a single phase was observed.

TEST FACILITY SafePharm Laboratories (2003c)

Hydrolysis as a Function of pH The half-life at 25ºC is 34 days at pH 4, and greater than one year at pH 7 and 9.

METHOD EC Directive 92/69/EEC C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*Rate Constant*	*Estimated half-life 25 ºC*
4	$2.35X10^{-7}$	34.1 days
7	-	>1 year
9	-	>1 year

Remarks Sample solutions were prepared at a nominal concentration of $5.00 X 10^{-3}$ g/L in 3 buffered (filtered) solutions. A 1% co-solvent of acetonitrile was used to aid solubility. Solutions were subject to ultrasonication and degassing with nitrogen to minimise dissolved oxygen content. Sample solutions at pH 4, 7, and 9 were maintained in the dark at 50 ± 0.5°C. Testing was also performed at pH 4 with solutions maintained at 60 and 70 ± 0.5°C for 48 hours. Solution concentrations were determined by GC.

TEST FACILITY SafePharm Laboratories (2003a)

Partition Coefficient (n-octanol/water) Log10 Pow = 4.29 at 22.0 ± 0.5 °C

METHOD EC Directive 92/69/EEC A.8 Partition Coefficient (Shake Flask Method).

Remarks Six partitions were performed with stock solution mixed with six different volumes of n-octanol-saturated water at 22°C. Each solution was mixed for 5 mins and, after separation, aliquots of both phases were centrifuged. Organic phase samples were diluted methanol prior to analysis by GC.

Based on the hydrolysis study results (reported above) and the pH of the notified chemical in water the report indicated that negligible hydrolysis of sample solutions might have occurred during this study.

The high P_{ow} value is consistent with the low water solubility, indicating a high affinity for the organic component of soils and sediments.

TEST FACILITY SafePharm Laboratories (2003a).

Adsorption/Desorption Log K_{oc} = 3.78-4.44

METHOD Commission Directive 2001/59/EC C.19 HPLC screening method.

Remarks A sample solution was prepared in methanol. The HPLC dead time was determined by measuring the retention time of formamide. Solutions of 12 reference standards were also prepared in methanol. The sample, formamide and reference standard solutions were injected into the HPLC in duplicate.

According to the K_{oc} range (6026-27,542), the notified chemical is immobile (Mensink et al. 1995).

TEST FACILITY SafePharm Laboratories (2003a).

Dissociation Constant Not determined.

Remarks The notified chemical is expected to be stable in water and is not subject to dissociation, as it does not contain any dissociable group.

Particle Size Not determined

Remarks Imported as liquid and/or low melting solid at room temperature.

Surface Tension 62.2 mN/m at 21.0 ± 0.5°C for an 8.17 X 10^{-3} g/L solution).

METHOD EC Directive 92/69/EEC A.5 Surface Tension (Modified)
Remarks The determination was carried out using a White Electrical Institute Interfacial Tension Balance and a procedure based on the ISO 304 Ring Method, which complied with the above method except in that the surface tension result was not corrected using the Harkins-Jordan correction table as the correction is not applicable to the apparatus used. This deviation is not considered to have affected the integrity of the study. Negligible hydrolysis of the sample solution may have occurred during the study.

The notified chemical is not considered to be a surface-active substance.
TEST FACILITY SafePharm Laboratories (2003a)

Flash Point 141°C at 101.3 kPa

METHOD EC Directive 92/69/EEC A.9 Flash Point.
TEST FACILITY SafePharm Laboratories (2003b)

Flammability Limits Not highly flammable

METHOD EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks The notified chemical melted then ignited and propagated combustion over 200 mm in 4 minutes and 51 seconds in the preliminary test.
TEST FACILITY SafePharm Laboratories (2003b)

Autoignition Temperature 334 ± 5°C

METHOD 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).
TEST FACILITY SafePharm Laboratories (2003b)

Explosive Properties Predicted to be negative.

METHOD Predicted using EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks There are no chemical groups that would imply explosive properties, therefore the result has been predicted negative (SafePharm Laboratories (2003b). Based on an assessment of the chemical structure and the oxygen balance using the following calculation, the explosivity of Two-Eyed Musk was predicted to be negative by the notifier. Oxygen balance = [-1600(2X +Y/2-Z)]/MW = -275 where X = number of carbon atoms, Y = number of hydrogen atoms, Z = number of oxygen atoms and MW = the molecular weight.

Reactivity

Remarks Two-Eyed Musk is expected to be stable in water and air under normal conditions of temperature and pressure.

7. TOXICOLOGICAL INVESTIGATIONS

Endpoint and Result	*Assessment Conclusion*
Rat, acute oral LD50 >2500 mg/kg bw	low toxicity
Rat, acute dermal LD50 >2000 mg/kg bw	low toxicity
Rabbit, skin irritation	slightly irritating

Rabbit, eye irritation	slightly irritating
Mouse, Local Lymph Node Assay	evidence of sensitisation.
Rat, repeat dose oral toxicity – 28 days.	NOEL= 15 mg/kg bw/day
Genotoxicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro chromosomal aberration	non genotoxic
Genotoxicity – in vivo mouse micronuclei test	non genotoxic
Repeat Human Insult Patch test	No evidence of irritation nor sensitisation

7.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Sprague-Dawley CD
Vehicle	Arachis oil BP
Remarks - Method	No significant protocol deviations.

RESULTS

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

LD50	>2500 mg/kg bw
Signs of Toxicity	There were no signs of systemic toxicity. All animals showed expected gains in bodyweight over the study period.
Effects in Organs	No abnormalities were noted at necroscopy.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	SafePharm Laboratories (2003d)

7.2. 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) – Limit Test.
Species/Strain	Rat/Sprague-Dawley CD
Vehicle	None
Type of dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations.

RESULTS

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

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	There were no dermal reactions observed.
Signs of Toxicity - Systemic	There were no signs of systemic toxicity. All animals showed expected gains in bodyweight over the study period.
Effects in Organs	No abnormalities were noted at necroscopy.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.

TEST FACILITY Safepharm Laboratories (2003e)

7.3. Irritation – skin

TEST SUBSTANCE Notified chemical

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

Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	None
Observation Period	14 days
Type of Dressing	Semi-occlusive.
Remarks - Method	0.5 mL of the molten notified chemical was introduced under cotton gauze and placed in position on the shorn skin. For one animal separate patches were used for exposure times of 3 minutes, 1 hour and 4 hours. The results of the 4 hour patch are discussed below.

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	1.3	1.3	2	72 hours	0
Oedema	1	1.3	1	2	72 hours	0

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

Remarks - Results

At 1 hour and 24 hours well defined erythema was noted at all treated skin sites. Very slight or well-defined erythema was observed at all treated skin sites at 48 and 72 hours.

Very slight or slight oedema was noted at all treated skin sites at 1 hour and at 24 hours. Very slight oedema was noted at all treated skin sites at forty-eight hours and seventy-two hours.

Loss of skin elasticity was noted at one treated skin site at the 48 hours and all treated sites at 72 hours. Crust formation was noted at one treated skin site and severe desquamation was noted at two treated skin sites at 7 days.
Oedema and erythema had resolved by 7 days and all treated skin sites appeared normal at 14 days.

Similar results were seen for the 1 hour and 4 hour patches; 3 minute exposure did not result in observable irritation.

CONCLUSION The notified chemical is slightly irritating to skin.

TEST FACILITY Safepharm Laboratories (2003f)

7.4. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).

Species/Strain	Rabbit/New Zealand White

Number of Animals	3
Observation Period	72 hours
Remarks - Method	The notified chemical was melted in a 60°C water bath prior to instillation.

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	0.33	0.33	0.33	2	24 hours	0
Conjunctiva: chemosis	0	0	0.33	2	24 hours	0
Conjunctiva: discharge	0.33	0	0.33	1	24 hours	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	No corneal or iridial inflammation was observed during the study. Moderate conjunctival irritation was observed in all treated eyes at one hour with minimal conjunctival irritation in all treated eyes at 24 hours. All treated eyes appeared normal at 48 hours.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	Safepharm Laboratories (2003g)

7.5 Skin Sensitisation

7.5.1 Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay.
Species/Strain	Mouse/CBA/Ca
Vehicle	Acetone/olive oil 4:1
Remarks - Method	No significant deviations in protocol.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	1740.02	1
10	2325.24	1.34
25	5515.14	3.17
50	9077.71	5.22
Positive Control		
5	Not given	2.8
10	Not given	2.3
5.5	Not given	5.5

Remarks - Results	There were no deaths during the study. No signs of systemic toxicity were observed in the test or control animals during the study. The body weight changes of the test animals between Day 0 and Day 5 were comparable to those observed in the corresponding control group animals over the same period.

CONCLUSION	There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	Safepharm Laboratories (2003h)

7.5.2. Skin sensitisation – human volunteers

TEST SUBSTANCE	Notified chemical
METHOD	Patch test – repeated continuous induction with rest periods
Study Design	The study was conducted as single phase. No pilot phase was undertaken.
Study Group	79 males, 23 females; age range 18-69
Vehicle	Alcohol SD39C or [75:25] Alcohol SD39C:Diethylphthalate
Induction Procedure	Nine repeat, 24 hour applications of the notified chemical at 4% in vehicle under occluded patch conditions at three applications for 3 weeks to the upper back (between the scapulae).
Rest Period	14 days
Challenge Procedure	Challenge patches were applied to previously untreated sites on the back. After 24 hours, the patches were removed and sites were evaluated for dermal reactions. The test sites were re-evaluated at 48 hours and 72 hours.
Remarks - Method	No significant protocol deviations
RESULTS	
Remarks - Results	Two subjects discontinued study participation for reasons unrelated to the notified chemicals. One hundred subjects completed the study. One panellist had a barely perceptible reaction in the 8th and 9th inductions with the distilled water control; no responses to the notified chemical were reported.
CONCLUSION	A human repeat insult test was conducted using the notified chemical diluted with alcohol or [75:25] alcohol:diethylphthalate to 4% under occlusive dressing. The notified chemical was non-irritating and non-sensitising under the conditions of the test.
TEST FACILITY	Clinical Research Laboratories (2003).

7.6. 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	Rat/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	Doses selected on the basis of a 14 day preliminary study.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
I (control)	5/sex	0	0

II (low dose)	5/sex	15	0
III (mid dose)	5/sex	150	0
IV (high dose)	5/sex	1000	0
V (control recovery)	5/sex	0	0
VI (high dose recovery)	5/sex	1000	0

Mortality and Time to Death

There were no deaths observed during the study.

Clinical Observations

Increased salivation was observed around the time of dosing in both males and females treated at 1000 mg/kg bw/day from Day 6. As the study progressed, excessive salivation was detected one hour after dosing, noisy respiration, and wet/soiled fur. In the final week of treatment, hunched posture and tiptoe gait were observed in females treated at the high dose. The tiptoe gait resolved in the high dose recovery animals, at the cessation of treatment, while the hunched posture resolved by Day 33.

All the remaining inter and intra group differences in behavioural scores were considered to be a result of normal variation for rats of the strain and age used and were, therefore, of no toxicological significance.

No treatment related changes were detected in the functional performance parameters measured and the sensory reactivity. All inter and intra group differences in sensory reactivity scores were considered to be a result of normal variation for rats of the strain and age used and were not of toxicological significance. Statistical analysis of both functional performance and sensory reactivity quantitative revealed no significance intergroup differences.

No treatment related clinical observations were detected in animals treated at 15 or 150 mg/kg bw/day.

There were no treatment related effects on body weight. Test animals showed weight gains similar to those of the controls throughout the study. Recovery high dose males showed a slight but statistically significant increase ($p<0.05$) in body weight gain during Week 6, of the study.

There was no adverse effect on food consumption throughout the study. Food efficiency in the test animals was similar to that of the controls. Daily visual inspection of water bottles, as indication of water consumption, throughout the study period revealed no intergroup difference.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology

No treatment related effects were detected in the haematological parameters measured. Statistical analysis revealed no significant intergroup differences.

Blood Chemistry

Males and females treated at the high dose showed statistically significant increases in plasma cholesterol concentrations (males; $p<0.01$, females; $p<0.001$) and bilirubin (males; $p<0.05$, females; $p<0.01$), this was accompanied in high dose males only by a slight increase ($p<0.05$) in alanine aminotransferase. It is possible that a slight alteration in liver function could have resulted in these change, however individual values for the parameters were invariably with the normal range and there was no evidence of degenerative or obstructive liver changes.

Females treated at high dose showed increased plasma levels of creatinine, calcium (both $p<0.05$) and inorganic phosphorous ($p<0.01$) with reductions in potassium ($p<0.01$) and chloride ($p<0.05$) compared to controls. In the absence of histological evidence of a change in renal function or similar changes in males, these changes were considered not be toxicological significant.

Females treated at high dose showed an increase in total protein ($p<0.01$) and reduction in plasma glucose ($p<0.05$) and an apparent reduction in the albumin/globulin ratio ($p<0.05$). The individual values for glucose were within the normal range and the intergroup difference was considered acceptable. The protein change indicated an elevation in globin levels and in the absence of any associated effects indicative of injection or inflammatory changes, this was not considered toxicological significant.

A statistically significant reduction ($p<0.01$) in aspartate aminotransferase was detected in females treated with

1000 mg/kg bw/day in comparison with the controls. However a reduction in this parameter is unlikely to be associated with a chemical injury and hence the finding may not be of toxicological significance.

Recovery high dose females showed a minimal increase in calcium ($p<0.05$) and cholesterol ($p<0.05$) levels compared to the controls. However, in the absence of histological evidence of associated histopathological changes such as renal function or bile duct proliferation, these findings were considered not to be of toxicological significance.

Urinalysis
No toxicologically significant abnormalities were detected in any of the urinalysis parameters measured. Males and females treated at the high dose micturated an increase volume of urine ($p<0.05$) of low specific gravity that achieved statistical significance ($p<0.05$) in males when compared to the controls. In the absence of convincing evidence of associated microscopic or blood chemical changes, the increased urine output was considered to be associated with a physiological response to increased salivation. Similar changes were not observed in either sex at 150 mg/kg bw/day or 15 mg/kg bw/day or in the 1000 mg/kg bw/day dose group, at the end of the recovery period.

Effects in Organs
Organ weight
A statistically significant increase ($p<0.001$) in both absolute and relative liver weight to bodyweight was observed in either sex treated at the high dose, with an increase ($p<0.05$) in the relative weight of this organ detected in males treated with 150 mg/kg bw/day. Males treated with 1000 mg/kg bw/day also showed an increase ($p<0.05$) in absolute and relative kidney weight. Similar observations were not observed for animals treated at 150 and 15 mg/kg bw/day.

Necropsy
Three males treated with 1000 mg/kg bw/day showed enlarged and pale kidneys. Macroscopic examination of the liver noted abnormality involving a dark red firm necrotic (at histology) caudal lobe in one recovery 1000 mg/kg bw/day male, this was considered to be spontaneous in nature. A dark red colouration of the thymus (affecting approximately half the tissue) was observed in one male, this was possibly associated with exsanguination of animals at study termination. No other treatment related macroscopic findings were observed.

Histopathology

Liver:
Treatment – related centrilobular hepatocyte enlargement was seen for female rats only dosed at 1000 mg/kg bw/day. One female rat receiving 150 mg/kg bw/day of the notified chemical was similarly affected. In the high dose recovery group, where was regression of the condition, following fourteen days without treatment. Scattered mononuclear cell foci were observed in the liver among all groups.

Kidneys:
Globular accumulations of eosinophilic material were observed in the tubular epithelium of three rats dosed at 1000 mg/kg bw/day. This finding is consistent with presence of hydrocarbon nephropathy, which results from excessive α_2-microglobulin in real proximal tubular epithelial cells. α_2-microglobulin is found only in the proximal tubular epithelium of adult male rats. Increased severity of groups of basophilic renal tubules were also observed among 1000 mg/kg bw/day rats, associated with some instances with tubular dilatation. Globular accumulations of eosinophilic material were also seen in relation to males rats dosed at 150 mg/kg bw/day. All conditions regressed among the high dose recovery male rats compared to the recovery control animals at the end of the recovery period.

Thyroids:
Increased severity of follicular cell hypertrophy were observed in males and females dosed at 1000 mg/kg bw/day. A treatment related higher incidence of condition was also seen in females rats at the high dose. A similar treatment related response was not observed at any of the remaining dose levels. Follicular cell hypertrophy was observed to have regressed among the high dose recovery group at the end of the recovery period. The most plausible explanation for these changes is a compensatory secondary response to liver changes, due to increased thyroxine excretion in bile as result of conjunction with glucuronyltransferase that

has been induced by treatment.

Stomach
Acanthosis and hyperkeratosis of the forestomach were observed for two females dosed at 1000 mg/kg bw/day. Such findings also represent a typical adaptive response of the stomach to repeat oral administration of a gastric irritant. Although this conditions is seen occasionally among untreated animals, the possibility of an association with treatment cannot be excluded in this instance. Animals from the remaining dose levels were unaffected but there was evidence of similar changes among recovery group animals.

Other observed histopathological lesions (heart, spleen, kidney and lung) were either scattered occurrences or occurred with similar frequency across all groups.

Remarks – Results
Following oral gavage administration of the notified chemical for 28 consecutive days treatment related changes were observed in males and females at 1000 mg/kg bw/day and males treated at 150 mg/kg bw/day.

The transient increased salivation and associated findings in male and females treated at the high dose from day 6, would indicate that the primary effect of treatment is irritancy. Excessive salivation is commonly observed following oral administration and is considered attributed to an unpalatable or local irritant test material, rather than an indication of systemic toxicity. The hunched posture and tiptoe gait seen in both clinically and during open field assessment during the final weeks of the treatment in high dose males and females are further indication of the irritant nature of the notified chemical. These changes were considered associated with abdominal discomfort and not to be neurotoxicological importance. At the cessation of treatment, all effects except hunched posture regressed. The hunched postured in the recovery group resolved by Day 33. Acanthosis and hyperkeratosis of the forestomach was observed in two females dosed at the high dose, when stomach sections were examined microscopically. Such findings represent a typical adaptive response to the stomach to repeated oral administration of a gastric irritant. Similar changes in the forestomach were not observed in the high dose recovery females.

Macroscopically, the kidneys of three rats dosed at 1000 mg/kg bw/day appeared enlarged and pallid at necropsy. Microscopic examination of the kidney revealed globular accumulations of eosinophilic material in the tubular epithelium among rats dosed at 1000 mg/kg bw/day. Globular eosinophilic material were also seen in males dosed at 150 mg/kg bw/day. There was an increase in the severity of basophilic renal tubules among the high dose rats. In some instances, this was associated with tubular dilatation. The incidence of the condition in females was not altogether convincing and is it is likely that in the males it was associated with hydrocarbon nephropathy. The slight increase in kidney weight detected in males treated at 1000 mg/kg bw/day is very likely due to hydrocarbon nephropathy.

An increase in absolute and relative liver weight was observed in males and females treated at 1000 mg/kg bw/day and to a lesser extent also in males receiving 150 mg/kg bw/day. Treatment related centrilobular hepatocyte enlargement was send in only females dosed at 1000 mg/kg bw/day and in one female dosed at 150 mg/kg bw /day. Regression of this adaptive change was observed in the 1000 mg/kg bw/day recovery group, following fourteen days without treatment.

CONCLUSION
The No Observed Effect Level (NOEL) was established as 15 mg/kg bw/day in males and females in this study, based on the observation of increased liver weights (absolute and/or relative) and hepatocyte enlargement at higher doses.

TEST FACILITY	SafePharm Laboratories (2003i)

7.7. 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.

	Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100. *E. coli*: WP2 uvrA$^-$
Metabolic Activation System	phenobarbitone and β−naphthoflavone induced rat liver S9 fraction
Concentration Range in Main Test	a) With metabolic activation: 50-5000 µg/plate. b) Without metabolic activation: 50-5000 µg/plate.
Vehicle	dimethyl sulphoxide (DMSO)
Remarks - Method	Two independent tests were performed in triplicate.

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 (TA100)	5000 (TA100)	-	Negative
Test 2		5000 (TA100)	-	Negative
Present				
Test 1	5000 (TA100)	5000(TA100)	-	Negative
Test 2		5000 (TA100)	-	Negative

Remarks - Results	A visible reduction in the growth of the bacterial background lawn and/or a significant decrease in the frequency of revertant colonies was seen at 5000 µg/plate both with and without S9. The notified chemical was, therefore, tested up to maximum recommended dose level of 5000 µg/plate. No precipitation of the notified chemical was observed on the plates at any of the dosed tested either in the presence or absence of S9. No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains at any dose level, either with or without metabolic activation. Appropriate positive controls were used and resulted in large increases in mutant frequency, confirming the sensitivity of the test system. The results from the negative controls were acceptable.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Safepharm Laboratories (2003j)

7.8. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosomal Aberration Test.
Cell Type/Cell Line	Chinese Hamster Lung/(CHL/IU)
Metabolic Activation System	phenobarbitone and β−naphthoflavone induced rat liver S9 fraction
Vehicle	dimethyl sulphoxide (DSMO)
Remarks - Method	No significant protocol deviations. The dose selection for the main experiments was based on toxicity for exposure groups in a range finding cell growth inhibition test.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			

Test 1	0*, 2.5, 5.0, 10*, 12.5*, 15*, 20*	6 hrs	24 hrs
Test 2	0*, 2.5, 5.0*, 10*, 15, 20*, 30	24 hrs	24 hrs
Present			
Test 1 (S9 - 5% final concentration)	0*, 5.0, 10, 20, 40*, 60*, 80*	6 hrs	24 hrs
Test 2 (S9 - 1% final concentration)	0*, 2.5, 5.0, 10*, 20*, 40*, 80	6 hrs	24 hrs

*Cultures selected for metaphase analysis.

RESULTS

Remarks - Results

Test 1

An approximate 50% growth inhibition was achieved in terms of the cell count at 20 μg/mL in the absence of S9 and at 80 μg/mL in terms of mitotic index data in the presence of S9. No precipitate of the notified chemical was observed at the end of the exposure period at the doses tested in the absence and presence of S9. The maximum dose selected for metaphase analysis was based on toxicity and was 20 μg/mL in the absence of S9 and 80 μg/mL presence of S9. Both the vehicle control groups had frequencies of cells with chromosome aberrations within the expected range. The positive control materials induced a statistically significant increase ($p<0.001$) in the frequency of cells with aberrations. The metabolic activation and test method were therefore satisfactory. The notified chemical did not induce any statistically significant increase in the frequency of cells with aberrations nor in the number of polypoid cells either in the presence or absence of metabolic activations

Test 2

An approximate 50% growth inhibition was achieved at 20 μg/mL in the 24-hour exposure group. There was greater toxicity observed when 1% (final concentration) S9 was used when compared to 5% S9 was used in Test 1. An ideal approximate 50% toxicity was therefore not achieved within the 6(18) hours pulse exposure group. However there was complete toxicity and no metaphases at 80 μg/mL. No precipitate of the notified chemical was observed at the end of the exposure period in either group. The maximum dose selected for metaphase analysis was based on toxicity and for the 24-hour continuous exposure was 20 μg/mL and the 6 (18) hour exposure with S9, it was 40 μg/mL. The frequencies of cells with chromosome aberrations were within the expected range for the vehicle control groups. The positive control materials induced a statistically significant ($p<0.001$) in the frequency of cells with aberrations. The test method was therefore satisfactory. The notified chemical did not induce any statistically significant increase in frequency of cells with aberrations in either the 24-hour continuous exposure group or the 6(18) hour pulse exposure group at a 1% final concentration of S9. The notified chemical did not induce any statistically significant increase in the numbers polypoid cells at any dose level in either exposure group.

CONCLUSION

The notified chemical was not clastogenic to CHL treated in vitro under the conditions of the test.

TEST FACILITY

Safepharm Laboratories (2003k)

7.9. Genotoxicity – in vivo

TEST SUBSTANCE

Notified chemical

METHOD

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

Species/Strain: Crl:CD-1(ICR)BR
Route of Administration: Intraperitoneal
Vehicle: Arachis oil

Remarks - Method

No significant protocol deviation.

A range finding test was used to determine a suitable dose level, and route of administration for the main test. The test was also used to determine if the main test was to be performed using both sexes or males only. Three groups of mice were used. Each group comprised of one male and one female mouse. Two of the groups were dosed via intraperitoneal route and the third group was dosed orally. One of the two groups dosed intraperitoneally was dosed at 2000 mg/kg bw and the other at 1000 mg/kg bw. The group dosed orally was dosed at 2000 mg/kg bw. No premature deaths were observed in the range-finding test. However, in mice dosed at 2000 mg/kg bw, via the intraperitoneal route, the clinical signs observed were so severe that the animals were killed *in extremis* in the interests of animal welfare. The clinical signs observed at 2000 mg/kg bw were: loss of righting reflex, decreased respiratory rate, laboured respiration, ptosis, hypothermia, dehydration, occasional body tremors, and increase salivation.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I Vehicle Control (Arachis oil)	7 males	-	48
II Vehicle Control (Arachis oil)	7 males	-	24
III Positive Control (CP)	5 males	50	24
IV	7 males	1000	48
V	7 males	1000	24
VI	7 males	500	24
VII	7 males	250	24

CP=cyclophosphamide.

RESULTS

Doses Producing Toxicity

There were no premature deaths or clinical signs seen in any of the dose groups.

Genotoxic Effects

The notified chemical was found not to produce a significant increase in the frequency of micronuclei in polychromatic erythrocytes (PCE) of mice under the test conditions. There was no statistically significant decrease in PCE/ normal chromatic erythrocytes (NCE) ratio in the 24 or 48 hour notified chemical group when compared to their concurrent controls. However, there was marked decrease in the PCE/NCE ratio in the 48 hour notified chemical group. This was taken to indicate that systemic absorption had occurred. There were no statistically significant increases in the frequency on micronucleated PCE in any groups dosed with the notified chemical when compared to their concurrent vehicle control groups. The positive control group showed a marked increase in the incidence of micronucleated polychromatic erythrocytes hence confirming the test system.

Remarks - Results

CONCLUSION

The notified chemical was not clastogenic in this in vivo micronucleus test in the mouse under the conditions of the test.

TEST FACILITY

Safepharm Laboratories (2003l)

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test.
Inoculum	A mixed population of activated sewage sludge micro-organisms from the aeration stage of a sewage treatment plant, which predominantly treats domestic sewage.
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Dissolved organic carbon (DOC; 0.45 μm filtered) was monitored in test solutions in order to calculate the inorganic carbon/total carbon ratio in the test media.
Remarks - Method	The notified chemical was dispersed directly in culture medium. The notified chemical was heated to 60°C in order to liquify the sample prior to weighing. The notified chemical was dispersed in culture medium with the aid of high shear mixing prior to dispersal in inoculated culture medium. The final concentration was 13 mg/L, equivalent to 10 mg C/L. The culture vessels were sealed and CO_2-free air was bubbled through the solution and stirred continuously. CO_2 produced through degradation was collected and analysed on most days throughout the test. In addition to the test sample, blank and toxicity control samples and samples containing a reference substance (sodium benzoate at 17.1 mg/L) were measured.

RESULTS

	% degradation	
Day	*Test substance*	*Sodium Benzoate*
1	0	9
2	0	31
3	5	44
6	10	60
10	13	72
14	38	77
16	37	78
20	43	85
22	46	82
27	55	83
28	57	87
29*	66	90

* Day 29 values corrected to include any carry-over of CO_2 detected in the second CO_2 analyser.

Remarks - Results	There was no undissolved notified chemical visible in the test vessels. Sodium benzoate degraded 87% after 28 days, validating the test conditions.. The total CO_2 evolution in the control vessels of 37.52 mg/L, the variability between test vessels for CO_2 evolution of less than 20%, and the inorganic carbon (IC) content of the notified chemical suspension in the mineral medium at the beginning of the test being less than 5% of the total carbon content, were all within acceptable OECD validation criteria. The notified chemical attained 57% degradation within 28 days and 66% in 29 days but failed to meet the OECD 10-day window criterion. It was not found to be inhibitory to activated sludge bacteria under the test conditions.
CONCLUSION	The notified chemical exhibited a potential for biodegradation, however,

it cannot be considered to be readily biodegradable according to the OECD criteria.

TEST FACILITY SafePharm Laboratories (2003m)

8.1.2. Bioaccumulation

Not determined. The low water solubility, and high Pow suggest a potential for the notified chemical to cross biological membranes and bioaccumulate. However, the low import volume and dispersed use suggest that exposure will not be significant and will limit this potential. Further, the notifier provided a BCF value calculated using the EPIWIN BCF Program (v2.15) of 401.1 that indicates a relatively low potential for bioaccumulation.

During the fish and algae acute toxicity tests (summarised in Sections 8.2.1 and 8.2.3), decline of the measured concentrations were observed. The testing facility attributed the reduction to adsorption and/or accumulation of the notified chemical to the tissues of the exposed fish and to adherence/absorption on or into the algal cells in the fish and algae tests, respectively, as other loss pathways were discounted through testing of stability, adherence to glass containers or hydrolysis.

8.2. Ecotoxicological investigations

8.2.1. Acute toxicity to fish

TEST SUBSTANCE Notified chemical

METHOD OECD (1992) TG 203 Fish, Acute Toxicity Test – Semi-static.

Species	Rainbow trout (*Oncorhynchus mykiss*). Mean length 4.4 cm. Mean weight 1.11 g.
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	Test water was pre-tested for impurities. The test concentrations were determined by GC using an external standard. The temperature(14.3-15.1°C), the dissolved oxygen levels (≥9.0 mg O_2/L) and pH (7.6-8.2) were all satisfactorily maintained.
Remarks – Method	Preliminary (range-finding) and definitive tests were performed. Preliminary work determined that filtration was acceptable method to remove dispersed notified chemical.

For the definitive test, an excess amount of notified chemical was stirred in dechlorinated tap water at 25°C for 24 hours and filtered through a pre-conditioned 0.2 µm filter to obtain a saturated solution (21 mg/L nominal) from which the test solutions were made.

Test solutions were clear and colourless during the period of the tests. Toxicity values were determined by the trimmed Spearman-Karber method using ToxCalc (1999) computer software.

RESULTS

Concentration mg/L		*Number of Fish*	*Cumulative Mortality*					
Nominal	*Time-weighted Mean Measured*		*3 h*	*6 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	<LOQ	10	0	0	0	0	0	0
1.0	0.8	10	0	0	0	0	0	0
1.8	1.4	10	0	0	0	0	0	0
3.2	2.5	10	0	0	0	0	0	0
5.6	4.4	10	0	0	0	0	1	1
10	8.6	10	10	10	10	10	10	10

LC50	Nominal: 7.1 mg/L at 96 hours (95% CI 6.3-7.9 mg/L). Time-weighted mean measured: 5.7 mg/L at 96 hours (95% CI 5.1-6.4 mg/L).
NOEC (or LOEC)	Nominal: 1.8 mg/L at 96 hours. Time-weighted mean measured: 1.4 mg/L at 96 hours.
Remarks – Results	Sublethal effects were observed after 96 h exposure at concentrations ≥3.2 mg/L. These responses included increased pigmentation, loss of equilibrium and the presence of moribund fish. After 2.5 h, all fish exposed to 10 mg/L were moribund, and after 51 h was moribund in the 5.6 mg/L test solution. These fish were killed and classed as mortalities for the 3 and 72-hour time points, respectively. The analytical procedure had an acceptable level of recovery of notified chemical; however, the measured concentrations in expired test solutions at 24, 48, 72 and 96 hours were between 54 and 74% of nominal concentration. This marked decline was attributed the loss to adsorption and/or accumulation of the notified chemical to the tissues of the exposed fish as other loss pathways were discounted through testing of stability, adherence to glass containers or hydrolysis.
CONCLUSION	The notified chemical is toxic to Rainbow trout.
TEST FACILITY	SafePharm Laboratories (2003n)

8.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test – Semi-static referenced as Method C.2 of EC Directive 92/69/EEC
Species	*Daphnia magna* (1st instar)
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	The pH (7.9-8.0), dissolved oxygen (8.8-8.9 mg O_2/L) and temperature (20.0-20.8°C) were maintained satisfactorily. The test concentrations were determined by GC using an external standard.
Remarks – Method	Based on a media preparation trial and a preliminary (range-finding) test the test solutions for the definitive tests were prepared using the same method used in the fish toxicity study. The nominal concentration of the saturated solution was 19 mg/L. Preliminary work determined that filtration was acceptable method to remove dispersed notified chemical. The EC50 values and associated confidence limits were calculated by the Probit method (Finney, 1971) using ToxCalc (1999) software.

RESULTS

Concentration mg/L (Nominal)	*Number of D. magna**	*Number Immobilised*	
		24 h	*48 h*
0	20	0	0
0.19	20	0	0
0.34	20	0	0
0.61	20	0	0
1.1	20	0	0
1.9	20	1	1
3.4	20	2	7
6.1	20	4	18

11	20	20	20
19	20	20	20

* 2 replicates of 10

LC50	3.8 mg/L at 48 hours (95% CI 4.2-9.6 mg/L)
NOEC	1.1 mg/L at 24 and 48 hours (NOEC based on zero immobilisation).
Remarks - Results	Test solutions were clear and colourless during the period of the tests. The analytical procedure had an acceptable level of recovery of notified chemical between 0 h and 48 h (86-117% for concentrations <11 mg/L and 75-78% for 11 and 19 mg/L, respectively). As these values approximated the lower limit of 80% of nominal value, the nominal values were used. The notified chemical was found to be stable in the test media for 48 h with no loss of notified chemical.
CONCLUSION	The notified chemical is toxic to *Daphnia magna*.
TEST FACILITY	SafePharm Laboratories (2003o)

8.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test referenced as Method C.3 EC Directive 92/69/EEC
Species	Green algae (*Scenedesmus subspicatus*)
Exposure Period	72 hours
Concentration Range	
Nominal	0, 1.25, 2.5, 5.0, 10 and 20 mg/L (3 replicate flasks per concentration).
Actual	0, 0.78, 1.73, 3.66, 7.01 and 14.4 mg/L (Geometric mean measured conc).
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	The pH range (7.1-7.9) and temperature (24 ± 1°C) were monitored. Notified chemical concentrations in the test media were determined analytically by GC using an external standard. At initiation, nominal algal cell density was ~10^4 cells/mL.
Remarks - Method	Based on a media preparation trial and a preliminary (range-finding) test the test solutions for the definitive tests were prepared using the same method used in the fish toxicity study. The nominal concentration of the saturated solution was 20 mg/L, which was further diluted to obtain the required test concentrations. Preliminary work determined that filtration was acceptable method to remove dispersed notified chemical. The percentage inhibition values were plotted against test concentration and a line fitted using Xlfit 3 software (IDBS 2000) and the EC50 value with respect to area under the growth curve, EbC50 (72 h) determined from the equation to the fitted line. One-way ANOVA was carried out using SAS software (SAS 1999-2001).

RESULTS

Test Concentration		*Biomass*	*Growth*
Nominal	*Actual*	*% Inhibition*	*% Inhibition*
0	0	-	-
1.25	0.78	1	2
2.5	1.73	23	9
5.0	3.66	49	19
10	7.01	69	31
20	14.4	77	38

Remarks – Results	ErC50 (0-72 h)	23 mg/L (95% CI 17-32 mg/L)*
	EbC50 (72 h)	3.8 mg/L (95% CI 3.1-4.7 mg/L)
	NOEC (72 h)	0.78 mg/L.

All test cultures were examined microscopically at 72 h, with no abnormalities observed. Analysis of the test concentrations at 72 hours showed a marked decline in concentration (37% to 53% nominal values). The results were based on the geometric mean measured text concentrations to give a 'worst-case' analysis.

* The ErC50 value and associated confidence limits should be interpreted with caution as these were obtained from the equation for the fitted line as no concentration tested resulted in 50% inhibition of the 0-72 h growth rate. It was not possible to test a higher concentration in order to obtain 50% inhibition of growth rate due to the limited solubility of the notified chemical in aqueous medium; 20 mg/L being the highest attainable nominal test concentration.

CONCLUSION — The notified chemical is toxic to green algae.

TEST FACILITY — SafePharm Laboratories (2003p)

8.2.4. Inhibition of microbial activity

TEST SUBSTANCE — Notified chemical

METHOD — OECD TG 209 Activated Sludge, Respiration Inhibition Test.

Inoculum	A mixed population of activated sewage sludge micro-organisms from the aeration stage of a sewage treatment plant, which predominantly treats domestic sewage.
Exposure Period	3 hours
Auxiliary Solvent	None
Concentration Range	
Nominal	32, 320, 1000 and 3200 mg/L
Remarks – Method	Two preliminary (range-finding) tests (soluble and dispersed solutions) and a definitive test were performed. The notified chemical was dispersed directly in water. For the definitive test, the notified chemical was heated to 60°C in order to liquefy the sample prior to weighing. Amounts of notified chemical were separately dispersed in of water and subjected to ultrasonication prior to the additon of synthetic sewage (16 mL), and activated sewage sludge (200 mL).

A reference material (3,5-dichlorophenol) was also tested at 3.2, 10 and 32 mg/L.

RESULTS

	Nominal Concentration (mg/L)	*% Inhibition*
Control	R1	-
	R2	-
Notified chemical	32	15
	100	9
	320	6
	1000	17
	3200	17
3,5-dichlorophenol	3.2	6
	10	45
	32	81

EC50	>3200 mg/L in 3 hours
NOEC	320 mg/L in 3 hours
Remarks – Results	Test dispersions were observed to contain visible waxy particles of notified chemical. The relatively large increase in inhibition at the 32 mg/L test concentration is considered to be due to the possible hormetric response (the stimulation of biological activity due to the presence of notified chemical at concentrations below its toxic concentrations). The results of the range finding test conducted at the limit of solubility under experimental conditions indicated no adverse effects on the respiration of activated sewage sludge micro-organisms at the maximum attainable dissolved notified chemical concentration of approximately 18 mg/L. However, dispersions containing greater than this amount exhibited a slight adverse effect on the respiration rate. It was not possible to obtain an EC50 value for the notified chemical from the test concentration range used due to the flat response. The reference material, 3,5-dichlorophenol, gave a 3 h EC50 of 12 mg/L (within the acceptable range of 5-30 mg/L) validating the test conditions.
CONCLUSION	The notified chemical is not inhibitory to microbial respiration.
TEST FACILITY	SafePharm Laboratories (2003q)

8.2.5. Inhibition of microbial activity

The 'ready biodegradability' study summarised in 8.1.1 indicated that the notified chemical showed no inhibitory effect on the microorganisms during the 28-day period.

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

Nearly all of the imported notified chemical will eventually be released into the aquatic environment via the sewerage systems through formulation and use (washing off the skin, hair etc or cleaning activities) of the cosmetic and household products. Less than 15 kg per annum is expected to be disposed of to landfill as residue in empty containers via domestic garbage.

The notified chemical is moderately volatile and therefore, in part, will dissipate into air from the surfaces to which the products containing the fragrance oil is applied (eg. skin, aquatic and terrestrial environments). Although classified as moderately soluble in water (Mensink 1995), it is marginally above the 'slightly soluble' class. It will not readily hydrolyse in natural waters at environmental pH values. The high Log Koc indicates that the notified chemical cannot be expected to be mobile in either the aquatic and terrestrial compartment, as the notified chemical has a potential to adsorb to particulate organic material and therefore accumulate in sediments due to sorption and settlement. It is not readily biodegradable, however, when disposed in landfill the chemical will eventually become associated with soil and sediment and will slowly degrade through biological and abiotic processes.

Based on maximum annual imports of 500 kg per annum, and assuming a worst-case scenario that all of this is eventually released to sewer and not removed during sewage treatment processes, the daily release on a nationwide basis to receiving waters is estimated to be 1.37

kg/day. Assuming a national population of 20 million and that each person contributes an average 200 L/day to overall sewage flows, the worst-case predicted environmental concentration (PEC) in sewage effluent on a nationwide basis is estimated as 0.3425 µg/L (Environment Australia 2003). Based on the respective dilution factors of 0 and 10 for inland and ocean discharges of effluents, the PECs of the notified chemical in freshwater and marine water may approximate 0.3425 and 0.0342 µg/L, respectively.

The notified chemical is not readily biodegradable. The results obtained using the SIMPLETREAT model (European Commission 1996) for modelling partitioning and losses in sewage treatment plants (STP) indicate that when the chemical is released into the aqueous phase of a STP, about 1% released to air through volatilisation, 45% (225 kg) partitioned to water and 54 % (270 kg) partitioned to biosolids.

Based on these results, assuming that 45% of the notified chemical (225 kg) remains in solution, the following revised worst-case PEC values were obtained (Environment Australia 2003). The worst-case PEC for the aquatic environment resulting from the nationwide release of the notified chemical into the sewage systems is reduced to 0.1541 µg/L prior to any dilution and the respective concentrations in freshwater and marine water may approximate 0.1541 and 0.0154 µg/L.

Partitioning to biosolids in STPs Australia-wide may result in an average biosolids concentration of 1.85 mg/kg (dry wt). Biosolids are applied to agricultural soils, with an assumed average rate of 10 t/ha/year. Assuming a soil bulk density of 1000 kg/m^3 and a soil-mixing zone of 0.1 m, the concentration of the notified chemical may approximate 1.85 x 10^{-2} mg/kg in applied soil. This assumes that no degradation of the notified chemical occurs in the soil within 1 year from application. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated biosolids application, the concentration of notified chemical in the applied soil in 5 and 10 years may approximate 9.25 x 10^{-2} mg/kg and 0.185mg/kg, respectively.

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 0.1 m of soil (density 1000 kg/m^3). Using these assumptions, irrigation with a concentration of 0.1541 mg/L may potentially result in a soil concentration of approximately 1.5 x 10^{-3} mg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 7.5 x 10^{-3} mg/kg and 1.5 x 10^{-2} mg/kg, respectively.

There is potential for the notified chemical to bioaccumulate due to its high log P_{ow} and the low water solubility but this will be limited due to the relatively low volume imported and diffuse release to the sewer Australia wide.

9.1.2. Environment – effects assessment

The results of the aquatic toxicity tests are listed below. The most sensitive species were daphnia and algae with 48 hour EC50 and 72 hour EbC50 values each of 3.8 mg/L.

Organism	*Duration*	*End Point*	*mg/L*
Fish	96-h	LC_{50}	5.7 (measured)
Daphnia	48-h	EC_{50}	3.8 (nominal)
Algae	72-h	E_bC_{50}	3.8 (geometric mean measured)
		E_rC_{50}	23 (geometric mean measured)

A predicted no effect concentration (PNEC - aquatic ecosystems) of 3.8 x 10^{-2} mg/L (38 µg/L) has been derived by dividing the end point of 3.8 mg/L by a worst-case scenario uncertainty (safety) factor of 100 (as toxicity data are available for three trophic levels).

9.1.3. Environment – risk characterisation

Location	PEC µg/L	PNEC µg/L	Risk Quotient (RQ)
Australia-wide STPs			
Ocean outfall	0.0342 (0.0154) #	38	9×10^{-4} (4.1×10^{-4})#
Inland River	0.3425 (0.1541) #	38	9×10^{-3} (4.1×10^{-3})#

PEC and the RQ values calculated assuming 54% of the notified chemical partitioned into biosolids and 45% partitioned to water during the STP process based on the SIMPLETREAT model.

The RQ values (PEC/PNEC) for the aquatic environment, assuming nationwide use and that the chemical is not removed in STP, are below 1 for both freshwater and marine water, indicating no immediate concern to the aquatic compartment. Further, a large part of the notified chemical can be expected to be adsorbed to sludge in STP considerably reducing the PEC and the risk quotients. The RQ values based on the SIMPLETREAT model (assuming 54% will partition to sludge) are also given in the table above and have been reduced considerably compared with those calculated without considering partition behaviour.

Based on the proposed use pattern the notified chemical is not expected to pose an unacceptable risk to the health of aquatic life. Bioaccumulation is not expected from the diffuse use pattern and low import volume.

9.2. Human health

9.2.1. Occupational health and safety – exposure assessment

Exposure to imported fragrance oil.

Transport and warehouse workers will be exposed to the fragrance oil (up to 10% notified chemical) only when there is an accidental spillage or packaging breach. At the notifier's warehousing site transport and warehouse workers will wear protective overalls, hard hats, chemical resistant gloves, and safety glasses.

Worker exposure to the imported fragrance oil (up to 10% notified chemical) may occur during the formulation of the consumer products. Dermal and accidental ocular exposure may occur during, drum handling, pre weighing, the transfer of the fragrance oil to the batch mixer, mixing, and QC sampling. Exposure may also occur during the cleaning and maintenance of equipment. Worker exposure will be minimised by use of the appropriate personal protection equipment. Workers involved the formulation process would wear coveralls, gloves, and safety glasses. Local exhaust ventilation will be used and self-contained breathing equipment will be available if required.

Exposure to finished product containing the notified chemical

Worker exposure to finished cosmetic and household products (up to 0.8% notified chemical) during the transport, storage, and distribution is unlikely to occur unless there is an accidental spillage or packaging breach.

Exposure for retail workers handling the finished products is not likely to occur, unless the packaging of the final product is breached.

9.2.2. Public health – exposure assessment

Public exposure will be widespread, based on the range of end use consumer products that the notified chemical will be incorporated into. The notified chemical will be present between 0.01 to 0.8% w/w in the final product. Thus, the typical the public will be exposed to low levels of the notified chemical via wide range of cosmetic and household products.

The public will normally be dermally exposed to the notified chemical, while accidental ocular exposure and ingestion of the notified chemical may also occur.

Direct public exposure during transport and storage or from manufacturing waste is unlikely.

9.2.3. Human health - effects assessment

The notified chemical is a solid at room temperature and melts between 38 and 46°C. It boils at 293°C @ 101.92 kPa and has a molecular weight of 250.38. The notified chemical has a vapour pressure of 0.0027 mmHg at 25°C, a water solubility of 13.3 mg/l at 20°C, and an octanol/water partition coefficient of 4.29 at 25°C. The molecular weight, water solubility and octanol/water partition indicate potential for dermal absorption and absorption following ingestion. The vapour pressure (0.0027 mmHg at 25°C), indicates that inhalation exposure will be very low. The major potential route of exposure is anticipated to be dermal (considering the intended use of the substance). The notified chemical has low acute oral or dermal toxicity. It is considered to be slightly irritating to the skin and to the eyes. Two-Eyed Musk is a skin sensitiser in the LLNA at 25 and 50%. It was, however, not found to be sensitising at 10% in the LLNA or in the human repeated insult patch test at 4% in alcohol/diethyl phthalate. No genotoxicity was observed in the Ames test, the in vitro metaphase analysis in Chinese Hamster Lung (CHL) cells and the in vivo mouse micronucleus test.

The NOEL established in a 28 day repeat dose study in rats was 15 mg/kg bw/day. Rat specific hydrocarbon nephropathy was observed in males at 150 and 1000 mg/kg bw/day with associated increase kidney weight and macroscopic renal changes in males treated with 1000 mg/kg bw/day. Elevated liver weights were observed in both sexes at 1000 mg/kg bw/day and males treated with 150 mg/kg bw/day. While centrilobular hepatocyte enlargement was observed in females dosed at 150 mg/kg bw/day and 1000 mg/kg bw/day. Histological evidence of gastric irritation was observed in females dosed at 1000 mg/kg bw/day and clinical observations,

adaptive liver and thyroid changes were observed in both sexes at 1000 mg/kg bw/day. The gastric irritation, liver and thyroid changes were reversible.

Based on the results form the LLNA study provided the notified polymer should be classified as a skin sensitiser (R43) according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (1999a).

9.2.4. Occupational health and safety – risk characterisation

Occupational exposure can occur when handling the imported finished fragrance oil (maximum 10%; typically 4% notified chemical). Formulation of notified chemical into end use consumer products will occur in fully automated and enclosed processes. However, some operations are not full automated. During the formulation process, dermal and accidental ocular exposure to notified chemical may occur when the imported solution is pumped into the mixer, from splashing or vortexing during the mixing of the batch, and during QC testing. Workers handling the finished consumer products will be exposed to notified chemical at levels normally between 0.01% and 0.8%. Based on the physicochemical data provided, dermal and oral absorption may occur humans. The low volatility of the notified chemical will restrict the possibility of exposure through inhalation. The notified chemical is a skin sensitiser, and also slightly irritating to the skin and eyes. Workers involved in the formulation process should wear gloves, safety glasses, and overalls.

Occupational exposure may also occur during the maintenance and cleaning of formulation equipment. The concentration of the notified chemical following formulation will be between 0.01 and 0.8 %. Exposure is minimised by the use of safety glasses, gloves, and overalls.

Once the final consumer product is packed, exposure should be low. Hence, exposure for warehousing and distribution workers and retail workers is unlikely unless the packaging is breached.

9.2.5. Public health – risk characterisation

The levels of the notified chemical in finished consumer products ranges from 0.01 to 0.8%. The public will be repeatedly exposed to low level of the notified chemical. Dermal absorption of the notified chemical is likely due to the physicochemical properties of the notified chemical. The notified chemical is a skin sensitiser and therefore repeated dermal exposure is of concern. However, the notified chemical results of local lymph node assay in mice and human repeat insult patch tests, indicate notified chemical is not a sensitiser at 10% and 4%, respectively. Therefore, at low levels of the notified chemical typically found in the final consumer products the risk of sensitisation risk would be low.

Based on information provided by the notifier the notified chemical is present in formulated product at a maximum level of 0.8 % w/w (typically 0.32%) in toilet waters. This has been used to calculate the worst case dermal exposure scenario for notified chemical

Systemic exposure = [da*c*mdu*ssa]/bw

Where:

c = concentration in toilet waters
da = dermal absorption
mda = maximum daily quantity applied
bw = body weight

The following assumptions were made:

c = 0.8%
da = 10% (assumed)
mda = 3750 mg (based on 0.75 g per use, 1-5 times/day)
bw = 60 kg

Systemic exposure = 0.05 mg/kg bw/day

Margin of Exposure calculations were undertaken, based on the NOEL from the subchronic study.

Margin of Exposure
Subchronic Study
MOE = NOEL/systemic exposure
= 15 mg/kg bw/day/0.05 mg/kg bw/day
= 300

The Margin of Exposure exceeds 100, and is hence acceptable.

However, the primary issue involved in public use of the notified chemical is skin sensitisation. While the notified chemical should be classified as a skin sensitiser based on the results of the LLNA study, the results of the patch testing in humans did not indicate sensitisation was occurring at 4%. Accordingly, the notified chemical is not expected to cause sensitisation in humans at a maximum of 0.8%.

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

10.1. Hazard classification

Based on the available data the notified chemical is classified as hazardous under the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2002). The classification and labelling details are:

R43 – May cause sensitisation by skin contact.

and

As a comparison only, the classification of 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.

For health hazard the notified chemical is classified as Sensitiser.

For environmental hazard the notified chemical is classified as Chronic II (toxic to aquatic life with long lasting effect).

10.2. Environmental risk assessment

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

10.3. Human health risk assessment

10.3.1. Occupational health and safety

There is Low Concern to occupational health and safety under the conditions of the occupational settings described.

10.3.2. Public health

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

11. MATERIAL SAFETY DATA SHEET

11.1. Material Safety Data Sheet

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

11.2. Label

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

12. RECOMMENDATIONS

REGULATORY CONTROLS
Hazard Classification and Labelling

- The NOHSC Chemicals Standards Sub-committee should consider the following health hazard classification for the notified chemical:
  R43 – May cause sensitisation by skin contact.

- The following safety phrases should be used for the notified chemical as introduced:
  - S24/25 Avoid contact with skin and eyes
  - S36/37/39 Wear suitable protective clothing, gloves, and eye/face protection.

CONTROL MEASURES
Occupational Health and Safety

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced,
  - Coveralls,
  - Gloves
  - Safety goggles

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

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

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

Environment

Disposal

- The notified chemical should be disposed of according to the local regulations.
- Avoid disposing into drainage systems and into the environment.
- Keep away from drains, surface and ground water and soil

Emergency procedures

- Spills/release of the notified chemical should be contained by use of sand or inert powder and disposed of according to local regulations.

- Prevent entering waterways and sewer.

12.1. Secondary notification

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

(1) Under Section 64(1) of the Act; if
  - the level of the notified chemical in perfumes exceeds 0.8%; or
  - the level of the notified chemical in leave on products exceeds 0.3%; or
  - any further sensitisation potential reports including clinical observations, become available.

or

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

The Director will then decide whether secondary notification is required.

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