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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

(No Exempt Information Claimed)

Appleide

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Director
NICNAS

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
International Flavours and Fragrances Australia Pty. Ltd. (ABN: 77 004 269 658)
301 Frankston-Dandenong Road
Dandenong South Victoria 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.

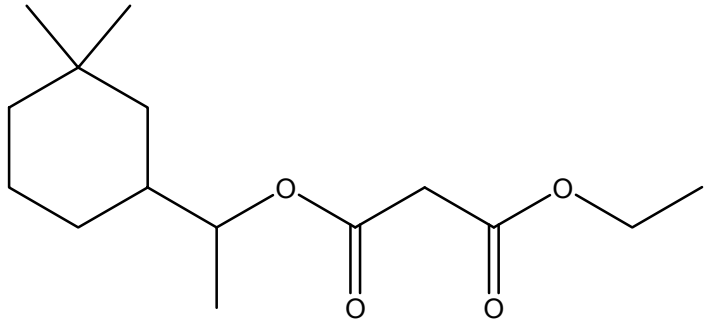
PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
No

NOTIFICATION IN OTHER COUNTRIES
US EPA: PMN (International Flavours and Fragrances)
EC - Spain: VIIA 2004, (International Flavours and Fragrances)
Environment Canada: Schedule II, 2005
Philippines: PMPIN, 2005

2. IDENTITY OF CHEMICAL

CHEMICAL NAME	Propanedioic acid, 1-(3,3-dimethylcyclohexyl)ethyl, ethyl ester
OTHER NAME(S)	Musk Nouvelle
OTHER NAME(S)	Fruity Musk
MARKETING NAME	Appleide
CAS NUMBER	478695-70-4
MOLECULAR FORMULA	$C_{15}H_{26}O_4$

STRUCTURAL FORMULA



MOLECULAR WEIGHT 270.37

SPECTRAL DATA
^{1}H NMR
Peaks 0.88 ppm, 0.91 ppm, 0.81-0.95 ppm, 0.99-1.10 ppm, 1.19 ppm, 1.29 ppm, 1.33 – 1.82 ppm, 3.35 ppm, 4.21 ppm, 4.75 ppm.

UV
Neutral pH (pH 7)
λ_{max} = 200 nm ε_{max}= 438.1 Lmol^{-1}cm^{-1}
Acid pH (pH ~2-3)
λ_{max} = 201 nm ε_{max}= 327.6 Lmol^{-1}cm^{-1}
Basic pH (pH~9-10)
λ_{max} = 204 nm ε_{max}= 275.5 Lmol^{-1}cm^{-1}

IR
Peaks at: 3463, 2945, 2864, 1734, 1462, 1412, 1368, 1324, 1270, 1187, 1152, 1134, 1102, 1069, 972, 846 cm^{-1}
1734 cm^{-1} Ester stretching, saturated, acyclic
2945, 2864 cm^{-1} C-H stretch, alkane
1462 cm^{-1} C-H bending, alkane, -CH_2-
3014 cm^{-1} C-H stretching, alkene, di-substituted
cis
1102 cm^{-1} O-H bending and C-O stretching, from secondary alcohol
1270, 1324 cm^{-1} O-H bending and C-O stretching, from primary alcohol

METHOD Nuclear Magnetic Resonance (NMR), Ultraviolet (UV), Infrared (IR) Spectroscopy

METHODS OF DETECTION AND DETERMINATION

ANALYTICAL METHOD Gas Chromatography

3. COMPOSITION

DEGREE OF PURITY
90% to 99.5%, typical = 97.5%

HAZARDOUS IMPURITIES
None

NON HAZARDOUS IMPURITIES (> 1% by weight)
Up to 10% Propanedioic acid, 3-(3,7-trimethylcycloheptyl)ethyl ester

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 formulation at maximum concentration of 10% or in end-use consumer products at concentrations ranging from 0.01% to 0.8%.

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

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

USE
The notified chemical will be used as an odourant in alcoholic perfumery, cosmetics, toiletries, household products, soaps and detergents at concentrations ranging from 0.01% to 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
The notified chemical will be imported into Australia as a component of finished fragrance oils in sealed, polypropylene lined, 205 L steel drums or as a component of finished consumer products in standard consumer packaging. The notified chemical will be transported from the docks by road to the notifier's warehouse. The finished fragrance oil will then be transported to customers, typically by road, when needed. The finished consumer product will be transported to retail stores for distribution.

STORAGE FACILITIES & STORAGE REQUIREMENTS
The finished fragrance oil will be stored in the original sealed containers. The drums should be stored in a cool, dry and ventilated area away from heat sources and protected from light.

The finished consumer product will be stored in the warehouse under appropriate conditions until distributed to retail stores.

5.2. Operation description

The drummed finished fragrance oil containing a 10% concentration of notified chemical will be used

in the cosmetic industry for production of toiletries, shampoos, soap and household cleaning agents and detergents. End-use consumer products will contain up to 0.8% notified chemical 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, 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 and Warehouse workers	5	None	Incidental Exposure only
Plant operators			
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

Exposure Details

Transport and Warehouse Workers

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 are required to wear protective overalls, hard hats, chemical resistant gloves and safety glasses.

Formulation workers

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.

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 mixtures containing the notified chemical.

5.4. Release

RELEASE OF CHEMICAL AT SITE

The notified chemical will not be manufactured in Australia. Local operations will include transport, storage and reformulation. Release to the environment may occur in the unlikely event of an accident during transport or if the packaging is damaged during handling and storage. Spilt notified chemical should be physically contained and collected, and will be disposed of according to local regulations, probably landfill. Losses from accidental spills arising from transport, handling and storage are expected to account for up to 1% or 10 kg of the annual import volume.

During reformulation and filling, there is the potential for release to the environment through spills. However, these should be contained by standard physical engineering means. Spilt notified chemical is expected to be recycled if not contaminated, or otherwise disposed of according to local regulations.

Reformulation will be done using batch processes. Following each batch, the cleaning of equipment may result in the generation of wastewaters containing the notified chemical. The quantity of notified chemical remaining in wash water is expected to account for up to 1% or 10 kg of the annual import volume. This may be disposed of to an on-site wastewater treatment plant or to the sewer as trade waste. Empty import drums containing residual notified chemical are expected to be rinsed, with the rinsings being disposed of to sewer, and the drum subsequently being recycled.

RELEASE OF CHEMICAL FROM USE
Since the notified chemical will be used in household, laundry and personal cleaning products, almost all (~97%) or 970 kg of the annual import volume will end up in the sewer. Residual notified chemical in end-use containers is expected to account for up to 1% or 10 kg of the annual import volume, which will be disposed of in domestic waste to landfill.

5.5. Disposal

Emptied import drums containing residual quantities of the notified chemical may be rinsed and re-used, or sent to landfill for disposal. Drum rinsate will be discharged to on-site wastewater treatment plants or to sewer. Following use, emptied product containers are expected to be disposed of through domestic garbage disposal and then to landfill or a recycling program.

5.6. Public exposure

End-use products are designed for general consumption. The public may be repeatedly exposed to low levels of the notified chemical via a number of different consumer products; typical concentrations will range between 0.01% and 0.8%. Due to its low levels and use patterns, the overall daily exposure to the notified chemical will be very low for a person using one or more consumer products.

Public exposure from transport, storage, reformulation or disposal is considered to be negligible.

6. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa Clear colourless liquid

Melting Point/Freezing Point < -20°C

METHOD	BS4633: Method for the Determination of Crystallizing Point Method A1 of Commission Directive 92/69/EEC
Remarks	A sample of the notified chemical was placed in a test tube in a dry ice/acetone bath and did not freeze at the limit temperature of –20°C. Test conducted in accordance with GLP standards.
TEST FACILITY	SafePharm Laboratories Limited (2003a)

Boiling Point 206°C to 265°C at 101.3 kPa

METHOD	OECD TG 103 Boiling Point. EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks	The contract laboratory used differential scanning calorimetry to determine the boiling point. The test substance decomposed and therefore, no specific boiling temperature could be measured. To obtain an estimate of boiling temperature, the vapour pressure data were acquired using a vapour pressure balance and the boiling temperature was determined by extrapolation from log vapour pressure versus temperature. Test conducted in accordance with GLP standards.
TEST FACILITY	SafePharm Laboratories Limited (2003a)

Density 1000 kg/m^3 at 20°C

METHOD OECD TG 109 Density of Liquids and Solids.
EC Directive 92/69/EEC A.3 Relative Density.
Remarks The relative density was determined using a gas comparison pycnometer.

Test conducted in accordance with GLP standards.
TEST FACILITY SafePharm Laboratories Limited (2003a)

Vapour Pressure 9.33×10^{-5} kPa at 25°C

METHOD EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks The vapour pressure was determined using a vapour pressure balance system. In relation to the environment, the notified chemical is moderately volatile (Mensink *et al* 1995).

Test conducted in accordance with GLP standards.
TEST FACILITY SafePharm Laboratories Limited (2004a)

Water Solubility 13.3 mg/L at 20ºC

METHOD EC Directive 92/69/EEC A.6 Water Solubility
Remarks The determination was carried out using the Flask method. In relation to the environment, the notified chemical is moderately soluble (Mensink *et al* 1995).

Test conducted in accordance with GLP standards.
TEST FACILITY SafePharm Laboratories Limited (2003a)

Hydrolysis as a Function of pH Half life of > 1-year at pH 4, 71.5-days at pH 7 and < 1-day at pH 9.

METHOD EC Directive 92/69/EEC C.7 Abiotic Degradation

PH	*T (°C)*	*$t_{½}$ < days>*
4	25	>365
7	25	71.5
9	25	<1

Remarks Sample solutions were prepared in stoppered glass flasks at a nominal concentration of 5.00×10^{-3} g/L in three buffer solutions. Solutions were maintained at 50ºC, with samples of pH 7 also at 60ºC and 70ºC. Aliquots of the sample solutions were taken from the flasks at various times and the pH of each solution was recorded. The concentration of the sample solution was determined by GC. The rate constants and estimated half-lives at 25ºC of the test material were determined at pH 4, pH 7 and pH 9 by extrapolation.

Test conducted in accordance with GLP standards.
TEST FACILITY SafePharm Laboratories Limited (2003a)

Partition Coefficient (n-octanol/water) Log K_{OW} = 4.90

METHOD EC Directive 92/69/EEC A.8 Partition Coefficient
Remarks The HPLC method was used in the test since the test material emulsified within the n-octanol/water system in a preliminary test thus making it unfeasible to use the shake-flask method.

Test conducted in accordance with GLP standards.
TEST FACILITY SafePharm Laboratories Limited (2003a)

Adsorption/Desorption Log K_{OC} = 3.68

METHOD EC Directive 2001/59/EC C.19 Adsorption Coefficient
Remarks HPLC method.

Test conducted in accordance with GLP standards.
TEST FACILITY SafePharm Laboratories Limited (2003a)

Dissociation Constant Not conducted

Remarks The notified chemical is not subject to dissociation by inspection of the structure.

Flash Point 141°C at 101.3 kPa

METHOD EC Directive 92/69/EEC A.9 Flash Point.
Remarks The test flame was introduced into the sample in a closed cup.

Test conducted in accordance with GLP standards.
TEST FACILITY SafePharm Laboratories Limited (2004a)

Autoignition Temperature 376°C

METHOD 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks Aliquots of the test substance were heated in a flask and observed for ignition over a 300-second period. This procedure was repeated with varying sample size until the lowest temperature at which ignition occurred within 300-seconds was determined.

Test conducted in accordance with GLP standards.
TEST FACILITY SafePharm Laboratories Limited (2004a)

Explosive Properties

METHOD Estimated by Calculation
Remarks Based on an assessment of the chemical structure and the oxygen balance using the following calculation, the explosivity of the notified chemical is predicted to be negative. Oxygen balance = [-1600(2X +Y/2-Z)]/MW = 0.144 where X = number of carbon atoms, Y = number of hydrogen atoms, Z = number of oxygen atoms and MW = the molecular weight.
TEST FACILITY Reference: W.C. Lothrop and G.R. Handrich, The Relationship between Performance and Constitution of Pure Organic Explosive Compounds, Chemical Reviews, 44 pp. 419-445 (1949).

Reactivity Not conducted

Remarks The notified chemical is expected to be stable in water and air under normal conditions of temperature and pressure.

Fat (or n-octanol) Solubility Miscible in all proportions with standard fat (HB307) at 37ºC

METHOD OECD TG 116 Fat Solubility of Solid and Liquid Substances
Remarks The test material was liquefied in standard fat to obtain test samples of 5%, 50% and 95% w/w test material. The samples were shaken at 37ºC for 3-hours and then visually assessed for miscibility.

Test conducted in accordance with GLP standards.
TEST FACILITY SafePharm Laboratories Limited (2003b)

Surface Tension 53.4 mN/m at 21ºC

METHOD	EC Directive 92/69/EEC A.5 Surface Tension
Remarks	This determination was carried out using an interfacial tension balance and a procedure based on the ISO 304 ring method. The surface tension of the sample was measured at intervals until a constant reading was obtained. The concentration of the test material in the sample was determined by gas chromatography. The surface tension result was not corrected using the Harkins-Jordan correction table, as the correction is not applicable to the apparatus used. The test material is considered to be a surface-active material. Test conducted in accordance with GLP standards.
TEST FACILITY	SafePharm Laboratories Limited (2003a)

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	mildly irritating
Rabbit, eye irritation	mildly irritating
Rat, repeat dose oral, 28-days	NOAEL = 1000 mg/kg/day
LLNA, Mice	non-sensitising
Genotoxicity – in vitro Ames test	non-mutagenic
Genotoxicity – in vitro Chromosome aberration test	clastogenic
Genotoxicity – in vivo mouse micronucleus test	non-genotoxic
Human Repeated Insult Patch test	not-sensitising

7.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Toxic Class Method. EC Directive 2001/59/EC
Species/Strain	Rat/Sprague-Dawley CD strain
Vehicle	Arachis oil BP
Remarks - Method	Test conducted in accordance with GLP standards.

RESULTS

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

LD50	> 2500 mg/kg bw
Signs of Toxicity	There were no deaths following the administration of a single oral dose of the notified chemical at 2000 mg/kg bodyweight in arachis oil. All animals showed expected gains in body weight over the study period. There were no deaths, no signs of systemic toxicity and no abnormalities at necroscopy.
Effects in Organs	Terminal autopsy revealed no macroscopic lesions
Remarks - Results	None
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	SafePharm Laboratories Limited (2004b)

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 strain
Vehicle	None, material was moistened with distilled water prior to application.
Type of dressing	Semi-occlusive.
Remarks - Method	Test conducted in accordance with GLP standards

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	10 (5 female, 5 male)	2000	0

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	None
Signs of Toxicity - Systemic	None
Effects in Organs	None
Remarks – Results	None

CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	SafePharm Laboratories Limited (2004c)

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	Test conducted in accordance with GLP standards.
RESULTS	Slight to well-defined erythema and very slight oedema was seen in all rabbits. The notified chemical was determined to be a mild irritant.

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0.33	2	1	2	72 hours	0
Oedema	0	1	0	1	72 hours	0

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

Remarks - Results	Loss of skin elasticity and slight crust formation was seen in one rabbit. However, all animals were normal at the day 7 and at the end of the observation period at day 14.
CONCLUSION	The notified chemical was mildly irritating to rabbit skin.

TEST FACILITY SafePharm Laboratories Limited (2004d)

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 3-days
Remarks - Method 0.1 mL of the test substance was placed into the conjunctival sac of the right eye of 3-rabbits. The left eye served as the untreated control. The animals were assessed after 1, 24, 48 and 72-hours following treatment using the Draize scale. Minimal conjunctival irritation was noted in all treated eyes 1-hour after treatment with minimal conjunctival irritation also at the 24-hour observation. All treated eyes appeared normal at the 72-hour observation.

Test conducted in accordance with GLP standards.

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	0.6	0.3	0.3	1	48 hours	0
	6	3	3			
Conjunctiva: chemosis	0	0	0	1	1 hour	0
Conjunctiva: discharge	0.3	0	0	1	24 hours	0
	3					
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

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

CONCLUSION The notified chemical was slightly irritating to rabbit eyes.

TEST FACILITY SafePharm Laboratories Limited (2004e)

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

TEST SUBSTANCE Notified chemical

METHOD OECD TG 429 Skin Sensitisation – Local Lymph Node Assay
EC Directive 2001/59/EC

Species/Strain Mouse/CBA/Ca

Number of Animals Test Group: 12 female
Control Group: 4
Remarks - Method Three groups, each of four mice [CBA/Ca(CBA/CaBkl)] were treated with 50 µL (25 µL per ear) of the test material as a solution in acetone/olive oil 4:1 at concentrations of 25%, 50% and 100% v/v. A further group of four animals were treated with the vehicle alone as the control group. Five days after the first topical application of the test material, all mice were injected via the tail vein with 250 µL of phosphate buffered saline containing ^{3}H-methyl thymidine. Five hours later the mice

were killed and the draining auricular lymph nodes from the four mice were excised and pooled for each experimental group. The simulation index for each treatment group was calculated as the mean radioactive incorporation for each group divided by the control. The SI's were 1.32, 2.27, and 2.24 at the 25%, 50% and 100% concentrations respectively.

Test conducted in accordance with GLP standards.

RESULTS

Test Group	*Concentration (%)*	*Proliferative Response (DPM/lymph node)*	*Stimulation Index (SI)*	*Result*
Group 1	25	1309.42	1.32	Negative
Group 2	50	2242.49	2.27	Negative
Group 3	100	2414.87	2.44	Negative

Remarks - Results None

CONCLUSION There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY SafePharm Laboratories Limited (2004f)

7.6. Skin sensitisation – human volunteers

TEST SUBSTANCE Notified chemical

METHOD Human Repeated Insult Patch Test/Adaptation of Draize Patch Test

Study Design

Induction Procedure

Repetitive application of sample to the same site on the skin for approximately three weeks. An alternative site was used if samples evoked irritation under conditions of the test.

Rest Period

Following the induction period, the subjects did not receive any application of sample for approximately 14–days.

Challenge Procedure

Application of sample to a naïve site to test for reaction indicative of contact sensitisation.

Rechallenge Procedure

Application of sample or sample components to naïve site to confirm reaction indicative of contact sensitisation.

Study Group 107 individuals

Vehicle Alcohol SD39C or [75:25] Alcohol SD39C:Diethylphthalate

Remarks - Method None.

RESULTS

Remarks - Results No significant reactions were seen to the sample in any of the 107 subjects tested.

CONCLUSION A human repeated insult patch test was conducted using the notified chemical diluted with alcohol or [75:25] alcohol:diethylphthalate 4% under occlusive dressing. The notified chemical was non-irritating and/or non-sensitising under the conditions of the test to all 107 panellists.

TEST FACILITY CRL (2004)

7.7. Repeat dose toxicity

TEST SUBSTANCE Notified chemical

METHOD EC Directive 96/54/EC B.7, OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. Repeated Dose (28-days) Toxicity (Oral). USEPA Health Effects Test Guidelines, OPPTS 870.3050 Repeated Dose

	28-Day Oral Toxicity Study in Rodents, July 2000. Japanese MHW Guidelines 1986 for a twenty-eight day repeat dose oral toxicity study as required by the Japanese Chemical Substances Control Law 1973 of the METI amended 1986.
Species/Strain	Rat/Sprague-Dawley Crl:CD (SD) IGS BR
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28-days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Arachis oil (BP)
Remarks - Method	Three groups of 10 rats (5 males, 5 females), strain Sprague-Dawley Crl:CD (SD) IGS BR were dosed for 28 days consecutively by gavage. Dose levels were 15 mg/kg/day, 150 mg/kg/day and 1000 mg/kg/day. A control group of 10 rats were dosed with vehicle only. Two recovery groups (each of 5 male and 5 female) were treated with the high dose (1000 mg/kg/day) or the vehicle alone for 28-days consecutively and then observed without treatment for a further 14-days. Test conducted in accordance with GLP standards.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
I (control)	5 male, 5 female	0	0
II (low dose)	5 male, 5 female	15	0
III (mid dose)	5 male, 5 female	150	0
IV (high dose)	5 male 5 female	1000	0
V (control recovery)	5 male, 5 female	0	0
VI (high dose recovery)	5 male, 5 female	1000	0

Mortality and Time to Death

There were no treatment-related deaths during the study.

Clinical Observations

Animals of either sex treated with the high dose developed transient increased salivation around the time of dosing from day 2 onwards together with associated signs of increased salivation approximately one hour after dosing as well as instances of noisy respiration and stained fur or fur loss. Hunched posture was seen in high dose animals of either sex from day 21 and episodes of tiptoe gait were observed in two high dose females on day 26 only. All findings regressed in the high dose recovery group on cessation of treatment. Increased salivation of short duration is often reported following the oral administration of a test material and the daily occurrence of this finding around the time of dosing is usually considered attributable to an unpleasant tasting or locally irritant formulation rather than an indication of systemic toxicity. No treatment-related clinical signs were detected in animals treated with mid or low doses.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Clinical Chemistry
No toxicologically significant effects were detected in the blood chemical parameters measured.

Haematology
No toxicologically significant effects were detected in the haematological parameters measured.

Urinalysis
No abnormalities were detected in any of the parameters measured.

Effects in Organs

Organ weights
An increase in liver weight was detected in either sex treated with the high dose. Males treated with the high dose also showed an increase in relative kidney weight with an increase in the absolute weight of this organ also seen in recovery group males treated with the high dose at the end of the fourteen-day recovery period. No such changes in organ weights were detected for animals of either sex treated with the mid or low doses.

Necroscopy
Four males treated with the high dose showed pale kidneys. No other treatment-related macroscopic findings were observed.

Histopathology
Centrilobular hepatocyte enlargement was observed in high dose animals. A similar effect was also observed for one male rat dosed at the mid dose but this condition is occasionally observed spontaneously in control rats and thus cannot be regarded convincingly as an effect of treatment at this dose level. Hepatocyte enlargement is commonly observed in the rodent liver following the administration of xenobiotics and, in the absence of associated inflammatory or degenerative changes, is generally considered to be adaptive in nature, as evidenced by significant regression of the condition among high dose recovery animals.

Kidney
Globular accumulations of eosinophilic material, with associated renal tubular basophilia in some instances, were observed in the tubular epithelium of male rats of all dose groups. There was partial regression of the condition among high dose recovery group animals after completion of the 14-day recovery period. This finding is consistent with the presence of hydrocarbon nephropathy, which results from the excessive accumulation of $\alpha_2\mu$-globulin in renal proximal tubular epithelial cells. $\alpha_2\mu$-globulin is found only in the proximal tubular epithelium of adult male rats.

Thyroid
A higher incidence of follicular cell hypertrophy was seen in relation to treatment for female rats at the high dose, but not at any other dose level. The condition was observed to have regressed among recovery group animals given the high dose following an additional fourteen days without treatment.

CONCLUSION

Oral administration of the notified chemical to rats by gavage for a period of 28 days consecutively, resulted in treatment-related but non-adverse changes in either sex at the high dose and in males treated with the mid dose or low doses. There was no effect of treatment in females treated with mid or low doses. A clear No Observed Effect Level (NOEL) for treatment-related histopathological changes was not established in respect of renal changes in mid or low dose treated males but the NOEL in females was considered to be 150 mg/kg/day.

Treatment-related renal changes seen in male rats treated with mid or low doses were consistent with well-documented condition known as hydrocarbon nephropathy. This only occurs in male rats and is not indicative of a hazard to human health. Furthermore, the effects seen in animals treated with the high dose were confined to minimal clinical observations and adaptive, reversible liver and thyroid changes.

Therefore the No Observed Adverse Effect Level (NOAEL) was considered to be 1000 mg/kg/day.

TEST FACILITY SafePharm Laboratories Limited (2004g)

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.
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. Coli*:WP2 *uvr*A
Metabolic Activation System	S9 mix from liver homogenate of Arochlor 1254 induced rats
Concentration Range in Main Test	a) With metabolic activation: 50 to 5000 µg/plate. b) Without metabolic activation: 50 to 5000 µg/plate.
Vehicle	Dimethyl sulfoxide
Remarks - Method	A preliminary assay was carried out to determine the toxicity of the test substance. The concentrations tested were 0, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate. The main test was conducted at concentrations ranging from 50 to 5000 µg/plate. There was no visible decrease in the bacterial lawn both in the presence and absence of S9 activation.Test conducted in accordance with GLP standards.

RESULTS

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

Remarks - Results: The positive control substances demonstrated the sensitivity of the test and the negative controls were within historical limits.

CONCLUSION: The notified chemical was non-mutagenic to bacteria under the conditions of the test.

TEST FACILITY: SafePharm Laboratories Limited (2003c)

7.8. Genotoxicity – in vitro

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 473 Chromosome Aberration Test *in vitro*.
Annex V B.10 CHL cells *in vitro*

Cell Type/Cell Line: Chinese Hamster Lung Cells
Metabolic Activation System: S9 mix from liver homogenate of Arochlor 1254 induced rats
Vehicle: Dimethyl sulfoxide
Remarks - Method: Test conducted in accordance with GLP standards.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 5.28, 10.56*, 21.13*, 42.25*, 63.38*, 84.5	6	24
Test 2	0*, 5.28, 10.56*, 21.13*, 42.25*, 63.38, 84.5	24	24
Present			
Test 1	0*, 84.5169, 338, 676*, 1352*, 2704*	6	24

*Dose levels selected for metaphase analysis

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in PreliminaryTest	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	> 42.25			
Test 1		> 2704	> 169	
Test 2		> 2704	> 169	
Present	> 2704			
Test 1		> 1352	> 169	

Remarks - Results: The test substance induced a statistically significant increase in the frequency of cells with chromosomal aberrations in the presence of metabolic activation at the 10 mM dose level (2704 µg/mL) (the maximum recommended dose) only. A small but non-significant increase in the frequency of cells with aberrations was noted in the intermediate dose level of 1352 µg/mL. There were no increases observed in the absence of metabolic activation groups but the dose levels scored were limited by toxicity and were lower concentrations than in the group with S9.

CONCLUSION: The notified chemical was clastogenic to Chinese Hamster lung cells treated in vitro under the conditions of the test.

TEST FACILITY SafePharm Laboratories Limited (2003d)

7.9. Genotoxicity – in vivo

TEST SUBSTANCE Notified chemical

METHOD OECD TG 474 Micronucleus Test *in vivo*.
EC Directive 2000/32 B.12
Species/Strain Albino Crl:CD-1^{TM}(ICR)BR strain mice
Route of Administration Oral – gavage
Vehicle Arachis oil (BP)
Remarks - Method Administration of the test substance was via the oral route to groups of seven male mice at doses of 2000 mg/kg, 1000 mg/kg and 500 mg/kg. Animals were killed 24-hours or 48-hours hours later and polychromatic (PCE) and normochromatic (NCE) erythrocytes were scored for the presence of micronuclei.

Test conducted in accordance with GLP standards.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	7 male	0	24
II (vehicle control)	7 male	0	48
III (low dose)	7 male	500	24
IV (mid dose)	7 male	1000	24
V (high dose)	7 male	2000	24
VI (high dose)	7 male	2000	48
VII (positive control, CP)	5 male	50	24

CP=cyclophosphamide

RESULTS
Doses Producing Toxicity In the range-finding test, the high dose (2000 mg/kg bw) reached the limit dose for a non-toxic test substance.
Genotoxic Effects Negative. The presence of clinical signs was taken to indicate that systemic absorption had occurred.
Remarks - Results The test material did not cause a statistically significant decrease in the polychromatic (PCE)/normochromatic (NCE) erythrocytes ratio in any of the dose groups. The positive control group (dosed with cyclophosphamide) showed a marked increase in micro-nucleated PCE, confirming the sensitivity of the system to the known mutagenic activity of cyclophosphamide.

CONCLUSION The notified chemical was found to be non-genotoxic under the conditions of the test.

TEST FACILITY SafePharm Laboratories Limited (2003e)

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

TEST SUBSTANCE: Notified Chemical

METHOD
- Inoculum: Activated Sewage Sludge bacteria
- Exposure Period: 28-days
- Auxiliary Solvent: None
- Analytical Monitoring: Shimadzu TOC-5050A TOC Analyser.
- Remarks - Method: The test material, at a concentration of 10 mg C/L, was exposed to activated sewage sludge micro-organisms with culture medium in sealed culture vessels in the dark at 21ºC for 28 days.

  Following the recommendations of the International Standards Organisation (ISO 1996) and in the published literature (Handley *et al*, 2002), the test material was adsorbed onto granular silica gel prior to dispersion in the test medium in order to aid dispersion of the test material in the test medium and to increase the surface area of the test material exposed to the test organisms.

  The degradation of the test material was assessed by the determination of carbon dioxide produced. Control solutions with inoculum and the standard material, sodium benzoate, together with a toxicity control were used for validation purposes.

  Test conducted in accordance with GLP standards.

RESULTS

Test substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
1	16	1	24
6	37	6	49
10	43	16	56
20	69	20	69
28	89	28	85

- Remarks - Results: The test material attained 89% degradation after 28 days. However, it did not meet the 10-day window criteria, i.e. 60% degradation within 10 days of reaching 10%.

  Sodium benzoate attained 85% degradation after 28 days and passed the 10-day window validation criteria thereby confirming the suitability of the inoculum and test conditions.

  The toxicity control attained 83% degradation after 28 days thereby confirming that the test material was not toxic to the sewage treatment micro-organisms used in the study.

CONCLUSION: The notified chemical cannot be classed as readily biodegradable within the strict requirements of the test.

TEST FACILITY: SafePharm Laboratories Limited (2003f)

8.1.2. Bioaccumulation

TEST SUBSTANCE	Notified Chemical
METHOD	Computer Model Estimation
Remarks - Method	Log K_{OW} = 4.90 was entered as a constant for the model using the following SMILES: C1(C)(C)CCCC(C(C)OC(=O)CC(=O)OCC)C1.
RESULTS	
Bioconcentration Factor	1183
CONCLUSION	The notified chemical has the potential to bioaccumulate.
TEST FACILITY	BCF Program (v2.15)

8.2. Ecotoxicological investigations

8.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-Static
Species	Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period	96-hours
Auxiliary Solvent	None
Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	GC
Remarks – Method	Following a preliminary range-finding test, fish were exposed, in groups of ten, to an aqueous solution of the test material over a range of nominal concentrations for a period of 96-hours at a temperature of approximately 14ºC in sealed vessels under semi-static conditions. The nominal test concentrations were based on the result of chemical analysis of a saturated solution prepared for the Acute Toxicity to *Daphnia magna* study (see below) where the measured concentration of the saturated solution was 14.1 mg/L. The test material solutions were prepared by stirring an excess (100 mg/L) of test material in dechlorinated tap water via propeller stirrer at 2000 rpm at a temperature of approximately 25ºC for 24-hours. After the stirring period the preparation was cooled to 14ºC and any undissolved test material present was removed by filtration (0.2 µm Gelman Suporcap filter, first approximate 2 L discarded in order to precondition the filter) to produce a saturated solution with a nominal concentration of 14.1 mg/L. A series of dilutions were made from this saturated solution to prepare the test series, all of which remained clear and colourless throughout. The number of mortalities and any sub-lethal effects of exposure in each test and control vessel were determined after 3 hours after the start of exposure and then daily throughout the test until termination after 96-hours. The acute fish test was conducted in vessels that were completely filled with minimal headspace and sealed with no aeration to minimise loss due to volatilisation, though the test material is not inherently a volatile substance. The test water used for both the range-finding and definitive tests was the same as that used to maintain the stock fish. Laboratory tap water was dechlorinated by passage through an activated carbon filter and partly softened giving water with a total hardness of approximately 100 mg/L as

$CaCO_3$. After dechlorination and softening, the water was passed through a series of computer controlled plate heat exchangers to achieve the require temperature. Physico-chemical measurements of pH and temperature were taken throughout the study and these parameters remained within the accepted limits.

An estimate of the LC50 values at 3-hours was given by inspection of the mortality data. The LC50 values and associated confidence limits at 24-hours were calculated by the trimmed Spearman-Karber method (Hamilton *et al* 1977) using the ToxCalc computer software package (ToxCalc 1999) and at 48, 72 and 96-hours the LC50 values were calculated using the geometric mean method.

Test conducted in accordance with GLP standards.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
0.14	0.10	10	0	0	0	0	0
0.25	0.18	10	0	0	0	0	0
0.45	0.35	10	0	0	0	0	0
0.78	0.57	10	0	0	0	0	0
1.40	1.10	10	0	8	10	10	10

LC50 > 1.1 mg/L at 3-hours.
0.86 mg/L at 24-hours.
0.79 mg/L at 48-hours.
0.79 mg/L at 96-hours (95% CI 0.57–1.10 mg/L)

NOEC (or LOEC) 0.57 mg/L at 96-hours.

Remarks – Results Analysis of the freshly prepared saturated solution at 0, 24, 48 and 72-hours showed measured concentrations to range from 97% to 119% of the nominal.

Analysis of the freshly prepared test preparations at 0, 24, 48 and 72-hours showed measured test concentrations to range from 99% to 152% of nominal value indicating the correct dosing of the test system and that the test material was present in the dissolved form. Some of the measured test concentrations of the freshly prepared test media at 0, 24, 48 and 72-hours were in excess of the acceptance limits of 80% to 120% of nominal. This was considered to be due to an unknown factor as the saturated solution, from which the dilutions were made to prepare the remainder of the test series, was measured to be 97% to 119% of nominal.

Analysis of the 24-hour old test media at 24, 48, 72 and 96-hours showed a decline in measured test concentrations with values observed to range from 22% to 68% of nominal value. This was contrary to the preliminary stability analysis, which showed the test material to be stable in the test diluent over 24-hours. The decline in measured concentrations was attributed to the adsorption of the test material to the glassware and fish. A similar decline in the algal inhibition test (see below) both in opened and unopened test vessels.

Given the decline in measured test concentrations it was considered justifiable to base the results on the time-weighted mean measured test concentrations of the test media to give a “worst case” analysis of the data.

There were no sub-lethal effects of exposure observed in the test.

CONCLUSION The notified chemical is very toxic to fish.

TEST FACILITY SafePharm Laboratories Ltd (2004h)

8.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified Chemical

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

Species	*Daphnia magna*
Exposure Period	48-hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	GC
Remarks - Method	Following a preliminary range-finding test, twenty daphnids (2 replicates of 10 animals) were exposed to solutions of the test material over a range of nominal concentrations for 48-hours at a temperature of approximately 21ºC under semi-static test conditions. The test material solutions were prepared by stirring an excess (100 mg/L) of test material in reconstituted water at approximately 2000 rpm at a temperature of 25ºC for 24-hours prior to removing any undissolved test material by filtration (0.2 µm) through a pre-conditioned filter to produce a saturated solution with a nominal test concentration of 14.1 mg/L. A series of dilutions was made from this saturated solution to prepare the remainder of the test series. The number of immobilised *Daphnia* were recorded after 24 and 48-hours. Physico-chemical measurements of pH and temperature were taken throughout the study and these parameters remained within the accepted limits. The EC50 values and associated confidence limits at 24 and 48-hours were calculated by the maximum-likelihood probit method (Finney 1971) using the ToxCalc computer software package (ToxCalc 1999). Probit analysis was used where two or more partial responses to exposure are shown. Test conducted in accordance with GLP standards.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	Not given	10	0	0
0.14		10	0	0
0.25		10	0	0
0.45		10	0	0
0.79		10	0	0
1.40		10	0	0
2.50		10	0	3
4.50		10	4	9
7.90		10	7	10
14.10		10	10	10

LC50	6.5 mg/L at 24-hours (95% CI: 5.5–7.6 mg/L) 3.4 mg/L at 48-hours (95% CI: 2.9–3.9 mg/L)
NOEC	1.4 mg/L at 48-hours
Remarks - Results	Analysis of the test preparations throughout the exposure period showed measured test concentrations to range from 84% to 119% of nominal with the exception of the 1.4 mg/L test concentration at 0 hours and the 2.5 and 7.9 mg/L test concentrations at 24-hours (fresh test media) which, showed measured test concentrations of 123, 121 and 127% of nominal respectively. Duplicate frozen samples were analysed and showed measured test concentrations of 96, 103 and 111% of nominal respectively. Given that the duplicate frozen samples and the corresponding old media samples all gave measured test concentrations below the upper acceptance limit of 120% of nominal the high values shown initially were considered not to represent the true measured test concentrations. There was no decline in the measured test concentrations over each media renewal period, indicating that the test material was stable in the test diluent during the definitive test. Therefore, the results are based on nominal test concentrations only. All samples remained clear and colourless throughout the test period.
CONCLUSION	The notified chemical is toxic to *Daphnia magna*.
TEST FACILITY	SafePharm Laboratories Ltd (2004i)

8.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test. EC Directive 92/69/EEC C.3 Algal Inhibition Test.
Species	Green algae (*Scenedesmus subspicatus*)
Exposure Period	96-hours
Concentration Range	Nominal: 0.125, 0.250, 0.500, 1.000, 2.000 mg/L Actual: 0.031, 0.062, 0.240, 0.640, 1.500 mg/L
Auxiliary Solvent	None
Analytical Monitoring	
Remarks - Method	Following a preliminary range-finding test, *Scenedesmus subspicatus* was exposed to solutions of the test material (three replicates per concentration) for 72-hours, under constant illumination and shaking at a temperature of 24 ± 1ºC. The test material solutions were prepared by stirring an excess (100 mg/L) of test material in culture medium at approximately 2000 rpm at a temperature of 25ºC for 24-hours prior to removing any undissolved test material by filtration through a preconditioned 0.2 µm filter to produce a saturated solution with a nominal concentration of 14 mg/L. A series of dilutions was made from this saturated solution to prepare the required test concentrations. Samples of the algal populations were removed daily and cell concentrations determined for each control and treatment group, using a Coulter® Multisizer Particle Counter. Statistical analysis of the area under the growth curve data was carried out for the control and the 0.031, 0.062, 0.240 and 0.64 mg/L test concentrations using one way analysis of variance incorporating Bartlett's test for homogeneity of variance (Sokal and Rohlf 1981) and Dunnett's multiple comparison procedure for comparing several treatments with a control (Dunnett 1955).

The 95% confidence limits were calculated using the method of Litchfield and Wilcoxon (Litchfield and Wilcoxon 1949).

Test conducted in accordance with GLP standards.

RESULTS

Biomass		*Growth*	
E_bC50	*NOE_bC*	*E_rC50*	*NOE_rC*
mg/L at72 h	*mg/L at72 h*	*mg/L at 72 h*	*mg/L at 72 h*
0.22 (CI: 0.18-0.27)	0.031	0.46 (CI: 0.40-0.52)	0.031

Remarks - Results

There were no statistically significant differences between the control and 0.031 mg/L test concentration ($P \geq 0.05$), however all other test concentrations were significantly different ($P < 0.05$) and, therefore the No Observed Effect Concentration (NOEC) was 0.031 mg/L.

Analysis of the test solutions at 0 hours showed the measured test concentrations to range from 125% to 141% of nominal. Analysis of the test solutions at 0 hours prepared in culture medium alone (no algal cells) showed measured test concentrations to range from 137% to 143% of nominal, thereby indicating that immediate adsorption of the test material to the algal cells present did not occur.

Analysis of the test solutions at 72-hours showed a marked decline in measured test concentrations in the range of 4% to 43% of nominal. Analysis of a fourth test replicate prepared alongside the test, which remained unopened for the test duration, showed measured test concentrations in the range of 4% to 48% of nominal. This decline in measured test concentrations in the unopened test vessels was similar to that observed in the opened test vessels thereby indicating that the decline in measured test concentrations was due to adsorption of the test material to the algal cells present, not the volatile nature of the test material. Although the results obtained from the test solutions prepared in the presence of algal cells at 0-hours showed no evidence of adsorption to algal cells, this did not preclude long-term adsorption over the test period.

Given this decline in measured test concentrations it was considered justifiable to base the results on the geometric mean test concentration to give a "worst case" analysis of the data.

CONCLUSION

The notified chemical was found to be very toxic to *Scenedesmus subspicatus*.

TEST FACILITY

SafePharm Laboratories (2004j)

8.2.4. Inhibition of microbial activity

TEST SUBSTANCE

Notified chemical.

METHOD

OECD TG 209 Activated Sludge, Respiration Inhibition Test.
EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test

Inoculum: Activated sewage sludge
Exposure Period: 3-hours
Concentration Range: Nominal: 1000 mg/L
Remarks – Method: Following preliminary range-finding tests, activated sewage sludge was exposed to an aqueous dispersion of the test material at a concentration of

1000 mg/L (three replicate flasks) for a period of 3-hours at a temperature of 21ºC, with the addition of a synthetic sewage as a respiratory substrate.

The rate of respiration was determined after 30-minutes and 3-hours contact time and compared to data for the control and a reference material 3,5-dichlorophenol.

The percentage inhibition values were plotted against concentration for the reference material only, a line fitted with the Xlfit3 software package (IDBS, 2002) and the EC50 values determined from the equation for the fitted line.

The EC50 values of the test material were determined by inspection of the inhibition of respiration rate data.

Test conducted in accordance with GLP standards.

RESULTS

IC50	> 1000 mg/L
NOEC	1000 mg/L
Remarks – Results	The effect of the test material on the respiration of activated sewage sludge gave a 3-hours EC50 of greater than 1000 mg/L. The No Observed Effect Concentration after 3-hours exposure was 1000 mg/L. The reference material gave a 3-hour EC50 value of 10 mg/L, thus validating the test.

CONCLUSION The notified chemical did not inhibit the respiration of bacteria up to the limit of the test (1000 mg/L).

TEST FACILITY SafePharm Laboratories (2004k)

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

Nearly all of the imported notified chemical would eventually be released into the aquatic environment via the sewerage systems through formulation and use. Less than 15 kg per annum is expected to be disposed of to landfill as residue in empty containers via domestic garbage.

Since nearly all of the notified chemical will be washed into the sewer, under a worst case scenario, with no removal of the notified polymer in the sewage treatment plant, the resultant predicted environmental concentration (PEC) in sewage effluent on a nationwide basis has been estimated as follows:

Amount entering sewer annually (Worst Case)	1000 kg/y
Number of days used per year	365 d/y
Amount entering sewer per day (Worst Case)	2.74 kg/d
Population of Australia	20,100,000 persons
Daily water use per person	200 L/person/d
Daily water entering sewer	4020 ML/d
Predicted Environmental Concentration	0.682 µg/L

Based on dilution factors of 1 and 10 for inland and ocean discharges of STP-treated effluents, the PEC's of the notified chemical in freshwater and marine water may approximate 0.682 and 0.068 µg/L respectively.

The notified chemical is not readily biodegradable. The results obtained using the

SIMPLETREAT model (European Commission 2003) 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% is lost through volatilisation, 27% partitions to water, 55% degrades and 17 % partitions to biosolids, based on a Henry's Law Constant = 1.872 x 10^{-5} atm-m^3/mole and using the tables for chemicals that pass levels within 28-days in a test on "ready biodegradability", 10-day window criterion is not fulfilled.

Based on these results assuming that 27% of the notified chemical (270 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 0.184 µg/L prior to any dilution and the respective concentrations in freshwater and marine water may approximate 0.18 and 0.02 µg/L.

Partitioning to biosolids in STP's Australia-wide may result in an average biosolids concentration of 1.159 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 0.01 mg/kg in applied soil. This assumes that 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 0.05 mg/kg and 0.10 mg/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 0.184 mg/L may potentially result in a soil concentration of approximately 1.8 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 0.01 mg/kg and 0.02 x 10^{-4} 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 will be limited due to the relatively low volume imported and diffuse release to the sewer Australia wide. Also, the notified chemical is likely to degrade while exposed to the alkaline pH conditions of STP's, further limiting its potential to bioaccumulate.

9.1.2. Environment – effects assessment

The results of the ecotoxicological studies indicate that the notified chemical is moderately to highly toxic to aquatic organisms with the highest toxicity being to algae (E_bC_{50} = 0.220 mg/L) Therefore, a PNEC has been calculated using a safety factor of 100 as being 2.200 µg/L.

9.1.3. Environment – risk characterisation

Given the above, the following have been calculated:

Location	PEC (µg/L)	PNEC (µg/L)	Risk Quotient (RQ)
Australia-wide STP's (worst case)			
Inland river	0.682	2.2	0.310
Ocean outfall	0.068	2.2	0.031
After mitigation using SIMPLETREAT model (27 % remains in solution)			
Inland river	0.184	2.2	0.084
Ocean outfall	0.018	2.2	0.008

As the PEC/PNEC ratios are all less than 1, there should be an acceptable risk to aquatic organisms, even for considerably higher volumes of import, keeping in mind that only about 25% of sewer effluent is released to fresh water.

9.2. Human health

9.2.1. Occupational health and safety – exposure assessment

Exposure to imported fragrance oil.

Transport and warehouse workers may be exposed to the imported fragrance oil at a concentration of up to 10% of the notified chemical in the event of an accidental spillage or packaging breach. At the notifier's warehousing site, transport and warehouse workers are required to wear protective overalls, hard hats, chemical resistant gloves and safety glasses.

Worker exposure to the imported fragrance oil at up to 10% notified chemical might also 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 quality control sampling. Exposure may also occur during the cleaning and maintenance of equipment. Worker exposure will be minimised or eliminated by use of the appropriate personal protection equipment. Workers involved the formulation process will 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 at a concentration of 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. The notified chemical will be present between 0.01% to 0.8 % w/w in the final product. Thus, typically the public will be exposed to low levels of the notified chemical via wide range of cosmetic and domestic products.

The public would experience dermal exposure 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 liquid at room temperature and boils between 206°C and 265°C at 101.92 kPa and has a molecular weight of 270.37. The notified chemical has a vapour pressure of 9.33×10^{-5} kPa 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 (9.33×10^{-5} at 25°C) indicates that the potential for inhalation exposure will be very low. Considering the intended use of the substance, the main potential route of exposure is anticipated to be dermal.

The notified chemical has low acute oral and dermal toxicity. It is considered to be mildly irritating to the skin and to the eyes. The notified chemical was determined to be negative for the LLNA or in the human repeated insult patch tests for skin sensitisation.

No genotoxicity was observed in the Ames Test. The notified chemical was found to be clastogenic under the conditions of the in vitro Chromosome Aberration Test, though only at the highest concentration of 10 mM. However, the notified chemical was found to be non-genotoxic for the in vivo mouse micronucleus test and therefore cannot be classified as genotoxic.

The No Observed Adverse Effect Level (NOAEL) was considered to be 1000 mg/kg bw/day in a 28-day oral repeat dose study. A clear No Observed Effect Level (NOEL) for treatment-related histopathological changes was not established in respect of renal changes in 15 mg/kg bw/day or 150 mg/kg bw/day treated males but the NOEL in females was considered to be 150 mg/kg/day.

9.2.4. Occupational health and safety – risk characterisation

Occupational exposure may occur when handling the imported finished fragrance oil at a maximum concentration of 10% notified chemical. Formulation of notified chemical into end use consumer products will occur in fully automated and enclosed processes. However, some operations are not fully 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 vortex mixing of the batch, and during quality control testing. Workers handling the finished consumer products may 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 in humans. The low volatility of the notified chemical will restrict the possibility of exposure through inhalation.

The notified chemical is a mild skin irritant and mild eye irritant. 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.

There is a low occupational health risk posed by the notified chemical due to low potential for exposure and low hazard.

9.2.5. Public health – risk characterisation

The concentration of notified chemical in finished consumer products ranges from 0.01% to 0.8%. The public would experience dermal exposure to low levels of the notified chemical through use in personal and cosmetic products. Dermal absorption of the notified chemical is possible due to the physicochemical properties of the notified chemical.

The notified chemical was determined to be a mild skin irritant under the conditions of the rabbit acute skin irritation test (Section 7.3). However, under the conditions of the Human Repeat Insult Patch Test (Section 7.6), was found to be non-irritating and therefore the notified chemical is likely to be a slight irritant at most. The notified chemical is not likely to be an eye irritant.

Although the notified chemical was clastogenic at one dose in the chromosomal aberration study, genotoxic effects are unlikely as clastogenicity occurred only at a high dose suggesting it was weak and the concentration in consumer products should preclude any effects.

The notified chemical is present in formulated products at a maximum level of 0.8% (w/w) in toilet waters. This has been used to calculate the worst case dermal exposure scenario for notified chemical as follows:

Systemic Exposure Dosage (SED) = [da*c*mda]/bw (SCCNFP/0690/03 Final)

Where:

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

The following assumptions were made:
da = 100 % (assumed worst case)
c = 0.8 % (concentration of notified chemical in toilet waters)
mda = 3,750 mg (based on 0.75 g per use, 1-5 times/day)
bw = 60 kg

SED = [100%/100*0.8%/100*3750]/60
= 0.5 mg/kg bw/day

Margin of Exposure calculations were undertaken, based on the NOAEL from the subchronic study:

Margin of Exposure (MOE)
Subchronic Study NOAEL = 1000 mg/kg bw/day

MOE = NOAEL/systemic exposure
= 1000 mg/kg bw/day/0.5 mg/kg bw/day
= 2000

The Margin of Exposure exceeds the accepted value of 100 and is therefore acceptable. Therefore, the notified chemical is not likely to be a health risk to consumers.

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

10.1. Hazard classification

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

10.2. Environmental risk assessment

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

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

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

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:
  - 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

- Avoid release of concentrated notified chemical to the aquatic environment.

Disposal

- The notified chemical should be disposed of by thermal decomposition in incinerators or to secure landfill.

Emergency procedures

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

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 importation volume is likely to exceed one tonne per annum of notified chemical
  - the level of notified chemical in end-use products reaches or exceeds 1 % w/w
  - any further information regarding genotoxic potential, including clinical observations becomes 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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