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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Helvetolide

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Director
Chemicals Notification and Assessment

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HELVETOLIDE

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Firmenich Ltd
73 Kenneth Road Balgowlah NSW 2093

NOTIFICATION CATEGORY
Limited-small volume: Chemical other than polymer (1 tonne or less per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication:
Chemical name
Other names
CAS number
Molecular formula
Structural formula
Molecular weight
Spectral data
Identity and weight percent of toxic or hazardous impurities
Identity and weight percent of toxic or non-hazardous impurities
Identity and weight percent of additives and adjuvants

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)
Low Volume Chemical: Permit 37, LVC 38 (1995), Permit 237, LVC 267 (1998), Permit 377, LVC 420 (2001).

NOTIFICATION IN OTHER COUNTRIES
USA (1995), Switzerland (1995), Canada (2003), EU (1994, 1998, 2003) (ELINCS 415-490-5).

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Helvetolide

METHODS OF DETECTION AND DETERMINATION

ANALYTICAL METHOD	UV/visible spectrophotometry Infrared (IR) spectroscopy 1H and 13C NMR spectroscopy
Remarks	
TEST FACILITY	Firmenich Laboratories, Europe

3. COMPOSITION

DEGREE OF PURITY
Minimum 73% (sum of main diastereoisomers)

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS

None.

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (>1% by weight)
All identified impurities were present in the sample of notified chemical used for toxicity testing.

ADDITIVES/ADJUVANTS
None.

4. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. It will be introduced as a small component (maximum 5%) of compounded fragrances.

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

Year	*1*	*2*	*3*	*4*	*5*
Kilograms	150	200	250	300	350

USE
The notified chemical will be used as a fragrance ingredient in a variety of cosmetic and domestic products. It will be imported in liquid compounded fragrances, which will be reformulated in Australia to produce the final consumer products. In the final products, the concentration of the notified chemical will be a maximum of 1% in fine perfumes, and a maximum of 0.025% in other cosmetic products and domestic products.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, transport and storage

PORT OF ENTRY
The notified chemical will be imported through Sydney, by wharf or airport, as a small component of perfume preparations.

IDENTITY OF MANUFACTURER/RECIPIENTS
The fragrance preparations containing the notified chemical will be imported by Firmenich Ltd and will be reformulated locally. The fragrance preparations containing the notified chemical will initially be stored and distributed from the notifier's site. Customers (manufacturers of cosmetics, toiletries and household products) will receive the perfume compositions for blending into a wide variety of cosmetics, toiletries and household products.

TRANSPORTATION AND PACKAGING
The fragrance preparations containing the notified chemical will be transported by road from the wharf or airport of entry to the Firmenich Ltd warehouse for storage. Firmenich Ltd forward them directly to the clients. These fragrance preparations will be imported and distributed in tightly closed lacquered drums, typically of 180 kg size, but also 100, 50, 25, 10 or 5 kg. Final consumer products will be sold in a variety of small package sizes, typical of consumer-sized containers that will be transported to retail stores for distribution.

5.2. Operation description
The fragrance preparations containing the notified chemical will be reformulated at customer sites to produce domestic products in a continuous mixing process, which will involve a regulated feed of the fragrance mixture into an automated system. Cosmetic products will be produced in a batch process, which may involve open vessels and manual addition of the fragrance preparations containing the notified chemical, but usually batches will be produced by blending all ingredients together in a large mixer, usually closed, followed by automatic filling in containers of various sizes.
The final consumer products will be distributed to retail outlets, displayed and sold to the public.

5.3. Occupational exposure

Number and Category of Workers

Category of Worker	*Number*	*Exposure Duration (Hours/Day)*	*Exposure Frequency (Days/Year)*
FIRMENICH workers:			
storage, maintenance and quality control (and, if need arises, repack containers)	**3**	**1**	**1**
Customers' workers:			
Drum handling/storage/transport	**5**	**2**	**2**
Mixer/weighing/formulation	**5**	**4**	**2**
Drum cleaning/washing	**8**	**2**	**2**
Maintenance	**3**	**2**	**1**
Quality assurance	**2**	**½**	**1**
Packaging	**8**	**4**	**2**

Exposure Details

Import; Transport to & from Warehouse

The notified chemical will be imported as a component of fragrance preparations. These fragrance preparations will be transported by road to the Firmenich warehouse, and then distributed to clients for reformulation. Transport and warehouse workers will only be exposed to the notified chemical in the event of container breakage and/or accidental spillage.

Formulation of consumer products

Following distribution to clients, import containers of fragrance preparations containing the notified chemical will be opened and re-formulated into consumer products. The major occupational exposure to the notified chemical will be during these processes. Workers at these sites may be exposed to the notified chemical during warehouse, production line, cleaning and sampling or analysis tasks.

The notifier identified two classes of consumer products that will be made using fragrance preparations containing the notified chemical: household cleaning products and cosmetics.

Household cleaning products will be formulated in a continuous mixing process, with a regulated feed of the fragrance mixture into an automated system, and automated packing lines. Cosmetics will be produced in large batches of several thousand kilograms, with mixing vessels that may be open or closed. However, for large batches employing a number of hazardous components, typically this will necessitate use of closed lines, local exhaust ventilation where vapours or aerosols are produced, and automated packing lines. PPE including suitable gloves, eye and face protection and protective clothing will further reduce worker exposure, and should be used for any manual handling (such as manual addition to the mixing vessel).

End Use Products

Worker exposure to end use products may include professional cleaners (household cleaning products) and beauticians (cosmetics). These workers can be expected to use minimal PPE. However, the final concentration of notified chemical in cleaning and cosmetic products (other than fine perfumes) will be less than 0.025%.

5.4. Release

No manufacturing of the notified substance will occur in Australia. Environmental release of the notified substance is unlikely during importation, storage and transportation, and accidental spills, leaks and catastrophic mechanical failure during a transport accident is the most likely reason for environmental release. Established engineering controls (eg. 180 kg sealed and lacquered drum specifications) and established emergency clean-up procedures will limit the impact on the environment of such incidents.

RELEASE OF CHEMICAL AT SITE

Release of the notified chemical to the environment during blending of the cosmetic and household products is expected to be minimal due to the relatively small import quantity and the enclosed automated processes used. Potential sources of release include spills, equipment washing, and container residues. The drum size of the fragrance preparation containing the new chemical will determine the amount of environmental release in the event of an accidental spill. The notifier estimates that up to 0.1% of waste may be generated as a result of spills. No release is anticipated from

cleaning of formulation equipment. It is expected that this equipment will be cleaned using water and the aqueous solution reused for new purposes. The average amount of residue in empty containers after removal by vacuum pump is estimated to be < 0.1%. Therefore a total of 0.2% or up to 0.7 kg of waste may be generated each year as a result of formulation activities. Spilled material will be either reclaimed and reused or disposed of by incineration or landfill. Emptied imported containers of perfume compositions containing the notified chemical will either be recycled or disposed of through an approved waste management procedure (eg. metal recycling, drum reconditioning).

RELEASE OF CHEMICAL FROM USE

The majority of the notified chemical will enter the sewerage system after use of the consumer products (cosmetics, toiletries, household products) into which it is incorporated, when these products are washed off the hair and skin (i.e. up to ~350 kg/y). A widespread and diffuse use and disposal pattern is expected. The notifier estimates that finished product container residues of the notified chemical will vary depending of size, construction material (glass, plastic, metal, paperboard, etc) of the containers, physical state and viscosity of the consumer products and the way the consumers finish their products; however, residues of 0.1-3% of the consumer product may be assumed (i.e.<200 g/yr of the notified chemical based on 1% content). These emptied consumer containers will be disposed of into domestic rubbish and ultimately landfill.

5.5. Disposal

Disposal via incineration or landfill is recommended for wastes generated during the formulation of the products containing the fragrance preparations. The majority of the notified chemical will ultimately be disposed of in the sewer, with a minor proportion to landfill. The emptied imported drums may potentially be rinsed and re-used, sent to a recycler, or sent to landfill for disposal. Drum rinse water may be reused in additional batches. Following use, emptied consumer product containers are disposed of through domestic garbage disposal and hence will enter landfill or recycling.

5.6. Public exposure

Public exposure to the notified chemical as imported as a component of fragrance compositions could only occur in the event of transport accident or spillage. Public exposure from the reformulation process is unlikely. Public exposure to the notified chemical will occur during day-to-day usage of consumer products (cosmetics, toiletries and household products) containing the notified chemical at a maximum concentration of 1%.

6. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa Colourless liquid

Melting/Freezing Point Less than -24°C

METHOD EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks
TEST FACILITY SafePharm Laboratories Ltd (1994a)

Boiling Point 133-303°C (range) at 101.3 kPa

METHOD EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks Results indicate that test substance is a mixture
TEST FACILITY SafePharm Laboratories Ltd (1994a)

Density 938.33 kg/m^3 at 20°C

METHOD EC Directive 92/69/EEC A.3 Relative Density.
Remarks Density was measured using the pycnometer method.
TEST FACILITY SafePharm Laboratories Ltd (1995a)

Vapour Pressure 0.0233 kPa at 25°C

METHOD EC Directive 92/69/EEC A.4 Vapour Pressure.

Remarks The vapour pressure was measured using an isoteniscope system. Vapour pressure was measured in replicate at several temperatures between 170-254°C using a mercury in glass manometer. The temperature of the sample was regulated by use of a silicone oil bath. The test material did not change in appearance during the test. The test substance is volatile (Mensink et al., 1995). SafePharm Laboratories (2003b) derived a Henry's Law Constant of 2218 (units not described), thus indicating a potential for significant losses due to volatility.

TEST FACILITY SafePharm Laboratories Ltd (1995b)

Water Solubility 2.95 mg/L at 20°C

METHOD EC Directive 92/69/EEC A.6 Water Solubility.

Remarks Flask Method. A preliminary test was performed to determine the quantity of test material required to achieve 20 times the saturation concentration (determined to be ~100 mg/L). Water solubility was determined in replicate by stirring excess test material into water at 30°C, equilibrating for 24 hours at 20°C, and then separating the aqueous and non aqueous layers by centrifugation and filtration. The concentration of the test substance in the aqueous phase was determined by gas chromatography (GC) and the average of 3 determinations taken as the solubility. Test solution pH range 6.9-7.4. The test substance is slightly soluble in water (Mensink et al., 1995).

TEST FACILITY SafePharm Laboratories Ltd (1994a)

Hydrolysis as a Function of pH

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

pH	*T (°C)*	*$t_{½}$*
4	25	>1 year
7	25	>1 year
9	25 & 50	224 hours & 19.4 hours

Remarks A preliminary test was performed at pH 4, 7 and 9; however, only in the pH 9 test did >10% hydrolysis of the test material occur after 5 days at 50°C. Consequently, a definitive test was performed only at pH 9 at 25°C and over a period of 411 hours. Aliquots of stock solution (test substance in acetonitrile) were used to prepare test solution concentrations for addition to pH buffered solutions of pH 4, 7 and 9. All solutions were shielded from light. Samples were analysed in duplicate by GC. Initial sample concentrations for pH 4, 7 and 9 solutions were $1.52x10^{-3}$, $1.53x10^{-3}$ and $1.86x10^{-3}$ g/L, respectively, in the range finding test and 1.61X10-3 g/L in the definitive test.

TEST FACILITY SafePharm Laboratories Ltd (1995a)

Partition Coefficient (n-octanol/water) log Pow = 4.68 at 22.5°C

METHOD EC Directive 92/69/EEC A.8 Partition Coefficient.

Remarks A preliminary assessment was performed based on the solubility in the pure solvents. The 3 definitive tests were in duplicate. Aliquots of test substance (25, 50 and 100 mL) were prepared in water saturated n-octanol (200 mL). After shaking and phase separation, the concentration of the test material in the aqueous and organic phases was determined by GC.

TEST FACILITY SafePharm Laboratories Ltd (1994a)

Adsorption/Desorption log Koc = 3.34 (estimated)

METHOD

Remarks The notifier indicates that the log Koc was calculated using QSAR for esters. The method is recommended by the EEC to calculate the Koc of various classes of

organic compounds (European Commission, 2003). For esters, the QSAR is calculated as follows: Log Koc = 0.49 Log Kow + 1.05 = 3.34. The notified chemical is expected to be slightly mobile in soils.

TEST FACILITY

Dissociation Constant Not determined. There are no acidic or basic groups on the molecule able to dissociate.

Particle Size Test not conducted as the notified chemical is a liquid.

Flash Point 139°C at 100.6 kPa

METHOD EC Directive 92/69/EEC A.9 Flash Point.
Remarks The determination was carried out using the closed cup equilibrium method.
TEST FACILITY SafePharm Laboratories Ltd (1994b)

Flammability Limits Test not conducted since experience in use indicate that negative results would be obtained.

Autoignition Temperature 294°C

METHOD 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks
TEST FACILITY SafePharm Laboratories Ltd (1995c)

Explosive Properties Not explosive

METHOD EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks A negative result was obtained for sensitivity to shock and to heat. The test for friction is not applicable to liquids.
TEST FACILITY SafePharm Laboratories Ltd (1995c)

Reactivity The notified chemical is expected to be stable under normal environmental conditions. No test of oxidising properties was performed, however the notified chemical has not structural indications of oxidising properties or other unusual reactivity.

ADDITIONAL TESTS

Surface Tension 62.4 mN/m at 18.5oC

METHOD EC Directive 92/69/EEC A.5 Surface Tension.
Remarks ISO 304 Ring Method using a surface tensiometer. Test solution was prepared by dilution of test substance (0.0542 g) in 500 mL of glass double distilled water. After mixing (~18 h at 30°C), standing (~3 h at 20°C) a centrifuging (2000 rpm for 10 minutes), the supernatant was filtered (0.45 µm) and diluted to 90% of its former concentration with the dilution water. Surface tension readings were repeated until constant values were obtained. Test substance concentration was determined (in duplicate) by GC (mean sample concentration was 3.91×10^{-3} g/L). The test substance (ST06C93) is not a surface active substance (<60 mN/m) at the concentration tested.
TEST FACILITY SafePharm Laboratories Ltd (1995a)

7. TOXICOLOGICAL INVESTIGATIONS

Endpoint	*Assessment Conclusion*
Rat, acute oral	low toxicity LD50 >2000 mg/kg bw
Rat, acute dermal	low toxicity LD50 >2000 mg/kg bw
Rabbit, skin irritation	Slightly irritating
Rabbit, eye irritation	Slightly irritating
Guinea pig, skin sensitisation – adjuvant test	slight evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL 250 mg/kg bw/day
Genotoxicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosome aberration	non genotoxic
Rat, reproductive toxicity-one generation	NOAEL on reproductive function > 1000 mg/kg bw/day NOAEL on parental toxicity > 1000mg/kg bw/day
Human, repeat insult patch test – skin sensitisation	No evidence of sensitisation to 20% preparation

7.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	EC Directive 92/69/EEC B.1 Acute toxicity (Oral)
Species/Strain	Rat/Crl:CD (SD) BR (VAF plus)
Vehicle	Water
Remarks - Method	Single dose by oral gavage. Observation period: 14 days.

RESULTS

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

LD50	>2000 mg/kg bw
Signs of Toxicity	No clinical signs of toxicity. No effect on bodyweight.
Effects in Organs	At necropsy, the submandibular lymph nodes were enlarged and the bladder was distended with fluid in one male. The thymus was red and swollen in one female. Due to their low incidence, these findings are considered not to be of toxicological significance.
Remarks - Results	

CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Toxicol Laboratories Ltd (1994a)

7.2. Acute toxicity - dermal

TEST SUBSTANCE	Notified chemical
METHOD	EC Directive 92/69/EEC B.3 Acute toxicity (Dermal)
Species/Strain	Rat/Crl:CD (SD) BR (VAF plus)
Vehicle	none
Type of dressing	Occlusive
Remarks - Method	Limit test in which the test article was dermally applied under an occlusive dressing to the clipped backs of rats at a dose level of 2000 mg/kg/bodyweight. After a contact period of 24 hours, the dressings were

removed and the treated skin cleansed with water. The animals were observed on the day of dosing and daily thereafter for a further 14 days at the end of which, they were killed and subjected to necropsy.

RESULTS

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

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	All animals maintained a healthy appearance throughout the observation period. A scab on the head of one male was noted on day 15 but this is considered not to be treatment related. No effect on bodyweight.
Signs of Toxicity - Systemic	None.
Effects in Organs	At necropsy, abnormal findings were confined to one male and consisted of moderate pelvic dilation of one kidney and a swollen submandibular lymph node.
Remarks - Results	

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

TEST FACILITY Toxicol Laboratories Ltd (1995a)

7.4. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD	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	Animals assessed at 24, 48 and 72 hours
Type of Dressing	Semi-occlusive.
Remarks - Method	One animal was treated initially as a pilot.

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	0	0	0	2	< 24 h	0
Oedema	0	0	0	1	< 24 h	0

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

Remarks - Results Skin reaction to the test article was confined to well defined erythema and/or barely perceptible oedema noted at the treated site on all rabbits one hour after patch removal. The skin on all 3 animals was free of signs of irritation at the 24 hour observation and remained so at all subsequent examinations.

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY Toxicol Laboratories Ltd (1994b)

7.5. Irritation - eye

TEST SUBSTANCE Notified chemical

METHOD — EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).
Species/Strain — Rabbit/New Zealand White
Number of Animals — 3
Observation Period — Animals assessed at 24, 48 and 72 hours
Remarks - Method — One animal was initially treated as a pilot.

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	0	0	1	< 24 h	0
Conjunctiva: chemosis	0	0	0	0	-	0
Conjunctiva: discharge	0	0	0	1	< 24 h	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

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

Remarks - Results — The test article produced very slight conjunctival hyperaemia in the treated eye of all 3 rabbits one hour after dosing. This was accompanied by a slight discharge from the treated eye in 2 animals. 24 hours after dosing, the treated eye of all 3 animals was free of signs of irritation and remained so for the remainder of the 72 hours observation period.

CONCLUSION — The notified chemical is slightly irritating to the eye.

TEST FACILITY — Toxicol Laboratories Ltd (1994c)

7.6. Skin sensitisation

TEST SUBSTANCE — Notified chemical

METHOD — EC Directive 96/54/EC B.6 Skin Sensitisation – Maximisation Test
Species/Strain — Guinea pig/Dunkin Hartley
PRELIMINARY STUDY — Maximum Non-irritating Concentration:
intradermal: 25% in liquid paraffin
topical: 100%
MAIN STUDY
Number of Animals — Test Group: 20 Control Group: 10
INDUCTION PHASE — Induction Concentration:
intradermal: 25%
topical: 100%
Signs of Irritation — No observations reported after Induction Phase
CHALLENGE PHASE
Topical induction only — Challenge concentration: 100% and 50% in ethanol
Remarks - Method — Only one challenge phase.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st challenge		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	100%	1/20	0/20	N/A	N/A
	50%	0/20	0/20	N/A	N/A
Control Group	100%	0/10	0/10	N/A	N/A
	50%	0/10	0/10	N/A	N/A

Remarks - Results — Following application of undiluted test article, one test animal exhibited a

	skin response at the 24 hour examination resulting in a response incidence of 5%.
CONCLUSION	There was slight evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Toxicol Laboratories Ltd (1994d)

7.7. 4 week repeat dose oral toxicity

TEST SUBSTANCE	Notified chemical
METHOD	EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/Crl:CD(SD)BR (VAF plus)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days; Dose regimen: 7 days per week; Post-exposure observation period: None
Vehicle	0.5% w/v carboxymethylcellulose
Physical Form	liquid
Remarks - Method	Haematology and blood chemistry were evaluated for all animals at the end of the study. All animals were subjected to a gross necropsy examination and histopathological evaluation of selected tissues from 1000 mg/kg/day and control animals was performed.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
I (control)	5 per sex	0	0/10
II (low dose)	5 per sex	250	0/10
III (mid dose)	5 per sex	500	0/10
IV (high dose)	5 per sex	1000	0/10

Mortality and Time to Death

There were no deaths during the study.

Clinical Observations

There were no treatment related clinical signs noted. The only clinical sign noted was dorsal cervical hair loss in one group (250 mg/kg/day) on days 7-29.

Bodyweights and bodyweight gains were unaffected by administration of the test article. Food consumption was not affected by treatment. There was no treatment related ocular findings.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematological evaluation revealed a higher mean neutrophil and lower mean lymphocyte counts in males of groups at dose levels of 500 and 1000 mg/kg/day. These values were at the upper or lower limit of the normal ranges respectively. The toxicological significance of these findings cannot be discounted. A small number of other statistically significant changes were noted, however all changes were within the normal ranges found in the laboratory.

Mean glucose levels were reduced in all treated male groups and female group treated at dose level of 1000 mg/kg/day. As these values were within the normal ranges found in the laboratory and/or did not occur at a dose related manner they were considered not to be of toxicological significance. All other statistically significant changes were within the normal ranges found in the laboratory and were considered to be unrelated to treatment. Urinary parameters were unaffected by administration of the test article, except one male of group dosed at 500 mg/kg/day and 2 males dosed at 1000 mg/kg/day which show moderate levels of ketones.

PATHOLOGY

1. Effects in Organs

Absolute and bodyweight related liver weights were increased in males dosed at 500 mg/kg/day and both sexes dosed at 1000 mg/kg/day.
A statistically significant increase in absolute and bodyweight related thyroid weight was apparent in males dosed at 1000 mg/kg/day. All treated females showed an increase in bodyweight related thyroid weight. Group mean values were within the normal ranges found in the laboratory.

2. Macroscopic Findings
There were no treatment-related macroscopic findings.

3. Histopathology
Microscopic examination revealed an increased incidence of minimal kidney tubular basophilia in animals dosed at 1000 mg/kg/day compared to the controls. The remaining small number of histopathological findings recorded were within the normal range seen in rats of this age and strain.

Remarks – Results
Administration of test substance orally by gavage at a dose level of 1000 mg/kg/day was associated with changes in neutrophil and lymphocyte counts and increased absolute and bodyweight related liver weights in both sexes. In the absence of any histopathological findings the increased liver weights were considered to be associated with the metabolism of the test article.
At 500 mg/kg/day, absolute and bodyweight related liver weights were higher in males only.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 250 mg/kg bw/day in this study, based on the liver weight increase and haematological changes at higher doses.

TEST FACILITY Toxicol Laboratories Ltd (1996)

7.8. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD
- Species/Strain: *S. typhimurium*: TA1538, TA1535, TA1537, TA98, TA100
- Metabolic Activation System: Aroclor 1254 activated S9 fraction
- Concentration Range in Main Test: a) With metabolic activation: 8-5000 µg/plate. b) Without metabolic activation: 8-5000 µg/plate.
- Vehicle: Acetone
- Remarks - Method: Two independent mutation tests were performed

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	No cytotoxicity observed	No cytotoxicity observed	No precipitation observed	No genotoxicity observed
Test 2	No cytotoxicity observed	No cytotoxicity observed	No precipitation observed	No genotoxicity observed
Present				
Test 1	No cytotoxicity observed	No cytotoxicity observed	No precipitation observed	No genotoxicity observed
Test 2	No cytotoxicity observed	No cytotoxicity observed	No precipitation observed	No genotoxicity observed

Remarks - Results: No cytotoxicity and no significant increase in the number of revertant colonies were recorded for any of the bacterial strains with any dose of the test article, either with or without metabolic activation.

CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	SafePharm Laboratories Ltd (1994c)

7.9. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Cell Type	Human peripheral blood lymphocytes
Metabolic Activation System	Aroclor 1254 activated S9 fraction
Vehicle	Ethanol
Remarks - Method	Test performed in duplicate

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Present				
Test 1	10, 20, 40*, 80*,160*	4 h	16 h	20 h
Test 2	20, 40*, 80*, 160*, 320	4 h	16 h	20 h
Test 2	20, 40, 80, 160*, 320	4 h	40 h	44 h
Absent				
Test 1	5, 10*, 20*, 40*, 80	20 h		20 h
Test 2	10, 20*, 40*, 60*	20 h		20 h
Test 2	10, 20, 40*, 60*	44 h		44 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Present				
Test 1	80	160	None reported	None observed
Test 2		No cytotoxicity observed up to 320	None reported	None observed
Absent				
Test 1	80	60	None reported	None observed
Test 2		60	None reported	None observed

Remarks - Results	No statistically significant increases in the frequency of cells with aberrations or polyploid cells were observed either in the presence or absence of metabolic activation, at any of the dose levels tested. Appropriate positive controls induced large increases in the number of aberrant cells, indicating that the test system responded appropriately.
CONCLUSION	The notified chemical was not clastogenic to human peripheral blood lymphocytes treated in vitro under the conditions of the test.
TEST FACILITY	SafePharm Laboratories Ltd (1995d)

ADDITIONAL INVESTIGATIONS

7.13T. Skin sensitisation – human volunteers

TEST SUBSTANCE	Notified chemical
METHOD	Human repeated insult patch test
Study Design	The study took 6 weeks and had 3 phases: induction, rest and challenge.

	The test material was the notified chemical diluted to 20% in diethyl phthalate.
Study Group	113 male and female subjects between the ages of 18 and 70, of which 108 completed the study.
Vehicle	Diethyl phthalate
Induction Procedure	9 consecutive applications of the study material with subsequent evaluation of patch sites. Patches were removed 24 hours after application. Subjects were assessed at 48 hour intervals.
Rest Period	10 to 15 days
Challenge Procedure	Identical patches were applied to sites previously unexposed to the study material. The patches were removed after 24 hours and the patch sites assessed after a further 24 and 48 hours.
Remarks - Method	No re-challenge.

RESULTS

Remarks - Results	There was no evidence of sensitisation in the 108 subjects who completed the study. No adverse events were reported.

CONCLUSION	The notified chemical was non-irritating and non-sensitising under the conditions of the test.
TEST FACILITY	TKL Research, Inc. (2001)

7.15. Toxicity to reproduction – one generation study

TEST SUBSTANCE	Notified chemical
METHOD	OECD 415 One-Generation Reproduction Toxicity Study
Species/Strain	Rat / Sprague-Dawley Crl:CD (SD) IGS BR
Route of Administration	Oral – gavage
Exposure Information	Exposure period - female: 17 days before pairing; up to 21 days during mating; throughout gestation; up to 21 days post partum Exposure period - male: 73 days before pairing, and up to 21 days during mating
Vehicle	0.5% carboxymethylcellulose
Remarks – Method	Histopathology was carried out on reproductive and target organs from control and high dose group parental animals. The target organs were examined from the low and intermediate dose groups.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
1	28 males & 28 females	0	0/56
2	28 males & 28 females	50	1/56
3	28 males & 28 females	250	0/56
4	28 males & 28 females	1000	0/56

Mortality and Time to Death

At 50 mg/kg one parental female was found dead during the gestation phase. There was no previous clinical history. Post mortem macroscopic findings showed significant changes within the gastro-intestinal tract including gaseous distension of the small intestine and discolouration of the contents of the intestines.

Effects on Parental (P) animals:

For all dose levels, particularly 250 mg/kg and above there was evidence of increased salivation, predominantly post dosing. The incidence and frequency were dosage-related. The finding was considered to be an adaptation to administration of an unpleasant-tasting material. There were no other significant effects upon adults during the in-life phase of the study.

There were no treatment-related effects upon reproductive performance or fertility.

Post-mortem findings showed no treatment-related effects upon the reproductive organs.

At 1000 mg/kg there were significantly increased liver and kidney weights for males only compared to control values. Histopathology showed hepatocyte enlargement and vacuolation for males and females. In addition eosinophilic accumulations were observed in the kidney of males only together with an increased prevalence of basophilic tubules.
At 250 mg/kg there was a significantly increased kidney weight for males only compared to controls. Histopathology showed similar findings in the liver and kidneys of male rats only to those observed at the highest dose levels. The incidence and severity was lower than the highest dose level indicating a dose response relationship.
At 50 mg/kg histopathological findings were restricted to accumulation of eosinophilic material in the renal tubules of male kidneys.

Effects on 1st Filial Generation (F1)
There were no treatment-related effects upon offspring viability, growth or development.

Remarks - Results
The histopathology findings in parental animals are commonly associated with adaptive responses to the metabolism and excretion of hydrocarbon based xenobiotics.

CONCLUSION
The administration of the test material to male and female rats throughout the reproductive cycle for one generation resulted in some evidence of toxicity to the adults.
There was no evidence of adverse effects on reproductive organs, reproductive performance, or offspring viability, growth and development during gestation or lactation.
The No Observed (Adverse) Effect Level (NO(A)EL) for reproduction and offspring viability development is > 1000 mg/kg/bodyweight/day.
As treatment-related effects (globular accumulations of eosinophilic material in the renal tubules) were observed at the lowest dose, no NOEL for parental toxicity was established. However, as the effects observed at all doses were adaptive rather than adverse, the NOAEL for parental toxicity was > 1000 mg/kg bw/day.

TEST FACILITY SafePharm Laboratories Ltd (2003a)

8. ENVIRONMENT

8.1. Environmental fate

8.1.1a. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test.
Inoculum	Mixed biological population of sewage treatment microbes, Severn Trent Water plc, UK.
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Degradation of the test material was assessed by measurement of daily dissolved oxygen depletion in standard test solutions on days 0, 3, 6, 9, 12, 15, 18, 21, 24 and 28 by means of a DO probe.
Remarks - Method	The test material (100 mg) was dispersed directly in culture medium (~1000 mL) to give a 100 mg/L stock solution. An aliquot (120 mL) of stock solution was dispersed in 6 L of culture media to give a test concentration of 2 mg/L. 60 mL of the stock solution and 9 mL of 1000 mg/L stock solution of sodium benzoate were dispersed in 6 L of inoculated culture media to give a test concentration of 1 mg/L of test substance and 1.5 mg/L of sodium benzoate to act as a toxicity control. Test chambers were inoculated at a rate of 1 drop of inoculum per litre of test solution. The test chambers were incubated for 28 days (21°C) in sealed 250-300 mL BOD bottles.

RESULTS

Test substance (2.0 mg/L)		*Sodium Benzoate Reference 1.5 mg/L*	
Day	*% Degradation*	*Day*	*% Degradation*
6	6	6	52
12	11	12	83
18	12	18	80
24	18	24	81
28	17	28	85

Remarks - Results	The biodegradation of the reference substance, sodium benzoate was 85% after 28 days, indicating the test conditions were valid. Analysis of the concentration, stability and homogeneity of the test material and preparations was not considered appropriate to the test guideline.
CONCLUSION	The test material achieved 17% degradation in 28 days and cannot be classed as readily biodegradable under the conditions of the test. The toxicity control achieved 36% degradation in 28 days indicating that the test substance was not toxic to the sewage microbes used in the study.
TEST FACILITY	SafePharm Laboratories (1994d)

8.1.1b. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: Modified Sturm Test (CO_2 Evolution Test).
Inoculum	Activated sludge acclimated microbial inoculum from duplicate semi-continuous activated sludge (SCAS) units dosed with test substance (10 and 20 mg/L nominal).
Exposure Period	28 days
Auxiliary Solvent	None

Analytical Monitoring	Degradation of the test material was assessed by measurement of soluble organic carbon (SOC) at the completion of the test..
Remarks - Method	The test apparatus consisted of four Erlenmeyer flasks (4 L) containing 2 L of modified BOD water. Test material (0.02 g, 0.04 g) was mixed in dilution water and microbial inoculum. CO2-free air was supplied at the flasks through a CO2-scrubbing train. Three bottles containing 100 mL of Ba(OH)2 were connected to each flask to trap the evolved CO2 from the flasks. The amount of CO2 produced was chemically analysed by titration of soluble organic carbon (SOC). The cumulative percentage of theoretical CO2 (TCO2) produced for the individual flasks was determined. The test chamber was incubated at ~23°C. A readily biodegradable reference substance (D-glucose) was also tested.

RESULTS

Substance	*ThoD (mgCO2*	*Final SOC (mg C/L)*	*Degradation (% of TCO2)*		
			Day 7	*Day 13*	*Day 28*
Blank control	58.68	0.8	48.1	56.9	65.3
A	58.68	0.9	48.1	56.9	65.3
B	52.60	5.1	12.2	13.5	20.3
C	105.2	10.5	9.5	12.2	16.3

A = D-glucose; B = Test material (10 mg/L); C = Test material (20 mg/L).

Remarks - Results	The biodegradation of the reference substance, D-glucose was ~65% after 28 days, indicating the test conditions were valid.
CONCLUSION	The test material achieved ~16-20% degradation in 28 days and cannot be classed as readily biodegradable under the conditions of the test.
TEST FACILITY	Roy F. Weston, Inc. (1995a)

8.1.1c. Inherent biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	Semi-continuous Activated Sludge (SCAS) Removability Test based on Soap and Detergent Association (1965) Procedure and Standards for the Determination of Biodegradability of Alkyl Benzene Sulfonate and Linear Alkylate Sulfonate.
Inoculum	Acclimated activated sludge, Downingtown Regional Water Pollution Control Center, Downingtown, USA. Filtered 2 mm. Total suspended solids 3280 mg/L. Acclimated 7 days pre-test to incremental additions up to 20 mg/L.
Exposure Period	14 days
Auxiliary Solvent	None
Analytical Monitoring	Soluble organic carbon (SOC) was monitored daily in test solution effluents
Remarks – Method	Test apparatus consisted of 4 SCAS aeration chambers containing 1.5 L of activated sludge (total suspended solids ~2500 mg/L). The units were aerated sufficient to maintain solids in suspension. Test substance was added by weight directly to test chambers (duplicated) for a 7 day acclimation period (nominal test concentration was 20 mg/L). A control was also tested. Throughout the test, all units were fed synthetic sewage daily. The test duration was extended from 7 to 14 days to obtain steady-state conditions.

RESULTS

Day	*Control Unit SOC (mg/L)*	*Test Unit SOC (mg/L)*	*Test Unit Carbon Removal (%)*

1	7.8	17.0	33
3	9.1	21.6	9.4
5	8.7	18.9	25.7
7	7.6	17.5	30.6
14	6.0	17.6	21.2
21	5.8	17.7	16.3
28	5.3	16.0	26.7

Remarks – Results

On day 3 of the test, an oily film/residue was present on the SCAS units containing test substance only above the sludge surface line, indicating incomplete dissolution and degradation. In addition, the settled sludge levels in the test substance units were higher than the settled sludge levels in the control, and the clarity of the supernatants in the settled test substance units was better than the clarity of the supernatants in the control units, suggesting greater settlement of suspended material in the test solutions containing the notified chemical. Average percent SOC removal was 25% (95% CL ±1.6%) after 14 days.

CONCLUSION

With a mean removal rate of only 25% SOC removal after 28 days contact time, the notified chemical is not inherently biodegradable under the conditions of the test.

TEST FACILITY

Roy F. Weston, Inc. (1995b)

8.1.2. Bioaccumulation

No bioaccumulation study was conducted. As the test substance has an octanol:water partition coefficient (log Pow) of 4.68, a high affinity to lipids is expected and bioaccumulation may potentially occur.

8.2. Ecotoxicological investigations

8.2.1. Acute toxicity to fish

TEST SUBSTANCE

Notified chemical

METHOD

Species	Rainbow trout (*Oncorhynchus mykiss*); juvenile, 4.2 cm length, 0.56 g.
Exposure Period	96 h
Auxiliary Solvent	10% v/v Tween 80-dimethylformamide (DMF)
Water Hardness	100 mg/L (as $CaCO_3$)
Analytical Monitoring	GC analysis of test solutions at 0, 24, 48, 72 and 96 h.
Remarks – Method	Range finding and definitive tests were performed. Aliquots of test material (0.350, 0.625, 1.10 and 1.975 g) were separately dissolved in solvent and the volume adjusted to 25 mL. Aliquots (2 mL) were then dispersed in ~750 mL reverse osmosis water with the aid of ultrasonication. The volume was then adjusted to 1 L in water prior to dispersal in 20 L (final volume) of dechlorinated tap water (activated carbon filtered) to give the required nominal test concentrations. To prepare the nominal test concentration of 14 mg/L, an amount of test material (0.28 g) was dissolved in 2 mL solvent and then dispersed in reverse osmosis water to 1 L then dispersed in 20 L of dechlorinated tap water. Control and solvent controls were also tested. Test aquaria consisted of 20 L glass containers (covered; not aerated; no replicates were used). Test temperature was 14±1°C, pH range 7.2-7.3, dissolved oxygen >9.8 mgO2/L. Photoperiod was 16 light: 8 h dark. Fish were not fed during exposure. The LC50 values and associated confidence limits were calculated by the moving average method of Thompson (1947). Observations of mortality and adverse effects were made at 0, 3 and 6 hours and daily thereafter.

RESULTS

Concentration mg/L		*Number of Fish*	*Cumulative Mortality*				
Nominal	*Actual**		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	<LOQ	10	0	0	0	0	0
Solvent control	<LOQ	10	0	0	0	0	0
1.4	0.771	10	0	0	0	0	0
2.5	1.479	10	0	0	0	0	0
4.4	3.165	10	0	0	0	2	5
7.9	8.577	10	1	7	10	10	10
14	15.477	10	8	10	10	10	10

* LOQ (Limit of Quantitation) = 0.013 mg/L.

LC50 (nominal)	3.6 mg/L at 96 hours (measured; 95% CI 2.7-4.8 mg/L)
NOEC	1.5 mg/L at 96 hours (measured).
Remarks – Results	No adverse water quality parameters were reported (eg. unclear, coloured water or presence of precipitate). Analysis of the test solutions showed a marked decline in concentration and therefore test values are based on 24 h old measured water concentrations. Sublethal effects (i.e. increased pigmentation, loss of equilibrium) were observed at test concentrations ≥4.4 mg/L.

CONCLUSION — The notified chemical is toxic (i.e. LC50 1-10 mg/L; United Nations, 2003) to rainbow trout under the conditions tested.

TEST FACILITY — SafePharm Laboratories (1995e)

8.2.2a. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE — Notified chemical

METHOD — OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – static conditions

Species	Cladoceran (*Daphnia magna*; 1st instar <24 hours old)
Exposure Period	48 hours
Auxiliary Solvent	10% v/v Tween 80-dimethylformamide (DMF)
Water Hardness	270 mg/L (as $CaCO_3$; hard water)
Analytical Monitoring	GC determined at 0 and 48 hours.
Remarks - Method	Range finding and definitive studies were performed. Test material (1.0 g mg) was dissolved in solvent and the volume adjusted to 10 mL. An aliquot (500 µL) was dispersed in reconstituted water and the volume adjusted to 5 L to give the 10 mg/L test concentration, from which serial dilutions were made. Test aquaria consisted of 250 mL flasks containing ~250 mL test solution. Effects were monitored for at 24 and 48 hours. Daphnids were immobilised when unable to swim for ~15 s after gentle agitation. Water temperature: 21°C, dissolved oxygen 7.9-8.0 mgO2/L and pH 7.6-7.8 (acceptable). Photoperiod 16 h light: 8 h dark. The EC50 values and associated confidence limits were calculated by the moving average method of Thompson (1947).

RESULTS

Concentration mg/L		*Number of D. magna*	*Number and Percent Immobilised*			
Nominal	*Actual*		*24 h*		*48 h*	
	(0 and 48 h)		*No.*	*%*	*No.*	*%*
Control	<LOQ*	20 (2 replicates of 10)	0	0	0	0
Solvent control	<LOQ	"	0	0	0	0
0.10	0.096-0.087	"	0	0	0	0

0.18	Not determined	"	0	0	0	0
0.32	0.295-0.274	"	0	0	0	0
0.56	Not determined	"	0	0	0	0
1.0	1.002-0.802	"	0	0	0	0
1.8	Not determined	"	0	0	3	15
3.2	2.869-2.569	"	3	15	9	45
5.6	Not determined	"	7	35	17	85
10	8.715-8.163	"	18	90	20	100

* LOQ (limit of quantitation) = 0.000095 mg/L.

EC50	3.3 mg/L (nominal) at 48 hours (95% CI 2.7-3.9 mg/L)
NOEC	1.0 mg/L (nominal) at 48 hours
Remarks - Results	Test concentrations were stable as determined by GC at 0 and 48 hours, and within 80-100% of nominal (acceptable), and consequently the results are based on nominal test concentrations only.
CONCLUSION	The notified chemical is toxic (i.e. EC50 1-10 mg/L; United Nations, 2003) to Daphnia magna under the conditions tested.
TEST FACILITY	SafePharm Laboratories (1995f)

8.2.2b. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 *Daphnia* sp. Reproduction Test – static renewal conditions
Species	Cladoceran (*Daphnia magna*)
Exposure Period	21 days
Auxiliary Solvent	10% v/v Tween 80-dimethylformamide (DMF)
Water Hardness	250 mg/L (as $CaCO_3$)
Analytical Monitoring	Chemical analysis by GC on days 0, 2, 5, 7, 9, 12, 14, 16 and 19.
Remarks - Method	Range finding and definitive studies were performed. Test solutions were prepared from a saturated solution prepared from an initial test material dispersion of 100 mg/L. An amount of test material (1100 mg) was dispersed in 11 L reconstituted water and stirred (2000 rpm for 24 h at 25°C) after which the mixture was allowed to settle at 21°C and then filtered (0.2 µm) to give a solution concentration of 3.0 mg/L (nominal). Aliquots were diluted is series in 2 L to give the other required nominal test solutions concentrations. Test aquaria consisted of 150 mL beakers containing 100 mL test solution (1 daphnid per aquaria). Daphnids were monitored daily. Daphnids were considered immobilised when unable to swim for ~15 s after gentle agitation. Water temperature: 21°C, dissolved oxygen 8.0-8.6 mgO2/L and pH 7.8-8.0 (acceptable). Photoperiod: 16 h light (416-565 lux): 8 h dark. Test solutions were renewed 3 times per week. EC50 values were based on parental daphnia (P1). Solubility trials were conducted prior to the test, and a saturation concentration of 3.3-3.7 mg/L was determined using GC analysis of filtrate. The EC50 values and associated confidence limits were calculated by the trimmed Spearman-Karber method using TOXCALC (Version 5.0.23C). NOEC values were calculated using ANOVA after Bartlett's test for homogeneity of variance and Dunnett's multiple comparison procedure for comparing several treatments with a control.

RESULTS

Concentration mg/L		*%*	*No. Live Young*		*No. Dead Young*		*No. Unhatched Eggs*	
Nominal	*Actual (time weighted mean)*	*Survival of P1*	*Total*	*Per Female (cumulative)*	*Total*	*Per Female (cumulative)*	*Total*	*Per Female (cumulative)*

Control	<LOQ*	90	812	89	0	0	0	0
0.030	0.023	90	824	90	0	0	0	0
0.095	0.063	100	827	83	0	0	0	0
0.30	0.27	80	753	78	0	0	0	0
0.95	0.66	0	423	47	0	0	1	<1
3.0	2.0	0	22	7	0	0	0	0

* LOQ (limit of quantitation) = 0.0045 mg/L.

EC50 (Parental immobilisation)	0.36 mg/L at 21 days (95% CI 0.28-0.46 mg/L; time-weighted average)
EC50 (Reproduction)	0.27-0.66 mg/L (nominal) at 21 days)
NOEC (Reproduction)	0.27 mg/L at 21 days (time-weighted average)
Remarks - Results	Analysis of 2-3 day old test solutions were within 11-59% of nominal, and consequently the results are based on time weighted average test concentrations unless specified otherwise. No mortality or other adverse effects were observed in the control groups.
CONCLUSION	The notified chemical is slightly chronically toxic (i.e. EC50 <1 mg/L; Mensink et al., 1995) to Daphnia magna under the conditions tested.
TEST FACILITY	SafePharm Laboratories (2003b)

8.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	Green algae (*Scenedesmus subspicatus*; freshwater unicellular alga).
Exposure Period	96 hours
Concentration Range	
Nominal	14 mg/L
Actual	13.6 mg/L (0 h) to ~1.14 mg/L (96 h).
Auxiliary Solvent	10% v/v Tween 80-dimethylformamide (DMF).
Water Hardness	Not measured
Analytical Monitoring	GC analysis of test solutions at 0 and 96 h (limit of detection 0.03 mg/L).
Remarks - Method	Range finding and definitive tests were performed. The test concentration of 14 mg/L was the highest that could be prepared due to the limit of water solubility. Six replicate 250 mL flasks containing 100 mL were tested. Test material (3.50 g) was dissolved in solvent and the volume adjusted to 25 mL. From this stock solution, an aliquot (200 µL) was dispersed in 2 L of algal suspension to give the nominal test concentration of 14 mg/L. Test containers were incubated at 24±1°C under continuous light (7000 lux) and mixing. At 0 h and 96 h cell densities were ~104 and ~105 cells/mL, respectively, in the controls and treatments based on observed mean cell density from the mean of cell counts from 3 fields of view for each of the replicate flasks. Statistically significant differences between test and control groups were determined using Students t-tests.

RESULTS

Biomass		*Growth*	
EbC50 mg/L at 96 h	*NOEC mg/L*	*ErC50 mg/L at 0-72 h*	*NOEC mg/L*
≥1.1	1.1*	≥1.1	1.1*

* Highest concentration tested

Remarks - Results	No abnormalities were detected in any of the control or test cultures. Analysis of test solutions throughout the study showed marked decline in concentration and test values are based on measured test solution concentrations. Test solution pH increased from 8.0-8.1 (0 h) to 10.1-10.3 (96 h) in controls and treatments, which is not unusual due to CO2

	formation in the test solution during the tests.
CONCLUSION	The test substance was not toxic to algae at the limit of its water solubility for the test conditions as determined during the test.
TEST FACILITY	SafePharm Laboratories (1995g)

8.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	ASTM Microbial Inhibition Testing Procedure: Modified BOD5 Test.
Inoculum	Activated sludge, Downingtown Regional Water Pollution Control Center, Downingtown, USA.
Exposure Period	72 hours
Concentration Range	
Nominal	0.7, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0 mg/L
Remarks – Method	Changes in oxidation of inoculated (5 mL) D-glucose solution (6 mg/L) were measured after 72 hours and compared to inoculated test solutions containing test substance at varying concentrations. Test substance was added directly to test bottles in duplicate. Sewage sludge prepared for the test by filtration (2 mm) and total suspended solids (TSS) determined. The sludge was distributed into a semi-continuous activated sludge (SCAS) unit at a TSS concentration of ~2500 mg/L. The unit was aerated to maintain solids in suspension. An aliquot of prepared sludge (25 mL) was diluted to 250 mL with BOD dilution water.
RESULTS	
IC50	>100 mg/L
NOEC	100 mg/L
Remarks – Results	The toxicity threshold concentration is defined as the lowest concentration of the test substance that produces a reduction in biochemical oxidation.
CONCLUSION	The toxicity threshold concentration of the test substance was > 100 mg/L, the highest concentration tested. The notified chemical is not toxic to activated sludge microbes under the conditions of the test.
TEST FACILITY	Roy F. Weston, Inc. (1995c)

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

The notified chemical is volatile (~23 Pa) and loss to the atmosphere is likely to be significant from spills/leaks, sewers and environment. It is not readily biodegradable (17% biodegradation after 28 days) or inherently biodegradable (25% SOC removal after 28 days). It is slightly soluble in water (~3 mg/L) and has a hydrolysis half-life of greater than 1 year in acidic (pH 4) or neutral pH solutions at 25°C; however, in alkaline solutions hydrolysis is expected to be more pronounced (i.e. half-life of ~9 days at pH 9 at 25°C, and with hydrolysis increasing as temperature increases). It has a log Pow of 4.68 and a log Koc of 3.34 indicating that it has the potential to bioaccumulate and the ability to bind tightly to organic matter in soil or solutions.

Following its widespread use in Australia, the notified chemical will eventually be released into the sewerage system through washing or cleaning activities. As a worst case, if all of the notified substance entered the sewerage system (up to $3.5x10^{11}$ µg/y), a national wastewater concentration of 0.24 µg/L may be calculated. This assumes an Australian population of 20.1 million people generates 200 L/person/day (i.e. $1.467x10^{12}$ L/y) and no attenuation within the sewerage system. Assuming dilution factors for freshwater and marine environments of 1 and 10, respectively, PEC(freshwater) and PEC(marine) of 0.24 µg/L and 0.024 µg/L, respectively, may be calculated. A biosolids concentration of <0.4 mg/kg has been estimated, which assumes Australian production of 100 kg/ML of effluent treated, and this is not considered to pose a risk to the environment.

Attenuation of the notified substance within the sewerage system by volatilisation as well as partitioning to sludge based on its affinity to organic matter and some biodegradation is expected. By using the SimpleTreat Model (European Commission, 2003) and assuming a Henry's constant of $2.247x10^{3}$ Pa/m3/mole (Log H = 3.35) based on measured vapour pressure and water solubility, and a Log Pow 4.68 and being not readily or inherently biodegradable, ~77% of the chemical may potentially volatilise and 16% partition to sludge, leading to an estimated potential effluent concentration of 0.017 µg/L (PEC(freshwater) and PEC(marine) of 0.017 µg/L and 0.0017 µg/L, respectively). This attenuation will further reduce the risk to the environment from the potential presence of the notified substance in effluent discharged. The notifier calculated a similar result using this model.

No bioaccumulation test report was provided. Although the notified chemical has a log Kow of 4.68 indicating a high affinity to lipids and a potential to bioaccumulate, bioaccumulation is not expected due to the small imported quantity, the volatility of the notified chemical and the low potential for environmental occurrence of the notified chemical after sewerage system treatment of domestic wastewaters containing the notified chemical.

9.1.2. Environment – effects assessment

Aquatic ecotoxicity data were available for 4 taxonomic groups (fish, invertebrates, algae and sewage microbes). The results of the ecotoxicological data indicate that the notified chemical is very toxic to aquatic life (i.e. L{E}C50 <1 mg/L; Mensink et al., 1995; United Nations, 2003). The most sensitive species of those tested are daphnids (chronic EC50 0.36 mg/L and NOEC 0.27 mg/L). A predicted no effect concentration (PNECfreshwater) of 0.027 mg/L (27 µg/L) has been derived by dividing the lowest available chronic NOEC by an assessment (uncertainty) factor of 10 to account for intra and inter species sensitivity to the notified chemical. In the absence of marine toxicity data, the PNECfreshwater is tentatively extrapolated to the marine environment, an approach is supported by a preliminary review of comparative data by ECETOC (2003). The NOEC for activated sewage sludge microbes is 100 mg/L at 3 hours; however, such concentrations are unlikely in the sewerage system.

9.1.3. Environment – risk characterisation

An indication of risk can be made by comparison between the PEC and the PNEC using a risk quotient (RQ) approach. RQ values for freshwater and marine environments receiving treated sewage effluent of 0.009 (i.e. 0.24 µg/L ÷ 27 µg/L) and 0.0009 (i.e. 0.024 µg/L ÷ 27 µg/L), respectively, may be calculated. As in both cases the RQ is <<1, taking into account the very

worst case natures of the PEC calculations, the risk from use of the notified chemical is expected to be low and much lower if the expected sewerage system attenuation processes are taken into consideration and most (~92%) partitioning to air or sludge. The notified substance is not expected to be mobile within a landfill, and is expected to degrade over time.

9.2. Human health

9.2.1. Occupational health and safety – exposure assessment

Transport & Storage

Occupational exposure to the notified chemical during transport and storage of fragrance preparations containing the notified chemical is only likely in the event of accidental container breakage and/or spillage. Exposure in these circumstances is expected to be infrequent and acute, and can be limited by use of gloves, goggles, masks and protective clothing during clean-up operations.

Formulation

During reformulation of fragrance preparations containing the notified chemical into cosmetics and domestic cleaning products, dermal exposure is the most likely route. Ocular exposure may occur due to accidental splashes. Exposure may occur when workers open the drums containing imported notified chemical at up to 5%, when weighing and transferring the imported fragrance preparations into mixing vessels, during blending operations and when cleaning up spills and equipment. Blending operations can be in open or closed systems, however, the process is often automated and local exhaust ventilation is usually employed.

Exposure to the notified chemical during filling of consumer product containers is expected to be minimal, as the filling of consumer containers is typically automated.

Dermal and inhalation exposure during formulation was estimated using the EASE model (HSE, 1994). Assuming non-dispersive use and intermittent direct handling, the estimated dermal exposure during formulation is 0.1-1 mg/cm^2/day of fragrance preparations containing up to 5% of the notified chemical. This equates to 0.005-0.05 mg/cm^2/day of the notified chemical. Absorption of the notified chemical may be significant, as the substance has a high Log P_{ow} and fat solubility so ready diffusion across membranes would be expected. Therefore, for a 70 kg worker with surface area for hands at 820 cm^2 and forearms at 1140 cm^2, and assuming 100% absorption, systemic exposure is estimated to be 0.14-1.4 mg/kg bw/day of the notified chemical. This exposure would be substantially reduced by the use of protective clothing and gloves.

The estimated atmospheric concentration of notified chemical during formulation is 1182-2364 mg/m^3 for an open system (non-dispersive use), with aerosol formation, and local exhaust ventilation. If no aerosols are formed, the estimated atmospheric concentration for an open system (non-dispersive use) with local exhaust ventilation is 6-12 mg/ m^3. For a closed system, even if aerosols are formed, the estimated atmospheric concentration is 0-1 mg/m^3. Therefore for a 70 kg worker, assuming an inhalation rate of 1.3 m^3/hour, a 4 hour exposure time and 100% bioavailability, inhalation exposure is estimated to be 88-176 mg/kg bw/day for an open system with aerosol formation and local exhaust ventilation; 0.5-0.9 mg/kg bw/day for an open system with local exhaust ventilation and no aerosol formation; and 0-0.1 mg/kg bw/day for a closed system.

Inhalation exposure to the notified chemical would be further reduced by the use of personal respiratory equipment.

End Use

Occupational exposure to end use consumer products may occur, for example, with professional cleaners using cleaning products, or beauticians using cosmetic products. These workers are less likely to use extensive PPE; however, the concentration of notified chemical in end use products will be less than 0.025% (except for fine fragrances, which have up to 1%).

Using the EASE model, and assuming wide dispersive use with extensive, direct handling, estimated dermal exposure to end use products is 5-15 mg/cm^2/day of end use products. This equates to 0.001-0.004 mg/cm^2/day of notified chemical at 0.025% in most end use products. (The exception is fine fragrances, which contain up to 1% of notified chemical, and therefore would expose workers to 0.05-0.15 mg/cm^2/day of notified chemical. However, fine fragrances are not likely to be used occupationally.) For a 70kg worker with 1960 cm^2 surface area and assuming 100% absorption (as above), systemic exposure is therefore estimated to be 0.035-

0.105 mg/kg bw/day of the notified chemical for cleaning products and cosmetics other than fine fragrances.

9.2.2. Public health – exposure assessment

It is expected that during import, transport, storage, reformulation of fragrance compositions containing the notified chemical, exposure of the general public will be limited, except in the event of an accidental spill.

Consumer products containing the notified chemical (cosmetics, toiletries, household cleaning products) will be sold in the public domain, consequently there is the potential for widespread public exposure. Exposure will be principally via dermal route. Exposure to the notified chemical is considered minimal given the small amount of notified chemical in the final consumer products (maximum 0.025% other than fine fragrances, which have maximum 1%).

9.2.3. Human health - effects assessment

The notified chemical is of low acute oral and dermal toxicity in rats. Acute inhalation toxicity data were not provided.

The notified chemical is a slight skin and eye irritant in rabbits. It is not a skin sensitiser in either adjuvant or non-adjuvant studies in guinea pigs or in humans exposed repeatedly to a 20% formulation over 9 weeks.

The notified chemical is not mutagenic in bacteriological testing, and not clastogenic to human lymphocytes.

In a 4-week repeat dose oral toxicity study in rats, the NOAEL was 250 mg/kg bw/day, based on liver weight increase and haematological changes at higher doses. In an oral gavage one-generation reproduction study in rats, the NOAEL for reproduction and offspring viability was 1000 mg/kg bw/day. As treatment-related effects (globular accumulations of eosinophilic material in the renal tubules) were observed at the lowest dose, no NOEL for parental toxicity was established. However, as the effects observed at all doses were adaptive rather than adverse, the NOAEL for parental toxicity was > 1000 mg/kg bw/day.

Based on the available data, the notified chemical is not classified as a hazardous substance in accordance with the NOHSC Approved Criteria for Classifying Hazardous Substances (NOHSC, 2002).

9.2.4. Occupational health and safety – risk characterisation

The notified chemical is of low acute toxicity (LD50 >2000 mg/kg for oral or dermal routes), and is a slight skin and eye irritant. The risk of acute toxic effects in workers is low.

During formulation, chronic dermal exposure to the notified chemical was estimated to be 0.005-0.05 mg/kg bw/day. The margin of exposure (MOE) for chronic toxicity is based on a NOAEL of 250 mg/kg bw/day. MOE greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. For dermal exposure, the MOE is calculated to be greater than 5000 during formulation. Therefore, the risk of chronic systemic toxicity using modelled worker data is acceptable for formulation workers handling fragrance preparations containing up to 5% notified chemical. Occupational risk due to dermal exposure can be further limited by the use of PPE specified in the MSDS.

Chronic inhalation exposure during formulation was estimated to be 88-176 mg/kg bw/day for an open system with aerosol formation and local exhaust ventilation, 0.5-0.9 mg/kg bw/day for an open system with local exhaust ventilation and no aerosol formation, and 0-0.1 mg/kg bw/day for a closed system. Based on a NOAEL of 250 mg/kg bw/day, the MOE for inhalation exposure is calculated to be 1.4-2.8 for an open system with aerosol formation and LEV, 278-500 for an open system with LEV and no aerosol formation, and more than 2500 for a closed system. Therefore, the risk using modelled worker data is acceptable for workers handling the notified chemical in an open system if no aerosols are formed; or, if aerosols are formed, in a closed system. The risk using modelled worker data is not acceptable for workers handling the

notified chemical in an open system if aerosols are formed. Occupational risk due to inhalation exposure can be further limited by the use of personal respiratory PPE. The risks of chronic exposure are also limited by the predicted exposure frequency, which for reformulation workers is up to 2 days/year.

Dermal exposure to end use products containing up to 0.025% notified chemical is estimated to be 0.035-0.105 mg/kg bw/day. Using the same toxicity data (NOAEL of 250 mg/kg bw/day), the MOE is calculated to be greater than 2300. Therefore the risk to workers handling end use products in the absence of PPE is acceptable.

9.2.5. Public health – risk characterisation

It is expected that public exposure to compounded fragrances containing up to 5% notified chemical for industrial use will be minimal except in the rare event of an accidental spill. There will be public exposure to the notified chemical from dermal, inhalation, oral and ocular exposure to cosmetics, toiletries, and household cleaning products containing up to 0.025% of the notified chemical. Consequently the public risk from exposure to the notified chemical through all phases of its life cycle is considered to be low.

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.*

The notified chemical is classified as dangerous for the environment according to the EU criteria with the following risk phrase :
R51/53 Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

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

	Hazard category	***Hazard statement***
Chronic hazards to the aquatic environment	2*	Toxic to aquatic life with long lasting effects

*Although the chronic *Daphnia* test report indicates that the notified chemical is very toxic (i.e. Hazard category 1), the GHS classification system only uses the results of acute toxicity tests.

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, provided closed systems or personal respiratory equipment are used for any reformulation operations in which aerosols are likely to be formed.

10.3.2. Public health

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

11. MATERIAL SAFETY DATA SHEET

11.1. Material Safety Data Sheet

The MSDS of the notified chemical and fragrance preparations containing 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 and fragrance preparations containing 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 notified chemical should be classified as follows under the ADG Code:
  - Class 9-Miscellaneous Dangerous Goods and Articles

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical:
  - Closed system during mixing and blending of ingredients with fragrance preparations containing the notified chemical, particularly if aerosol formation is likely.
  - Local exhaust ventilation if the mixing vessel is open.

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical:
  - Prevent splashes and spills.

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical during formulation of fragrance preparations containing it into consumer products:
  - Chemical resistant gloves, protective overalls and goggles/faceshield.
  - Personal respiratory equipment if aerosols are produced in an open system.

  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.

Disposal

- The notified chemical should be disposed of by incineration or landfill.

Emergency procedures

Spills/release of the notified chemical should be contained as described in the MSDS (i.e. by

sand or inert powder) and collected in labelled sealable containers for disposal in accordance with Government regulations.

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 subsection 64(1) of the Act; if
- the importation volume exceeds one tonne per annum notified chemical; or
- The notified chemical is itself manufactured locally or imported

or

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

The Director will then decide whether secondary notification is required.

No additional secondary notification conditions are stipulated.

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