File No: SN/10

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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Habanolide

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Director
Chemicals Notification and Assessment

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FULL PUBLIC REPORT

HABANOLIDE

1. INTRODUCTION

The assessment of Habanolide was carried out as a Limited Notification (<1 tonne/yr) under the *Industrial Chemicals (Notification and Assessment)* Act 1989, as NA/577, with the Summary Report of the assessment published in the *Chemical Gazette* of 3 March 1998.

Secondary notification of Habanolide was required as the notifier applied to exceed the import volume of 1 tonne per year and also to import neat notified chemical. In the original assessment, Habanolide was imported as a minor ingredient (<1%) in concentrated fragrance preparations. In accordance with Section 65 of the Act, a notice requiring the secondary notification of Habanolide was published in the *Chemical Gazette* of 2 May 2000, which stipulated the following data were required to undertake further assessment of Habanolide:

1. Uses, quantity to be imported, occupational health and safety, public health and environment impact information, partition coefficient data, labels and Material Safety Data Sheets (MSDS); and,

2. Toxicology data: acute oral and dermal toxicity, skin and eye irritation, sensitising potential, repeated dose toxicity, genotoxicity and ready biodegradability.

This report, SN/10 addresses the impact of usage volume and changes in use pattern (ie, importation of neat notified chemical) on the occupational health and safety, public health and environmental risk assessment. New toxicology studies submitted by the applicant were considered in this secondary notification assessment. Toxicology data were provided in the original assessment (NA/577), however, the results were provided in summary form only.

2. APPLICANT AND NOTIFICATION DETAILS

APPLICANT
Firmenich Limited
73 Kenneth Road
Balgowlah 2093

NOTIFICATION CATEGORY
Secondary notification

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication:
Identity of the chemical
Molecular formula
Structural formula
Molecular weight
Spectral data

3. IDENTITY OF CHEMICAL

MARKETING/OTHER NAME(S)
Habanolide

4. INTRODUCTION AND USE INFORMATION

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

Maximum introduction volume of notified Chemical (100%) was stated in the original assessment (NA/577) as being 850 kg in year 5. In the current submission, import volumes where the chemical is a component of preparations will be up to 10 tonnes per year. The increase in importation volume of neat chemical is estimated with 0.1 tonne per year expected initially and up to 1 tonne per year in the future.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	<10.1				11

USE

Habanolide is to be used as an ingredient of an aromatic concentrated formulation. It will be imported both as a component of perfume preparations and as neat chemical into Australia. The formulated perfume concentrates (containing up to 10% Habanolide) will be incorporated into cosmetics and household cleaning products at a maximum level of 0.5% (0.05% notified chemical in the finished consumer product) or in fine perfumes at a maximum of 20% (2% notified chemical in the fine perfume).

5. PROCESS AND RELEASE INFORMATION

IDENTITY OF MANUFACTURER/RECIPIENTS

Firmenich Limited

TRANSPORTATION AND PACKAGING

The notified chemical as well as the fragrance preparations containing it will be imported into Australia in tightly lacquered closed drums; standard size for fragrance preparations: 180 kg, sometimes, 100 kg, 50 kg, 25 kg, 10 kg or 5 kg.

5.1. Operation Description

Habanolide as a pure substance will be sold in small quantities to fragrance manufacturers in Australia. They will blend it with other raw materials in order to formulate perfumes. The notified chemical (liquid form) is weighed and then either manually or automatically charged to mixers to be blended into formulated perfumes at a concentration of up to 10%, which is then automatically filled into containers. The batch sizes may vary between a few kg to 1 tonne. The concentrated perfumes containing Habanolide will be incorporated into cosmetics and household cleaning products at a level of maximum of 0.5%, hence a maximum of 0.05% of Habanolide in the finished consumer products.

Habanolide imported as a minor ingredient (≤10%) in formulated perfume concentrates, will be incorporated in cosmetic and cleaning products at a maximum of 0.5% (maximum of 0.05% notified chemical) or 20% in fine perfumes (maximum of 2% notified chemical). The perfume concentrate will be weighed and charged into a mixer, and then packed off into various sized containers after formulation.

Formulation of fragrance composition and final consumer products may be carried out in closed or open systems.

5.2. Occupational exposure

Categories of workers likely to be exposed to the notified chemical are:

- Transport and store workers
- Workers handling the notified chemical in the fragrance formulation plant
- Workers involved in the formulation of the consumer products containing the notified chemical

The notifier indicates that between 5 to 20 workers, including warehouse workers, production workers and laboratory workers, could be potentially exposed to the notified chemical (as a pure form, an ingredient in perfume concentrates, and as a consumer product). The duration of worker exposure could be estimated at several minutes per batch.

Waterside workers, forklift workers and truck drivers will handle closed containers of the notified chemical. During transport or storage of drums containing the notified chemical or perfume compositions containing the notified chemical, exposure is likely only in the event of accidental spillage.

Workers may be exposed by skin contact to the notified chemical or perfume preparations containing the notified chemical (maximum 10% Habanolide), when opening and closing drums, weighing and charging them to the blending vessel, mixing in open vessels and during cleaning and maintenance operations. The concentration of the notified chemical in the final consumer products ranges from 0.05 to 2%. Worker exposure is minimised during the use of closed systems, where the opportunity for respiratory or dermal exposure is low; however, if open mixing tanks are used, aerosols may be generated. Personal protective equipment (gloves, eye and face protection and protective clothing) will be worn by formulators. The notifier indicates that it is unlikely for the fragrance product containing the notified chemical to be added to the mixing vessel manually.

5.3. Release

RELEASE OF CHEMICAL AT SITE

The chemical is used to prepare perfume blends containing up to 10% of the chemical. These preparations are subsequently blended into cosmetics and household cleaning products by a number of companies.

During blending operations, the notifier estimates 0.05% of the notified chemical will be lost through washing out of mixing vessels. Based on an importation volume of 10 tonnes, this equates to 5 kg per annum. While there are no definitive local estimates for release, release estimations based on European guidance, suggest up to 2% of the chemical could be lost to waste water during formulation operations. This suggests a maximum release of around 200 kg per annum. However, in this case, overseas data indicate a loss of approximately 100 g Habanolide per 180 kg drum, and formulation will probably occur in a closed system, so this release may be considered worst case.

This waste is likely to be sent to on site waste treatment facilities. Due to its low water solubility and high affinity for the organic phase, the notified chemical is unlikely to remain in the water column in significant quantities. Most would be expected to partition to the sludge component where incineration is the likely fate.

RELEASE OF CHEMICAL FROM USE

The end use products are in the public domain and consist of cosmetics and cleaning products. Through this use pattern it may be assumed almost the entire import volume of the chemical will be released to the sewer in a dispersive manner. Assuming a maximum annual import volume of 10 tonnes per annum (accounting for the previous maximum amount entering Australia prior to this notification), and use averaged over 300 days per annum, this corresponds to a release to sewer of around 33 kg per day.

5.4 Public exposure

Minor public exposure may result from disposal of unused fragrance containing the notified chemical, accidental spillage of the fragrance during transport and storage, or during reformulation. However, adequate measures are described by the notifier to minimise the risk of public exposure during disposal, reformulation or in the event of accidental spillage.

Since the perfumes and cosmetics containing the notified chemical will be applied to the skin of the face, hands and other areas, and cleaning products may be widely used, significant and prolonged public exposure is expected. Dermal exposure is considered to be the most likely route of exposure, but some accidental ocular exposure is also possible.

Assuming that 750 mg of toilet water containing 0.5% notified chemical is applied at up to 5 times per day, then it is estimated that people may be exposed to the notified chemical at doses of up to 0.27 mg /kg bw/day. Similarly, if a person applies 800 mg of a face cream containing 0.05% notified chemical two times per day, then the person may be exposed to approximately 0.01 mg/kg bw/day. Once per day use of 5000 mg of a shower gel containing 0.05% notified chemical may result in exposure to up to

approximately 0.071 mg/kg bw/day. Use of about 100g of a household cleaning product containing 0.05% notified chemical could result in exposure to up to 0.71 mg/kg bw/day. Based on these model calculations of exposure to individual products containing the notified chemical, exposure to several products (some on several occasions during a day) could result in a total daily exposure in the order of several milligrams per kilogram body weight per day.

6. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa Colourless to pale yellow liquid

BOILING POINT 283.5 to 331.3°C at 94.84 kPa
METHOD Distillation Method EC Directive 84/449/EEC
TEST FACILITY Toxicol Laboratories Limited (1992)

FREEZING POINT < -20°C
METHOD EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
TEST FACILITY Safepharm Laboratories (1995a)

DENSITY 964 kg/m^3 at 20.5°C
METHOD Pyncometer method
EC Directive 92/69/EEC A.3 Relative Density.
TEST FACILITY Safepharm Laboratories Limited (1995a)

VAPOUR PRESSURE 1.6×10^{-4} kPa at 25°C
METHOD EC Directive 92/69/EEC A.4
TEST FACILITY Safepharm Laboratories Limited (1995b)

WATER SOLUBILITY 9.64×10^{-4} g/L at 20°C
METHOD EC Directive 92/69/EEC A.6
Remarks Analytical Method: Shake Flask Method. The concentration of test material in the sample solutions was determined by gas chromatography and recovery analysed to prove its adequacy. Recoveries of between 72% and 88% were found.

The preliminary water solubility test indicated that the column elution method should have been performed instead of this shake flask method as the solubility was less than 1×10^{-2} g/L. However, due to the physical nature of the test material, it was not possible to use this method as liquid test materials generally coat the glass beads causing them to adhere and form a plug within the column thus preventing water circulation.
TEST FACILITY Safepharm Laboratories Limited, (2001a)

HYDROLYSIS AS A FUNCTION OF pH
Remarks Hydrolysis was not tested due to the low solubility of the chemical. Some hydrolysis at the ester linkage is possible but unlikely in the environmental pH range and conditions.

PARTITION COEFFICIENT (n-octanol/water) log Pow at 20°C >6.20
METHOD EC Directive 92/69/EEC A.8
Remarks Analytical Method: HPLC. The determination was performed at an approximately neutral pH since the test material has no dissociating groups. The log Pow was determined to be >6.20 as retention times were longer than the highest standard (DDT) tested which has a log Pow of 6.20.
TEST FACILITY Safepharm Laboratories Limited (2000)

ADSORPTION/DESORPTION – screening test log K_{oc} = 4.65 at 30°C.
METHOD OECD TG 121 Estimation of the Adsorption coefficient on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC).

Remarks	Testing was carried out at approximately neutral pH. Retention times for the substance of around 18 minutes were between the two highest standards of diclofop-methyl (log Koc 4.2) and DDT (log Koc 5.63).
TEST FACILITY	Safepharm Laboratories Limited, 2001b

DISSOCIATION CONSTANT	The substance will not dissociate in the environmental pH range.
Remarks	Not determined.

PARTICLE SIZE	
Remarks	Not applicable as the notified chemical is a liquid

FLASH POINT	157 ºC (closed cup)
METHOD	Pensky- Marten Method EC Directive 84/449/EEC
TEST FACILITY	Toxicol Laboratories Limited (1992)

FLAMMABILITY LIMITS	
Remarks	Test not conducted

AUTOIGNITION TEMPERATURE	252 ± 5 ºC
Remarks	Method complied with A15 of Commission Directive 92/69/EEC
TEST FACILITY	Safepharm Laboratories Limited (1995c)

EXPLOSIVE PROPERTIES	Non explosive
Remarks	The explosive properties were determined using the BAM Fall Hammer test and the Koenen Steel tube test.
METHOD	Method complied with A14 of Commission Directive 92/69/EEC
TEST FACILITY	Safepharm Laboratories Limited (1995c)

REACTIVITY	
Remarks	chemical structure indicates relatively stable, non-oxidising

FAT (OR N-OCTANOL) SOLUBILITY	Soluble in all proportions
METHOD	Official Journal of the European Communities Method A.7. (Flask)
TEST FACILITY	Toxicol Laboratories Limited (1992)

7. TOXICOLOGICAL INVESTIGATIONS

Some toxicology studies were submitted and assessed under NA/577 (a summary of the results is tabulated). The additional studies are described in detail below the table:

Test*	***Species***	***Outcome***
Acute oral toxicity	Rat	LD_{50} > 2000 mg/kg
Acute dermal toxicity	Rat	LD_{50} > 2000 mg/kg
Skin irritation	Rabbit	Slight irritant
Eye irritation	Rabbit	Slight irritant**
Skin sensitisation	Guinea pig	Non sensitising
4-Week oral (gavage)	Rat	NOAEL 1 000 mg/kg/day
Reverse mutation	*S. typhimurium*	Not mutagenic
Chromosome aberration	Human lymphocytes	Non-clastogenic
***Oral repeated dose toxicity (7-week)	Rat	NOAEL of 1000 mg/kg/day

***Oral repeated dose toxicity (90-day)	Rat	NOAEL of 250 mg/kg/day
***Skin sensitisation	Insult patch repeated test (human volunteers)	No evidence of skin sensitisation.
***Genotoxicity – in vitro	Mouse lymphoma assay	Non genotoxic
***Toxicokinetic studies-	Residue analysis in abdominal fat	No residues of notified chemical were detected at levels above 50 µg/kg (limit of quantitation)

* All tests were performed according to OECD protocols

** The notified chemical was described as non irritant in NA/577, however, conjunctival redness was observed at one hour in the rabbit study

*** Studies not assessed in the original assessment

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7.1.a 7-Day Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	Seven Day (Gavage) Dose Range Finding (carried out prior to the 28-day test) EC Directive 92/69/EEC
Species/Strain	Rats
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 7 days; Dose regimen: daily;
Vehicle	0.5% w/v carboxymethyl cellulose
Remarks - Method	24 rats were divided into 4 groups (3 males and 3 females). Three groups received Habanolide orally by gavage for 7 days at dose levels: 500, 750 and 1000 mg/kg/day. One group received vehicle only. There was only one amendment to the protocol, which was not considered to compromise the study.

RESULTS

Mortality and Time to Death

No mortality reported

Clinical Observations

All animals were unremarkable throughout the treatment period. Body weights and body weight gains were those expected for animals of this age and strain. Food consumption was unaffected by treatment.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No analysis in this range-finding study

Effects in Organs

Females dosed at 750 and 1000 mg/kg/day exhibited a slightly higher absorption and body weight-related liver weights. These changes were not considered to be treatment related.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 1000 mg/kg bw/day in this study, based on lack of treatment related effects at the highest dose.

TEST FACILITY	Toxicol Laboratories Limited (1995)

7.1.b. 90-Day Repeat Dose Toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD No. 408: Subchronic Oral Toxicity-Rodent: 90-day study. EC Directive 87/302/EEC Ninety day repeated dose oral (gavage) toxicity study in the rat Residue analysis in the abdominal fat
Species/Strain	Rats/Sprague Dawley
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 90 days; Dose regimen: daily;
Vehicle	0.5% carboxymethyl cellulose
Remarks – Method	15 males and 15 female rats received the notified chemical at 50, 250 and 1000 mg/kg/day. One control group received vehicle only. Recovery groups: ten males and ten females at 1000 mg/kg/day were maintained for a further 28 days. Fat samples were measured using gas chromatography. Samples were pooled from two animals in the same treatment group. Certain deviations to the protocol were noted, which did not compromise the study.

RESULTS

Mortality and Time to Death

No treatment –related deaths during the study.
2 males treated with 1000 mg/kg/day died on days 34 and 85.
One female treated with 250 mg/kg/day developed a large mass around the abdominal region.

Clinical Observations

No clinical observable signs of toxicity were detected during the study.

No adverse effect on body weight development and food consumption was reported during the study. The female recovery group treated at 1000 mg/kg/day showed a slight statistically significant reduction in body weight gain during the first week

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis, Residue Analysis

No treatment related effects on haematology, and urinalysis. The recovery 1000 mg/kg/day group showed a statistically significant reduction in monocyte count with males also showing a reduction in neutrophil count in comparison with controls.

Females treated with 1000 mg/kg/day showed a statistically significant reduction in plasma triglyceride concentration compared with controls. Males treated with 250 mg/kg/day showed a statistically significant reduction in plasma alkaline phosphatase compared with controls.

Residue Analysis

The limit of quantitation for the analyte in the abdominal fat is 50 µg/kg. The notified chemical was not detected at levels above the limit of quantitation in any of the fat samples

Pathology

Speckled kidneys in males at 250 (1/5) and 1000 (5/5) mg/kg/day

Histopathology
Statistically significant kidney changes in males at 1000 mg/kg/day

Effects in Organs
Females treated with 250 mg/kg/day showed a statistically significant increase in thymus weight compared with controls. A slight but statistically significant increase in relative thymus weight was also evident among 1000 mg/kg/day in females.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established at 250 mg/kg bw/day in this study, based on kidney effects seen at the next higher level.

Fat Samples
The test material was not detected at levels above the limit of quantitation in any of the fat samples.

TEST FACILITY Safepharm Laboratories Ltd (1998a and b)

ADDITIONAL INVESTIGATIONS

7.2. Skin sensitisation– human volunteers

TEST SUBSTANCE	Habanolide 15% in diethyl phthalate
METHOD	
Study Design	Repeated Insult Patch Study Six weeks: (1) Induction, (2) Rest and (3) Challenge
Study Group	104 volunteers completed the study
Vehicle	diethyl phthalate
Induction Procedure	Nine consecutive applications to the back Subjects were required to remove the patches approximately 24 hours after application. At 48 hour intervals, the sites were evaluated and identical patches reapplied. Patches were evaluated after 72 hours if applied on Friday, and 96 hours if applied on Thursday.
Rest Period	10-12 days
Challenge Procedure	Challenge: initiated during the 6th week of the study. Patches applied to unexposed sites were removed after 24 hours and sites graded after 48 and 72 hours after application. Rechallenge: Conducted whenever there was evidence of possible sensitisation. Conducted on naïve sites on the back under occlusive and semi occlusive conditions approximately one or two weeks after challenge had been completed. Patches applied for 24 hours and evaluated at 48, 72 and 96 hours.
Remarks - Method	Subjects discontinuing in the study did so for reasons unrelated to treatment with the notified chemical.

RESULTS	
Remarks – Results	No reactions were reported during the induction and challenge phase. The test did not provide evidence of sensitisation.
CONCLUSION	A Repeated Insult Patch Test was conducted using the notified chemical at 15% in diethyl phthalate using occlusive dressing. The notified chemical did not show evidence of skin sensitisation under the conditions of the test.

TEST FACILITY	TKL Research Inc. (1997)

7.3. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	L5178Y TK +/- Mouse Lymphoma Assay GLP compliant Protocol meets OECD (476), EC Method B17, Commission Directive 2000/32/EC.
Species/Strain	L5178Y TK +/- 3.7.2c mouse lymphoma cells (heterozygous at the thymidine kinase locus)
Dose range	Expt (1) 2.5 to 40 μg/mL in the absence of S9 5 to 80 μg/mL in the presence of S9 Expt (2) 10 to 70 μg/mL in the presence of S9 5 to 50 μg/mL in the absence of S9
Vehicle	Dimethyl sulphoxide
Remarks – Method	Mouse lymphoma cells were treated with the test material at six dose levels, in duplicate. 3 hour exposures were used with and without activation (S9); except for expt (2), exposure time without activation was increased to 24 hours. Positive controls: without activation- Ethylmethanesulphonate- 800μg/mL (expt 1); 150 μg/mL (expt 2) with activation- Cylophosphamide- 2.5 mg/mL (expt 1 and 2)
RESULTS	
	Toxicity at 80 μg/mL with S9 (in Expt 1). A precipitate was not observed at any dose level. There was a steep dose-related reduction in the % Relative Suspension Growth values for cultures dosed with the test material in both the absence and presence of metabolic activation. The test material did not induce any statistically significant or dose related increases in the mutant frequency at any dose level either with or without metabolic activation in Expt (1) and (2).
CONCLUSION	The test substance is not mutagenic under the conditions of the test.
TEST FACILITY	Safepharm Laboratories (2001c)

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

Ready Biodegradability was investigated during the NA/577 assessment using a Manometric Respirometry Test and the CO_2 Production (Modified Sturm) test according to OECD Guidelines 301F and 301B respectively. The former showed 61% and 95% degradation within 10 and 28 days respectively. The latter showed 72% and 86% CO_2 yield within 10 and 28 days respectively. These results indicate that the notified chemical is readily biodegradable. Although not required from the results of the ready biodegradability tests, an inherent biodegradability has also been carried out by measuring the Soluble Organic Carbon (SOC) removal in a Semi-Continuous Activated Sludge (SCAS) system (OECD Test Guideline No 302A). This showed 99% removal confirming the ultimate biodegradable nature of the chemical. This test however is specific for water soluble organic compounds and the low solubility of the present chemical casts some doubt on the validity of the results.

A further degradation study has been provided with this submission and is presented below:

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I).
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	BOD
Remarks - Method	

RESULTS

Test substance		*Reference Substance - Aniline*	
Day	*% degradation*	*Day*	*% degradation*
28	92	28	79

Remarks - Results

CONCLUSION	These results concur with previous biodegradation studies and show the chemical to be degraded by microorganisms.
TEST FACILITY	Kurume Research Laboratories, 1998.

8.1.2. Bioaccumulation

CONCLUSION

The bioaccumulation potential for this chemical has not been tested. However, the low water solubility and high Log Pow indicate the potential for bioconcentration. This is supported using QSAR predictions for chemicals with a log Kow>6 as described in European Commission guidance documentation (EC, 1996) where a log BCF of 4.6 is calculated.

However, the readily biodegradable nature of the chemical and its low concentration in the water compartment indicate exposure should be limited, and the potential for bioaccumulation of the substance is not expected to be significant.

8.2. Environmental Effects

8.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test - flow through.
Species	Rainbow trout (*Oncorhynchys mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	Tween 80-dimethylformamide (5%)

Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	Analysis of the test preparations showed a marked decline in the measured test concentrations over the 96 hours of the study.
Remarks – Method	

RESULTS

Concentration mg/L	*Number of Fish*	*Mortality*				
Nominal		*3h*	*24h*	*48h*	*72h*	*96h*
Control	10	0	0	0	0	0
Solvent Control	10	0	0	0	0	0
1.0	10	0	0	0	0	0
1.8	10	0	0	0	0	0
3.2	10	0	10	10	10	10
5.6	10	4	10	10	10	10
10	10	8	10	10	10	10

LC50	2.4 (1.8-3.2) mg/L at 96 hours (nominal concentration). 2.0 (1.3-3.0) mg/L at 96 hours (measured concentration).
NOEC (or LOEC)	0.52 mg/L at 96 hours (measured).
Remarks – Results	Sub-lethal effects to exposure were observed at test concentrations of greater than 1.0 mg/L. These responses were swimming at the surface, swimming at the bottom, loss of equilibrium and the presence of moribund fish.

CONCLUSION — This chemical can be considered moderately toxic to fish (Mensink *et al* 1995).

TEST FACILITY — Safepharm Laboratories, 1995d.

8.2.2. Acute/chronic toxicity to aquatic invertebrates

TEST SUBSTANCE — Notified chemical

METHOD — OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – static.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Tween 80-dimethylformamide (5%)
Water Hardness	270 mg $CaCO_3$/L
Analytical Monitoring	There was a decline in test substance detected in the water at 48 hours with analysis detecting between 24 and 98% of the nominal concentration.
Remarks - Method	

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*	(2 x replicates of 10 each)	*24 h*	*48 h*
Controls		20	0	0
0.1-0.32		20	0	0
0.56		20	0	4
1.0		20	0	13
1.8		20	0	18
3.2		20	5	20
5.6		20	10	20
10		20	17	20

LC50	0.88 (0.73-1.1) mg/L at 48 hours (nominal) 0.48 (0.42-0.54) mg/L at 48 hours (measured)

NOEC (or LOEC)	0.32 mg/L at 48 hours (nominal) 0.24 mg/L at 48 hours (measured)
Remarks - Results	No other adverse reactions to exposure were observed.
CONCLUSION	The chemical can be considered highly toxic to aquatic invertebrates (Mensink *et al* 1995).
TEST FACILITY	Safepharm Laboratories, 1995e.

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
Species	*Scenedesmus subspicatus* (Green alga)
Exposure Period	72 hours
Concentration Range	0.625-10 mg/L (nominal)
Auxiliary Solvent	Tween 80-dimethylformamide (5%)
Water Hardness	Not reported
Analytical Monitoring	Test substance concentrations decreased markedly throughout the experiment. At the start of the experiment, between 84-130% of nominal concentration was found. After 72 hours, this had fallen to below the limit of quantitation (9 ppb) at all doses.
Remarks - Method	

RESULTS

Biomass		*Growth*	
EbC50 *mg/L at 72h*	*NOEC* *... mg/L*	*ErC50* *mg/L at 24-48 h*	*NOEC* *... mg/L*
2.4 (nominal)		5.0	0.625

Remarks - Results	At the highest test concentration (10 mg/L), algal cells were observed to be clumped together at 72 hours. The pH values of the control and test cultures were observed to increase from 7.9-8.1 to 8.3-10.3 over the course of the study. This effect is considered to be due to the large number of cells in the exponential growth phase respiring oxygen and producing carbonates and bicarbonates which give rise to alkaline conditions in solution.
CONCLUSION	The chemical is moderately toxic to algae (Mensink *et al* 1995).
TEST FACILITY	Safepharm Laboratories, 1995f.

8.2.4. Inhibition of microbial activity – Not tested.

8.2.5. Acute Toxicity to Earthworms

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 207 Earthworm Acute Toxicity Tests.
Species	Earthworm (*Eisenia foetida*)
Exposure Period	14 days
Remarks - Method	A defined artificial soil was used containing 69% sand; 20% clay; 10% moss peat and 1% calcium carbonate to bring the pH to about 6. The moisture content was around 29%.

A range finding test was performed with exposure at nominal concentrations of 10, 100 and 1000 mg/kg with 10 worms per concentration and control. No mortalities at any concentration were found.

Based on the range finding test, a definitive test was conducted at a concentration of 1000 mg/kg soil to confirm no mortalities or sub-lethal effects were observed. Six test vessels were used with 10 worms in each. The weight of each worm was recorded on day 0 and at the end of the test. Further, the approximate burrowing time of the worms in each test group was recorded on Days 0 and 7.

A positive control study using chloroacetamide as the reference material was also conducted.

Results

Mortality – there were no mortalities or sub-lethal effects of exposure observed. The LC50 >1000mg/kg; NOEC =1000 mg/kg.

Weight data – there were no significant differences between the control and exposed worms in terms of weight on days 0 and 14.

Remarks – results

CONCLUSION: The notified chemical is very slightly toxic to earthworms.

TEST FACILITY: Safepharm Laboratories Limited, 2001d

8.2.6. Effects on Terrestrial Plants

TEST SUBSTANCE: Notified chemical

METHOD: OECD Draft Guideline 208 - Part A. Seedling Emergence and Seedling Growth Test.

Species: 1 monocotyledon - Oat (*Avena sativa*); 2 dicotyledons - soybean (*Glycine max*) and - tomato (*Lycopersicon esculentum*).

Exposure Period: 24-26 days.

Remarks – Method: Exposure vessels consisted of polypropylene pots. 10 replicate pots were maintained for the control and application rate for each species. The study was conducted in a greenhouse with the temperature maintained between 15 and 35ºC and a 16:8 light:dark ratio.

The notified chemical was prepared in a 99:1 deionised water/acetone mix. The resultant solution was observed to be slightly yellow and very oily.

Prior to planting, test treatments, solvent controls and controls were moistened with the appropriate solution. Exposure levels were 3.9, 12, 36, 110, 330 and 1000 mg/kg soil. These levels were determined following a range finding study where an apparent toxicant-related reduction in emergence and dry weight was observed for all species.

Observations were carried out to determine percent emergence, mortality and the morphological abnormalities (eg chlorosis) of the emerged shoots. Dry weights were determined at test termination.

Results (mg/kg soil)

	OATS		SOYBEAN		TOMATO	
	NOEC	EC50	NOEC	EC50	NOEC	EC50
Post emergence	1000	>1000	1000	>1000	1000	>1000
Shoot weight	1000	>1000	1000	>1000	1000	>1000

Remarks - results	**Oats**: Twelve non-emerged or dead plants were observed in the 1000 mg/kg treatment compared with four non-emerged or dead plants in the control and six non-emerged or dead plants in the solvent control. No plants with morphological abnormalities were observed in the control or any of the treatment rates. **Soybean**: Ten non-emerged or dead plants were observed in the 36 mg/kg treatment compared with six non-emerged or dead plants in the control and seven non-emerged or dead plants in the solvent control. No plants with morphological abnormalities were observed in the control or any of the treatment rates. **Tomato**: Three non-emerged or dead plants were observed in the 1000 mg/kg treatment compared with one non-emerged or dead plants in the control and one non-emerged or dead plants in the solvent control. Chlorosis was observed on between 12-24 plants in all test concentrations and one plant with necrosis was observed in the 1000 mg/kg treatment. It is difficult to count chlorosis as a morphological abnormality in this case, however, as 21 plants in the solvent control also exhibited chlorosis.
CONCLUSION:	The notified chemical is very slightly toxic to terrestrial plants.
TEST FACILITY:	Springborn Laboratories, 2002.

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

Section 5.3 above gives expected releases to sewer resulting from importation and use. With an assumption of 100% release to sewer averaged over 300 days of the year, daily release of 33 kg is estimated. This will occur in a diffuse manner around the country.

Given the properties of this chemical, significant removal would be expected in the sewage treatment plant prior to release to receiving waters. Based on the water solubility of 0.96 mg/L, the Henry's Law Constant, H, is calculated to be 39.56 $Pa.m^3/mol$. Therefore, for a readily degradable chemical with the parameters of Log Kow >6.2 and Log H 1.6, it is estimated, based on SIMPLETREAT (EC, 1996), that 3% will volatilise; 6% will remain in the water; 64% will partition to the sludge; and 27% will degrade during the residence time in the sewage treatment plant.

This suggests out of an import volume of 10 tonnes, with 100% release to sewer, ultimately 6.4 tonnes per annum will partition to sewage sludge. While the fate of this sludge is often incineration, significant amounts can be put to beneficial use, for example, through application to agricultural land or use in compost and horticulture. Therefore, the opportunity exists for release of the chemical to the terrestrial compartment.

The amount of chemical remaining in the water column is estimated to be 6%, or 600 kg. This will be released to receiving waters over the course of the year in a diffuse pattern around the country.

The following predicted environmental concentrations (PEC) for water and soil have been made:

PEC_{water}: 0.35 µg/L (ppb).

Assumptions include

- release over 300 days of the year;
- daily release to receiving waters is 2 kg (6% of import volume over 300 days of

the year);
- due to the diffuse use pattern, release is spread evenly throughout the country;
- daily water use averages 150 L per person; and
- Population consists of 19 million people.
- A worst case dilution of 2:1 exists on release as this chemical will be used inland and so release to inland river systems will result.

PEC_{soil}: 0.75 µg/kg (ppb)
Assumptions include
- the PEC_{soil} is based on sludge application to agricultural land;
- 0.05 kg sludge produced per litre of wastewater processed in a sewage treatment plant (based on 1997 Sydney Water data)
- $1.4x10^8$ kg sludge produced daily with the notified chemical present at a concentration of 0.15 mg/kg
- an application rate of 5000 kg/ha dry weight per annum occurs (EC, 1996)
- the applied sludge is mixed to a depth of 10 cm in a soil with a bulk density of 1 g/cm^3.

9.1.2. Environment – effects assessment

Effects on aquatic organisms have been summarised above. The most sensitive species was *Daphnia magna* with a 48 h EC50=0.48 mg/L. Based on OECD guidance an assessment factor of 100 should be applied to this as there were no chronic data available for fish or *Daphnia*. Therefore, a **$PNEC_{aqua}$ = 5 ppb** can be derived.

Terrestrial effects data were provided for plants and earthworms and are summarised above. For both, no effects were seen up to 1000 mg/kg of soil. With no chronic data or testing on impacts on microbial activity, an assessment factor of 1000 is applied which results in a **$PNEC_{soil}$ = 1 mg/kg**.

9.1.3. Environment – risk characterisation

Having undertaken the exposure assessment and environmental effects assessment for both the aquatic and terrestrial compartments, the risk characterisations can be carried out by comparing the PEC with the PNEC for these compartments.

Aquatic: PEC/PNEC = 0.35/5 = **0.07**

Terrestrial: PEC/PNEC = 0.75/1000 = **0.00075**

In both these instances, the PEC/PNEC ratio is less than 1 and so it can be concluded the notified chemical is unlikely to present a risk to the environment based on its reported use pattern.

9.2. Human Health

9.2.1. Human health – hazard assessment

SUMMARY OF TOXICOLOGICAL INVESTIGATIONS

Endpoint and Result	*Assessment Conclusion*
Acute oral LD_{50}>2 000 mg/kg (rats)	Low toxicity
Acute dermal LD_{50}>2000 mg/kg (rats)	Low toxicity
Skin irritation (rabbits)	Slightly irritating
Eye irritation (rabbits)	Slightly irritating

Skin sensitisation: • Guinea pig Magnusson-Kligman maximisation • Insult patch repeated test (human volunteers)	No evidence of skin sensitisation.
Rat, oral repeated dose toxicity (7-day and 28-day)	NOAEL of 1000 mg/kg/day
Rat, oral repeated dose toxicity (90-day)	NOAEL of 250 mg/kg/day
Genotoxicity – bacterial reverse mutation	Non mutagenic
Genotoxicity – in vitro: • Chromosomal aberration in human lymphocytes • Mouse lymphoma assay	Non genotoxic
Toxicokinetic studies-residue analysis in abdominal fat	No residues of notified chemical were detected at levels above 50 µg/kg (limit of quantitation)

DISCUSSION

Acute oral and dermal studies, skin and eye irritation studies, skin sensitisation study and genotoxic studies were submitted in the original assessment (NA 577). For this assessment, the applicant submitted additional studies, namely, a repeated insult patch study, a 90-day rat oral repeat dose study, an in vitro genotoxic study and a residue study.

The notified chemical is found to be of low acute oral and dermal toxicity. It is slightly irritating to the skin and eyes and not a skin sensitiser. Inhalation studies were not provided. Considering that the notified chemical has low vapour pressure, inhalation hazard is not expected to be significant.

Repeat dose toxicity studies conducted on rats by the oral route for 4 weeks, and 7 days demonstrated that the NOAEL is 1000 mg/kg/day. The 90-day repeated dose study in rats established a NOAEL at 250 mg/kg/day, based on kidney effects seen at the higher dose.

HEALTH HAZARD CLASSIFICATION

Based on the available data the notified chemical is not classified as hazardous under the National Occupational Health and Safety Commission's (NOHSC) *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999).

The notifier provided MSDS for three products containing the notified chemical. One product was classified by the notifier as non hazardous based on NOHSC criteria. The other two products were classified as hazardous based on NOHSC criteria. The MSDS for these products states that they may cause sensitisation by skin contact. The classification of these products is due to ingredients other than the notified chemical.

9.2.2. Human health – risk characterisation

OCCUPATIONAL HEALTH AND SAFETY

The toxicological test results indicate no foreseeable hazard from exposure to the notified chemical in its pure state.

The incorporation of the fragrance concentrate into final consumer products will generally occur in automated processes at industrial sites. Dermal and inhalation exposure may result during the formulation operations. The blending process (pure notified chemical with raw materials) is usually carried out in closed systems and personal protective equipment is expected to be used during mixing. Considering the low toxicity of the notified chemical, engineering controls and the use of ventilation or respiratory protection if mixing is open, the risk of adverse effects from exposure to the notified chemical will be low.

The consumer product contains 0.05 to 2% of notified chemical, and is expected to be manufactured on a large industrial scale using fully automated technology. No identifiable risk is expected to

exposed workers.

During transport and storage, the health risk to workers is not considered significant given the packaging and the amount of notified chemical in the fragrance preparations/products.

PUBLIC HEALTH

The notified chemical will be used to modify the organoleptic properties of fragrances. The chemical will be incorporated (as part of fragrance preparations) into products such as cosmetics and household cleaners, which will contain the notified chemical at concentrations ranging from 0.05% to 2%. Use of the products which incorporate the notified chemical will result in considerable dermal exposure and some accidental ocular exposure. Estimated total daily exposure to the notified chemical was several orders of magnitude lower than the NOAEL obtained in animal studies. Given that the concentration of the chemical in the products, and its potential toxicity are low, the chemical poses a very low public health risk. The potential for minor public exposure exists during transport, disposal and reformulation of the notified chemical. This is minimised by the recommended practices during storage, disposal, formulation and transportation.

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

10.1 Environment

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.

10.2 Human health – Occupational health and safety

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

10.3 Human health – public

There is Negligible Concern to public health when used according to the set conditions.

11. MATERIAL SAFETY DATA SHEET

The MSDS for the notified chemical and products containing the notified chemical were provided in a format consistent with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 1994a).

The MSDS were provided by the applicant as part of the notification statement. They are reproduced here as a matter of public record. The accuracy of this information remains the responsibility of the applicant.

12. LABEL

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

13. RECOMMENDATIONS

REGULATORY CONTROLS

- There are no regulatory requirements in Australia with regard to environmental classification. However, if this chemical were to be classified according to the Globally Harmonised System of Classification and Labelling (GHS), the following would apply:

  Category: Acute I. Hazardous to the aquatic environment.

Habanolide is classified as dangerous to the environment in accordance with Directives 67/548/EC and 88/379/EC with the following risk and safety phrases:

R50 Very toxic to aquatic organisms
S60 This material and its container must be disposed of as hazardous waste
S61 Avoid release to the environment. Refer to special instructions/Safety data sheets

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 the ingredients with the notified chemical. Good general ventilation should be provided 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 the fragrance concentrate and consumer products:
  - Chemical resistant gloves, protective overalls, and goggles/faceshield.

  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

Further testing

Given the high toxicity of this chemical to aquatic invertebrates, it is highly desirable that a chronic test on *Daphnia magna* be undertaken.

Disposal

- The use pattern of the chemical is such that release will almost entirely be to the sewer system. No specific recommendations with respect to disposal are suggested.

Emergency procedures

Given the high toxicity of the notified chemical to aquatic invertebrates, any spillage should be prevented from entering streams, rivers or waterways. Spillages may be cleaned by using absorbent material and collecting for disposal to landfill.

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
  - additional information becomes available on adverse environmental effects of this

chemical;

- annual import levels of the chemical exceed 100 tonnes, as safety margins are much narrower above that level;

- additional fate data becomes available, such as field monitoring performed at sewage treatment plants in the Netherlands; 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.

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