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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Butanoic acid, 3a,4,5,6,7,7a-hexahydro-4,7-methano-1H-indenyl ester [Cyclobutanate]

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Director
Chemicals Notification and Assessment

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
International Flavours & Fragrances (Australia) Pty Ltd (ABN 77 004 269 658)
310 Frankston-Dandenong Road Dandenong South VIC 3175.

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

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

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None.

NOTIFICATION IN OTHER COUNTRIES
USA TSCA (2000), EU ELINCS (2002), Canada Schedule 1 (2002) Philippines PICCS (PMPIN under review, 2002).

2. IDENTITY OF CHEMICAL

CHEMICAL NAME
Butanoic acid, 3a,4,5,6,7,7a-hexahydro-4,7-methano-1H-indenyl ester

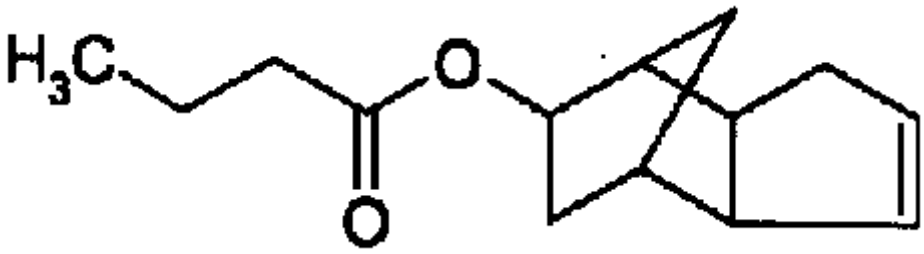
OTHER NAME(S)
Methanoindene

MARKETING NAME(S)
Cyclobutanate

CAS NUMBER
113889-23-9

MOLECULAR FORMULA
$C_{14}H_{20}O_2$

STRUCTURAL FORMULA Extracted from SciFinder (2003)



MOLECULAR WEIGHT
220.31

SPECTRAL DATA

ANALYTICAL METHOD	UV, IR, NMR, GC-MS, GC
Remarks	UV: λmax = 200 nm, ε = 2716 (pH 7, 10% H_2O/Methanol); λmax = 200 nm, ε = 3051 (pH 2-3, 10% 0.1N HCl/Methanol); λmax = 207 nm, ε = 317 (pH 9-10, 10% 0.1N NaOH/Methanol) IR peaks: 3047, 2963, 2845, 1733, 1619, 1465, 1445, 1381, 1353, 1304, 1257, 1182, 1155, 1089, 1056, 989, 949, 875, 849, 795, 741, 699 cm^{-1}. Readings of ^1H NMR, GC-MS, and GC spectra were consistent with the accepted structural molecular properties of the notified chemical.

3. COMPOSITION

DEGREE OF PURITY
98%

HAZARDOUS IMPURITIES
None.

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (>1% by weight)
Related isomers (weight % not individually specified).

ADDITIVES/ADJUVANTS
None.

4. INTRODUCTION AND USE INFORMATION

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

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	0.15	0.15	0.15	0.15	0.15

USE
As a 1% component of a fragrance oil which is a mixture of various aroma chemicals for use as a fragrance enhancer in cosmetic and household products.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, Transport and Storage

PORT OF ENTRY
Melbourne.

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

TRANSPORTATION AND PACKAGING
The imported fragrance oil containing 1% notified chemical will be shipped to the IFF Dandenong facility in 205 L drums for storage prior to road delivery to customers as needed.

5.2. Operation Description

The drummed fragrance oil will be used in the cosmetic industry for production of toiletries, shampoos, soap, and household cleaning agents and detergents (containing <0.001% cyclobutanate) following mixing with other ingredients. The production process mainly involving a blending operation will be highly automated and will occur in a fully enclosed environment. Plant operators will only be involved in opening and closing drums, weighing and charging the mixing vessel, and cleaning and maintenance tasks. Waste will generally be disposed of by incineration or through a wastewater treatment plant prior to release to the environment.

5.3. Occupational exposure

Number and Category of Workers

Category of Worker	*Number*	*Exposure Duration*	*Exposure Frequency*
Transport and warehouse workers	Not stated.	Not stated.	Not stated.
Plant operators			
Mixing	5	4 hours/day	2 days/year
Drum handling	5	4 hours/day	2 days/year
Drum cleaning/washing	10	4 hours/day	2 days/year
Maintenance	5	4 hours/day	2 days/year
Quality control worker	2	0.5 hour/day	2 days/year
Packager	10	4 hours/day	2 days/year

Exposure Details

At the time of submission, the notifier indicated that it has not found customers for cyclobutanate yet and thus details on customer blending operations, worker exposure and life cycle of the notified chemical were not available. Number and category of workers will vary depending on the nature of the customers' business. However, it is anticipated that typical practices by cosmetic and consumer product manufacturers will include the use of adequate local ventilation, appropriate PPE, enclosed mixing vessels and filling areas as well as a high degree of process automation to protect workers.

At the IFF facility, transport and warehouse workers will be exposed to the 1% fragrance oil only in the event of a spill due to an accident or leaking drum. Workers will wear protective overalls, hard hats, chemical resistant gloves and safety glasses.

At customer facilities (cosmetic and consumer product manufacturers), exposure is possible during handling of the drums, cleaning and maintenance of the equipment. Skin, inhalation and eye contact (due to splashing) are likely to be the main route of exposure. Good personal hygiene practices (eg wash hand after any contact, before breaks and meals, etc) and industrial standard PPE will be used. The plant will have adequate ventilation and self-contained breathing apparatus if required. The production process will be in compliance with the good manufacturing practices, including the availability of eyewash fountains and/or safety showers in the vicinity of the blending area.

Only workers qualified and trained in the safety of working with chemicals and chemical mixtures will be permitted to handle the cyclobutanate mixtures. A copy of MSDS will be easily accessible to employees. Atmospheric monitoring will be conducted every two years at the manufacturing facilities or when significant changes have been made. Employees will routinely undergo medical surveillance every two years.

5.4. Release

RELEASE OF CHEMICAL AT SITE

No release is anticipated at the IFF's storage facility and during distribution and transportation to customer sites, except in the event of an accident. In the event of a transport accident, the type and size (205 L steel drums) of the containers and the concentration of the notified chemical (≤1%) would limit the release of the chemical to the environment. The MSDS provides clean-up procedures.

At the formulation facilities, the batch process will be used. Following each batch, cleaning the blending equipment may result in the generation of wastewaters containing the notified chemical. This

equipment may include automated mixing tank and filling machines. The quantity of notified chemical remaining in wash waters has not been specified by the notifier but may approximate 1% of the import volume (~1.5 kg/year). The disposal route for these wastewaters may include disposal to on-site wastewater treatment plants and/or sewer.

The quantity of notified chemical remaining in the emptied import containers (205 L steel drums) has also not been specified by the notifier but may potentially approximate 1% of the import volume (~1.5 kg/year). The disposal route for container rinsate may include disposal to on-site wastewater treatment plants and/or sewer.

RELEASE OF CHEMICAL FROM USE
Following use, most of the notified chemical is expected to eventually be discharged to sewers. When applied as a skin preparation, a fraction of the notified chemical applied will be absorbed through skin and be metabolised. A fraction of this may be washed off, and a proportion will volatilise to the atmosphere following application or spray as an aerosol. A proportion may also enter stormwater from incorrect disposal of cleaning products or as run-off of cleaning products from cleaned surfaces.

5.5. Disposal

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

5.6. Public exposure

As the notified chemical is used in a range of cosmetic and household products, there will be widespread public contact via dermal, inhalational and possibly eye contact. However, exposure would be low due to the low concentration of the notified chemical in the final products.

6. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20ºC and 101.3 kPa Clear colourless liquid with a typical aroma.

Freezing Point <-20ºC

METHOD	EC Directive 92/69/EEC A.1 Determination of Crystallizing Point - BS4633.
Remarks	Cyclobutanate became slightly viscous during cooling.
TEST FACILITY	SPL (2002a)

Boiling Point 274.4ºC at 103.84 kPa

METHOD	EC Directive 92/69/EEC A.2 Boiling Temperature - Differential Scanning Calorimetry - ASTM E537-86.
Remarks	Decomposition concurred with the boiling.
TEST FACILITY	SPL (2002a)

Density 1030 kg/m^3 at 20ºC

METHOD	EC Directive 92/69/EEC A.3 Relative Density – Pycnometer Method.
Remarks	None.
TEST FACILITY	SPL (2002a)

Vapour Pressure 1.12 x 10^{-2} kPa at 25ºC

METHOD	EC Directive 92/69/EEC A.4 Vapour Pressure - Isoteniscope System.
Remarks	Cyclobutanate did not change in appearance under the conditions of the test. The notified chemical is regarded as moderately volatile (Mensink et al., 1995).

The significant reductions (eg up to 68% reduction in 24 h for initial concentration of 12.9 mg/L) in the concentrations of the notified chemical used in the aquatic toxicity tests (SPL, 2002f-h) could be attributed to volatilisation of the chemical.

TEST FACILITY SPL (2002b)

Surface Tension 65.1 mN/m at 20°C (1.30 x 10^{-2} g/L)

METHOD EC Directive 92/69/EEC A.5 Surface Tension – Ring Method ISO 304.

Remarks Result was not corrected using the Harkins-Jordan correction table as it was deemed not applicable to the apparatus used. This was considered not to have affected the integrity of the study.
Cyclobutanate is considered not to be a surface-active material.

TEST FACILITY SPL (2002a)

Water Solubility 1.15 x 10^{-2} g/L at 20°C

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

Remarks Preliminary test result was 1.40 x 10^{-2} g/L.
The notified chemical is regarded as moderately soluble in water (Mensink et al., 1995). However, observations made during the Microbial Inhibition Tests (SPL, 2002i) indicate that at the concentration of 1000 mg/L, oily globules of the undissolved notified chemical became visible on the water surface, and this could be dispersed by ultrasonication.

TEST FACILITY SPL (2002a)

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)*	*$t_{½}$ days*
4	25	>1 year
7	25	>1 year
9	25	13 days

Remarks Stable to hydrolysis at pH 4 and 7. The notified chemical will hydrolyse in alkaline solutions (at pH 9 rate constant 6.17 x 10^{-7} s^{-1}).

TEST FACILITY SPL (2002a)

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

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

Remarks No significant protocol deviations.

TEST FACILITY SPL (2002a)

Partition Coefficient (n-octanol/water) Kow = 3.05 x 10^4, log Pow = 4.48 at 21°C

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

Remarks Preliminary test result showed Kow >1.57 x 10^4, log Pow >4.20 at 21°C Substances having a log Pow >3 are regarded as having the potential to bioaccumulate in the environment. Estimated Bioconcentration Factor (BCF) = 562-1280 using QSAR equation: Log BCF = 0.77 x Log Kow – 0.70 (Meylan & Howard, 2000) and Log BCF = 0.85 x Log Kow – 0.70 (Veith et al., 1979).

TEST FACILITY SPL (2002a)

Adsorption/Desorption Koc 1.51 x 10^3, log Koc = 3.18 at 30°C

METHOD OECD TG 121 Estimation of the Adsorption Coefficient on Soil and on Sewage Sludge Using HPLC

Remarks The adsorption coefficient of the notified chemical was determined in an unionised

form only at an approximately neutral pH as the chemical contained no dissociating functional groups. The notified chemical has a low mobility potential due to low water solubility and adsorption to organic material.
TEST FACILITY SPL (2002a)

Dissociation Constant Not determined.

Remarks No testing was performed by the OECD TG 112 (Dissociation Constants in Water) as the structure of the notified chemical displayed no mode of dissociation.
TEST FACILITY SPL (2002a)

Particle Size Not applicable.

Remarks The notified chemical is imported as 1% fragrance oil.

Flash Point 134 ± 2ºC at 101.3 kPa

METHOD EC Directive 92/69/EEC A.9 Flash Point – Closed Cup Equilibrium Method.
Remarks No significant protocol deviations.
TEST FACILITY SPL (2002c)

Flammability Limits Not flammable.

Remarks Test not conducted.

Autoignition Temperature >400ºC

METHOD 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks No ignition, but emission of grey fumes was observed.
TEST FACILITY SPL (2002b)

Explosive Properties Predicted negative

METHOD EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks The structure contains no groups that would infer explosive properties. The oxygen balance is –261.4.
TEST FACILITY SPL (2002b)

Reactivity Not determined.

Remarks Cyclobutanate is not anticipated to be reactive as the oxygen balance is negative.

7. TOXICOLOGICAL INVESTIGATIONS

The toxicological studies submitted for cyclobutanate are summarised and detailed below:

Endpoint and Result	*Assessment Conclusion*	*References ([1]SPL, 2002d & [2]HTR, 2002)*
Rat, acute oral LD50 >5000 mg/kg bw	Low toxicity	[1]Project no. 1543/021
Rat, acute dermal LD50 >2000 mg/kg bw	Low toxicity	[1]Project no. 1543/022
Rabbit, skin irritation	Moderately irritating	[1]Project no. 1543/023
Rabbit, eye irritation	Slight irritating	[1]Project no. 1543/024
Guinea pig, skin sensitisation - adjuvant test	Limited evidence of sensitisation.	[1]Project no. 1543/025
Human, skin sensitisation - Repeated insult patch test (5% in ethanol/DEP 75:25)	No evidence of sensitisation	[2]Study no. 01-110250-74
Rat, repeated dose oral toxicity – 28 days	NOEL = 15 mg/kg/day (males) and 1000 mg/kg/day (females) NOAEL = 1000 mg/kg/day (males)	[1]Project no. 1543/026
Genotoxicity - bacterial reverse mutation	Non-mutagenic	[1]Project no. 1543/028

Genotoxicity – in vitro mammalian chromosomal aberration test	Non-clastogenic	[1]Project no. 1543/027

([1]SPL, 2002d & [2]HTR, 2002)

7.1. Acute toxicity – oral

TEST SUBSTANCE: Cyclobutanate

METHOD: OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
EC Directive 92/54/EEC B.1 tris Acute Oral Toxicity – Acute Toxic Class Method.

Species/Strain	Rat/Sprague-Dawley CD
Vehicle	None.
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	3 females	2000	0
II	3 males	2000	0

LD50	>5000 mg/kg bw (according to the Globally Harmonised System for LD50 cut-off values)
Signs of Toxicity	Hunched posture, ataxia and lethargy were noted in all females. They recovered one or two days after dosing. No signs of systemic toxicity were noted in males. The body weight gain in all animals was as expected.
Effects in Organs	No abnormalities were noted at necropsy.

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

TEST FACILITY: SPL (2002d)

7.2. Acute toxicity – dermal

TEST SUBSTANCE: Cyclobutanate

METHOD: OECD TG 402 Acute Dermal Toxicity.
EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal).

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

RESULTS

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

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	Slight erythema was noted in all females one to three days after dosing. There were no signs of dermal irritation in male animals.
Signs of Toxicity - Systemic	No signs of systemic toxicity were noted. The weight gain in all animals was as expected.
Effects in Organs	No abnormalities were noted at necropsy.

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

TEST FACILITY SPL (2002d)

7.3. Irritation – skin

TEST SUBSTANCE Cyclobutanate

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

Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	None
Observation Period	14 days
Type of Dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema	1.3	2	1.3	2	72 h	0
Oedema	0.3	1.3	1	2	72 h	0

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

Remarks - Results Very slight to well-defined erythema and oedema were observed up to 72 hrs after patch removal. At the 72 h observation, loss of skin elasticity was also noted at all treated skin sites. Slight desquamation was noted at two treated skin sites and crust formation at one treated skin site at the 7-day observation. All treated skin sites appeared normal at 14-day observation. Primary Irritation Index = 2.09.

CONCLUSION The notified chemical is moderately irritating to skin.

TEST FACILITY SPL (2002d)

7.4. Irritation – eye

TEST SUBSTANCE Cyclobutanate

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

Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Observation Period	72 h
Remarks - Method	No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0.3	0.3	0.3	2	24 h	0
Conjunctiva: chemosis	0.3	0	0	1	24 h	0
Conjunctiva: discharge	0.3	0	0	2	24 h	0
Corneal opacity	0	0	0	0	0 h	0
Iridial inflammation	0	0	0	0	0 h	0

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

Remarks - Results	No corneal or iridial effects were noted during the study. Moderate conjunctival irritation was observed in all treated eyes 1 h after treatment and the irritation reduced to minimal at the 24 h observation. All treated eyes appeared normal at the 48 h observation.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	SPL (2002d)

7.5. Skin sensitisation – guinea pig

TEST SUBSTANCE	Cyclobutanate
METHOD	OECD TG 406 Skin Sensitisation – Maximisation Test. EC Directive 96/54/EC B.6 Skin Sensitisation – Maximisation Test.
Species/Strain	Guinea pig/Albino
PRELIMINARY STUDY	Concentration causing mild to moderate skin irritation: intradermal: 1% & 5% in arachis oil BP topical: 25%-100% (undiluted) in arachis oil BP
MAIN STUDY	
Number of Animals	Test Group: 20 Control Group: 10
INDUCTION PHASE	Induction Concentration: intradermal injection 5% v/v arachis oil BP topical application undiluted as supplied
Signs of Irritation	After intradermal induction, discrete or patchy to moderate and confluent erythema was noted in test and control group animals. After topical induction, discrete or patchy erythema was noted in test and control group animals. Bleeding from the intradermal injection sites was noted in 9 test group animals at 1 h observation. Dried blood was noted at the topical induction sites in 7 test group animals at 24 h observation.
CHALLENGE PHASE	
1st challenge	topical application: 75% v/v in arachis oil BP topical application: undiluted as supplied
Signs of Irritation	Discrete or patchy erythema was noted at 24 h observation in 6 test and 2 control group animals, and in 8 test and 1 control group animal following the challenge with 75% and undiluted test substance. These skin reactions were not apparent at 48 h observation and thus considered not to be attributed to contact sensitisation.
Remarks – Method	The highest non-irritant concentration was not appropriately selected.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: 1st challenge*	
		24 h	*48 h*
Test Group	75% in arachis oil BP	6/20	0/20
	undiluted	8/20	0/20
Control Group	75% in arachis oil BP	2/10	0/20
	undiluted	1/10	0/20

CONCLUSION	There was limited evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	SPL (2002d)

7.6. Skin sensitisation – human

TEST SUBSTANCE	Notified chemical, designated as Test Article A (01-218-01) (5% notified chemical) and Test Article B (01-218-02) (negative control)
METHOD	Repeated Insult Patch Test
Study Design	In-house protocol.
Study Group	114 of 129 completed the study.
Patch Condition	Occlusive
RESULTS	
Remarks - Results	Reactions during induction to Test Article A (5% notified chemical) consisted of an isolated instance of mild erythema. Reactions at challenge consisted of 2 instances of mild, and one of moderate erythema at 24 h evaluation, all of which resolved by the 48 h evaluation. Reactions during induction to Test Article B (negative control) consisted of mild erythema at the original patch site after application 2, but resolved by application 6. There was no irritation at the adjacent site. Reactions at challenge consisted of a single instance of mild erythema at 24 h evaluation which resolved by the 48 h evaluation. There was no sensitisation indicated at challenge. Reports of chest pains/numbness and a possible malignant melanoma on upper right chest were considered unrelated to the test substances.
CONCLUSION	There was some irritation effects observed after induction. No sensitisation was indicated at the challenge phase.
TEST FACILITY	HTR (2002)

7.7. Repeat dose toxicity

TEST SUBSTANCE	Cyclobutanate
METHOD	OECD TG 407 Repeated Dose 28-Day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose 28 Days Toxicity (Oral). USA EPA OPPTS 870.3050 Repeated Dose 28-Day Oral Toxicity Study in Rodents. Japanese MHW Guidelines 1986 Repeated Dose 28 Days Oral Toxicity.
Species/Strain	Rat/Sprague-Dawley Crl:CD
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days; Dose regimen: 7 days per week; Post-exposure observation period: 14 days for recovery groups
Vehicle	None
Remarks - Method	No significant protocol deviations.

RESULTS

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

Mortality and Time to Death
There were not deaths during the study.

Clinical Observations
No treatment related clinical signs were detected in low and mid dose animals. High dose animals of either sex showed increased salivation of short duration from day 6 onwards. On occasions during the final week of treatment, salivation was up to 1 h after dosing. This finding was absent in recovery high dose animals following cessation of treatment. The report indicated that excessive salivation of short duration is often reported following the oral administration of a test material and the daily occurrence of these findings around the time of dosing is usually considered attributable to an unpleasant tasting or locally irritant formulation rather than an indication of systemic toxicity. No treatment related changes in bodyweight, behavioural assessment, functional performance, and sensory reactivity assessment were noted.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
No toxicologically significant effects were detected in parameters of blood chemistry, haematology and urinalysis.

Effects in Organs
No treatment related macroscopic abnormalities detected. In microscopic studies, treatment related renal changes involving a higher incidence of globular accumulations of eosinophilic material in the tubular epithelium was observed in male rats dosed at 1000 mg/kg/day or at 150 mg/kg/day. The condition regressed in recovery 1000 mg/kg/day male rats following an additional 14 days without treatment. This finding is consistent with the presence of hydrocarbon nephropathy, which only occurs in the male rat resulting from the excessive accumulation of α2-microglobulin in renal proximal tubular epithelium. No treatment related microscopical changes were observed in female animals.

Remarks – Results
None.

CONCLUSION
The No Observed Effect Level (NOEL) was established as 15 mg/kg bw/day for males and 1000 mg/kg bw/day for females in this study, based on the histological renal changes. The NOAEL for males was considered to be 1000 mg/kg/day.

TEST FACILITY SPL (2002d)

7.8. Genotoxicity – bacteria

TEST SUBSTANCE	Cyclobutanate
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Japanese METI/MHLW/MAFF Guidelines for Bacterial Mutagenicity Testing. Plate incorporation procedure.
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100. *E. coli*: WP2uvrA.
Metabolic Activation System	S9 rat liver fraction
Concentration Range in Main Test	a) With metabolic activation: TA1535, TA 1537, TA98, TA100: 15, 50, 150, 500, 1500, 5000 µg/plate. WP2uvrA: 50, 150, 500, 1500, 5000 µg/plate. b) Without metabolic activation: TA1535, TA 1537, TA98, TA100: 1.5, 5, 15, 50, 150, 500 µg/plate. WP2uvrA: 50, 150, 500, 1500, 5000 µg/plate.
Vehicle	Dimethyl sulphoxide
Remarks - Method	The concentrations tested were depending on bacterial strain type and the presence or absence of S9-mix.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in PreliminaryTest	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
S. typhimurium	≥150	≥150	≥5000	>5000
E. coli	>5000	>5000	≥5000	>5000
Present				
S. typhimurium	≥1500	≥1500	≥5000	>5000
E. coli	>5000	>5000	≥5000	>5000

Remarks - Results

The test material at high dose levels caused a visible reduction in the growth of the bacterial background lawn to all of the *Salmonella* tester strains. This cytotoxic responses were greater on plates dosed in the absence of S9-mix with weakened lawns initially observed at 150 µg/plate. Plates dosed in the presence of S9 exhibited variable toxicity at higher dose levels (1500 µg/plate or higher). No toxicity was exhibited in *E. coli* tester strain either with or without S9-mix.

A slight, oily precipitate was observed at 5000 µg/plate under an inverted microscope, however, this was considered not to prevent the scoring of revertant colonies.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation.

Negative (vehicle) controls were within historical limits. Positive controls confirmed the sensitivity of the test system.

CONCLUSION

The notified chemical was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY

SPL (2002d)

7.9. Genotoxicity – in vitro

TEST SUBSTANCE

Cyclobutanate

METHOD

OECD TG 473 In vitro Mammalian Chromosomal Aberration Test.
EC Directive 2000/32/EC Annex V B.10 Mutagenicity – In vitro Mammalian Chromosome Aberration Test.
Japanese METI Guidelines for Chromosomal Mutagenicity Testing.

Cell Type/Cell Line: Chinese hamster lung (CHL) cells
Metabolic Activation System: S9 rat liver fraction
Vehicle: Dimethyl sulphoxide
Remarks - Method

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 4.35, 8.7*, 17.3*, 34.5*, 51.7, 68.9 Control: mitomycin C, 0.1*	6 h	18 h
Test 2	0*, 2.175, 4.35, 8.7*, 17.3*, 34.5*, 51.7 Control: mitomycin C, 0.05*	24 h	24 h

	0*, 2.175, 4.35*, 8.7*, 17.3*, 34.5, 51.7 Control: mitomycin C, 0.025*	48 h	48 h
Present			
Test 1	0*, 68.9, 137.7*, 275.4*, 550.8*, 826.15, 1101.5 Control: cyclophosphamide, 5.0*	6 h	18 h
Test 2	0*, 68.9, 137.7*, 275.4*, 550.8*, 661.0, 826.15 Control: cyclophosphamide, 5.0*	6 h	18 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*		
	Cytotoxicity in Main Test	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	≥ 34.5	> 51.7	Negative
Test 2	≥ 34.5	none	Negative
	≥ 17.3	none	Negative
Present			
Test 1	≥ 550.8	> 826.15	Negative
Test 2	≥ 550.8	≥550.8	≥ 550.8 (ns)

ns – no toxicological significance.

Remarks – Results

The notified chemical was shown to be toxic to CHL cells in vitro and optimal levels of toxicity were achieved in all exposure groups.
The notified chemical did not induce any toxicologically significant, dose-related increases in the frequency of cells with aberrations, either in the presence or absence of a liver metabolising system, or after various exposure times. Negative (vehicle) controls were within historical limits. Positive controls confirmed the sensitivity of the test system.

CONCLUSION

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

TEST FACILITY

SPL (2002d)

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test

Inoculum

Non-adapted activated sludge from Severn Trent Water Plc sewage treatment plant, Loughborough, Leicestershire, UK, with culture medium. The tests were performed at a concentration of 10 mg Carbon/L in sealed culture vessels in the dark at 21°C. About 300 mg of test material was dispersed into a culture medium with the aid of high sheer mixing (30 mins, 7500 rpm) and the volume adjusted to 1 litre to give a stock concentration of 300 mg/L. An aliquot of stock solution (131 mL) was dispersed in inoculated culture medium and the volume adjusted to 3 litres to give a final concentration of 13.1 mg/L, equivalent to 10 mg Carbon/L. A standard material (sodium benzoate) was used. Toxicity control was assessed using a solution containing the test and standard material.

Exposure Period

28 days

Auxiliary Solvent

None

Analytical Monitoring

CO_2 was monitored and the percentage degradation or percentage of

	Theoretical Amount of Carbon Dioxide ($ThCO_2$) produced was calculated. Dissolved organic carbon was analysed
Remarks – Method	None

RESULTS

Test substance (13.1 mg/L or 10 mg Carbon/L)		*Sodium Benzoate (17.1 mg/L or 10 mg Carbon/L)*		*Notified Chemical plus Sodium Benzoate Toxicity Control*	
Day	*% degradation*	*Day*	*% degradation*	*Day*	*% degradation*
0	0	0	0	0	0
1	15	1	12	1	12
2	18	2	23	2	23
3	17	3	28	3	28
6	14	6	36	6	36
8	19	8	48	8	48
10	21	10	53	10	53
13	21	13	53	13	53
15	19	15	56	15	56
17	24	17	54	17	54
20	25	20	55	20	55
22	28	22	64	22	64
24	26	24	66	24	66
27	30	27	69	27	69
28	38	28	73	28	73
29*	43	29*	74	29*	74

* Day 29 values corrected to include any carry-over of CO_2 detected in Absorber 2.

Remarks - Results	The test material attained 38% degradation after 28 days and therefore cannot be considered to be readily biodegradable. The toxicity control attained 73% degradation after 28 days thereby confirming that the notified chemical was not toxic to the sewage treatment micro-organisms used in the study. Sodium benzoate attained 77% degradation in 28 days thereby confirming the suitability of the inoculum and test conditions.
CONCLUSION	Not Ready Biodegradable
TEST FACILITY	SPL (2002e)

8.1.2. Bioaccumulation

No biological study data available. The partition co-efficient (Log Kow) of the notified chemical was measured in n-octanol to be 4.48 at 25°C. The notified chemical has a strong potential to bioaccumulate in animals.

8.2. Ecotoxicological investigations

8.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Freshwater EC Directive 92/69/EEC C.1 Acute Toxicity for Fish - Freshwater
Species	Rainbow Trout (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	~176 mg $CaCO_3$/L
Analytical Monitoring	Temperature, dissolved oxygen and pH were measured daily in fresh and old (24-h) test media. Actual concentrations were measured at 0 (fresh media), 24, 48, 72 (fresh and old media) and 96 (old media) hours by GC.
Remarks – Method	• Juvenile trout used (mean length 4.1 cm). • Dissolved oxygen >9.5 mg/L. • Water pH 7.4-7.6. Temp. 14°C.

- Light:dark 16:8 hours with 20 minute dawn and dusk transition period. Reference substance: none used.
- Semi-static test with daily renewal of test solution.
- Range finding and definitive tests were performed.

Test material was prepared by stirring an excess (100 mg/L) of notified chemical) in dechlorinated tap water for 48 hours prior to cooling (14°C for 24 hours) and filtration (0.2 μm) to give a saturated solution of the test material (time-weighted mean concentration of 11.0 mg/L). This solution was diluted to the required test concentrations.

RESULTS

Mortality

Concentration mg/L		*Number of Fish*	*Cumulative Mortality (N)*						*Mortality*
Nominal	*Actual**	*N*	*3 h*	*6h*	*24 h*	*48 h*	*72 h*	*96 h*	*% 96 h*
Control	0	10	0	0	0	0	0	0	0
Not stated	0.85	10	0	0	0	0	0	0	0
Not stated	1.4	10	0	0	0	0	0	0	0
Not stated	2.7	10	0	0	0	0	0	0	0
Not stated	4.9	10	0	0	0	10	10	10	100
Not stated	11	10	10	10	10	10	10	10	100

* Time-weighted mean test concentration.

Sub-lethal Effects

Time-weighted Mean Test Conc. (mg/L)	*Sublethal Effects*	*Time (Hours)*					
		3	*6*	*24*	*48*	*72*	*96*
0	No abnormalities detected						
0.85	No abnormalities detected						
1.4	No abnormalities detected						
2.7	Swimming at bottom				6/10		
	Swimming at surface with exopthalmus (popeye)					5/10	3/10
	Swimming at bottom with exopthalmus (popeye)					5/10	7/10
4.9	Swimming at bottom	5/10	6/10	6/10	6/10	A/D*	A/D*
	Swimming at surface	5/10	4/10	4/10	4/10		
11	All dead	10/10	10/10	10/10	10/10	10/10	10/10

*A/D: All dead

LC50	3.6 mg/L (95% C.I. 2.7 mg/L-4.9 mg/L) at 96 hours (time-weighted mean conc.).
NOAEC (mortality)	1.4 mg/L at 96 hours (time-weighted mean conc.).
Remarks – Results	Time-weighted mean test concentration used in this study because of a decline in the concentration of the notified chemical during the tests that was possibly due to bioaccumulation in the fish. Volatilisation was considered very low due to the use of sealed vessels with minimal headspace and no aeration; however, some losses may be attributed to volatilisation. At the 4.9 mg/L test concentration between 24 and 48 hours, 7 fish were observed to be moribund. These 7 fish were included in the mortality estimate for the 48 hour time point. At the test concentration of 11 mg/L, after 1.5 hours all 10 fish were moribund with arched spines. These 10 fish were in the mortality values for the 3 hour time point.
CONCLUSION	Toxic to Fish (LC50 1 mg/L-10 mg/L; OECD, 2002).
TEST FACILITY	SPL (2002f)

8.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test – Freshwater. EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - Static Test
Species	*Daphnia magna* (Waterfleas)
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	Approximate theoretical hardness of 250 mg/L as $CaCO_3$.
Analytical Monitoring	Temperature, dissolved oxygen and pH were measured at 0 and 48 hours. Actual concentrations were measured at 0 and 48 hours. Volatilisation of notified chemical was not significant due to the use of sealed test containers with minimal headspace. The results were based on the mean measured test concentration and not the time-weighted mean measured test concentrations.
Remarks - Method	Due to the low solubility and high purity of the notified chemical, the test concentrations used in the definitive tests were prepared by diluting (with reconstituted water) a saturated solution. An initial test material dispersion was stirred (25°C at 2000 rpm) for 48 hours to give an initial test material dispersion of 100 mg/L. The dispersion was cooled to 21°C and filtered (0.2 µm) to give a saturated solution of the test material (mean measured concentration of 12.4 mg/L at the end of the test). Aliquots of the saturated solution were dispersed in reconstituted water (2 litres) to provide the remaining measured test concentrations of 0.12, 0.26, 0.43, 0.68, 1.1, 2.1, 3.8 and 6.6 mg/L (mean measured test concentrations at the end of the test). The test vessels were completely filled to minimise headspace and volatilisation. Water pH range was between 7.6 to 7.7 at 0 hours and 7.7 to 7.9, at 48 hours (Temp. 21°C). Dissolved oxygen range was from 8.1 to 8.4 mg/L. In this study immobilisation referred to incapability of swimming.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised (%)*	
Nominal	*Actual (0-48 h)*		*24 h*	*48 h*
Not determined	0	10 per replicate	0	0
0.13	0.12	10 per replicate	0	0
Not determined	0.26	10 per replicate	0	0
Not determined	0.43	10 per replicate	0	0
Not determined	0.68	10 per replicate	0	0
1.3	1.1	10 per replicate	0	0
Not determined	2.1	10 per replicate	0	0
Not determined	3.8	10 per replicate	0	15
Not determined	6.6	10 per replicate	40	95
12.9	12.4	10 per replicate	100	100

EC50	7.1 mg/L at 24 h (95% C.I. 6.2 mg/L-8.1 mg/L) (mean measured conc.) 4.7 mg/L at 48 h (95% C.I. 4.1 mg/L-5.3 mg/L) (mean measured conc.)
NOAEC (zero immobilisation)	3.8 mg/L at 24 hours (mean measured conc.) 2.1 mg/L at 48 hours (mean measured conc.)
Remarks - Results	None
CONCLUSION	Toxic to Aquatic Invertebrates (EC50 1 mg/L-10 mg/L; OECD, 2002).
TEST FACILITY	SPL (2002g)

8.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test – Static Test
Species	*Scenedesmus subspicatus* (unicellular)
Exposure Period	72 hours
Concentration Range Nominal	0, 0.81, 1.63, 3.25, 6.5, and 12.9 mg/L
Time-weighted Mean	0, 0.10, 0.17, 0.34, 0.77, and 1.2 mg/L
Measured Concentration	The saturated solutions were prepared by stirring an excess (100 mg/L) of notified chemical in culture medium using a propeller stirrer prior to removal of the solid phase by filtration (0.2 μm). This saturated solution was then diluted to produce the required test concentrations. The detection system was assessed and found to have an acceptable linearity. The method of analysis was validated and proven to be suitable for use. The test material was unstable in the test medium over the test period.
Auxiliary Solvent	None
Water Hardness	Not stated.
Analytical Monitoring	Temperature and pH were measured at 0 and 72 hours. Actual concentrations of notified chemical were also measured at 0 and 72 hours.
Remarks – Method	Preparation of stock solutions followed similar procedures to SPL (2002g). Water pH increased from 7.7 at 0 hours to pH 9.3 at 72 hours. This increase in pH is considered to be due to the amount of CO_2 required by algal cells in the log phase of growth exceeding the transfer rate of CO_2 from the gaseous phase. In this situation, the CO_2 required for synthesis and growth would be derived from bicarbonate in solution which results in an increase in pH of the culture. The increase in pH after 72 hours exceeded EEC test guideline criteria (1.5 units after 72 hours); however, this was considered to have no adverse effect on the results of the study given that the increase in cell concentration in the control cultures exceeded the validation criterion given in the EEC guidelines. • Temp. 24°C ± 1°C. • Light:dark 24:0 hours. • Initial cell density ~1 x 10^4 [cells/mL]. • Three replicate flasks per treatment concentration. Chemical analysis at 72 hours showed a marked decline in measured concentration of the notified chemical, with all concentrations being less than the limit of quantitation (LOQ) of the analytical method of 0.00098 mg/L. Losses were attributed to adsorption to algal cells and volatility. As such, the results of the test were based on the time-weighted average measured test concentrations in order to give a worst-case analysis of the data.

RESULTS

*Biomass**		*Growth**	
NOAEC	*E_bC50*	*NOAEC*	*E_rC50*
mg/L at 72h	*mg/L at 72 h*	*mg/L at 72 h*	*mg/L at 0-72 h*
0.17	0.29	0.17	0.39
	95% CI: 0.28 to 0.30		95% CI: 0.35 to 0.43

* Based on time-weighted mean measured concentrations.

Remarks - Results	Algal growth was completely inhibited at 0.77 mg/L (time-weighted mean concentration) at 72 hours. No inhibition effects observed at 0.17

mg/L as the growth increased relative to the control at this concentration.

CONCLUSION Very Toxic to Algae (EC50 ≤1 mg/L; OECD, 2002).

TEST FACILITY SPL (2002h)

8.2.4. Inhibition of microbial activity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 209 Activated Sludge, Respiration Inhibition Test.
EC Directive 87/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test
US EPA TG Draft Ecological Effects OPPTS 850.6800.

The notified chemical (500 mg) was dispersed in 250 mL of dechlorinated tap water and subjected to ultrasonication (30 mins). Synthetic sewage (16 mL), activated sewage sludge (200 mL) and water were added to a final volume of 500 mL to give the required concentration of 1000 mg/L. Synthetic sewage (16 g peptone, 11 g meat extract, 3 g urea, 0.7 g NaCl, 0.4 g $CaCl.2H_2O$, 0.2 g $MgSO_4.7H_2O$, 2.8 g K_2HPO_4) was added to each test vessel to act as a respiratory substrate. The tests were replicated (n=3). A control and reference material (3,5-dichlorophenol) were also tested. Range-finding and definitive tests were performed. Oxygen consumption rates were monitored during the tests.

Inoculum A mixed population of activated sewage sludge micro-organisms was from the aeration stage of the Severn Trent Water Plc sewage treatment plant, Loughborough, Leicestershire, UK, which treats mainly domestic sewage.

Exposure Period 0.5 and 3 hours

Remarks – Method Test was conducted April 2002. Initial and final dissolved oxygen concentrations (6.5 mg O_2/L and 2.5 mg/L) were below those recommended in the test guidelines which was not considered to adversely affect the results of the study.

RESULTS

IC50 >1000 mg/L (30 mins)
>1000 mg/L (3 hours)

NOEC 1000 mg/L (3 hours; the highest concentration tested).

Remarks – Results In the range-finding tests, no significant effect on respiration was observed at any of the test concentrations employed. At the test concentration of 11.15 mg/L (the approximate limit of water solubility of the notified chemical), no undissolved notified chemical was observed in the dark brown dispersion and no inhibition was observed therefore it was considered unnecessary to test at this concentration in the definitive test. The validation criteria for the control respiration rates and reference material EC50 values have been satisfied.

CONCLUSION No microbial inhibition was observed at the approximate level of water solubility of the notified chemical.

TEST FACILITY SPL (2002i)

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

The notified chemical is moderately volatile (11.2 Pa at 25°C) and loss to the atmosphere is likely to be a relatively significant process from surfaces to which the fragrance oil is applied. In environments where volatilisation to the atmosphere is not able to occur (eg. deep waters, groundwater, subsoils), the notified chemical is expected to be relatively persistent. It is not readily biodegradable but will likely biodegrade over time as in biodegradability tests the notified chemical attained 38% degradation after 28 days and it would likely have degraded further with more time. The notified chemical will not readily hydrolyse in natural waters at environmentally relevant pH values, but in alkaline waters (eg pH 9 or greater) hydrolysis may be a significant degradation process (half life 13 days). It is moderately soluble in water (11.5 mg/L; Mensink et al., 1995) and has a density similar to water, but it has a potential to adsorb to particulate organic material and therefore accumulate in sediments due to this sorption and settlement. It is not expected to be very mobile in soils and groundwater due to its high sorption potential (log Koc 3.18) and moderate water solubility. With a log Kow of 4.48 and calculated bioconcentration factor (BCF) range of 562 (Meylan & Howard, 2000) to 1280 (Veith et al., 1979), the notified chemical has the potential to bioconcentrate in exposed aquatic organisms; however, no test data were available on depuration rates or actual bioaccumulated residues.

The main fate pathways for the notified chemical following its uses in Australia include dissipation in air due to its volatility, discharge into sewage treatment systems following its use, and there is a potential for discharges from these systems to aquatic environments to contain the notified chemical (refer below). Relatively minor quantities may potentially be released during formulation, storage, handling and transportation (eg uncontained spills and leaks) resulting in discharges to land and aquatic environments; however, the majority of the wastes generated are expected to be discharged to sewer or sent to landfills for disposal.

In landfills, the notified chemical may be present in residues in disposed containers or in sludges derived from wastewater treatment plants and formulation and drum recycling facilities. These residues may potentially constitute only a fraction of the total product (eg ~3% or 4.5 kg of the notified chemical per annum). Over time, residues of the notified chemical in containers and unstabilised sludges may dissolve and mobilise in leachate. However, sorption to organic matter may occur and biodegradation of the notified chemical is likely over time. In addition, hydrolysis of the notified chemical is expected to occur if in contact with alkaline leachate.

Using the worst-case scenario, it has been assumed that all 150 kg of the notified chemical used is discharged to sewerage systems annually throughout Australia and none is attenuated within these systems. Australia has a population of 19.5 million people, and an average value for water consumption of 200 L/person/day has been adopted for this national-level assessment (3900 ML/day for total population). Therefore, the concentration of notified chemical in the Australian sewerage network may approximate 1×10^{-4} mg/L. Based on dilution factors of 0 and 10 for inland and ocean discharges of STP (sewage treatment plant)-treated effluents, outfalls, predicted environmental concentrations (PECs) of the notified chemical in freshwater and marine surface waters may approximate 1×10^{-4} mg/L and 1×10^{-5} mg/L, respectively.

Partitioning to biosolids in STPs Australia-wide may result in an average biosolids concentration of 1 mg/kg (dry wt) assuming 100% attenuation in sludge during the STP process. This is based on the assumption that 0.1 tonnes of biosolids is generated for each ML of STP effluent. Biosolids are applied to agricultural soils, with an assumed average rate of 10 t/ha/year. Assuming a soil bulk density of 1000 kg/m^3 and a soil mixing zone of 0.1 m, the concentration of the notified chemical may approximate 0.1 mg/kg in the applied soil, assuming accumulation of the notified chemical in soil for 10 years under repeated biosolids application. Thus, 0.1 mg/kg is an estimated worst case PEC for the notified chemical in soils following application of biosolids.

The effluent re-use concentration of the notified chemical may potentially approximate 1×10^{-4} mg/L, assuming no attenuation during the STP process. STP effluent re-use for irrigation in Australia occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 0.1 m of soil (density 1000 kg/m^3). Using these assumptions, irrigation with a concentration of 1×10^{-4} mg/L may potentially result in a soil concentration of approximately 0.01 mg/kg assuming accumulation of the notified chemical in

soil for 10 years under repeated irrigation. Thus, 0.01 mg/kg is an estimated worst case PEC for the notified chemical in soils following effluent irrigation.

Using the SIMPLETREAT model for modelling partitioning and losses in STPs (EC, 1996) the percent removal from solution by STPs may potentially approximate 82% (ie 123 kg of notified chemical) of the quantity entering the STPs, of which 25% released to air through volatilisation and 57% partitioned to biosolids. Approximately 18% (27 kg) of the inflow concentration of the notified chemical may potentially remain in solution, passing through the STP. These partition estimates assume that no degradation of the notified chemical occurs during the STP process. Thus, the PEC concentrations in surface waters and irrigation waters may be 18% of that estimated with allowance for potential STP removal, and ~82% of that estimated for soils following biosolids application.

A BCF of 1280 has been calculated using QSAR (Veith et al., 1979) based on the log Kow of 4.48. This suggests the potential for bioconcentration of the notified chemical in exposed aquatic organisms; however using the abovementioned calculated PECs, tissue concentrations in aquatic organisms are unlikely to exceed 1 mg/kg on a worst-case scenario, and would most likely be at least 2-3 orders of magnitude lower than this estimate.

9.1.2. Environment – effects assessment

In summary, the aquatic toxicity data indicate:

Fish: 96-h LC50	3.6 mg/L (95% CI: 2.7 mg/L-2.9 mg/L; time-weighted mean measured conc.)
Invertebrate 48-h EC50	4.7 mg/L (95% C.I: 4.1 mg/L-5.3 mg/L; mean measured test conc.)
Alga 72-h E_bC50 (biomass)	0.29 mg/L (95% C.I: 0.28 mg/L-0.30 mg/L; time-weighted mean measured conc.)
Alga 72-h E_rC50 (growth)	0.39 mg/L (95% C.I. 0.35 mg/L-0.43 mg/L; time-weighted mean measured conc.)

Using the lowest acute toxicity datum (ie IC50 of 0.29 mg/L for alga biomass), a predicted no effect concentration (PNEC) for aquatic ecosystems of 2.9 x 10^{-3} mg/L (2.9 µg/L) has been derived by dividing the IC50 value by an uncertainty (safety) factor of 100. The notified chemical should be classified and labelled under the Globally Harmonised System for the Classification and Labelling of Chemicals (OECD, 2002) as *Chronic Hazard Category 1: Very Toxic to Aquatic Life with long Lasting Effects*. The chronic toxicity classification is based on acute toxicity to algae of <1 mg/L (SPL, 2002h), the lack of ready biodegradability and potential for bioconcentration (calculated log Kow 4.48 and calculated BCF 1280).

No aquatic toxicity data of the notified chemical were available for Australian endemic aquatic species or marine species. No chronic data were available. Acute aquatic toxicity data for three freshwater species (fish, invertebrate, alga) were available for this assessment. Rainbow trout are naturalised in temperate Australian freshwater systems. In accordance with Australian guidance (ANZECC/ARMCANZ, 2000), the freshwater data have tentatively been adopted to assess risks to marine life.

9.1.3. Environment – risk characterisation

Location	*PEC*	*PNEC*	*Risk Quotient (RQ)[a]*	
Australia-wide STPs				
Ocean outfall	1 x 10^{-5} mg/L	2.9 x 10^{-3} mg/L	0.003	(0.004)[c]
Inland river	1 x 10^{-4} mg/L	2.9 x 10^{-3} mg/L	0.03	(0.04)[c]
Soils				
Irrigation reuse	0.01 mg/kg	1.0 mg/kg[b]	0.01	(0.013)[c]
Biosolids reuse	0.1 mg/kg	1.0 mg/kg[b]	0.1	(0.4)[c]

(a) RQ = PEC ÷ PNEC. (b) No data available - trigger level estimation. (c) RQ values calculated assuming 57% attenuation of notified chemical in biosolids and 25% loss through volatilisation during STP process based on SIMPLETREAT model (EC, 1996).

On the basis of the low volumes used (ie. 150 kg/year) and nationwide use pattern of the notified

chemical, and the likelihood for attenuation of the notified chemical in sewage treatment plant processes, it is not considered to pose an unacceptable risk to the health of aquatic life based on its reported use and estimated disposal patterns. The low RQ value for marine life based on freshwater data indicates that the absence of marine ecotoxicity data is unlikely to affect this conclusion.

Based on low exposure potential, reuse of biosolids is unlikely to pose an unacceptable risk to the health of soil organisms. Reuse of effluent for agricultural purposes is unlikely to result in unacceptable health risks to soil organisms.

The low toxicity to mammalian species and low exposure potential indicates that the notified chemical is unlikely to pose an unacceptable risk to the health of wildlife.

9.2. Human health

9.2.1. Occupational health and safety – exposure assessment

During transport and storage, workers are unlikely to be exposed to the notified chemical. Accidental spills will be handled as outlined in the MSDS and in compliance with the local waste management regulations.

Dermal, inhalational, and possible ocular exposure can occur during certain mixing processes at the formulation sites. However, exposure to significant amounts of the notified chemical is limited because of the engineering controls, good personal hygiene practices and PPE worn by the plant operators. In addition, the low concentration of the chemical in the formulated products would preclude any exposure due to splashing.

9.2.2. Public health – exposure assessment

There will be widespread and repeated public contact with the notified chemical via skin and inhalation. However, given the small amounts per application and the low chemical concentration (<001% cyclobutanate) in the consumer products, the public exposure is determined to be low.

9.2.3. Human health - effects assessment

Cyclobutanate has low acute toxicity via oral and dermal routes. It was shown to be a slight eye irritant and a moderate skin irritant with no evidence of sensitisation found in the toxicological studies submitted. Cyclobutanate is not mutagenic or clastogenic in genotoxicity tests. However, the relatively high log oil/water partition coefficient suggests that cyclobutanate can pass across biological membranes. Based on its structure, it is anticipated that cyclobutanate will undergo hydrolysis of the carboxylic acid ester, or through oxidation of the carbon-carbon double bond or aliphatic hydroxylation to from an alcohol, followed by glucuronic acid conjugation and excretion in the urine or bile (Parkinson, 1996). The primary route of excretion is expected to be via the renal system, based on its metabolism and the likely formation of water-soluble excretion products.

Since introduced in 2000, there has been no reports of injury or illness associated with the manufacture and handling of cyclobutanate. Taken all together, cyclobutanate is unlikely to be classified as hazardous according to the *NOHSC Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999).

9.2.4. Occupational health and safety – risk characterisation

The imported 1% fragrance oil will be shipped in secure steel drums. In the event of an accident, damaged/leaking containers and spills will be contained and disposed of in accordance with the MSDS and government regulations. Transport and storage workers would not experience any significant exposure, therefore the risk of adverse health effects is minimal.

At the formulation site, plant operators will wear protective gloves, safety goggles, industrial coveralls and footware. They will also be trained in safe handling and sampling of chemicals.

Exposure to cyclobutanate therefore would be negligible. In addition, with the use of fully enclosed and automated blending systems, appropriate spot vents in the area, and the regular review of good personal hygiene practices and health monitoring at the industrial sites, it is suggested that the notified chemical will not pose a significant occupational health risk to the workers.

9.2.5. Public health – risk characterisation

Contact with cyclobutanate may result in slight skin and moderate eye irritation. However, on the basis of low exposure and low systemic toxicity expected for the notified chemical, it is considered that the chemical will not pose a significant risk to public health when used in the proposed manner.

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*.

Based on the available data, the notified chemical is classified as Chronic Hazard Category 1: Very Toxic to Aquatic Life with Long Lasting Effects in accordance with the *OECD Globally Harmonised System for the Classification and Labelling of Chemicals* (OECD, 2002).

10.2. Environmental risk assessment

On the basis of the PEC/PNEC ratio, the low volumes used, the proposed nationwide, diffuse use pattern, and the potential re-use/disposal pattern the chemical is not considered to pose a risk to the environment.

10.3. Human health risk assessment

10.3.1. Occupational health and safety

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

10.3.2. Public health

There is No Significant Concern to public health when used in the proposed manner.

11. MATERIAL SAFETY DATA SHEET

11.1. Material Safety Data Sheet

The MSDS of the notified chemical provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 1994a). It is published here as a matter of public record. The accuracy of the information on the MSDS remains the responsibility of the applicant.

11.2. Label

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

12. RECOMMENDATIONS

REGULATORY CONTROLS
Hazard Classification and Labelling

- The NOHSC Chemicals Standards Sub-committee should consider the following [health, environmental and physico-chemical] hazard classification for the notified chemical:
  - The notified chemical should be classified and labelled as follows under the OECD (2002) Globally Harmonised System for the Classification and Labelling of Chemicals: **Chronic Hazard Category 1: Very Toxic to Aquatic Life with long Lasting Effects**

CONTROL MEASURES

Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical introduced as a 1% fragrance oil mixture:
  - Adequate ventilation, process automation and use of enclosed systems for the blending operation, including enclosed and automatic transfer lines/pumps for loading and emptying of the mixing vessels.

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical introduced as a 1% fragrance oil mixture:
  - Documented standard operating instructions and procedures, including the observation of good personal hygiene practices;
  - Adequate induction and training programs for workers handling the notified chemical;
  - Implementation of general health surveillance and monitoring programs at regular intervals.

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical introduced as a 1% fragrance oil mixture:
  - Coveralls;
  - Protective gloves;
  - Safety glasses; and
  - Safety boots.

  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

- The following water quality assessment benchmark may be used by the notifier and regulatory agencies for assessment of accidental release of the notified chemical to the aquatic environment:
  - 2.9×10^{-3} mg/L (3 µg/L; based on PNEC calculations).

- The following control measures should be implemented by the notifier and end users to minimise environmental exposure of the notified chemical:
  - DO NOT dispose of unused product to sewer. Do not allow unused product or used containers to contaminate drains and watercourses.

Disposal

- The notified polymer should be disposed of to landfill in accordance with the methods described in the Material Safety Data Sheet, including by licensed waste contractor and in accordance with local jurisdiction waste management guidance.

Emergency procedures

- Spills/release of the notified polymer should be handled by trained personnel in accordance with the material safety data sheet provided by the manufacturer.
- Spills/release of the notified chemical should be contained using sand or inert powder and earth. Collect and seal in properly labelled drums for disposal in accordance with relevant Government regulations.
- Avoid disposing to natural waterways or stormwater.

Transport and Packaging

- The following precautions should be taken by the manufacturer regarding storage of the notified polymer:
  - Australian Code for the Transport of Dangerous Goods by Road and Rail (DOTARS, 1996).

  International Maritime Dangerous Goods Code (IMO, 2000).

12.1. Secondary notification

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

(1) Under Section 64(1) of the Act;
  - if over 150 kg per annum of the notified chemical is used in Australia, a chronic *Daphnia* toxicity test report and a bioaccumulation test report, or evidence showing a low potential for bioaccumulation, for the notified chemical are required to be submitted.

or

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

The Director will then decide whether secondary notification is required.

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