File No: STD/1367

May 2010

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Cyclopropanecarboxylic acid, 2-[1-(3,3-dimethylcyclohexyl)ethoxy]-2-methylpropyl ester

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health and Ageing, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment, Water, Heritage and the Arts.

For the purposes of subsection 78(1) of the Act, this Full Public Report may be inspected at our NICNAS office by appointment only at 334-336 Illawarra Road, Marrickville NSW 2204.

This Full Public Report is also available for viewing and downloading from the NICNAS website or available on request, free of charge, by contacting NICNAS. For requests and enquiries please contact the NICNAS Administration Coordinator at:

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Director
NICNAS

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FULL PUBLIC REPORT

Cyclopropanecarboxylic acid, 2-[1-(3,3-dimethylcyclohexyl)ethoxy]-2-methylpropyl ester

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Givaudan Australia Pty Limited (ABN 87 000 470 280)
Unit 36, 5 Inglewood Place
Baulkham Hills 2153

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

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

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
LVC/719 (2006)
LVC/784 (2009)

NOTIFICATION IN OTHER COUNTRIES
EU (2005), USA (2005), Switzerland (2005) and China (2007)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Serenolide

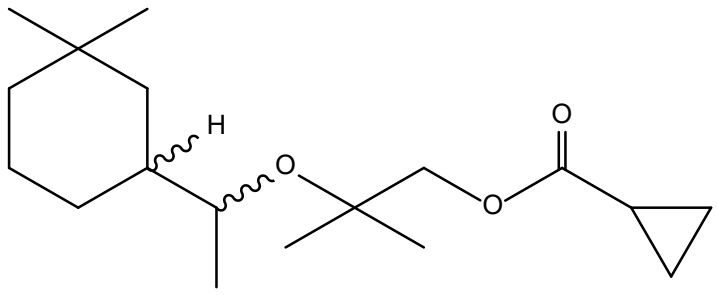
CAS NUMBER
477218-42-1

CHEMICAL NAME
Cyclopropanecarboxylic acid, 2-[1-(3,3-dimethylcyclohexyl)ethoxy]-2-methylpropyl ester

OTHER NAME(S)
GR-85-4287

MOLECULAR FORMULA
$C_{18}H_{32}O_3$

STRUCTURAL FORMULA



(S,R/R,S) 2[1-(3,3-dimethylcyclohexyl)ethoxy]-2-methylpropylcyclopropanecarboxylate (72-82%)
(S,S/R,R) 2[1-(3,3-dimethylcyclohexyl)ethoxy]-2-methylpropylcyclopropanecarboxylate (10-15%)

MOLECULAR WEIGHT
296.4 Da

ANALYTICAL DATA
Reference ^{1}H-NMR, 13C-NMR, infra red (IR) spectroscopy, GC/MS and UV spectra were provided.
IR peaks at 2924, 1730, 1454, 1399, 1381, 1271, 1160, 1118, 1100, 1068, 970, 922, 900, 849, 823, 744 and 661 cm^{-1}.

3. COMPOSITION

DEGREE OF PURITY > 93%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (> 1% by weight)

Chemical Name Cyclopropanecarboxylic acid 2-methyl-2-(2,6,6-trimethyl-cycloheptyloxy)-propyl ester (Cis isomer)
CAS No. - *Weight %* 1.16

Chemical Name Cyclopropanecarboxylic acid 2-methyl-2-(2,6,6-trimethyl-cycloheptyloxy)-propyl ester (Trans isomer)
CAS No. - *Weight %* 4.01

ADDITIVES/ADJUVANTS

Chemical Name 2*H*-1-Benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-
CAS No. 10191-41-0 *Weight %* 0.05

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Almost colourless, pale yellow liquid

Property	Value	Data Source/Justification
Freezing Point	< -50°C	Measured
Boiling Point	331°C (pressure unknown)	Estimated
Density	964 kg/m^3 at 20°C	Measured
Vapour Pressure	1.5×10^{-3} kPa at 20°C	Measured
Water Solubility	3.8×10^{-3} g/L at 20°C	Measured
Hydrolysis as a Function of pH	$t_{½}$ > 1 year at pH 4, $t_{½}$ = 7104 hour at pH 7 and 1226 hour at pH 9, 25˚C.	Measured
Partition Coefficient (n-octanol/water)	log Pow = 5.6	Measured
Adsorption/Desorption	log K_{oc} = 3.88	Measured
Dissociation Constant	Not determined	The notified chemical does not contain any groups that will ionise at environmental pH.
Particle Size	Not determined	Liquid
Flash Point	158°C at 101.3 kPa	Measured
Flammability	Not expected to be highly flammable.	Based on measured flash point.
Autoignition Temperature	340°C	Measured
Explosive Properties	Not determined	A negative result is expected on structural grounds.

DISCUSSION OF PROPERTIES
For full details of tests on physical and chemical properties, refer to Appendix A.

Reactivity
The notified chemical is expected to be stable in water and air under normal conditions of temperature and pressure. The notifier states that rags impregnated with similar fragrance materials (particularly aldehydes and terpenes) left unattended in a dustbin have caught fire. In light of these two facts, precautions should be taken to prevent combustion. The notifier states flushing rags impregnated with fragrance materials with water should prevent this risk.

Dangerous Goods classification
Based on the submitted physical-chemical data in the above table the notified chemical is not classified according to the Australian Dangerous Goods Code (NTC, 2007). However the data above do not address all Dangerous Goods endpoints. Therefore consideration of all endpoints should be undertaken before a final decision on the Dangerous Goods classification is made by the introducer of the chemical.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. It will be introduced as a component (< 5.1%) of fragrance compounds.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	0.2	0.4	0.6	0.8	1

PORT OF ENTRY
Sydney (by sea or air)
Perth (by air)

TRANSPORTATION AND PACKAGING
The fragrance blends containing up to 5.1% of the notified chemical are imported in glass, lacquer-lined containers. The proposed standard packaging sizes are: 1, 5, 10, 25, 100 and 190 kg. The blends will be transported by road in sealed containers to formulators. The finished product containing < 1% of the notified chemical will be transported to industrial customers or retail outlets.

USE
The notified chemical will be used as an aroma chemical in alcoholic perfumery, cosmetics, toiletries, household products, soaps, detergents and industrial perfumery. The concentration of the notified chemical in fragrance compound is up to 5.1%. The concentration of the notified chemical in end use consumer products will be < 1% in alcohol-based perfumes, and typically 0.15% in soaps and 0.05% in detergents.

OPERATION DESCRIPTION
Details on how the notified chemical is to be used are not available to the notifier. The following is a typical operation description for similar chemicals in fragrance compounds.

The notified chemical will be imported as a component of a liquid fragrance mixture at up to 5.1%.

Formulation
If imported as a component of a liquid fragrance mixture, the mixture will be blended with other ingredients at customer formulation sites, to make end use consumer products, such as alcoholic perfumes, cosmetics, toiletries, household products, detergents and soaps. While the formulation process will vary with the product type and formulation site, it is expected that most sites will have closed, automated mixing and dosing equipment. The packaged consumer products will be transported to retail outlets for sale to the public.

End use
There is potential for the formulated products to be used occupationally, for example by professional cleaners using cleaning products (containing 0.05%) or beauticians using cosmetic products (containing < 1%).

Cleaning products are generally applied with a cloth or sponge, by mop or brush or by spray followed by wiping. In some cases, the cleaning product will be diluted with water prior to application. The dilution factor, which is often on the label, depends on the type of surface to be cleaned, the soil loading, and the type and method of application.

Depending on the nature of the cosmetic product these could be applied a number of ways such as by hand, using an applicator or sprayed.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Details of occupational exposure are not available to the notifier. The following occupational exposure table is given as an example of the likely exposure based on similar chemicals in fragrance compounds.

Category of Worker	*Number*	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and Warehouse workers	5	None	Incidental Exposure only
Plant operators			
Mixer	5	4	2
Drum handling	5	4	2
Drum cleaning/washing	10	4	2
Maintenance	5	4	2
Quality control worker	2	0.5	2
Packager	10	4	2
End users (professionals)	> 1000	1-8	200

EXPOSURE DETAILS

Details on customer blending operations, worker exposure and life cycle of the notified chemical are not available to the notifier. The 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 personal protective equipment (PPE), enclosed mixing vessel and filling areas as well as a high degree of process automation to protect workers.

At the customer facilities, transport and warehouse workers will be exposed to the fragrance mixture (up to 5.1% notified chemical) 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 to the fragrance mixture (up to 5.1% notified chemical) or products containing the notified chemical (0.05-1%) 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 routes of exposure. The level of exposure would vary from site to site depending on the level of automation of the formulation process. The worst case dermal exposure is expected to be to workers directly handling the imported fragrance mixture, and is estimated to be 0.005-0.05 mg/cm^2/day, based on EASE model (EASE) using reasonable worst case defaults for the exposure scenario 'manual addition of liquids' (European Commission, 2003) and assuming the notified chemical is present at concentration of 5.1%. Therefore, assuming a surface area of 420 cm^2 (one hand) for a 70 kg worker and a 100% dermal absorption factor, systemic exposure is estimated to be 0.03-0.3 mg/kg bw/day. Exposure is likely to be minimised by good personal hygiene practices (eg. washing hands after any contact, before breaks and meals, etc) and use of industrial standard PPE.

According to EASE (1997) modelling of this work environment, in which it is assumed that non-dispersive use occurs in the presence of local exhaust ventilation, the estimated atmospheric concentration during handling of the imported fragrance oil (5.1% notified chemical) is 0.025-0.05 ppm (0.19-0.39 mg/m^3). Therefore for a 70

kg worker, assuming an inhalation rate of 1.3 m^3/h, and 4 hour exposure, systemic exposure after inhalation is estimated to be 0.014-0.029 mg/kg bw/day. This estimate assumes that no respiratory protection is worn.

The worst-case total systemic exposure from the dermal and inhalation routes is therefore estimated as 0.044-0.33 mg/kg bw/day.

End use
Exposure to no more than 1% notified chemical could occur during final application of the cleaning/cosmetic products or during their addition to water if dilution is required. The main route of exposure is expected to be dermal, although ocular exposure to splashes is possible and inhalation of aerosols could occur where application is by spray. Although the level and route of exposure will vary depending on the method of application and work practices employed, exposure is considered to be low due to the low concentration of the notified chemical in end use products.

6.1.2. Public exposure

End use products containing the notified chemical at 0.01-1% are designed to be sold to consumers. The general public will be repeatedly exposed to low-levels of the notified chemical via a number of different consumer products.

Acute dermal exposure
Use of perfumery products are expected to give the highest single exposure because of the relatively high concentration of the products applied to the skin, and the "leave-on" nature of these products. The maximum dermal exposure is estimated as shown below using consumer exposure data from two different sources. In all calculations the retention factor for these products is assumed to be 1.

	Perfumery products	
Data Source	Cadby/SCCP[a]	Tozer[b]
Quantity product applied (mg)	750	not indicated
Surface Area (cm^2)	100	not indicated
Exposure to product ($\mu g/cm^2$)	7500	2210
Concentration of notified chemical (%)	1.0	1.0
Exposure to notified chemical ($\mu g/cm^2$)	75	22.1

[a] Amount per application taken from data presented in Cadby et al. (2002); surface area taken from data given in SCCP's Notes of Guidance (SCCP, 2006).
[b] Measured and modelled data presented in Tozer et al. (2004);

Chronic dermal exposure
The worst-case long-term dermal exposure to the notified chemical can be estimated by assuming that the notified chemical is present in the maximum amount in all cosmetic products used by the consumer, and that there is 100% dermal absorption. The estimated skin surface residue to fragrances due to use of a number of cosmetic products is estimated as 2.547 mg/kg bw/day assuming a body weight of 60 kg (Cadby, 2002). Therefore based on a concentration of 5% notified chemical in the fragrance compound the long-term dermal exposure to the notified chemical is estimated as 0.127 mg/kg bw/day.

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

6.2. Human health effects assessment

The results from toxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw, low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw, low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	non-irritating
Mouse, skin sensitisation – Local Lymph Node Assay	evidence of sensitisation
Human, skin sensitisation – Repeat Insult Patch Test	no evidence of sensitisation at 15% concentration
Rat, repeat dose oral toxicity – 28 days	NOEL = 30 mg/kg bw/day

Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro <Mammalian Chromosome Aberration Test>	non genotoxic
Genotoxicity – in vitro <Mammalian Cell Gene Mutation Test>	non genotoxic

Toxicokinetics, metabolism and distribution
Given the low molecular weight of the notified chemical (296 Da) and its log P_{ow} of 5.6, it is likely to be significantly absorbed following oral and dermal exposure. The octanol-water partition coefficient suggests that distribution of the notified chemical is unlikely to be bioaccumulate. Hydrolysis is likely to minimise the amount of the notified chemical available for distribution. Metabolism is likely to be extensive.

Acute toxicity
The notified chemical is of low acute toxicity *via* the oral and dermal routes.

Irritation
Based on the studies provided, the notified chemical is considered to be slightly irritating to the skin and non-irritating to the eye.

Sensitisation
The notified chemical is expected to have the potential to cause skin sensitisation based on the Mouse Local Lymph Node Assay.

In a human repeat insult patch test conducted using a 15% solution of the notified chemical, there were no reactions indicative of irritation or sensitisation observed in any of the 97 subjects.

Repeated dose toxicity
The No Observed Effect Level (NOEL) was established as 30 mg/kg bw/day in this study, based on effects observed in animals treated with 150 and 750 mg/kg bw/day.

Genotoxicity
The notified chemical tested was not mutagenic in a bacterial reverse mutation study and not genotoxic in an *in vitro* mammalian chromosome aberration test or an *in vitro* mammalian cell gene mutation test.

Health hazard classification
Based on the skin sensitisation test using the Mouse Local Lymph Node Assay the notified chemical is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:
Xi; R43 May cause sensitisation by skin contact

6.3. Human health risk characterisation

6.3.1. Occupational health and safety

The notified chemical is a skin sensitiser and is slightly irritating to the skin and non-irritating to the eye. It is not a mutagen but may cause systemic toxicity.

The highest occupational exposure is expected to occur to workers directly handling the imported fragrance mixture when it is added to the mixing vessel during formulation of the end use products. Based on EASE modelling the worst-case total systemic exposure (without PPE) is estimated as 0.044-0.33 mg/kg bw/day. A dermal NOAEL was not available, however a NOEL of 30 mg/kg bw/day was established in a 28-day oral study in the rat. The use of this NOEL results in a margin of exposure (MOE) of 91-682. MOE greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. The MOE is based on conservative assumptions (100% dermal absorption, no PPE use) and likely to overestimate the risk.

The risk of sensitisation and irritation effects in workers handling the notified chemical is expected to be low due to the PPE expected to be worn.

The risk to transport and storage workers is expected to be low due to the negligible exposure expected.

The risks of skin sensitisation and irritation to workers using the end use products (cleaners, beauticians) are not expected due to the low concentrations of the notified chemical (< 1 %) in the end use products.

Overall, the risk to the health of workers from use of the notified chemical is not considered unacceptable given the expected use of PPE when handling the imported fragrance mixture and low concentration (< 1%) in end use products.

6.3.2. Public health

The public may come into contact with the notified chemical (< 1%) through the use of a range of cosmetic and consumer products. No skin sensitisation or irritation risks are expected due to the very low concentrations.

The worst-case long-term dermal exposure to the notified chemical is estimated as 0.127 mg/kg bw/day using an oral NOEL of 30 mg/kg bw/day. A margin of exposure (MOE) is calculated as 236. MOE greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. The MOE is based on conservative assumptions and is likely to overestimate the risk.

Overall, the risk to public health from use of products containing the notified chemical at < 1% is not considered unacceptable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported as a component of a liquid fragrance mixture. The mixture will be further blended with other ingredients at customer formulation sites to make end use consumer products, such as alcoholic perfumes, cosmetics, toiletries, detergents and soaps.

The reformulation of the imported product will be done by a batch process and it is expected that most sites will have closed, automated mixing and dosing equipment. The amount of waste notified chemical from cleaning of mixing equipment and empty import containers is estimated to be < 2% of the import volume and will be disposed of with the wash water to either the on-site waste water treatment plants or the sewage.

RELEASE OF CHEMICAL FROM USE

While there will be some releases of this moderately volatile fragrance chemical to the atmosphere, most of the release will be to the sewer as a result of its use pattern.

RELEASE OF CHEMICAL FROM DISPOSAL

It is estimated that < 1% of the imported notified chemical will be lost as residues in consumer containers, which will most likely be sent to landfill.

7.1.2 Environmental fate

The notified chemical is not expected to be readily biodegradable or inherently biodegradable. It is expected to have potential for bioaccumulation in the aquatic organisms given its low molecular weight and high log P_{OW} (5.6). For the details of the environmental fate studies refer to Appendix C. If released to the atmosphere, the notified chemical is expected to degrade through reaction with hydroxyl radicals. Most of the notified chemical is expected to be released to the sewage system after application. In the waste water treatment processes in the sewage treatment plant, most of the notified chemical is expected to partition to sludge or to suspended solids due to its low water solubility and log K_{oc}, where it will be removed for disposal to landfill. In landfill it is expected to slowly decompose by abiotic and biotic processes to form water and oxides of carbon. No significant amount of the notified chemical is expected to be released to the water environment. Therefore, the notified chemical is not expected to be bioavailable to the aquatic organisms despite its potential for bioaccumulation.

7.1.3 Predicted Environmental Concentration (PEC)

The PEC has been calculated assuming 100% release of the notified chemical to sewage after application and no removal of it from the STP, which is for the most conservative worst case consideration.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	1,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	2.74	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	21.161	million
Removal within STP	0%	
Daily effluent production:	4,232	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.65	µg/L
PEC - Ocean:	0.06	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate

and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a hypothetical worst case concentration of 0.65 µg/L may potentially result in a soil concentration of approximately 4.3 x 10^{-3} mg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 2.2 x 10^{-2} mg/kg and 4.3 x 10^{-2} mg/kg, respectively.

7.2. Environmental effects assessment

The results from ecotoxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	EC50 > 1.9 mg/L	Not harmful up to the limit of its solubility in water
Daphnia Toxicity	EC50 > 1.49 mg/L	Toxic
Algal Toxicity	E_rC50 > 1.9 mg/L	Not harmful up to the limit of its solubility in water
Inhibition of Bacterial Respiration	EC50 > 100 mg/L	Not harmful

The results of the studies indicate that the notified chemical is expected to be toxic to daphnids but not harmful to fish and algae (up to its limit of solubility in water) and bacteria.

7.2.1 Predicted No-Effect Concentration

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
Invertebrates	> 1.49	mg/L
Assessment Factor	100.00	
PNEC:	> 14.90	µg/L

A PNEC has been calculated by using the most sensitive endpoint of EC50 > 1.49 mg/L for daphnids. A safety factor of 100 has been used since more than three endpoints are available for the environmental risk assessment.

7.3. Environmental risk assessment

Risk Assessment	**PEC µg/L**	**PNEC µg/L**	**Q**
Q - River:	0.65	> 14.9	< 0.043
Q - Ocean:	0.06	> 14.9	< 0.004

The Risk Quotients (Q=PEC/PNEC) for the worst case scenario consideration have been calculated to be < 0.1 for both river and ocean water environment. This indicates the notified chemical is not expected to pose an unacceptable risk to the aquatic environment based on its reported use pattern.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available data the notified chemical is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)]. The following risk phrase applies to the notified chemical:

− Xi; R43 May cause sensitisation by skin contact

and

As a comparison only, the classification of the notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations 2009) is presented below. This system is not mandated in Australia and carries no legal status but is presented for information purposes.

	Hazard category	*Hazard statement*
Skin sensitisation	Category 1	May cause an allergic skin reaction
Environment	Acute Category 2	Toxic to aquatic life
	Chronic Category 2	Toxic to aquatic life with long lasting effects.

Human health risk assessment
Under the conditions of the occupational settings described, the notified chemical is not considered to pose an unacceptable risk to the health of workers.

When used in the proposed manner, the notified chemical is not considered to pose an unacceptable risk to public health.

Environmental risk assessment
On the basis of the PEC/PNEC ratio calculated and the reported use pattern, the notified chemical is not expected to pose a risk to the environment.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- Safe Work Australia should consider the following health hazard classification for the notified chemical:
  - Xi: R43 May cause sensitisation by skin contact

- Use the following risk phrase for products/mixtures containing the notified chemical:
  - Conc ≥ □1%: R43

Health Surveillance

- As the notified chemical is a potential skin sensitiser, employers in product formulation plants should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of skin sensitisation.

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical at or above 1% concentration (product formulation plant workers):
  - Avoid contact with skin

- Employers should ensure that the following personal protective equipment is used by workers in product formulation plants to minimise occupational exposure to the notified chemical at ≥ 1% concentration:
  - Protective gloves
  - Coveralls

  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 *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)]

workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

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

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director of NICNAS must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(1) of the Act; if
  - the chemical is used in consumer products at or above 1%;

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from as an aroma chemical in alcoholic perfumery, cosmetics, toiletries, household products, soaps, detergents and industrial perfumery, or is likely to change significantly;
  - the amount of chemical being introduced has increased from 1 tonne per year, or is likely to increase, significantly;
  - the chemical has begun to be manufactured in Australia;
  - additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

Material Safety Data Sheet
The MSDS of the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point < -50°C

Method OECD TG 102 Melting Point/Melting Range.
EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Test Facility Givaudan Sussie SA (2004a)

Boiling Point 331°C (pressure unknown)

Method OECD TG 104 Vapour Pressure.
Remarks The vapour pressure curve as a function of temperature could not be determined with the notified chemical according to OECD method 104 (dynamic method) and thus could not be used to extrapolate the normal boiling temperature. The boiling point was estimated using Meissner's method.
Test Facility Givaudan Sussie SA (2004b)

Density 964 ± 1 kg/m^3 at 20°C

Method OECD TG 109 Density of Liquids and Solids.
EC Directive 92/69/EEC A.3 Relative Density.
Remarks Oscillating densitometer method was used.
Test Facility Givaudan Sussie SA (2004c)

Vapour Pressure $1.5 \pm 0.1 \times 10^{-3}$ kPa at 20˚C

Method OECD TG 104 Vapour Pressure,
EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks The static technique was used. The vapour pressures measured at three temperatures 37.61˚C, 32.24˚C and 25.10˚C were between 2.2 and 6.2 Pa. The vapour pressure at 20˚C was determined to be 1.5 ± 0.1 Pa.
Test Facility NOTOX B.V. (2004a)

Water Solubility 3.8×10^{-3} g/L at 20˚C

Method OECD TG 105 Water Solubility.
EC Directive 92/69/EEC A.9 Water Solubility.
Remarks Flask Method. The notified chemical was in contact with water at 20˚C under still conditions for up to 552 hours. Aqueous samples were withdrawn and analysed by Gas Chromatography (GC) method. The aqueous concentration reached equilibrium after 432 hours at 3.8×10^{-3} g/L.
Test Facility Givaudan Sussie SA (2005a)

Hydrolysis as a Function of pH $t_{½}$ > 1 year at pH 4, $t_{½}$ = 7104 hour at pH 7 and 1226 hour at pH 9, 25˚C.

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

pH	*T (°C)*	*$t_{½}$ <hours >*
4	25	> 1 year
7	25	7104
9	25	1226

Remarks Following a preliminary test at 50˚C and pH 4, 7 and 9, a main test was conducted by incubating the test solutions of 0.25 mg/L at pH 4 and 90˚C; pH 7 and 75˚C, 90˚C; and pH 9 and 75, 90˚C. GC was used for determination of the solution concentrations. Since linear relationships (logarithm of concentration vs. time) were found at all pH conditions, the data were analysed using pseudo-first order kinetics and the half-life time at 25˚C was determined.

Test Facility NOTOX B.V. (2006)

Partition Coefficient (n-octanol/water) log Pow = 5.6

Method OECD TG 117 Partition Coefficient (n-octanol/water).
EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks HPLC Method. The determination was performed at 35˚C (column temperature). The chromatograms of the notified chemical showed three peaks at average retention times of 21.39 minutes (7.6% relative peak area), 23.13 minutes (85.1% relative peak area) and 24.32 minutes (7.3% relative peak area), corresponding to partition coefficients (log values) of 5.5, 5.6 and 5.6, respectively.
Test Facility Givaudan Sussie SA (2009a)

Adsorption/Desorption – screening test log K_{oc} = 3.88

Method OECD 121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography
Remarks HPLC Method. The determination was performed at 35˚C (column temperature). The notified chemical showed one peak at an average retention time of 10.91 minutes, corresponding to an adsorption coefficient (log value) of 3.88.
Test Facility Givaudan Sussie SA (2009b)

Flash Point 158ºC at 101.3 kPa

Method DIN 51578 (closed cup)
Test Facility Givaudan Sussie SA (2005b)

Autoignition Temperature 340ºC

Method EC Directive 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).
DIN 51794
Remarks The lowest measured auto-ignition temperature was 343ºC at an injection volume of 125 µL. In accordance with DIN 51794, the temperature was round down to the nearest 5ºC giving the auto-ignition temperature of 340ºC.
Test Facility NOTOX B.V. (2004b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method. EC Directive 92/69/EEC B.1tris Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/HanBrl: WIST (SPF)
Vehicle	Polyethylene glycol 300
Remarks - Method	No deviations from the protocol.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity	The three animals of the first group were observed with hunched posture from the 1- to the 5-hour reading as well as with slightly ruffled fur from the 1-hour reading to test day 6. Slightly ruffled fur was also noted in two animals of the second group at 2- and 3 hour examination.
Effects in Organs	No macroscopic findings were recorded at necropsy.
Remarks - Results	The body weight of the animals was within the range commonly recorded for this strain and age.

CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	RCC Ltd (2004a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 402 Acute Dermal Toxicity. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/HanBrl: WIST (SPF)
Vehicle	None
Type of dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Slight scales were observed in all male animals from test day 5 or 6 to 8 and persisted in one animal up to test day 10 and in two animals up to test day 11. Slight crusts were noted in one animal from test day 8 to 11 and in another animal from test day 9 to 10.
Effects in Organs	No macroscopic findings were recorded at necropsy.
Remarks - Results	The body weight of the animals was within the range commonly recorded for this strain and age.

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

TEST FACILITY RCC Ltd (2004b)

B.3. Irritation – skin

TEST SUBSTANCE Notified Chemical

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

Species/Strain	Rabbit/New Zealand White
Number of Animals	1 M, 2 F
Vehicle	None
Observation Period	72 hours
Type of Dressing	Semi-occlusive.
Remarks - Method	No deviations from the protocol.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0.3	0.3	1	2	< 72 hours	0
Oedema	0	0	0	1	< 24 hours	0

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

Remarks - Results

No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred.

Very slight to well-defined erythema was observed in all animals at the 1- and 24-hour reading and very slight erythema persisted in one animal up to 48 hours after treatment.

Very slight swelling (oedema) was noted in one animal at the 1-hour examination.

No abnormal findings were observed on the treated skin of any animal 72 hours after treatment (at the end of the observation period).

No staining produced by the test substance of the treated skin was observed.

The body weights of all rabbits were considered to be within the normal range of variability.

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY RCC Ltd (2004c)

B.4. Irritation – eye

TEST SUBSTANCE Notified Chemical

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

Species/Strain	Rabbit/New Zealand White
Number of Animals	1 M, 2 F
Observation Period	72 hours

Remarks - Method No deviations from the protocol.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0	0	0	1 (1 hour)	< 24 hours	0
Conjunctiva: chemosis	0	0	0	1 (1 hour)	< 24 hours	0
Conjunctiva: discharge	0	0	0	0	-	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

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

Remarks - Results No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred.

No abnormal findings were observed in the cornea of iris of any animal at any of the measurement intervals.

Slight reddening of the conjunctivae was noted one hour after treatment in all animals.

Slight swelling (chemosis) of the conjunctivae was observed in one of these animals at the 1-hour examination.

Slight reddening of the sclerae was present in all animals at the 1-hour reading.

No abnormal findings were observed in the treated eye of any animal 24 hours after treatment.

No corrosion of the cornea was observed at any of the reading times.
The body weights of all rabbits were considered to be within the normal range of variability.

CONCLUSION The notified chemical is non-irritating to the eye.

TEST FACILITY RCC Ltd (2004d)

B.5. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Notified Chemical

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain Mice/CBA/CaOlaHsd
Vehicle Ethanol:water 7:3 (v/v)
Remarks - Method No deviations from the protocol.

RESULTS

Concentration (% w/v)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	236	-
1	294	1.2
10*	426	1.8*
30*	1809	7.7*
50	3471	14.7

Positive Control (alpha-hexyl cinnamaldehyde)		
0	334	-
5	504	1.5
10*	744	2.3*
25*	2804	8.4*

*This value was used in calculation of EC3.

Remarks - Results

No death occurred during the study period.

No clinical signs were observed in any animals of the control group, group 2 (1%) or group 3 (10%). On the second application day, a slight erythema was observed at both dosing sites in all mice of group 4 (30%) and group 5 (50%), persisting for a total of four days. In addition, on the second and the third application days, a slight ear swelling was observed at both dosing sites in all mice of group 5 (50%) and group 4 (30%), separately, persisting for the remainder of the in-life phase of the study and for a total of three days.

A clear dose-response relation was observed and an EC3 value of 14.1% (w/v) was derived.

The body weight of the animals recorded on the test day 1 (prior to the first application) and on the test day 6, was within the range commonly recorded for animals of this strain and age.

The EC3 value for the positive control was ~11.7% (w/v) in this study.

CONCLUSION

There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY

RCC Ltd (2004e)

B.6. Skin sensitisation – human volunteers

TEST SUBSTANCE

Notified Chemical (15%)

METHOD

Human Repeated Insult Patch Test (In-house method)

Study Design

Induction Procedure: A sufficient amount of the test substance (approximately 0.2 mL) was placed onto a modified Parker-Davis Readi-Bandage occlusive patch and applied to the upper arm of each subject. This procedure was performed and repeated every Monday, Wednesday, and Friday until 9 applications of the test substance had been made.

The subjects were instructed to remove the patch 24 hours after application. Twenty-four hour rest periods followed Tuesday and Thursday removals and 48-hour rest periods followed each Saturday removal. Subjects returned to the testing Facility and the site was scored just prior to the next patch application.

If a subject developed a positive reaction of a level 2 erythema or greater during the induction phase or if, at the discretion of the study director, the skin reaction warranted a change in site, the patch was applied to a previously unpatched, adjacent site for the next application. If a level 2 reaction or greater occurred at the new site, no further applications were made. However, any reactive subjects were subsequently challenge patch tested.

Rest Period: 14 days

	Challenge Procedure: After a rest period of approximately 2 weeks (no applications of the test substance), the challenge patch was applied to a previously unpatched test site. The site was scored 24 and 72 hours after application. All subjects were instructed to report any delayed skin reactivity that occurred after the final challenge patch reading. When warranted, selected test subjects were called back to the clinic for additional examinations and scoring to determine possible increases in challenge patch reactivity.
Study Group	20 males and 90 females ranging in age from 18 to 74 years - exclusive panel
Vehicle	Not known
Remarks - Method	No deviations from the protocol.

RESULTS

Remarks - Results	Ninety-seven (97/110) subjects satisfactorily completed the test procedure using 15% notified chemical. Thirteen (13/110) subjects discontinued for personal reasons unrelated to the conduct of the study. Discontinued panellist data are shown up to the point of discontinuation, but are not used in the conclusion of the final report. There was no skin reactivity observed at any time during the course of the study.
CONCLUSION	A repeated insult patch test was conducted using the notified chemical diluted to 15% (vehicle unknown) under occlusive dressing. The notified chemical was non-irritating and non-sensitising under the conditions of the test.
TEST FACILITY	Essex Testing Clinic, Inc. (2006)

B.7. Repeat dose toxicity

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rats/SPF-bred Wistar
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Polyethylene glycol 300
Remarks - Method	No deviations from the protocol.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 per sex	0	1
low dose	5 per sex	30	0
mid dose	5 per sex	150	0
high dose	5 per sex	750	0
control recovery	5 per sex	0	0
high dose recovery	5 per sex	750	0

Mortality and Time to Death

One male control rat was found dead on treatment day 12. All other rats survived until scheduled necropsy.

Clinical Observations

Episodes of sedation (transient in males and persistent in females) were noted during daily observations in rats treated with 750 mg/kg bw/day. These changes were considered to be test substance related.

Emaciation was recorded in one female treated with 750 mg/kg bw/day and in two females during the first week of recovery.

No findings of toxicological relevance were noted during functional observation battery during week 4.

At 750 mg/kg bw/day, reduced mean hindlimb grip strength was noted in males whereas females had reduced mean fore- and hind-limb grip strength. These differences were considered to be test substance related. All other differences were considered to be incidental.

Test substance-related reductions of locomotor activity were noted in males and females treated with 750 mg/kg bw/day. All other differences were sporadic and did not differ appreciably from the control values and were considered incidental.

In female treated with 750 mg/kg bw/day, the mean daily food consumption values were slightly lower than those of controls. These differences were considered to be test substance related. The remaining females and males were unaffected and no differences were seen during recovery.

The mean body weights of the females treated with 750 mg/kg bw/day were slightly lower (-5%) during the treatment period than those of control females and were considered to be test substance related, whereas the main body weights of the test substance-treated males were unaffected. During the recovery period, no late affects attributed to the test substance were noted.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Test substance-related findings were noted only in rats treated with 750 mg/kg bw/day. The mean and absolute and relative reticulocyte counts of the females treated with 750 mg/kg bw/day were reduced after four weeks of treatment. Both values exceeded the lower limit of the historical control values. The man reticulocyte maturity indices were shifted towards cells of low fluorescence (increased) from middle fluorescence (reduced) and high fluorescence (reduced), which generally indicates retention of older cells and slower/impaired replacement of younger cells. This finding was considered to be test substance related, but was largely reversible after 2 weeks of recovery. All other differences noted after 2 weeks of recovery were within the ranges of the historical control data and considered incidental.

Aspartate aminotransferase activity was increased in both sexes treated with 750 mg/kg bw/day, and lactate dehydrogenase activity was elevated in females treated with 750 mg/kg bw/day when compared with controls. These values exceeded the ranges of the historical control data and were considered related to metabolic activation caused by the test substance. These findings were, however, reversible during the recovery period and therefore considered to be non-adverse. All differences noted after 2 weeks of recovery were within the ranges of the historical control data and considered incidental.

Elevated ketone noted in the urinalysis parameters of males and females treated with 750 mg/kg bw/day after 4 weeks of treatment, was considered possibly related to metabolism of the test substance. After 2 weeks of recovery, this finding was no longer observed. All other differences were considered to be incidental.

Effects in Organs

In males treated with 150 mg/kg bw/day, elevated liver-to-body weight ratios and elevated kidney-to-body weight ratios were noted after four weeks of treatment. Males treated with 750 mg/kg bw/day had elevated absolute and relative liver weights, as well as elevated kidney-to-body weight ratios.

In females treated with 150 mg/kg bw/day, elevated absolute and relative liver weight, and reduced absolute spleen weights were noted after weeks of treatment. The females treated with 750 mg/kg bw/day had elevated absolute and relative liver and kidney weights and reduced absolute and relative spleen weights. Females treated with 750 mg/kg bw/day had elevated heart-to-body weight ratio when compared with controls.

These changes were considered to be test substance related. All other differences were considered to be incidental.

After two weeks recovery, no test substance-related changes were noted in males or females previously treated with 750 mg/kg bw/day.

At the end of treatment period and subsequent recovery period, test substance-related macroscopic findings were recorded in the Harderian Glands (black foci: one female after treatment, and five females at 750 mg/kg bw/day), and at the liver (clay-coloured: 4 females at 150 mg/kg bw/day).

Under the conditions of this study, the treatment with the notified chemical induced histopathological changes in the heart, skeletal muscle, kidneys, Harderian gland, liver and thyroid in animals treated with 150 or 750 mg/kg bw/day.

In the heart, minimal to severe sarcoplasmic vacuolation, minimal to moderate single cell necrosis, increase incidence and mean grade of mononuclear foci, and minimal mycocardial (interstitial) fibrosis were recorded in main study animals treated with 150 or 750 mg/kg bw/day, reflecting a cardiotoxic potential of the test substance. After 14-day treatment-free recovery period, minimal mycocardical fibrosis were recorded in two females previously treated with 750 mg/kg bw/day.

In the skeletal muscle, minimal to slight mixed-cellular, interstitial inflammation, accompanied by minimal to slight interstitial oedema, minimal single fibre necrosis, and partly minimal muscle cell regeneration was recorded in some main study females treated with 750 mg/kg bw/day.

In the kidneys, increased incidence and mean grade of hyaline droplets and of tubular basophilia was recorded in male animals treated with 150 and 750 mg/kg bw/day. The incidence and mean grade of tubular basophilia was still increased in recovery males.

In the Harderian gland, minimal acinar degeneration, minimal to slight acinar hyperplasia, and increased porphyrin deposition was recorded in females treated with 150 or 750 mg/kg bw/day.

In the liver, an increased incidence of liver fatty change was recorded in main study females treated with 150 or 750 mg/kg bw/day. After recovery this finding showed tendency to regression. The toxicological relevance of this finding is unclear. Additionally an increased incidence of hepatocellular hypertrophy was recorded.

In the thyroid an increased incidence of minimal follicular hypertrophy was recorded in male animals at 750 mg/kg bw/day.

CONCLUSION
The No Observed Effect Level (NOEL) was established as 30 mg/kg bw/day in this study, based on the effects observed in animals treated with 150 and 750 mg/kg bw/day.

TEST FACILITY	RCC Ltd (2004f)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure (test 1)/Pre incubation procedure (test 2)
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, TA102
Metabolic Activation System	S9 was prepared from the livers of phenobarbital/β-naphthoflavone induced male Wistar Hanlbm rats.
Concentration Range in Main Test	a) With metabolic activation: 0, 33, 100, 333, 1000, 2500 and 5000 µg/plate b) Without metabolic activation: 0, 33, 100, 333, 1000, 2500 and 5000 µg/plate
Vehicle	Ethanol
Remarks - Method	E. coli was not used. No significant protocol deviations.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	> 5000			
Test 1		≥ 5000	> 5000	negative
Test 2		≥ 333	> 5000	negative
Present	> 5000			
Test 1		≥ 2500	> 5000	negative
Test 2		≥ 333	> 5000	negative

Remarks - Results

The plates incubated with the test substance showed normal background growth up to 5000 µg/plate with and without S9 mix in all strains used in test 1. In test 2 reduced background growth was observed in strains TA 1535, TA 1537, and TA 100 at 333 µg/plate and above with metabolic activation, in strains TA 98 (with metabolic activation) and TA 100 (without metabolic activation) at 1000 µg/plate and above, and in strain TA 102 at 33 µg/plate and above with and without metabolic activation.

No substantial increases in revertant colony numbers of any of the five tester strains were observed at any concentration level, neither in the presence nor absence of metabolic activation. There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged boarder of biological relevance.

Appropriate reference mutagens were used as positive controls. They showed a distinct increase in induced revertant colonies. The historical range of positive controls was exceeded in strain TA 1535 (test 1) without metabolic activation. This effect indicates the sensitivity of the strains rather than compromising the assay. In strain TA 102 of the test 1 without metabolic activation the historical range of positive controls was not reached. The effect was judged to represent fluctuations. The threshold of three times the corresponding solvent control was exceeded by far (factor of 4.1), so the test was considered valid.

CONCLUSION

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

TEST FACILITY

RCC Ltd (2004g)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE

Notified Chemical

METHOD

OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.

Cell Type/Cell Line: Chinese hamster/ V79 Cells

Metabolic Activation System: S9 was prepared from the livers of phenobarbital/β-naphthoflavone induced male Wistar Hanlbm rats.

Vehicle: Ethanol

Remarks - Method: No deviations from the protocol.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0, 0.4, 0.8, 1.6, 3.1*, 6.3*, 12.5*, 25.0, 50.0	4	18
Test 2a	0, 0.6, 1.3*, 2.5*, 5.0*, 10.0, 20.0	18	18
Test 2b	0, 2.5, 5.0*, 10.0, 20.0	28	28

Present			
Test 1	0, 62.5, 125.0, 250.0*, 500.0*, 1000.0, 1500.0*, 2000.0*, 2500.0	4	18
Test 2	0, 125.0, 250.0*, 500.0*, 1000.0*, 1500.0*, 2000.0	4	28

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	≥ 25.4			
Test 1		≥ 12.5	> 50.0	negative
Test 2a		≥ 20.0	> 20.0	negative
Test 2b		≥ 10.0	> 20.0	negative
Present	≥ 406.3			
Test 1		≥ 500.0	≥ 1000.0	negative
Test 2		≥ 500.0	≥ 1000.0	negative

Remarks - Results

Toxic effects indicated by reduced cell numbers and/or mitotic indices of about or below 50% of control were observed in all experiments.

In all tests, in the absence and presence of S9 mix, no biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed. The aberration rates of the cells after treatment with the test substance (0.0-3.5% aberrant cells, exclusive gaps) were close to the range of solvent control values (0.0-1.0% aberrant cells, exclusive gaps) and within the range of the historical control data: 0.0-4.0% aberrant cells, exclusive gaps.

Statistically significant ($p < 0.05$) increases were observed in the presence of S9 mix, in test 1 at preparation interval 18 hours after treatment with 250 to 2000 µg/mL (3% aberrant cells, each) and in test 2 at preparation interval 28 hours after treatment with 500 µg/mL (3.5% aberrant cells). Although these increases were statistically significant compared to the low responses (0.5% and 0.0% aberrant cells, respectively) in the solvent control data, the responses are within the historical control data range (0.0-4.0% aberrant cells). Therefore, the statistically significant increases have to be regarded as being biologically irrelevant.

In all tests, no biologically relevant increase in the rate of polyploid metaphases was found after treatment with the test substance (1.8-3.5%) as compared to the rates of the solvent controls (1.1-4.3%).

In all tests, EMS (200 and 300 µg/mL, respectively) and CPA (0.7 and 1.0 µg/mL, respectively) were used as positive controls and showed distinct increases in cells with structural chromosome aberrations.

CONCLUSION

The notified chemical was not clastogenic to the V79 Chinese hamster cell line treated in vitro under the conditions of the test when tested up to cytotoxic concentrations.

TEST FACILITY

RCC Ltd (2004h)

B.10. Genotoxicity – in vitro

TEST SUBSTANCE

Notified Chemical

METHOD

OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.

	EC Directive 2000/32/EC B.17 Mutagenicity - In vitro Mammalian Cell Gene Mutation Test.
Cell Type/Cell Line	Mouse lymphoma thymidine kinase locus using the cell line L5178Y
Metabolic Activation System	S9 was prepared from the livers of phenobarbital/β-naphthoflavone induced male Wistar Hanlbm rats.
Vehicle	DMSO
Remarks - Method	Since the recommended cytotoxic range of approximately 10 to 20% of relative total growth was not covered without metabolic activation a repeat experiment was performed in the absence of metabolic activation with a treatment time of 24 hours (test 2a).

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression and Growth Time*	*Selection Time*
Absent				
Test 1	0, 2.9, 5.9, 11.8, 23.5, 47.0	4 hours	48 hours	n/a
Test 2	0, 5.9, 11.8, 23.5, 47.0, 70.5	24 hours	48 hours	n/a
Test 2a	0, 50, 70	24 hours	48 hours	n/a
Present				
Test 1	0, 5.9, 11.8, 23.5, 47.0, 94.0	4 hours	48 hours	n/a
Test 2	0, 23.5, 47.0, 94.0, 117.3, 141.0	4 hours	48 hours	n/a

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	≥ 375.0			
Test 1		≥ 47.0	> 47.0	negative
Test 2		≥ 70.5	> 70.5	negative
Test 2a		≥ 50	> 70	negative
Present	≥ 375.0			
Test 1		≥ 94.0	> 94.0	negative
Test 2		≥ 117.3	> 141.0	negative

Remarks - Results

Relevant cytotoxic effects indicated by a relative total growth of less than 50% of survival were observed in test 1 at 94.0 µg/mL with and at 47.0 µg/mL without metabolic activation, following 4 hours of treatment. The cultures were not analysable at even higher concentrations due to exceedingly severe cytotoxic effects. In test 2 toxic effects as described above occurred at 70.5 µg/mL in the absence and at 117.3 µg/mL and above in the presence of metabolic activation. In the repeat test 2a strong toxic effects were determined at both evaluated concentrations of 50 and 70 µg/mL. Higher concentrations were not analysable due to exceedingly severe cytotoxic effects.

No substantial and reproducible dose dependent increase of the mutation frequency was observed in both main tests up to the maximum concentration with and without metabolic activation. In the first test the threshold of 126 plus each solvent control count was exceeded in culture 1 at 47.0 µg/mL. However, no comparable increase was noted in the parallel culture under identical conditions so this isolated increase was judged as not reproducible.

A linear regression analysis (least square) was performed to assess a possible dose dependent increase of mutant frequencies using SYSTAT statistics software. A significant dose dependent trend of the mutation frequency indicated by a probability value of < 0.05 was determined in the second culture of test 1 without metabolic activation. Since the mutation frequency neither exceeded the historical range of solvent

controls nor the threshold as indicated above, the statistically significant result is considered as biologically irrelevant fluctuation.

In this study the range of the solvent controls was from 85 up to 198 mutant colonies per 10^6 cells: the range of groups treated with the test substance was from 116 to 304 mutant colonies per 10^6 cells.

The highest solvent control value (198 colonies per 10^6 cells) exceeded the recommended 50-170 × 10^6 control range as stated in acceptability of the assay of this report. However, the number of mutant colonies per 10^6 cells in the parallel culture (158) was acceptable. The cloning efficiency exceeded the upper limit of 120% in the second culture of test 2 and in both cultures of test 2a without metabolic activation. Cloning efficiency values above 100% occasionally occur since even suspension cell cultures do not form an ideal solution in medium. The cells tend to form transient aggregates that are counted as single cells during determination of the cell density. The aggregation does not compromise the validity of the data however, since the absolute values of the cloning efficiency are used to calculate the mutation frequency. The total suspension growth just fell short of the lower limit of 8.0 in the solvent controls of the second culture of test 1 with and without metabolic activation. The data are judged as valid.

MMS (methyl methane sulfonate) and CPA (cyclophosphamide) were used as positive controls and showed a distinct increase in induced total mutant colonies with at least one of the concentrations of the controls. The relative total growth of the positive controls in the first experiment without metabolic activation fell short of the 10% limit. The data are judged as acceptable however since the relative suspension growth of both controls remained above 10%.

CONCLUSION

The notified chemical was not clastogenic to mouse lymphoma thymidine kinase locus using the cell line L5178Y treated in vitro under the conditions of the test.

TEST FACILITY

Harlan (2010)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test.
Inoculum	Activated sludge from a biological water treatment plant, Bois-de-Bay, Satigny, Switzerland.
Exposure Period	61 days
Auxiliary Solvent	None
Analytical Monitoring	The amount of oxygen taken up, Biological Oxygen Demand (BOD), during the biodegradation of the notified chemical was expressed as a percentage of ThOD (Theoretical Oxygen Demand). GC was used for analyses of the notified chemical and the metabolites.
Remarks - Method	The study was conducted at pH 7.6 and 22˚C at a nominal test concentration of 20 mg/L for the notified chemical. The water used during the study was deionised water containing less than 10 mg/L dissolved organic carbon. A blank control, a reference control using sodium benzoate (100 mg/L) and a toxicity control (20 mg/L notified chemical and 100 mg/L sodium benzoate) were conducted. All the tests were performed in duplicates.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% degradation*	*Day*	*% degradation*
7	1	5	64
13	5	7	73
21	6	13	82
28	13	21	87
61	29	28	82

Remarks - Results	The toxicity control showed that the notified chemical is not toxic to the micro-organisms at the nominal test concentration (20 mg/L). The notified chemical is not considered to be readily biodegradable based on the test results. However, quantitative analysis for the notified chemical suggested a rapid primary biodegradation of the chemical. 2-(1-(3,3-dimethylcyclohexyl) ethoxy)-2-methylpropan-1-ol (Serenol) was identified as the primary metabolite by comparison to the authentic material. Serenol further degraded to 2-(1-(3,3-dimethylcyclohexyl) ethoxy)-2-methylpropanoic acid (GR-87-4834), which was identified by comparison to the authentic material. At the end of the test (day 61) only GR-87-4834 was observed in the test solutions.
CONCLUSION	The notified chemical is not classed as readily biodegradable.
TEST FACILITY	Givaudan Suisse SA (2010)

C.1.2. Bioaccumulation

CONCLUSION	A test for bioaccumulation was not conducted for the notified chemical. Considering the notified chemical is not readily biodegradable, has a low molecular weight (296.4 g/mol)

and an estimated log P_{OW} of 5.6, the notified chemical is predicted to have potential to bioaccumulate and bioconcentrate.

C.1.3. Inherent biodegradability

TEST SUBSTANCE: Notified Chemical

METHOD: OECD TG 302C Inherent Biodegradability: Modified MITI Test (II)

Inoculum: Fresh activated sludge from a biological water treatment plant (City of Geneva, Peney-Dessous).

Exposure Period: 29 days

Auxiliary Solvent: Not reported

Analytical Monitoring: The Biological Oxygen Demand (BOD) during the biodegradation of the notified chemical was expressed as a percentage of ThOD (Theoretical Oxygen Demand).

Remarks – Method: The test was conducted at a nominal concentration of 30 mg/L, pH 7.3 and 22˚C. The water used during the study was deionised water containing less than 10 mg/L dissolved organic carbon.

The activity of the inoculum was tested by performing a simultaneous ready biodegradability test according to OECD TG 301F: Manometric Respirometry. The test conditions were the same as for the main test except that concentration of sodium benzoate was 100 mg/L and the inoculum concentration was 30 mg/L (dry matter).

A blank control and a reference control using sodium benzoate (100 mg/L, nominal) and a toxicity control (20 mg/L notified chemical and 100 mg/L sodium benzoate, nominal) were conducted. All the tests were performed in duplicates.

Oxygen uptakes are corrected to account for the small differences between actual and nominal concentrations of test and reference substances.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	-9.6	5	73
14	9.4	14	80
21	10.3	21	81
29	11.6	29	81

Remarks – Results: Degradation of sodium benzoate exceeded 75% after 7 days thus verifying the activity of the inoculum and validating the test.

The notified chemical can be classified as not inherently biodegradable based on the test results.

CONCLUSION: The notified chemical cannot be considered to be inherently biodegradable.

TEST FACILITY: Givaudan Suisse SA (2004d)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Notified Chemical

METHOD: OECD TG 203 Fish, Acute Toxicity Test - Semi-Static.

	EC Directive 92/69/EEC C.1 Acute Toxicity for Fish - Semi-Static.
Species	Carp
Exposure Period	96 h
Auxiliary Solvent	Acetone
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	Gas Chromatography for concentration analyses
Remarks – Method	For the static range-finding test, three fish were exposed to the notified chemical at levels up to 5 mg/L. At this level an oily layer floated on the surface and was present throughout the test period. A semi-static final test was conducted by exposing fish (seven carp per test group) to a solvent-control and nominal concentrations of 0.5, 0.8, 1.3, 2.0 and 3.2 mg/L at pH 7.9. Acetone was used in the preparation of stock solution. The test solutions were renewed daily. Samples for analytical confirmation of actual exposure concentrations were taken from freshly prepared solutions at the start and after 72 hours of exposure and from 24-hour old solutions after 24 and 96 hours of exposure.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*			
Nominal	*Actual*		*24 h*	*48 h*	*72 h*	*96 h*
Solvent control	NM	7	0	0	0	0
0.5	NM	7	0	0	0	0
0.8	NM	7	0	0	0	0
1.3	1.2*	7	0	0	0	0
2.0	1.7*	7	0	0	0	0
3.2	1.9**	7	0	0	0	0

NM: Not Measured
* Measured concentration at the start.
** Average exposure concentration

LC50	> 1.9 mg/L at 96 hours (nominal).
NOEC	1.9 mg/L at 96 hours.
Remarks – Results	Under the conditions of the test the notified chemical induced no visible effects in carp at any of the concentrations tested including a concentration (nominal 3.2 mg/l) exceeding the water solubility limit. The 96h-LC50 was above the solubility limit of the notified chemical in the test medium, i.e. above an average exposure concentration of 1.9 mg/L. It was noted during the range-finding test that two out of three tested fish were dead at the end of the test at the highest nominal concentration 5 mg/L, suggesting that some physical effects were involved due to the presence of the floating layer of the undissolved chemical. Therefore this test is considered invalid and the notified chemical is considered not harmful to fish up to the limit of its solubility.

CONCLUSION	The notified chemical is not harmful to fish up to the limit of its solubility.
TEST FACILITY	NOTOX B.V. (2004c)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test and Reproduction Test - Semi-static. EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - Semi-static.
Species	*Daphnia magna*
Exposure Period	48 hours

Auxiliary Solvent	Acetone
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	Gas Chromatography for concentration analyses
Remarks - Method	For the static range-finding test, 10 daphnids were exposed to the notified chemical at levels up to 5 mg/L. At this level an oily layer floated on the surface and was present throughout the test period. A semi-static final test was conducted by exposing daphnids (in four replicates, 5 animals per vessel) to a solvent-control and nominal concentrations of 0.5, 0.8, 1.3, 2.0 and 3.2 mg/L. Acetone was used in the preparation of stock solutions. The test solutions were renewed after 24 hours of the test. Samples for analytical confirmation of actual exposure concentrations were taken from freshly prepared solutions at the start and after 24 hours of exposure and from 24-hour old solutions after 24 and 48 hours of exposure.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Solvent control	NM	20	0	0
0.5	NM	20	0	0
0.8	NM	20	0	0
1.3	0.97*	20	0	1
2.0	1.48*	20	0 (2)**	3 (1)**
3.2	1.49*	20	3 (13)**	5

NM: Not Measured

* Average exposure concentration

** Bracketed values are numbers of daphnids trapped at the surface. These organisms were re-immersed into the respective solutions before recording of mobility.

EC25	1.49 mg/L at 48 hours
EC50	> 1.49 mg/L at 48 hours
NOEC	0.97 mg/L at 48 hours
Remarks - Results	Under the conditions of the test, the notified chemical did not induce significant immobilisation (i.e. < 10%) of *Daphnia magna* at a nominal concentration of 1.3 mg/L, corresponding to an average exposure concentration of 0.97 mg/L after 48 hours of exposure (NOEC). The 48h-EC50 was above the solubility limit of the notified chemical in the test medium, i.e. above an average exposure concentration of 1.49 mg/L. It was noted during the range-finding test that a total of 90% of the daphnids became immobilised at the highest nominal concentration 5 mg/L, suggesting that some physical effects were involved due to the presence of a floating layer of the undissolved chemical. Therefore this test is considered invalid. Considering up to 25% mortality was observed at the level of its solubility in the test medium (1.49 mg/L), the notified chemical is considered at worst toxic to daphnids.

CONCLUSION	The notified chemical is at worst toxic to daphnids.
TEST FACILITY	NOTOX B.V. (2004d)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test - Static EC Directive 92/69/EEC C.3 Algal Inhibition Test.
Species	*Pseudokirchneriella subcapitata*

Exposure Period	72 hours
Concentration Range	Nominal: 0.5 – 3.2 mg/L Actual: 0.5 – 1.9 mg/L
Auxiliary Solvent	Acetone
Water Hardness	240 mg $CaCO_3$/L
Analytical Monitoring	Gas Chromatography for concentration analyses
Remarks - Method	For the static range-finding test, algae were exposed to the notified chemical at levels up to 5 mg/L. A static final test was conducted by exposing algae (initial density 10,000 cells/L) to a blank control, a solvent-control and nominal concentrations of 0.5, 0.8, 1.3, 2.0 and 3.2 mg/L. Acetone was used in the preparation of stock solution.

RESULTS

Biomass (average exposure)		*Growth (average exposure)*	
E_bC50	*NOEC*	*E_rC50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
> 1.9*	1.9*	> 1.9*	1.9*

* Average exposure concentration

Remarks - Results	Under the conditions of the study with *Pseudokirchneriella subcapitata*, no inhibition of cell growth or reduction of growth rate was recorded at any of the concentrations tested including a concentration exceeding the water solubility limit. Hence the 72h-EC50 for both growth inhibition and growth rate reduction exceeded a nominal concentration of 3.2 mg/L (corresponding to an average measured exposure concentration of 1.9 mg/L).
CONCLUSION	The notified chemical is not harmful to algae up to its solubility limit in water.
TEST FACILITY	NOTOX B.V. (2004e)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. ISO 8192-1986 EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test
Inoculum	Activated sludge from a biological water treatment plant (City of Geneva, Peney-Dessous)
Exposure Period	3 hours
Concentration Range	Nominal: 1.0, 3.16, 10.0, 31.6, 100 mg/L
Remarks – Method	Activated sludge was exposed to the notified chemical at 5 nominal concentrations ranging 1.0 – 100 mg/L, 23˚C and pH 7 – 8. A blank control and a reference control with 3,5-dichlorophenol (at 5 mg/L and 30 mg/L) were performed.
RESULTS	
IC50	> 100 mg/L (nominal)
Remarks – Results	The IC50 for 3,5-dichlorophenol was between 5 and 30 mg/L. In the tested range, 1-100 mg/L, the inhibition rate did not exceed 20%. Therefore, the IC50 for the notified chemical is > 100 mg/L.
CONCLUSION	The notified chemical is considered not to be harmful to sewage sludge microorganisms.
TEST FACILITY	Givaudan Suisse SA (2005c)

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