File No: LTD/1582

April 2012

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Benzoic acid, 2-hydroxy-, (3Z)-1-methyl-3-hexen-1-yl 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 Sustainability, Environment, Water, Population and Communities.

For the purposes of subsection 78(1) of the Act, this Public Report may be inspected at our NICNAS office by appointment only at Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

This 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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SUMMARY

The following details will be published in the NICNAS *Chemical Gazette:*

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS SUBSTANCE	INTRODUCTION VOLUME	USE
LTD/1582	Givaudan Pty Ltd	Benzoic acid, 2-hydroxy-, (3Z)-1-methyl-3-hexen-1-yl ester	Yes	≤1 tonne per annum	Component of cosmetic and household cleaning products

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

R43 May cause sensitisation by skin contact

and

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. The environmental classification under 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
Aquatic Environment	Acute Category 1	Very toxic to aquatic life
	Chronic Category 1	Very 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 unreasonable risk to the health of workers.

When used at ≤0.20% in deodorants, ≤0.37% in fine fragrances, ≤0.51% in other leave-on cosmetic products,≤0.96% in rinse-off cosmetics and ≤ 0.12% in fabric care and household cleaning products, the notified chemical is not considered to pose an unreasonable risk to public health.

Environmental risk assessment
On the basis of the PEC/PNEC ratio, the reported use pattern and maximum annual import volume, the notified chemical is not considered to pose an unreasonable 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 phrases for products/mixtures containing the notified chemical:
  - Conc. ≥1%: Xi; R43

- The Delegate (and/or the Advisory Committee on Chemicals Scheduling) should consider the notified chemical for listing on the SUSMP.

Health Surveillance

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

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following isolation and engineering controls to minimise occupational exposure to the notified chemical during reformulation processes:
  - Enclosed, automated processes, where possible
  - Ventilation system including local exhaust ventilation

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical during reformulation processes:
  - Avoid contact with skin

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical during reformulation processes:
  - Coveralls, impervious gloves

  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.

Public Health

- The following measures should be taken to minimise public exposure to the notified chemical:
  - The notified chemical should only be used at ≤0.20% in deodorants, ≤0.37% in fine fragrances, ≤0.51% in other leave-on cosmetic products and ≤0.96% in rinse-off cosmetics, fabric care and household cleaning products.

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 importation volume exceeds one tonne per annum notified chemical;
- the concentration of the notified chemical exceeds or is intended to exceed 0.20% in deodorants, 0.37% in fine fragrances, 0.51% in other leave-on cosmetic products and 0.96% in rinse-off cosmetics,fabric care and household cleaning products.

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component of cosmetic and household cleaning products, or is likely to change 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.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Givaudan Pty Ltd (ABN: 87 000 470 280)
36/5 Inglewood Place
Baulkham Hills, NSW 2153

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
EU (2010), USA (2011)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Karmaflor

CAS NUMBER
873888-84-7

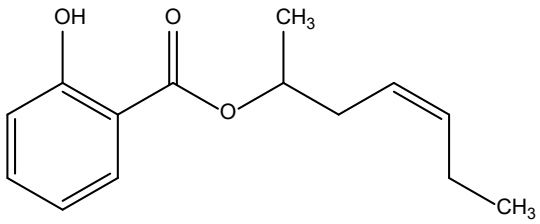
CHEMICAL NAME
Benzoic acid, 2-hydroxy-, (3Z)-1-methyl-3-hexen-1-yl ester

OTHER NAME(S)

(Z)-hept-4-en-2-yl 2-hydroxybenzoate
GR-86-3792

MOLECULAR FORMULA
$C_{14}H_{18}O_3$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
234 Da

ANALYTICAL DATA
Reference NMR, IR, GC/MS and UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY ~95%

KNOWN IMPURITIES

Chemical Name	Benzoic acid, 2-hydroxy-, 1-methylhexyl ester		
CAS No.	837364-32-6	*Weight %*	1.5
Chemical Name	Benzoic acid, 2-hydroxy-, (3E)-1-methyl-3-hexen-1-yl ester		
CAS No.	873888-85-8	*Weight %*	2.4
Chemical Name	Benzoic acid, 2-hydroxy-, (3Z)-2-methyl-3-hexen-1-yl ester		
CAS No.	-	*Weight %*	0.95
Chemical Name	Benzoic acid, 2-hydroxy-, (3Z)-1-methyl-3-hexyn-1-yl ester		
CAS No.	-	*Weight %*	0.26

ADDITIVES/ADJUVANTS None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Colourless liquid

Property	Value	Data Source/Justification
Freezing Point	<-50 °C	Measured
Boiling Point	282 °C at 101.3 kPa	Measured
Density	1037 kg/m^3 at 20 °C	Measured
Vapour Pressure	1.0 x 10^{-4} kPa at 25 °C	Measured
Water Solubility	1.0 x 10^{-3} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Contains functionality that is expected to hydrolyse slowly under environmental conditions
Partition Coefficient (n-octanol/water)	log Pow = 5.0	Measured
Surface Tension	58.7 mN/m at 20 °C	Measured
Adsorption/Desorption	log K_{oc} = 3.50	Calculated (chemical class ‘esters’; European Commission, 2003a)
Dissociation Constant	pKa ~ 10	Estimated by analogy

Flash Point	153 ºC at 101.3 kPa (closed cup)	Measured. Classified as a C2 combustible liquid (NOHSC, 2001).
Flammability	Not determined	Based on the flash point, not classified as flammable (NTC, 2007)
Autoignition Temperature	354 ± 5 ºC	Measured
Explosive Properties	Predicted negative	Contains no functional groups that would imply explosive properties.
Oxidising Properties	Predicted negative	Contains no functional groups that would imply oxidative properties.

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 under normal conditions of use.

Dangerous Goods classification
Based on the submitted physico-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 be imported into Australia as a component (≤24%) of compounded fragrances.

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

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

PORT OF ENTRY
Sydney and Perth

IDENTITY OF MANUFACTURER/RECIPIENTS
Givaudan Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a component (≤24%) of compounded fragrances in 1, 5, 10, 25, 100 and 190 kg glass lacquer-lined containers. The fragrance preparations will be transported by road from the wharf or airport of entry to reformulation sites. The end-use products will be packaged in containers suitable for retail sale.

USE
The notified chemical is intended to be used as a component of fragrances for a variety of cosmetic and household cleaning products (proposed usage concentration: ≤4.8% in fine fragrances, ≤0.96% in other cosmetic products and ≤0.12% in fabric care and household cleaning products).

OPERATION DESCRIPTION
The procedures for incorporating the imported preparations (containing ≤24% of the notified chemical) into end-use products will vary depending on the nature of the cosmetic and household products being formulated. The reformulation process will likely involve blending operations that are highly automated and occur in a fully enclosed environment, followed by automated filling of the reformulated end-use products (containing ≤4.8% notified chemical) into containers of various sizes.

The end-use products (containing ≤4.8% notified chemical) may be used by consumers and professionals, such as hairdressers, workers in beauty salons or cleaners. Depending on the nature of the product, these could be applied in 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

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	Unspecified	Unspecified
Cosmetic and detergent manufacture	1	240
Quality assurance	1	4
Salon workers	Unspecified	Unspecified
Cleaners	Unspecified	Unspecified

EXPOSURE DETAILS

Transport and storage workers may come into contact with the notified chemical, as a component of the imported preparations (≤24%) or end-use products (≤4.8%), only in the event of accidental rupture of containers.

During reformulation, dermal, ocular and perhaps inhalation exposure of workers to the notified chemical (≤24%) may occur during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. Exposure is expected to be minimised through the use of mechanical ventilation and/or enclosed systems and through the use of personal protective equipment (PPE) such as coveralls, safety goggles and impervious gloves.

Exposure to the notified chemical in end-use products (at ≤4.8% notified chemical) may occur in professions where the services provided involve the application of cosmetic and personal care products to clients (e.g. hairdressers and beauty salon workers) or in the cleaning industry. Such professionals may use some PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemical (at ≤4.8% concentration) through the use of fabric care and household cleaning products and the rinse-off and leave-on cosmetic and personal care products. The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible.

Data on typical use patterns of cosmetic product categories in which the notified chemical may be used are shown in the following table (SCCS, 2010; Cadby *et al.*, 2002). For the purposes of the exposure assessment, Australian use patterns for the various product categories are assumed to be similar to those in Europe. In the absence of dermal absorption data, the default dermal absorption of 100% was assumed for calculation purposes (European Commission, 2003b). Although, the actual level of dermal absorption may be lower than 100%, it may vary with the formulation type. Considering that there may be penetration enhancers in some cosmetic formulations, 100% was used in the estimation of the systemic dose. An adult bodyweight of 60 kg has been used for calculation purposes.

Product type	Estimated Daily Amount Applied (A) (mg)	Concentration (C) (%)	Retention Factor (RF) (unitless)	Daily systemic exposure (D) (mg/kg)
Body lotion	7820	0.96	1	1.25
Face cream	1540	0.96	1	0.25
Hand cream	2160	0.96	1	0.35
Fine fragrances	750	4.8	1	0.60
Deodorant spray	1430	0.96	1	0.23
Shampoo	10460	0.96	0.01	0.017
Hair conditioner	3920	0.96	0.01	0.0063
Shower gel	18670	0.96	0.01	0.030
Hand wash soap	20000	0.96	0.01	0.032
Hair styling products	4000	0.96	0.1	0.064
Total				2.82

D* = A × C × RF / BW
BW = body weight (kg)
*Calculations assume 100% dermal absorption

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above table that contain the notified chemical. This would result in a combined internal daily dose of 2.82 mg/kg.

A quantitative exposure estimate from use of the notified chemical in fabric care and household cleaning products (at ≤0.12% concentration) was not deemed necessary, based on the limited expected exposure to the notified chemical from use of these products.

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	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
Mouse, skin sensitisation – Modified Local lymph node assay	evidence of sensitisation
Human, skin sensitisation – repeat insult patch test (15%)	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days	NOAEL = 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro chromosome aberration	non genotoxic

Toxicokinetics, metabolism and distribution
Passive diffusion of the notified chemical across dermal and gastrointestinal membranes is expected based on its low molecular weight (234 Da), water solubility (1.0×10^{-3} g/L at 20 °C) and partition coefficient (log Pow = 5.0). Additionally, systemic absorption via the lungs is expected. The potential for absorption is supported by the observed systemic effects in animal studies following oral and dermal exposure to the notified chemical.

Acute toxicity
The notified chemical was found to have low acute oral (LD50 >2000 mg/kg bw) and dermal toxicity (LD50 >2000 mg/kg bw) in rats. In the dermal study, delayed local reactions were noted with slight erythema, scabs and/or scaling observed in 9/10 animals from day 3, which persisted in some animals until the end of the observation period.

No acute inhalation toxicity data on the notified chemical was provided.

Irritation and Sensitisation

The notified chemical was slightly irritating to the skin of rabbits, with slight erythema noted one hour post patch removal. In addition, the notified chemical was a slight irritant to the eyes of rabbits, with minimal to moderate conjunctival redness observed. The treated eyes appeared normal within 7 days. The irritation scores in these studies did not warrant classification of the chemical as a skin or eye irritant.

The notified chemical was a skin sensitiser in a local lymph node assay (LLNA) in mice, with reported stimulation indices of 2.21, 2.85 and 10.06 at 0.5, 5 and 25% concentration, respectively (EC_3 = 5.4%). In addition, the notified chemical was determined to be skin sensitiser in mice using a modified LLNA protocol (involving flow cytometry), with reported stimulation indices of 2.4, 4.9 and 9.7 at 10, 25 and 50% concentration, respectively (EC_3 = 13.8%). The potential for the notified chemical to be a mild to moderate irritant was also noted, based on ear thickness measurements. The notified chemical was not a skin sensitiser at 15% concentration in a human repeat insult patch test (HRIPT).

Repeated Dose Toxicity
In a 28 day oral toxicity study, rats (5/sex/dose) were administered the notified chemical at 0, 100, 300 or 1000 mg/kg bw/day. There were no mortalities during the study and the treatment related effects were, in general, limited to increased liver weights and minimal to mild hepatoceullar hypertrophy in 1000 mg/kg bw/day males (2/5) and females (2/5), and in 300 mg/kg bw/day males (2/5). The liver effects were not considered by the study authors to be adverse, thus the NOAEL established by the study authors was 1000 mg/kg bw/day.

Mutagenicity
The notified chemical was not mutagenic in a bacterial reverse mutation assay and was not clastogenic in an *in vitro* chromosome aberration assay in Chinese hamster V79 cells.

Health hazard classification
Based on the data provided, the notified chemical is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase: R43 May cause sensitisation by skin contact

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Reformulation
The main toxicological effect of concern is skin sensitisation. There is the potential for exposure to the notified chemical during reformulation of the imported product containing the notified chemical at ≤24% concentration. As the potential for skin sensitisation is of concern for workers handling the notified chemical at >1% concentration, caution should be exercised during reformulation processes to prevent skin contact.

Therefore, provided that control measures are in place to minimise worker exposure, including the use of PPE and automated reformulation processes, the risk to the health of workers is not considered to be unreasonable.

End-use
Workers involved in professions where the services provided involve the application of cosmetic products containing the notified chemical to clients (e.g. hairdressers and beauty salon workers) and/or professional cleaners, may be exposed to the notified chemical. The risk to these workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical (for details of the public health risk assessment, see Section 6.3.2.).

6.3.2. Public Health
Repeated dose toxicity
Members of the public may experience repeated exposure to the notified chemical through the use of cosmetic, fabric care and household cleaning products containing the notified chemical at the proposed concentration of up to 4.8%.

The repeat dose toxicity potential was estimated by calculation of the margin of exposure (MoE) using the worst case exposure scenario of 2.82 mg/kg bw/day (see Section 6.1.2) and a NOAEL of 1000 mg/kg bw/day that was established by the study authors in a 28 day oral toxicity study in rats. A MoE of 100 is considered acceptable to account for intra- and inter-species extrapolation. Based on the estimated exposure of 2.82 mg/kg bw/day and a NOAEL of 1000 mg/kg bw/day, a MOE of 355 is estimated.

Skin sensitisation
There is a risk of potential skin sensitisation associated with use of the notified chemical in cosmetics, fabric care and household cleaning products at the proposed usage concentration (up to 4.8% in fine fragrances and up to 0.96% in other cosmetics, fabric care and household cleaning products).

Proposed methods for the quantitative risk assessment of dermal sensitisation have been the subject of significant discussion (see for example, Api *et al.*, 2008 and RIVM, 2010). As is shown in the table below, the Consumer Exposure Level (CEL) from use of the notified chemical in a number of different cosmetics may be estimated (SCCS, 2010 and RIVM, 2006). When tested in an LLNA study, the notified chemical was a skin sensitiser with an EC_3 value of 5.4%. Although this value has been used for the purposes of a quantitative risk assessment of the notified chemical given the standard protocol followed in the LLNA study, the availability of additional information on the sensitisation potential of the notified chemical (i.e., the modified LLNA and the HRIPT) was taken into account when determining the safety assessment factors that should be applied. Thus, consideration of the study details and application of appropriate safety factors allowed the derivation of an Acceptable Exposure Level (AEL) of 14 μg/cm^2. In this instance, the safety factors employed include an interspecies factor (1), intraspecies factor (10), a matrix factor (3.16) and a use and time factor (3.16), giving an overall safety factor of 100.

Product type	Proposed usage concentration (%)	CEL (μg/cm^2)	AEL (μg/cm^2)	Recommended usage concentration (%)
Deodorant spray	0.96	69	14	≤0.20
Fine fragrances	4.8	180	14	≤0.37
Other leave-on cosmetics (assumed face cream)	0.96	26	14	≤0.51
Rinse-off cosmetics (assumed hand wash soap)	0.96	2	14	≤0.96

As the CEL>AEL, the risk to the public of the induction of sensitisation that is associated with the use of the notified chemical in deodorants (at ≤0.96%), fine fragrances (at ≤4.8%) and other leave-on cosmetic products (using face cream as a worst case example; at ≤0.96%) is considered to be unreasonable. Reducing the concentration of the notified chemical in deodorants to ≤0.20%, fine fragrances to ≤0.37% and other leave-on cosmetic products to ≤0.51% allows recalculation of the consumer exposure to acceptable levels. With regards to rinse-off cosmetic products, as the AEL>CEL (using hand wash soap as a worst case example), the risk of induction of sensitisation associated with the use of rinse-off cosmetic products at ≤0.96% concentration is not considered to be unreasonable. Based on the significantly lower expected exposure level from use in fabric care and cleaning products (at ≤0.96%), by inference, the risk of induction of sensitisation associated with use of these products is also not considered to be unreasonable. Furthermore, the notifier indicated that the notified chemical is only proposed to be used in fabric care and cleaning products at concentration ≤0.12%. It is acknowledged that consumers may be exposed to multiple products containing the notified chemical, and a quantitative assessment based on aggregate exposure has not been conducted.

Therefore, based on the information available, the risk to the public associated with use of the notified chemical at ≤0.20% in deodorants, ≤0.37% in fine fragrances, ≤0.51% in other leave-on cosmetic products and ≤0.96% in rinse-off cosmetics, fabric care and household cleaning products is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will not be manufactured in Australia; therefore there will be no release from this activity. Environmental release during importation, transport and distribution may occur as a result of accidental spills. In the event of a spill, the notified chemical is expected to be contained and collected with an inert absorbent material and disposed of in accordance with local regulations.

During reformulation of imported fragrance compounds containing the notified chemical to make consumer products, up to 0.09% of the import volume is estimated to be lost to waste water from cleaning of formulation

equipment and is expected to be released to sewers.

RELEASE OF CHEMICAL FROM USE
The notified chemical is expected to be released to sewers in domestic situations across Australia as a result of its use in cosmetic and household cleaning products, which are either washed off the hair and skin of consumers, or disposed of following cleaning activities.

RELEASE OF CHEMICAL FROM DISPOSAL
It is expected that some of the product containing the notified chemical will remain in end-use containers. These will be disposed of through domestic garbage disposal and will enter landfill or be recycled.

7.1.2. Environmental Fate
Following its use in Australia, the majority of the notified chemical is expected to enter the sewer system. An estimated 84% of the notified chemical is predicted to be removed during sewage treatment plant (STP) processes (SimpleTreat; European Commission, 2003c), with 30% removal by degradation, 51% removed through partitioning to sludge and 3% volatilisation, before discharge to surface waters on a nationwide basis. The notified chemical is expected to be hydrolytically stable under the environmental conditions ($t_{½}$ = 283 days at pH 8, 25 ºC, HydroWIN v2.00; US EPA, 2011). The notified chemical is not readily biodegradable as it did not meet the 10-day window criteria (see Appendix C) but it is considered rapidly degradable as it reached the pass level within 28 days (United Nations, 2009). Therefore, the notified chemical is not likely to persist in the aquatic environment. The notified chemical has the potential to be bioaccumulative based on its high partition coefficient (log P_{ow} = 5.0). In surface waters, the notified chemical is expected to disperse and degrade through biotic and abiotic processes to form water and oxides of carbon.

The notified chemical is moderately volatile from water (log H = 1.37 Pa/m^3/mol; European Commission, 2003a) and may volatilise to air during use or sewage treatment. The half-life of the notified chemical in air is calculated to be 1.4 h and 2.1 h, based on reactions with hydroxyl radicals and ozone respectively (AOPWIN v1.92; US EPA, 2011). Therefore, in the event of release to atmosphere, the notified chemical is not expected to persist in the air compartment.

A proportion of notified chemical may be applied to land when treated sewage effluent is used for irrigation or when sewage sludge is used for soil remediation, or disposed of to landfill. Notified chemical residues in landfill and soil are expected to have slight mobility based on its predicted sorption coefficient (log Koc = 3.5), and are expected to degrade to form water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)
Since most of the chemical will be washed into the sewer, under a worst case scenario assuming no removal of the notified chemical in the sewage treatment plant (STP), the Predicted Environmental Concentration (PEC) for release of sewage effluent on a nationwide basis would be 0.61 µg/L in rivers and 0.06 µg/L in oceans.

However, a more realistic exposure scenario includes mitigation by 84% removal of the notified chemical during STP processes. Therefore, the resultant PEC in sewage effluent on a nationwide basis are estimated as follows:

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)	22.613	million
Removal within STP	84%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1	
Dilution Factor - Ocean	10	
PEC - River:	0.10	µg/L
PEC - Ocean	0.01	µg/L

Partitioning to biosolids in STPs Australia-wide may result in an average biosolids concentration of 3.09 mg/kg (dry wt). Biosolids are applied to agricultural soils, with an assumed average rate of 10 t/ha/year. Assuming a soil bulk density of 1500 kg/m^3 and a soil-mixing zone of 10 cm, the concentration of the notified chemical may approximate 0.021 mg/kg in applied soil. This assumes that degradation of the notified chemical occurs in the soil within 1 year from application. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated biosolids application, the concentration of notified chemical in the applied soil in 5 and 10 years may approximate 0.105 mg/kg and 0.21 mg/kg, respectively.

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.097 µg/L may potentially result in a soil concentration of approximately 0.646 µg/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 3.23 µg/kg and 6.46 µg/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	96h LC50 >0.996 mg/L	Not expected to be harmful to fish up to the limit of solubility in water
Daphnia Toxicity	48h EC50 = 0.382 mg/L	Very toxic to aquatic invertebrates
Algal Toxicity	72h EC50 >0.490 mg/L	Not expected to be harmful to algae up to the limit of solubility in water

Under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009) the notified chemical is not expected to be harmful to fish and algae up to the limit of solubility in water. As the notified chemical is considered very toxic to aquatic invertebrates, it is formally classified as 'Acute Category 1: Very toxic to aquatic life'. The notified chemical is formally classified for long-term hazard on the basis of its acute ecotoxicity, rapid biodegradability and log Kow ≥4 as 'Chronic Category 1: Very toxic to aquatic life with long term effects'.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) has been calculated from the endpoint of the most sensitive species tested and an assessment factor of 100 to account for chronic toxicity and laboratory-to-field extrapolation.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Invertebrates)	0.382	mg/L
Assessment Factor	100	
PNEC:	3.82	µg/L

7.3. Environmental Risk Assessment

Based on the above PEC and PNEC values, the following Risk Quotient (Q) has been calculated:

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.10	3.82	0.025
Q - Ocean	0.01	3.82	0.003

The risk quotient for discharge of treated effluents containing the notified chemical to the aquatic environment indicates that the notified chemical is unlikely to reach ecotoxicologically significant concentrations based on its reported use pattern and annual importation quantity. The notified chemical has a low potential for bioaccumulation and is unlikely to persist in surface waters, air or soils. Therefore, on the basis of the PEC/PNEC ratio, maximum annual import volume and assessed use pattern in cosmetic and household cleaning products, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point <-50 ºC

Method	OECD TG 102 Melting Point/Melting Range.
Remarks	Determined by cooling the test substance in a dry ice/isopropanol bath to ~-50 ºC. The test substance did not show any indication of freezing.
Test Facility	Givaudan (2010a)

Boiling Point 282 ºC at 101.3 kPa

Method	OECD TG 103 Boiling Point.
Remarks	Determined using the Siwoloboff method.
Test Facility	Givaudan (2010b)

Density 1037 kg/m^3 at 20 ºC

Method	OECD TG 109 Density of Liquids and Solids.
Remarks	Determined using an oscillating density meter.
Test Facility	Givaudan (2010c)

Vapour Pressure 1.0 x 10^{-4} kPa at 25 ºC

Method	OECD TG 104 Vapour Pressure.
Remarks	Determined using the gas saturation method.
Test Facility	Harlan (2010a)

Water Solubility 1.0 x 10^{-3} g/L at 20 ºC

Method	OECD TG 105 Water Solubility. EC Directive 92/69/EEC A.6 Water Solubility.
Remarks	Flask method, determined by HPLC with UV detection.
Test Facility	Givaudan (2010d)

Partition Coefficient (n-octanol/water) log Pow = 5.0

Method	OECD TG 117 Partition Coefficient (n-octanol/water). EC Directive 2008/440/EC A.8 Partition Coefficient.
Remarks	HPLC method. Although the notified chemical has surface active characteristics, the HPLC conditions appear to be appropriate for this structure as there was no peak broadening observed
Test Facility	Givaudan (2010e)

Surface Tension 58.7 mN/m at 20 ºC

Method	OECD TG 115 Surface Tension of Aqueous Solutions.
Remarks	Concentration: 90% saturation solution. The test material is considered to be a surface active material.
Test Facility	Harlan (2010b)

Flash Point 153 ºC at 101.3 kPa

Method	EC Directive 2008/440/EEC A.9 Flash Point.
Remarks	Determined using a Pensky-Martens closed cup flash point apparatus.
Test Facility	Givaudan (2010f)

Autoignition Temperature 354 ± 5 ºC

Method	EC Directive 2008/440/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).

Remarks	Determined by heating aliquots of the test substance in a flask (flask heater) and observing for any signs of ignition.
Test Facility	Harlan (2010c)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/RccHan: WIST(SPF)
Vehicle	Polyethylene glycol 300
Remarks - Method	Test material administered at 20% (w/v) in vehicle. The selection of the vehicle was based on a preliminary solubility study. No significant protocol deviations.

RESULTS

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

LD50	>2000 mg/kg bw
Signs of Toxicity	Slightly ruffled fur was observed in all animals up to day 3.
Effects in Organs	None
Remarks - Results	Body weights were within normal limits.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Harlan (2010d)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test.
Species/Strain	Rat/RccHan: WIST(SPF)
Vehicle	Polyethylene glycol 300
Type of dressing	Semi-occlusive
Remarks - Method	Test material administered at 50% (w/v) in vehicle. The selection of the vehicle was based on a preliminary solubility study. No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5M	2000	0/5
2	5F	2000	0/5

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	Slight erythema, scabs and/or scaling were observed in 9/10 animals during the observation period. The responses were not evident until at least day 3 and in some animals persisted until the end of the observation period.
Signs of Toxicity - Systemic	None
Effects in Organs	None
Remarks - Results	The body weights of one male and one female were reduced after 14 days. However, despite the losses, the weights were considered by the study authors to be within normal ranges.. All other animals gained

weight over the study period.

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

TEST FACILITY Harlan (2010e)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 3 (1M + 2F)
Vehicle None
Observation Period 72 hours
Type of Dressing Semi-occlusive
Remarks - Method No significant protocol deviations.

RESULTS

Remarks - Results Slight erythema was observed in two animals one hour post-patch removal. No further signs of erythema or oedema were noted at the remaining observations.

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY Harlan (2010f)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 3 (1M + 2F)
Observation Period 7 days
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	1.0	1.0	1.0	2	<7 days	0
Conjunctiva: chemosis	0.3	0	0	1	<48 hours	0
Conjunctiva: discharge	0	0	0	1	<24 hours	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 iridial or corneal effects were reported. Minimal to moderate conjunctival irritation was noted in treated eyes 1 hour post-instillation, with treated eyes appearing normal after 7 days. Slight to moderate reddening of the sclerae was also noted up to an including the 24 hour observation.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY Harlan (2010g)

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	Mouse/CBA/CaOlaHsd
Vehicle	Acetone:olive oil (4:1)
Remarks - Method	A preliminary study was conducted using two mice, but the results were not reported. The main study was conducted at 0, 0.5, 5 and 25 % (w/v) concentration (5 mice/group). A concurrent positive control study was not run, but had been conducted previously in the test laboratory using α-hexylcinnamaldehyde (HCA).

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	372.5	1.00
0.5	822.8	2.21*
5	1062.1	2.85*
25	3746.4	10.06*
Positive Control (HCA)		
0 (vehicle control)	727.6	1.00
5	1303.6	1.79
10	1518.4	2.09
25	4976.6	6.84

*Significantly higher than control group (ANOVA, $p<0.001$)

Remarks - Results	No systemic toxicity or irritation were observed. The stimulation index values recorded indicate a dose response relationship. The stimulation index at 25% concentration was >3, indicating a positive response. Based on these results, the EC_3 value was calculated to be 5.4%.
CONCLUSION	There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	Harlan (2009a)

B.6. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Notified chemical
METHOD	Modified OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain	Mouse/J
Vehicle	Acetone:olive oil (4:1)
Remarks - Method	A preliminary study was conducted using three groups of mice (2/group; tested at 25%, 50% and 100% concentartion). Based on ear thickness measurements, the notified chemical was determined to be irritating at 100% concentration. On day 6 (and 5 hours prior to sacrifice), the animals were given an intraperitoneal injection of 5-bromo-2'-deoxy-uridine (BrdU). The auricular lymph nodes were then isolated and single-cell suspensions of lymph node cells generated. Flow cytometry was then used to analyse the

suspensions for BrdU incorporation and the total number of lymph node cells. In addition, aliquots of the suspension were stained with antibodies for immunophenotyping and activation marker evaluation.

A concurrent positive control study was run using 1-chloro-2,4-dinitrobenzene (DNCB).

RESULTS

Concentration (% w/w)	*Proliferative response (number of BrdU+ cells)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	34004	1.0
10	80456	2.4
25	165805	4.9
50	329206	9.7
Positive Control (DNCB)		
0.1	258093	7.6

Remarks - Results

Mild to moderate irritation was noted at 50% concentration of test substance, based on ear thickness measurements. In addition, body weight losses were noted during the study, but were reported by the study authors to be not significant.

The stimulation index values recorded indicate a dose response relationship. The stimulation indices at 25% and 50% concentration were >3, indicating a positive response. Based on these results, the EC_3 value was calculated to be 13.8%. The immunophenotyping results confirmed the sensitising potential.

CONCLUSION

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

TEST FACILITY

MB (2010)

B.7. Skin sensitisation – human volunteers

TEST SUBSTANCE

Notified chemical (applied at 15% concentration)

METHOD

Repeated insult patch test with challenge

Study Design

Induction Procedure: 9 induction applications made on Monday, Wednesday, Friday of three consecutive weeks. Skin was assessed 24 hours after patch removal (or 48 hours for patches applied on Friday).
Rest Period: Approximately 2 weeks.
Challenge Procedure: 1 challenge application to a naïve site, followed by skin assessment 24, 48 and 72 hours after application.

Study Group

105 (86 F, 19 M) ranging in age from 18-70 years.

Vehicle

3:1 diethyl phthalate:ethanol

Remarks - Method

Occlusive 2×2 cm patches containing 0.2 mL of test material (that had been allowed to volatilise for 10-40 minutes), were held in place for 24 hours before removal by the applicants. 4 subjects voluntarily withdrew (≤6 induction readings were noted for these subjects).

RESULTS

Remarks - Results

Scores of zero were noted at all induction and challenge observations indicating no irritation or sensitisation.

CONCLUSION

The test substance was non-sensitising under the conditions of the test.

TEST FACILITY

ETC (2010)

B.8. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain	Rat/RccHan:WIST(SPF)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: None
Vehicle	Polyethylene glycol
Remarks – Method	No significant protocol deviations. Behavioural observations were made weekly and a functional operational battery screen was conducted during week 4. Vaginal smears were taken during the final week of treatment to determine the stage of oestrus. Urinalysis was not conducted.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5M + 5F	0	0/10
low dose	5M + 5F	100	0/10
mid dose	5M + 5F	300	0/10
high dose	5M + 5F	1000	0/10

Clinical Observations

There were no clinical signs of toxicity noted for animals of the 100 or 300 mg/kg bw/day. However, slight to moderate salivation was observed in three 1000 mg/kg bw/day females. Dyspnea was observed in one 1000 mg/kg bw/day male. The study authors considered these changes to be a result of the gavage dosing procedure. There were no effects on body weight, food consumption or oestrous cycle at any treatment level.

Laboratory Findings – Clinical Chemistry and Haematology

There were some sporadic statistically significant changes in some measured parameters, which are unlikely to be treatment related due to the lack of a dose response, with the exception of reticulocyte count, which was increased in males (↑42% at the high dose). This effect was not observed in females. Total bilirubin was decreased in high dose males (↓45%) and females (↓46%). Overall, these effects do not appear to be adverse.

Effects in Organs

There were no treatment related macroscopic findings in any organ at necropsy. The mean absolute and relative liver weights were statistically increased in the 1000 mg/kg bw/day treated males (↑22%/↑26%, absolute/relative) and females (↑15%/↑19%, absolute/relative), and in the 300 mg/kg bw/day treated males (↑19%/15%, absolute/relative). The trend in males appeared to be dose related, as there were also minor increases at the lower doses. These findings were accompanied by minimal to slight diffuse hepatocellular hypertrophy in 1000 mg/kg bw/day males (2/5) and females (2/5), and minimal diffuse hepatocellular hypertrophy in 300 mg/kg bw/day males (2/5). The hepatocellular hypertrophy is consistent with the increased liver weights. Although the effects in the liver appear to be treatment related due to their absence in concurrent controls, they were considered by the study authors to be adaptive and not considered to be adverse.

There were statistically significant increases in male kidney weights at 1000 mg/kg bw/day, and in thymus and testes weights in males, and in pituitary weights in females. However, these effects were considered by the study authors to be incidental due to a lack of a dose response and/or associated histopathological findings.

Remarks – Results

The No Observed Effect Level was established as 100 mg/kg bw/day.

CONCLUSION

The effects noted in the livers of animals of the high and mid dose group were not considered by the study authors to be adverse. Therefore, the No Observed Adverse Effect Level (NOAEL) was established by the study authors as 1000 mg/kg bw/day, based on the lack of toxicologically adverse effects.

TEST FACILITY Harlan (2010h)

B.9. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
Plate incorporation procedure (Test 1)
Pre incubation procedure (Test 2)

Species/Strain *S. typhimurium*: TA1535, TA1537, TA98, TA100
E. coli: WP2uvrA

Metabolic Activation System S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver

Concentration Range in Main Test a) With metabolic activation: 3-5000 µg/plate
b) Without metabolic activation: 3-5000 µg/plate

Vehicle Dimethyl sulfoxide

Remarks - Method A preliminary toxicity test (0-5000 µg/plate) was performed to determine the toxicity of the test material (all strains). The results are reported as Test 1.

Vehicle and positive controls were conducted in parallel with the test material. Without metabolic activation, sodium azide for TA1535 and TA100, 4-nitro-o-phenylene-diamine for TA1537 and TA98, and methyl methane sulfonate for WP2uvrA; 2-aminoanthracene was used for all strains with metabolic acitivation.

RESULTS

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

Remarks - Results Toxicity was observed at 2500 and 5000 µg/plate in some strains, as indicated by a reduction in the number of revertants (50% reduction compared to controls).

There were no significant increases in the frequency of revertant colonies recorded for any of the bacterial strains up to and including the maximum dose, either with or without metabolic activation.

The positive controls gave satisfactory responses, confirming the validity of the test system.

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

TEST FACILITY Harlan (2009b)

B.10. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD: OECD TG 473 In vitro Mammalian Chromosome Aberration Test.

Species/Strain	Chinese Hamster
Cell Type/Cell Line	V79 cells
Metabolic Activation System	S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver
Vehicle	Dimethyl sulfoxide
Remarks - Method	No significant protocol deviations.

A preliminary toxicity test (9.4 to 2400 µg/mL) was performed to define the toxicity of the test material. The results obtained in the presence of metabolic activation are reported as Test 1. However, due to strong test item induced cytotoxicity, the assay was repeated in the absence of metabolic activation at lower concentrations (0.08 to 20 µg/mL).

Vehicle and positive controls (ethylmethane sulfonate and cyclophosphamide) were used in parallel with the test material.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 0.08, 0.16, 0.3, 0.6*, 1.3*, 2.5*, 5, 10, 20	4 hrs	18 hrs
Test 2a	0*, 0.04, 0.08, 0.16, 0.3, 0.6, 1.3, 2.5*, 5*, 10*	18 hrs	18 hrs
Test 2b	0*, 0.63, 1.25, 2.5, 5, 10*, 20*, 40*, 80*, 160, 320	18 hrs	18 hrs
Present			
Test 1	0, 9.4*, 18.8*, 37.5*, 75, 150, 300, 600, 1200, 2400	4 hrs	18 hrs
Test 2a	0, 2.5, 5, 10, 20*, 40*, 80*, 160	4 hrs	18 hrs
Test 2b	0, 5, 10*, 20*, 40*, 80, 120, 160, 320	4 hrs	18 hrs

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*		
	Cytotoxicity	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	≥5	none	Negative
Test 2a	>10	none	Negative
Test 2b	≥80	none	Negative
Present			
Test 1	≥75	≥300	Negative
Test 2a	≥160	none	Negative
Test 2b	≥80	none	Negative

Remarks - Results

There was no indication of clastogenic effects in test 1, with or without metabolic activation, or in test 2a without metabolic activation. There was a single statistically significant increase in the frequency of cells with chromosome aberrations in Test 2a in the presence of metabolic activation, which resulted in the confirmatory experiment (2b) being performed. In this confirmatory test, no statistically significant increase in the number of cells with aberrations was noted at any concentration, with and without metabolic activation.

The positive controls gave satisfactory responses, confirming the validity of the test system.

CONCLUSION: The notified chemical was not clastogenic to Chinese Hamster V79 cells treated in vitro under the conditions of the test.

TEST FACILITY: Harlan (2010i)

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 (domestic sewage)
Exposure Period	49 days
Auxiliary Solvent	None specified
Analytical Monitoring	Biological oxygen demand
Remarks - Method	The test was conducted in accordance with guidelines above and the principles of good laboratory practice (GLP). An earlier biodegradation study showed a difference of 19% between replicate test flasks so the test was repeated. Only the results of the second test were reported. The concentration of the inoculum was 30 mg/L (dry weight). Test conditions: temperature: 22 ºC; pH: 7.31-8.03.

RESULTS

Test substance (100 mg/L)		*Sodium Benzoate (100 mg/L)*		*Toxicity test (100 mg/L each of test substance and reference substance)*	
Day	*% Degradation*	*Day*	*% Degradation*	*Day*	*% Degradation*
4	22				
14	54	14	93	14	44
28	67				
49	71				

Remarks - Results	The reference substance (sodium benzoate) reached the pass level (60% ThOD) by Day 14. Oxygen uptake of the inoculums blank was ≤49.9 mg O_2/L in 28 days meeting the validity criteria (≤60 mg O_2/L). The toxicity test, containing 100 mg/L of the test substance and 100 mg/L of the reference substance, reached 44% degradation after 14 days exceeding the 25% (ThOD) criteria for the toxicity test. Therefore, the test substance is not assumed inhibitory. The validity criteria of the test were met. The test substance reached the pass level (60%ThOD) for ready biodegradability within 28 days, but failed to meet the 10-day window criterion (22% degradation on Day 4 and 54% on Day 14).
CONCLUSION	The notified chemical is not readily biodegradable under the conditions of the test.
TEST FACILITY	Givaudan (2010g)

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	Zebra Fish (*Brachydanio rerio*)
Exposure Period	96 hours
Auxiliary Solvent	None

Water Hardness	125 mg CaCO3/L
Analytical Monitoring	HPLC with UV/VIS-detection
Remarks – Method	The test was conducted in accordance with guidelines above and the principles of good laboratory practice (GLP). After a range finding test a limit test was performed was performed up to the limit of solubility of the test substance in water. A dispersion of the test substance in the test media with a loading rate of 100 mg/L was stirred for 24 hours and the undiluted filtrate was used as a single test concentration in parallel with a control. Due to the volatility of the test substance, the test system was sealed and the test medium was renewed daily. The test was performed without light due to the photolytic instability of the test substance. Actual concentrations were measured at the start and end of the first and last renewal periods only. Test conditions: temperature: 22-23 ºC; pH: 6.9-7.3; dissolved oxygen concentrations: >6.3 mg/L.

RESULTS

Loading Rate mg/L	*Actual Concentration mg/L*	*Number of Fish*	*Mortality*				
			1 h	*24 h*	*48 h*	*72 h*	*96 h*
Control	Not determined	7	0	0	0	0	0
100	0.996	7	0	0	0	0	0

LC50	> 0.996 mg/L at 96 hours.
NOEC	0.996 mg/L at 96 hours.
Remarks – Results	The test substance concentrations in fresh filtered media with a loading of 100 mg/L were 1392 and 1305 µg/L on Day 0 and 4, respectively. At the end of the 24-hour test medium renewal period the concentrations of the test substance were 724 and 660 mg/L. The results are based on the arithmetic mean of the measured concentration of test substance as determined at the start and end of the first and last renewal periods. There was no sign of film floating on the surface was observed on the test medium. No effects were observed in the course of the study. The no-observed effect concentration (NOEC) and median lethal concentration (LC50) were determined directly from the raw data.

CONCLUSION The notified chemical is not expected to be harmful to fish up to its limit of solubility in water

TEST FACILITY Harlan (2010j)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 *Daphnia sp*. Acute Immobilisation Test – Semi-static
EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - Semi-static.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	150 mg $CaCO_3$/L
Analytical Monitoring	HPLC with UV/VIS-detection
Remarks - Method	The test was conducted in accordance with guidelines above and the principles of good laboratory practice (GLP). After a range finding test a definitive test was performed. A dispersion of the test substance in the test media with a loading rate of 100 mg/L was stirred for 24 hours. The undiluted filtrate and series of dilutions were used in parallel with a control. Due to the volatility of the test substance, the test system was sealed and the test medium was renewed every 24 hours. The test was performed without light due to the photolytic instability of the test substance. Test conditions: temperature: 21ºC; pH: 7.6-8.1; dissolved

oxygen concentrations: 8.2-8.5 mg/L.

RESULTS

Dilution	*Actual Concentration µg/L*	*Number of D. Magna*	*Number Immobilised*	
			24 h	*48 h*
(Control)	Not determined	20	0	0
1:22	55	20	0	0
1:10	124	20	0	0
1:4.6	265	20	0	0
1:2.2	551	20	6	20
Undiluted filtrate (loading rate 100 mg/L)	1274	20	20	20

EC50	0.642 mg/L at 24 hours (95% confidence interval: 0.542 to 0.968 mg/L) 0.382 mg/L at 48 hours (95% confidence interval: 0.265 to 0.551 mg/L)
NOEC	0.265 mg/L at 48 hours
Remarks - Results	All test media were clear solutions for the duration of the test. Actual concentrations of the test substance were measured at the start and end of each renewal period. The test substance was stable for the duration of the renewal period under the conditions of the test. The reported test results are based on the mean measured concentrations. Due to the steep concentration effect relationship, the median effect concentration at 48 hours (EC50) was determined as the arithmetic mean of the two consecutive concentrations with 0% and 100% immobilisation. The 24-hour EC50 and 95% confidence intervals were calculated by Weibull analysis. The no observed effect concentration (NOEC) was determined directly from the raw data.

CONCLUSION The notified chemical is very toxic to aquatic invertebrates

TEST FACILITY Harlan (2010k)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD	OECD TG 201 Alga, Growth Inhibition Test. EC Directive 92/69/EEC C.3 Algal Inhibition Test.
Species	*Pseudokirchneriella subcapitata*
Exposure Period	96 hours (results reported for 72 hours)
Concentration Range	Dilution (100 mg/L loading rate): undiluted filtrate, 1:1.18, 1:3.2, 1:5.8, 1:10.5, 1:18.9 and 1:34 Actual: 409, 220, 123, 62, 38, 17 and 13 mg/L
Auxiliary Solvent	None
Water Hardness	25 mg $CaCO_3$/L
Analytical Monitoring	Electronic particle counter. Determination of the test substance by HPLC with UV/Vis detection.
Remarks - Method	The test was conducted in accordance with guidelines above and the principles of good laboratory practice (GLP). After a range finding test a definitive test was performed. A dispersion of the test substance in the test media with a loading rate of 100 mg/L was stirred for 24 hours. The undiluted filtrate and a series of filtrate dilutions were used in parallel with a control. The test system was closed due to the volatility of the test substance. Therefore, the buffering capacity of the test medium was increased by adding 6 mmol/L HEPES-buffer and increasing the content of $NaCO_3$ by 200 mg/L to 250 mg/L. Test conditions: temperature: 21 ºC; initial pH: 8.1-8.2; final pH: 9.2-10.1; mean measured light:4900 Lux.

RESULTS

Biomass		*Growth*	
E_bC50 *mg/L at 72 h*	*NOEC* *mg/L at 72 h*	*E_rC50* *mg/L at 72 h*	*NOEC* *mg/Lat 72 h*
>0.409	0.123	>0.409	≥0.409

Remarks - Results

The validity criteria were met. However, the final pH increased by more than 1.5 units from the initial value (ranging 1.7 to 2.0 units) for all test solutions except the undiluted filtrate (increase of 1.0 unit) despite the additional buffering capacity of the test medium. It was also necessary to stabilise the samples prior to algae separation due to poor recovery of the test substance following sample work up. These variations to the study protocol were not considered to affect the reliability of the study.

The reported test results at 72 hours are based on the geometric mean of the measured concentrations to account for loss of the test substance during the test duration. The concentration of the test substance was measured for all test solutions at 0 hours. The concentration of the test substance was also determined in the undiluted, 1:5.8 and 1:34 dilution test solutions at 72 hours. For the undiluted and 1:5.8 dilutions, an average 13.4% of the Day 0 concentration of the test substance remained in the test solution. The concentration in the 1:34 dilution was below the limit of quantification (LOQ = 10.1 µg/L). The geometric mean concentration in the remaining test solutions were extrapolated assuming that 13.4% of the initial concentration remained after 72 hours.

Investigations determined that loss of test substance over the duration of the study could be attributed to uptake/adsorption by algal cells (5-25% of Day 0 concentration), aqueous photolysis (40-50% of the Day 0 concentration) and potential loss through volatilisation (due to repeated opening for sampling).

The 72-hour no observed effect concentrations (NOEC) for growth rate and biomass were statistically determined according to a one-sided Dunnett t-test ($\alpha = 0.05$). The results for growth rate after 72 hours in the 1:1.8 dilution (220 µg/L) and undiluted filtrate (409 µg/L) test solutions were statistically different from the control due to very low variability in the results. However, the results were not estimated as a biologically relevant toxic effect since the inhibition was less than 10% ($\alpha = 0.05$, one-sided smaller).

CONCLUSION

The notified chemical is not expected to be harmful to algae up to its limit of solubility in water.

TEST FACILITY

Harlan (2010l)

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