File No: LTD/1730

June 2014

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Oils, patchouli, patchoulol synthase-modified *Saccharomyces cerevisiae*-fermented, from carbohydrates

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 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.

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

TABLE OF CONTENTS

SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/1730	Firmenich Limited	Oils, patchouli, patchoulol synthase-modified *Saccharomyces cerevisiae*-fermented, from carbohydrates	Yes	≤ 1 tonne per annum	Fragrance ingredient for cosmetics and household products

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the table below.

Hazard classification	*Hazard statement*
Aspiration Toxicity Category 1	H304 – May be fatal if swallowed and enters airways

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:

R65 – Harmful: May cause lung damage if swallowed

The environmental hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals* (GHS) is presented below. Environmental classification under the GHS is not mandated in Australia and carries no legal status but is presented for information purposes.

Hazard classification	*Hazard statement*
Acute (Category 2)	H401 - Toxic to aquatic life
Chronic (Category 2)	H411 - 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 in the proposed manner, 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 and the assessed use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Aspiration Toxicity Category 1: H304 – May be fatal if swallowed and enters airways

The above should be used for products/mixtures containing the notified chemical, if applicable, based on the concentration of the notified chemical present and the intended use/exposure scenario.

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical during reformulation:
  - Enclosed systems

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure to the notified chemical during reformulation:
  - Avoid skin and eye contact

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical during reformulation:
  - Impervious gloves
  - Eye protection
  - Protective clothing

  Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

- A copy of the (M)SDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should 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 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 is intended to exceed 4% as an ingredient in cosmetics and household products;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a fragrance ingredient for cosmetics and household products, or is likely to change significantly;
- the amount of chemical being introduced has increased, 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 (M)SDS of the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT
Firmenich Limited (ABN: 86 002 964 794)
73 Kenneth Road
BAGOWLAH NSW 2093

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: other names, molecular and structural formulae, molecular weight, analytical data, use details, import volume and analogue details.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: hydrolysis as a function of pH, adsorption/desorption, dissociation constant, flammability limits, explosive properties, and oxidising properties.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
USA (2013)

2. IDENTITY OF CHEMICAL

MARKETING NAME
Clearwood

CAS NUMBER
1450625-49-6

CHEMICAL NAME
Oils, patchouli, patchoulol synthase-modified *Saccharomyces cerevisiae*-fermented, from carbohydrates

MOLECULAR WEIGHT
The notified chemical is a substance in the category of Unknown or Variable compositions, Complex reaction products and Biological materials (UVCB). The molecular weight of the components of the notified chemical is < 500 Da.

ANALYTICAL DATA
Reference GC-MS spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
100%

HAZARDOUS IMPURITIES
None identified

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (> 1% BY WEIGHT)
None

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Colourless/extremely pale yellow liquid

Property	Value	Data Source/Justification
Freezing Point	< -20 °C	Measured
Boiling Point	273 °C at 96.9 kPa	Measured
Density	957 kg/m^3 at 20 °C	Measured
Viscosity	20.1 mm^2/s at 20 °C 8.65 mm^2/s at 40 °C	Measured
Vapour Pressure	Approximately 2.0×10^{-3} kPa at 25 °C	Measured
Water Solubility	5.8×10^{-3} g/L at 20 °C (Loading rate 0.1 g/L) 1.17×10^{-2} g/L at 20 °C (Loading rate 1.0 g/L)	Measured
Hydrolysis as a Function of pH	Not determined	Not expected to contain hydrolysable functionalities
Partition Coefficient (n-octanol/water)	log Pow = 1.99 to > 5.7	Measured
Surface Tension	55.2 mN/m at 21.2 ± 0.5 °C	Measured
Adsorption/ Desorption	log K_{oc} = 3.63, 4.58 and 4.63	Analogue, measured
Dissociation Constant	Not determined	Not expected to contain ionisable functionalities
Flash Point	125 °C	Measured
Flammability	Not determined	C1 combustible liquid based on measured flash point
Autoignition Temperature	236 °C	Measured
Explosive Properties	Not determined	Contains no functional groups that would imply explosive properties
Oxidising Properties	Not determined	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.

Physical hazard classification
Based on the submitted physico-chemical data depicted in the above table, the notified chemical is not recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

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 imported in the following forms:

1. in neat form to be reformulated into fragrance formula or end-use cosmetic and household products;
2. as a component in fragrance formula to be reformulated into end-use cosmetic and household products; and/or
3. as a component of formulated end-use cosmetic and household products.

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

IDENTITY OF MANUFACTURER/RECIPIENTS
Firmenich Limited

TRANSPORTATION AND PACKAGING
The notified chemical, either in its neat form or as a component in a fragrance formula, will be imported and distributed in tightly closed lacquered drums of varying sizes including 180 kg, 100 kg, 50 kg, 25 kg, 10 kg or 5 kg drums. From the port of entry, the notified chemical will be transported by road via commercial carrier truck to warehouses where it will be unloaded and stored in its original packaging until further distribution to customer reformulation sites. The notified chemical will be distributed typically by road. It is also possible that the notified chemical will directly be transported from the port of entry to customer facilities.

At the reformulation sites, the finished end-use products will be re-packaged into various small size consumer containers and distributed nationwide by road for retail.

USE
The notified chemical is a fragrance ingredient that will be used in consumer products ranging from cosmetics (including personal care products and fine fragrance) to household products (including laundry liquid, fabric softener, dishwashing liquid, air freshener and all-purpose cleaner).

OPERATION DESCRIPTION
At customer sites the notified chemical will be reformulated into either fragrance formula or finished end-use cosmetic/household products. The fragrance formula will be used for further reformulation of consumer products.

The procedures for reformulating the notified chemical or the fragrance formula containing the notified chemical will likely vary depending on the nature of the cosmetic/household products, and may involve both automated and manual transfer steps. In general, it is expected that the reformulation processes will involve blending operations that will normally be automated and occur in an enclosed system, followed by automated filling of the finished products into consumer containers of various sizes.

The finished products containing the notified chemical may be used by consumers and professionals such as hairdressers, workers in beauty salons or cleaners. Depending on the nature of the products, these could be applied in a number of ways, such as by hand, using an applicator or by spray.

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	Unknown	Unknown
Mixing	4	2
Drum handling	4	2
Drum cleaning	4	2
Maintenance	4	2
Quality control	0.5	1
Packaging	4	2

EXPOSURE DETAILS
Transport and storage workers may come into contact with the notified chemical, either in neat form or at various concentrations (in intermediate fragrance formulae and finished consumer products) up to 40%, only in the event of accidental rupture of packaging.

At reformulation sites, dermal, ocular and inhalation exposure of workers to the notified chemical (up to 100% concentration) may occur when handling the notified chemical or products containing it. 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 glasses and impervious gloves.

Dermal, ocular and inhalation exposure to the notified chemical in the finished end-use products (up to 4% concentration) may occur in professionals where the services provided involve the application of products to clients (e.g. hair dressers, 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% concentration) through the use of the household products and the rinse-off/leave-on cosmetic or personal care products. The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible, particularly if products are applied by spray.

Data on typical use patterns of cosmetic and household cleaning product categories in which the notified chemical may be used are shown in the following tables (SCCS, 2012; Cadby *et al.*, 2002; SDA, 2005). For the purposes of the exposure assessment via the dermal route, 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, 2003). For the inhalation exposure assessment, an adult inhalation rate of 23 m^3/day (enHealth, 2004) and an airspace volume of 2 m^3 were used. The bioavailability of the notified chemical in the inhalation exposure was assumed as 100%. An adult average bodyweight of 60 kg was used for the calculations.

Based on the above information, the daily systemic exposure to the notified chemical through the use of the cosmetic and household products is estimated as following:

Product type	Daily systemic exposure (mg/kg bw/day)	
Cosmetic products (dermal exposure)		
Body lotion	1.419	
Face cream	0.052	
Hand cream	0.053	
Fine fragrances	0.500	
Deodorant spray	0.148	
Shampoo	0.006	
Shower gel	0.026	
Hand soap	0.030	
Hair styling	0.021	
Subtotal		**2.255**
Household products (dermal exposure from wearing clothes)		
Laundry liquid	0.004	
Fabric softener	0.002	
Subtotal		**0.006**
Household products (direct dermal exposure)		
Laundry liquid	0.000	
Dishwashing liquid	0.000	
All-purpose cleaner	0.002	
Subtotal		**0.002**
Cosmetic products (inhalation exposure)		
Hairspray	0.125	
Subtotal		**0.125**
Total		**2.388**

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 dose of 2.39 mg/kg bw/day. It is acknowledgeable that inhalation exposure to the notified chemical from use of other cosmetic and household products (in addition to hairspray) may occur. However, it is considered that the combination of the conservative hairspray inhalation exposure assessment parameters, in particular assuming an airspace volume of 2 m^3, and the aggregate exposure from use of the dermally applied products, which assumes a conservative 100% absorption rate, is sufficiently protective to cover additional inhalation exposure to the

notified chemical from the use of other spray cosmetic and household products with lower exposure factors (e.g. air fresheners).

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical and a close analogue chemical are summarised in the following table. The analogue and the notified chemical are considered to be very similar in chemical composition and physico-chemical properties and therefore the endpoints presented below are likely to reflect the toxicity of the notified chemical.

For full details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*	*Test substance*
Rat, acute oral toxicity	LD50 > 5,000 mg/kg bw; low toxicity	Analogue
Rabbit, acute dermal toxicity	LD50 > 5,000 mg/kg bw; low toxicity	Analogue
Rabbit, skin irritation	irritating	Analogue
Eye irritation – *in vitro* BCOP	not corrosive or severely irritating	Analogue
Human, skin sensitisation – RIPT	no evidence of sensitisation	Analogue (2%)
Rat, repeat dose oral (dietary) toxicity – 28 days	NOAEL = 977 mg/kg bw/day	Analogue
Mutagenicity – bacterial reverse mutation	non mutagenic	Notified chemical
Genotoxicity – *in vitro* mammalian chromosome aberration test	non genotoxic	Analogue
Rat, reproductive/developmental toxicity	NOEL = 277 mg/kg bw/day	Analogue

Toxicokinetics, metabolism and distribution

No toxicokinetic information on the notified chemical was provided.

Based on the water solubility (11.7 mg/L at 20 °C), partition coefficient (log Kow = 1.99 to > 5.7) and the low molecular weight (< 500 Da) of the notified chemical, passive diffusion of some components of the notified chemical across the skin and the gastrointestinal (GI) tract may occur. The notified chemical may also be absorbed across the respiratory tract, if inhaled.

Aspiration toxicity

The notified chemical is a mixture of hydrocarbons and has a kinematic viscosity of < 20.5 mm^2/s at 40 °C. It is therefore considered to be an aspiration toxicity hazard.

Acute toxicity

Acute toxicity studies on the notified chemical were not provided.

In an acute oral toxicity study on the analogue chemical, a LD50 was established as > 5,000 mg/kg bw in rats, indicative of low toxicity for the analogue chemical via the oral route.

A limited summary of an acute dermal toxicity study in rabbits for the analogue chemical was also provided. LD50 of the analogue was determined to be > 5,000 mg/kg bw, indicative of low toxicity via the dermal route.

Based on the information provided on the analogue, the notified chemical is considered to be of low toxicity via the oral or dermal route.

Irritation

An *in vitro* bovine corneal opacity and permeability (BCOP) assay was conducted on the analogue chemical to evaluate its eye irritation properties. The results indicated that the analogue chemical was neither corrosive nor severely irritating to the eye. However given this study is limited to the identification of chemicals that are severely irritating to the eye, the potential for the analogue chemical to cause non-severe eye irritation effects cannot be ruled out

A summary of a rabbit skin irritation study on the analogue was provided. Three animals each treated with 500 mg of the analogue chemical in a semi-occlusive patch showed slight to distinct erythema after 1 hour. Two of the three animals also showed slight oedema. These effects intensified to show marked erythema together with slight to marked oedema in all 3 animals at 24 to 72 hours. The analogue was considered to be a moderate

irritant to the skin and a risk phrase of R38 (irritating to skin) was recommended by the study authors. However, no irritation scores were provided.

Based on the information available, the notified chemical is likely to be a skin irritant and is not expected to be severely irritating to the eye. However, eye irritation effects cannot be ruled out.

Sensitisation
A sensitisation study for the notified chemical was not provided.

The analogue chemical was tested at a concentration of 2% in human volunteers for sensitisation potential, using the repeated insult patch test (RIPT). In a total of 51 subjects, no skin reactions to the analogue chemical were recorded during the induction period and at the challenge stage, indicating no evidence of sensitisation for the analogue chemical when tested up to 2% concentration.

No structural alert for skin sensitisation was found for the notified chemical and further evaluation of available information indicates that the analogue chemical is not a skin sensitiser.

Repeated dose toxicity
A 28-day repeated dose oral (dietary) toxicity study on the analogue was provided and did not show clinically significant evidence of systemic toxicity for the analogue. A No Observed Adverse Effect Level (NOAEL) of 977 mg/kg bw/day was established for the chemical based on the highest dose tested.

Mutagenicity/Genotoxicity
The notified chemical was not mutagenic in a bacterial reverse mutation assay. The analogue chemical was not clastogenic in an *in vitro* chromosome aberration using Chinese hamster ovary (CHO) cells

Based on the results of the available studies, the notified chemical is not expected to be genotoxic.

Toxicity for reproduction/development
A reproduction/developmental toxicity screening test was conducted on the analogue chemical. The results showed no clinically observable signs of toxicity to adults and offspring. There were no treatment-related effects on mating, fertility, gestation, offspring sex ratio and offspring viability.

At the high dose level of 13,000 ppm (810 mg/kg bw/day), reductions of adult body weight, litter size, offspring weight and offspring surface righting reflex were noted in the study. A small number of treatment-related effects on offspring *pre* and *post-partum* with equivocal results on the nature of some *in utero* parameters were also recorded in this treatment group.

A No Observed Effect Level (NOEL) was established for the analogue chemical as 4,000 ppm (277 mg/kg bw/day), based on the fact that treatment-related effects in adults were noted at the higher dose of 13,000 ppm.

Health hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

Hazard classification	***Hazard statement***
Aspiration Toxicity Category 1	H304 – May be fatal if swallowed and enters airways

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase:

R65 – Harmful: May cause lung damage if swallowed

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Reformulation

Exposure of workers to the notified chemical at various concentrations up to the neat form (100%) may occur during blending operations. While the notified chemical is considered to be hazardous if swallowed and entering into airways, ingestion of the chemical is unlikely under the occupational settings described. The notified chemical is considered to be a moderate skin irritant. Harmful effects following inhalation exposure to the notified chemical cannot be ruled out. Caution should be exercised when handling the notified chemical during reformulation processes.

Provided that control measures stated by the notifier are in place to minimise worker exposure, including the use of automated processes and PPE, the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

End-use
Cleaners and beauty care professionals may come into contact with products containing the notified chemical at ≤ 4% concentration. These products will also be available to the public. The risk to workers who regularly use these products 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

Risk of repeated exposure
Members of the public may experience repeated exposure to the notified chemical (at ≤ 4% concentration) through the use of the cosmetic and household products.

The repeat dose toxicity potential was estimated by calculation of the margin of exposure (MoE) of the notified chemical using the worst case exposure scenario from use of multiple products possibly involving spray applications (see Section 6.1.2). A person who uses all the products simultaneously would result in a combined systemic internal dose of 2.39 mg/kg bw/day of the notified chemical, inclusive of possible inhalation. Using a NOEL of 277 mg/kg bw/day, which was derived in a reproductive/developmental screening test on the analogue chemical, the margin of exposure (MoE) was estimated to be 116. A MoE value ≥ 100 is considered to be acceptable to account for intra- and inter-species differences.

Irritation
The notified chemical is likely to be moderately irritating to the skin. However, irritation effects are not expected from use of the notified chemical at the proposed use concentrations in cosmetic and household products.

Based on the information available, the risk to the public associated with the use of the consumer products containing the notified chemical at ≤ 4% in concentrations 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 is no release of the notified chemical to the environment 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 processes, limited release of the notified chemical is expected from cleaning of equipment as washings will be reused. A total of up to 0.2% of the import volume is estimated to be generated as waste from residues in empty containers and spills during reformulation. Empty containers containing the notified chemical will either be recycled or disposed of through an approved waste management facility.

RELEASE OF CHEMICAL FROM USE
The majority of the notified chemical is expected to be released to sewers across Australia as a result of its use in cosmetic and domestic products, which are washed off the hair and skin of consumers as well as from washings

of the cleaning activities and disposed of to the sewer. A small percentage of up to 3% of the total import volume of the notified chemical, as residues in empty end use containers, is expected to be disposed of to landfill.

RELEASE OF CHEMICAL FROM DISPOSAL
It is expected that some of the product containing the notified chemical will remain in end-use containers. The containers are expected be disposed of through domestic garbage disposal and will enter landfill, or be subjected to recycling processes.

7.1.2. Environmental Fate

Following its use in Australia, the majority of the notified chemical is expected to enter the sewer before potential release to surface waters on a nationwide basis. The majority of the notified chemical will enter the sewer system as a result of the use of this chemical as component of fragrance preparations such as cosmetic and household care products. The notified chemical has potential for biodegradation and hence, it is expected to be degraded during the wastewater treatment process. Based on its low adsorption coefficient (log Koc = 1.9-2.4), only limited partitioning to sludge is expected. In surface waters, the notified chemical is expected to disperse and degrade through biotic and abiotic processes to form water and oxides of oxygen.

The 10-day window criterion is not applicable for the notified chemical as it is a complex mixture (see Appendix C). Therefore, it is considered readily biodegradable 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 potential to be bioaccumulative based on its high partition coefficient (1.99 to > 5.7). However, it is expected to have limited release due to its readily biodegradability and estimated efficient removal in sewage treatment plants. 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 expected to be moderately volatile from water (log H = 2 Pa/m^3/mol) and may volatilise to air during use or sewage treatment based on calculation for a representative component of the notified chemical. In the event of release to atmosphere, the notified chemical is not expected to persist in the air compartment based on calculations (AOPWIN v1.92; US EPA, 2011) for a representative component of the notified chemical.

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 be slightly mobile to immobile based on its predicted adsorption coefficient (log K_{oc} = 3.63, 4.58 and 4.63), and are expected to degrade to form water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)

The calculation for the Predicted Environmental Concentration (PEC) is summarised in the table below. Based on the reported use in cosmetics and household cleansing products, it is assumed that 100% of the total import volume of the notified chemical is released to the sewer. The release is assumed to be nationwide over 365 days per year. It is conservatively assumed that 0% of the notified chemical will be removed during sewage treatment processes.

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	200.0	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	0%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.61	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 1,000 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 1,500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.6 µg/L may potentially result in a soil concentration of approximately 4.039 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 20.2 µg /kg and 40.4 µg/kg, respectively.

7.2. Environmental Effects Assessment

No ecotoxicological data were submitted for the notified chemical. The results from ecotoxicological investigations conducted on a close analogue of the notified chemical are summarised in the table below. The analogue and the notified chemical are considered to be very similar in chemical composition and physico-chemial properties and therefore the endpoints presented below are likely to reflect the ecotoxicity of the notified chemical. Details of the studies of the analogue can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity (96 h)	LL50 = 5.7 mg/L (WAF)	Expected to be toxic to fish
Daphnia Toxicity (48 h)	EL50 = 21 mg/L(WAF)	Expected to be harmful to aquatic invertebrates
Algal Toxicity (96 h)	E_rL50 = 73 mg/L (WAF)	Expected to be harmful to algae

WAF: Water Accommodated Fraction

The notified chemical is expected to be toxic to fish, and harmful to aquatic invertebrates and algae. On the basis of the acute toxicity data of the analogue chemical, the notified chemical is expected to be toxic to aquatic organisms. Therefore, Under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009), the notified chemical is formally classified as Acute Category 2; Toxic to aquatic life. Based on the acute toxicity and ready biodegradability of the analogue chemical, and the log Kow value of the notified chemical ≥ 4, the notified chemical has been formally classified under the GHS as Chronic Category 2; Toxic to aquatic life with long lasting effects.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) for the notified chemical has been calculated and is presented in the table below. The PNEC is calculated based on the endpoint for the most sensitive species (fish, EL50) for an analogue chemical similar to the notified chemical. Three acute ecotoxicity endpoints for aquatic species from three trophic levels are available. Therefore, an assessment factor of 100 has been used.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EL50 (fish)	5.70	mg/L
Assessment Factor	100	
PNEC	57	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	0.61	57	0.011
Q - Ocean	0.06	57	0.001

The risk quotient for discharge 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 potential for bioaccumulation; however, it 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 domestic products, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Freezing Point < -20 ± 0.5 °C

Method	OECD TG 102 Melting Point/Melting Range. EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks	The notified chemical changed in appearance during cooling.
Test Facility	Firmenich (2013)

Boiling Point 273 ± 2 °C at 96.9 kPa

Method	OECD TG 103 Boiling Point. EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks	Determined using the Siwoloboff method
Test Facility	Firmenich (2013)

Density 957 kg/m^3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids. EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks	Determined using the Oscillating density meter method
Test Facility	Firmenich (2013)

Viscosity 20.1 mm^2/s (kinematic), 19.3 mPa.s (dynamic) at 20 °C
8.65 mm^2/s (kinematic), 8.28 mPa.s (dynamic) at 40 °C

Method	OECD TG 114 Viscosity of Liquids.
Remarks	Determined using a capillary viscometer method
Test Facility	Harlan (2013a)

Vapour Pressure Approximately 2.0×10^{-3} kPa at 25 °C

Method	OECD TG 104 Vapour Pressure. EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks	Determined using the gas saturation method. One preliminary test and two main tests were conducted. Due to the UVCB nature of the notified chemical, the composition of the vapour phase differed significantly in three tests. The results of the preliminary test were considered as the most representative and were used as an approximate value for the notified chemical.
Test Facility	Harlan (2013b)

Water Solubility 5.8×10^{-3} g/L at 20 °C (Loading rate 0.1 g/L)
1.17×10^{-2} g/L at 20 °C (Loading rate 1.0 g/L)

Method	OECD TG 105 Water Solubility.
Remarks	Flask Method. Water solubility of the notified chemical was measured using two loading rates, 0.1 g/L and 1.0 g/L, as recommended for substances of variable composition. After reaching equilibrium, all replicates were clear and colourless solutions which contained excess, undissolved test substance. However, after sampling, all replicates were clear and colourless solutions, visually free from excess, undissolved test substance. The concentrations of the test substance in the samples were determined by gas chromatography. Despite significant variations in concentration between replicates, the mean results were considered to be in agreement.
Test Facility	Harlan (2013a)

Partition Coefficient (n-octanol/water) log Pow = 1.99 to > 5.7

Method	OECD TG 117 Partition Coefficient (n-octanol/water).
Remarks	HPLC Method. The test was carried out at approximately neutral pH, with the test substance

considered to be tested in a non-ionized form. The test substance is a substance of variable composition, and the main compound had a calculated log Pow of 4.0. The test substance eluted as many different peaks which indicate a range in log Pow from 1.99 to 5.7. However, due to the large differences in absorptivity between many components of the test substance, it was concluded that any assessment of partition coefficient based on area normalization would be inappropriate.

Test Facility Harlan (2013a)

Surface Tension 55.2 mN/m at 21.2 ± 0.5 °C

Method OECD TG 115 Surface Tension of Aqueous Solutions.
EC Council Regulation No 440/2008 A.5 Surface Tension.
Remarks Concentration: ~5.2 mg/L
Test Facility Harlan (2013a)

Adsorption/Desorption – main test log K_{oc} = 3.63, 4.58 and 4.63

Method OECD TG 121 Adsorption - Desorption Using a Reverse – Phase HPLC Method.
Remarks The test substance is a substance of variable composition, and the chromatograms of the test substance showed three main peaks, corresponding to adsorption coefficients (log value) of 3.63, 4.58, and 4.63.
Test Facility Givaudan (2011)

Flash Point 125 ± 2 °C

Method EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks One preliminary test and two main tests were conducted. The result was the average of all three tests.
Test Facility Firmenich (2013)

Autoignition Temperature 236 ± 5 °C

Method EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks Orange flame and grey fumes were noted at the autoignition.
Test Facility Harlan (2013c)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Analogue

METHOD: Similar to OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure
- Species/Strain: Rat/Crl:CD(SD)BR
- Vehicle: None
- Remarks - Method: The purity of the test substance was not provided. Two dose levels at 2.07 and 5.17 mL/kg bw (corresponding to 2,000 and 5,000 mg/kg bw respectively) were tested. All animals were observed for 14 days after dosing.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 M/3 F	2,000	0/6
2	3 M/3 F	5,000	0/6

- LD50: > 5,000 mg/kg bw
- Signs of Toxicity:

3 h after dosing:
- Pilo-erection
- Abnormal body carriage (hunched posture)
- Abnormal gait (waddling)
- Lethargy
- Increased salivation

4 h after dosing:
- Decreased respiratory rate in all rats dosed at 2,000 mg/kg bw
- Pallor of the extremities in all rats dosed at 5,000 mg/kg bw
- Diarrhoea in females dosed at 2,000 mg/kg bw and in all rats dosed at 5,000 mg/kg bw

All rats were recovered on Day 3.

Slightly low bodyweight gains were noted on Day 8 for 5 of the 6 rats dosed at 5,000 mg/kg bw.

- Effects in Organs: Terminal autopsy findings were normal.
- Remarks - Results: The clinical signs observed on the treatment may be related to the irritating nature of the test substance.

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

TEST FACILITY: Huntingdon Research Centre (1988)

B.2. Irritation – skin

TEST SUBSTANCE: Analogue

METHOD: Similar to OECD TG 404 Acute Dermal Irritation/Corrosion.
- Species/Strain: Rabbit/New Zealand White
- Number of Animals: 3
- Vehicle: None
- Observation Period: 72 hours
- Type of Dressing: Semi-occlusive.
- Remarks - Method: 500 µL of the test substance was applied to each test sites using a 25 mm × 25 mm gauze pad. After 4 hours of exposure, the test substance was removed from the test animals and the test sites were observed for skin

reactions for 72 hours after patch removal.

RESULTS

Remarks - Results: Irritation scores were not provided.

The study summary stated that all 3 animals showed slight to distinct erythema at the 1-hour observation period. Two of the three animals also showed slight oedema. These effects intensified to show marked erythema together with slight to marked oedema in all 3 animals at 24 to 72 hours. A risk phrase of R38 (irritating to skin) for the test substance was recommended by the study authors.

CONCLUSION: The analogue chemical is irritating to the skin.

TEST FACILITY: Unilever (1988)

B.3. Irritation – eye (*in vitro*)

TEST SUBSTANCE: Analogue

METHOD: OECD TG 437 Bovine Corneal Opacity and Permeability Assay

Vehicle: None

Remarks - Method: Purity of the test substance was not provided.

Decreased light transmission through the cornea (opacity) and increased passage of sodium fluorescein dye through the cornea (permeability) were combined in an empirically derived formula to generate an *in vitro* irritancy score (IVIS).

Sodium chloride solution (0.9% w/v) and undiluted ethanol were used as negative control and positive control, respectively.

RESULTS

Test material	*Mean opacity of triplicate tissues*	*Mean permeability of triplicate tissues*	*IVIS*
Negative control	0.7	0.044	1.3
*Test substance**	0.7	0.006	0.8
*Positive control**	22.7	0.895	36.1

IVIS = *in vitro* irritancy score
*Corrected for background values

Remarks - Results: The IVIS for the positive control was in the acceptable range of 30.9 to 67.7.

CONCLUSION: The analogue chemical was not considered to be corrosive or a severe eye irritant under the conditions of the test.

TEST FACILITY: Harlan (2012d)

B.4. Skin sensitisation – human volunteers

TEST SUBSTANCE: Analogue (2%)

METHOD: Repeated insult patch test with challenge

Study Design:

- Induction Procedure: Left upper back area of each subject was patched for induction. A series of 9 induction patches were applied for a period of 3 weeks. Each patch was kept on the site for 24 hours. Rest time between the treatments was set at least 24 hours and at most 48 hours. Skin reactions were recorded for each treatment.
- Rest Period: 2 weeks

- Challenge Procedure: The challenge patch was applied to a virgin site for 24 hours and skin reactions were recorded after the exposure.

Study Group	44 F, 14 M; age range from 18 to 58 years
Vehicle	Dimethyl phthalate
Remarks - Method	Occluded patch. The concentration of the test substance was 2% in the vehicle and 200 µL of the solution was used for each treatment.

RESULTS

Remarks - Results	A total of 51 subjects, 14 males and 37 females, completed the test. Seven subjects did not complete the test for either no reasons or personal reasons. The original induction patch sites exhibited no skin reactions during induction and rest phases or at the challenge. The challenge patch sites showed no skin reactions at the challenge.

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

TEST FACILITY Harrison (1983)

B.5. Repeat dose toxicity

TEST SUBSTANCE Analogue

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	Rat/Wistar Han™:RccHan™: WIST
Route of Administration	Oral – diet
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: None
Vehicle	Dietary mixture
Remarks - Method	No significant deviation of protocol was noted.

RESULTS

Group	*Number and Sex of Animals*	*Dietary concentration (ppm)*	*Mean achieved dose (mg/kg bw/day)*	*Mortality*
Control	5 M/5 F	0	0	0/10
Low dose	5 M/5 F	500	41	0/10
Mid dose	5 M/5 F	4,000	323	0/10
High dose	5 M/5 F	13,000	977	0/10

Mortality and Time to Death
No death was recorded during the study.

Clinical Observations
A statistically significant increase in overall activity was evident in males treated at 13,000 ppm. In the absence of other supporting clinical observations, this finding was considered to be of no toxicological significance.

Animals treated at 13,000 ppm showed statistically significant reductions in body weight gain, possibly related to decreased dietary intake and food efficiency of the animals.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Females treated at 4,000 and 13,000 ppm showed statistically significant reductions in aspartate and alanine aminotransferase levels. These findings were not consistent with the histopathological effects seen in the liver in autopsy. In the absence of dose-related response, the authors did not consider these findings to have toxicological importance.

Effects in Organs
Rats treated with the test substance were found to have the following changes in the organs:

Liver - increased organ weight, centrilobular hepatocellular hypotrophy and vacuolation (fatty change)
Thyroid - increased incidence and/or severity of follicular hypertrophy
Kidney - increased organ weight, exacerbation of hyaline droplets and increased incidence of tubular degeneration/regeneration (dose-dependent)

The authors considered these changes to be adaptive in nature and not to represent serious damage to health.

Remarks – Results
The dose-dependent changes in kidneys of the male rats were consistent with the presence of hydrocarbon nephropathy resulted from the excessive accumulation of α2-microglobulin in renal proximal tubular epithelial cells.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 977 mg/kg bw/day in this study, based on the highest dose tested.

The No Observed Effect Level (NOEL) was established as 41 mg/kg bw/day in this study for females only, based on the histopathological effects observed in the livers, thyroids and kidneys of the males rats dosed with the test substance.

TEST FACILITY Harlan (2013d)

B.6. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Regulation Number 440/2008 B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.
Plate incorporation procedure and Pre incubation procedure

Species/Strain *S. typhimurium*: TA1535, TA1537, TA98 and TA100
E. coli: WP2*uvrA*

Metabolic Activation System Phenobarbitone/β-naphthoflavone induced rat liver homogenate (S9, 10%)

Concentration Range in Main Test
a) With metabolic activation: 1.5-5000 µg/plate
b) Without metabolic activation: 0.5-5000 µg/plate

Vehicle Acetone

Remarks - Method Positive controls:

a) N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) – for WP2*uvrA*, TA100 and TA1535
b) 9-Aminoacridine (9AA) – for TA1537
c) 4-Nitroquinoline-1-oxide (4NQO) – for TA98

Tests 1 and 2 were conducted using the plate-incorporation and pre-incubation method, respectively.

A preliminary cytotoxicity test was not conducted.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	-	≥ 1500	> 5000	Negative
Test 2	-	≥ 500	> 5000	Negative
Present				

Test 1	-	≥ 1500	> 5000	Negative
Test 2	-	≥ 1500	> 5000	Negative

Remarks - Results: A small statistically significant increase of revertant frequency in TA1537 strain was observed in the presence of metabolic activation at treatment level of 15 µg/plate. As no dose-response and reproducibility were noted, this result was not considered to be biologically relevant.

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

TEST FACILITY: Harlan (2013e)

B.7. Genotoxicity – *in vitro*

TEST SUBSTANCE: Analogue

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

Species/Strain: Chinese hamster
Cell Type/Cell Line: Ovary tissue/CHO K_1-BH_4
Metabolic Activation System: Aroclor 1254 induced rat liver homogenate (S9, 10%)
Vehicle: Ethanol
Remarks - Method: Positive control:

a) Mitomycin C (0.4 µg/mL) in the absence of metabolic activation
b) Cyclophosphamide (20 µg/mL) in the presence of metabolic activation

The test substance was mixed with ethanol at a concentration of 500 mg/mL. Maximum of 1% (v/v) was added to the aqueous cell culture to produce the highest final test concentration of 5,000 µg/mL.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	78.1, 156.3, 312.5, 625, 1250, 2500, 5000	24 h	24 h
Test 2	1.6*, 3.1*, 6.3*, 12.5*, 25, 50	24 h	24 h
Present			
Test 1	78.1, 156.3, 312.5, 625, 1250, 2500, 5000	6 h	24 h
Test 2	1.6, 3.1, 6.3, 12.5, 25, 50	6 h	24 h
Test 3	12.5, 25, 50, 58, 66, 75	6 h	24 h
Test 4	7.5*, 15*, 30*, 40, 50*, 60	6 h	24 h

* Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 78.1	-	≥ 625	-
Test 2	-	≥ 3.1	> 50	Negative
Present				
Test 1	≥ 78.1	-	≥ 625	-
Test 2	-	> 50	> 50	-
Test 3	-	≥ 50	> 75	-
Test 4	-	≥ 50	> 60	Negative

Remarks - Results: In the presence of metabolic activation, a statistically significant increase of chromosomal aberration rate was observed in the cultures treated with

15 µg/mL of the test substance. This increase of aberration was not considered to be treatment-related due to the following reasons:

a) The increased aberration frequency of 4.5% was within the historical range of background damage observed in the test facility.
b) The increase was only statistically significant when compared with untreated cells. It was not statistically significant when compared with vehicle control.
c) No increases of aberration rate were observed in cells treated with higher dose levels (i.e. 30 and 50 µg/mL) and there was no dose-related response.

CONCLUSION The analogue chemical was not considered as clastogenic to CHO cells treated *in vitro* under the conditions of the test.

TEST FACILITY Huntingdon Research Centre (1989)

B.8. Reproduction/Developmental toxicity

TEST SUBSTANCE Analogue

METHOD OECD TG 421 Reproduction/Developmental Toxicity Screening Test
Species/Strain Rat/Wister Han™:RccHan™:WIST
Route of Administration Oral – diet
Exposure Information Exposure period: up to 8 weeks
Vehicle Dietary mixture
Remarks - Method Pairing of animals within each dose group was undertaken on Day 15 of the study. Adult males were terminated on Day 43. Females were allowed to litter and rear their offspring until Day 5 *post partum* before being terminated.

RESULTS

Group	*Number and Sex of Animals*	*Dietary concentration (ppm)*	*Mean achieved dose (mg/kg bw/day)*	*Mortality*
Control	10 M/10 F	0	0	0/20
Low dose	10 M/10 F	1300	91.4	0/20
Mid dose	10 M/10 F	4,000	277	0/20
High dose	10 M/10 F	13,000	810	0/20

Mortality and Time to Death
There were no unscheduled animal deaths during the study.

Effects on Dams
Reductions in overall mean body weights, maybe co-related to reductions of food consumption and efficiency, were evident in all treated animals.

No clinically observable signs of toxicity were detected. No treatment-related effects on mating, fertility and gestation were noted during the study. No toxicologically significant macroscopic abnormalities were detected at the necropsy.

Effects on Foetus
Litter size and weight, offspring weights and surface righting reflex values were reduced in group treated at dose level of 13,000 ppm.

No treatment-related effects were evident in sex ratio and viability of the offspring. No clinically observable signs of toxicity were detected in the offspring.

Remarks – Results

The high dose level of 13,000 ppm also caused a small number of treatment-related effects on offspring *pre* and *post partum* with equivocal results on the nature of some *in utero* parameters.

CONCLUSION

The No Observed Effect Level (NOEL) was established as 4,000 ppm (277 mg/kg bw/day) in this study, based on treatment-related effects detected in adults in the 13,000 ppm (810 mg/kg bw/day) group.

TEST FACILITY Harlan (2013f)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE: Analogue

METHOD: OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test.

Inoculum	Activated sludge
Exposure Period	35 days
Auxiliary Solvent	Not reported
Analytical Monitoring	A respirometer, SAPROMAT D 12, was used for measurement of the consumption of oxygen.
Remarks - Method	The test was conducted according to the guidelines above using good laboratory practice (GLP). No significant deviations from the test guidelines were reported.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
6	15	5	64
16	58	10	72
28	61	21	75
35	66	28	76

Remarks - Results: All validity criteria for the test were satisfied. The reference compound, aniline, reached the 60% pass level by day 5 indicating the suitability of the inoculum. The toxicity control exceeded 25% biodegradation within 14 days showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the analogue chemical after the cultivation period was 64% and 66%, after 28 and 35 days, respectively. The percent degradation of the analogue chemical reached 58% at the end of the 10-day window. According to the nature of the test substance, 10-day window is not applicable as stated in OECD Guidelines for Testing Chemicals, Section 3. However, a criterion, which is that the test substance actually degrades biotically in the environment by > 60% in 28 days, is required to be demonstrated to meet the rapid biodegradation according to the GHS (GHS; United Nations, 2009). Therefore, the test substance can be classified as readily biodegradable according to the OECD (301 F) guideline.

CONCLUSION: The analogue and, by inference, the notified chemical are readily biodegradable.

TEST FACILITY: Givaudan-Roure (1995)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Analogue

METHOD: OECD TG 203 Fish, Acute Toxicity Test – Static Test

Species	Rainbow Trout (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	Not reported
Water Hardness	140 mg $CaCO_3$/L
Analytical Monitoring	Gas Chromatography (GC) Analysis

Remarks – Method

The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported.

The fish ecotoxicity test was conducted in Water Accommodated Fractions (WAFs) of the analogue chemical as it is a complex mixture and has low water solubility. WAFs of nominal loading rate were prepared by stirring the test substance in water by using a magnetic stirrer for 23 hours followed by a 1-hour settlement period. WAF treatment solutions were separated from mixtures by siphoning. Due to the light sensitive nature of the test substance, all WAF preparations were performed under laboratory safety lighting and/or shielded from the light. The chemical analysis results indicated that the test substance concentrations were relatively consistent, and the loss of chemical was due to volatility despite every precaution was taken to minimise volatilisation.

RESULTS

Nominal Concentration (WAF;mg/L)	*Number of Fish*	*Mortality (%)*				
		3 h	*24 h*	*48 h*	*72 h*	*96 h*
Control	7	0	0	0	0	0
1.0	7	0	0	0	0	0
3.2	7	0	0	0	0	0
10	7	0	0	100	100	100
32	7	14.3	100	100	100	100
100	7	100	100	100	100	100

LL50 5.7 (3.2 – 10) mg/L at 96 hours

NOEL 3.2 mg/L at 96 hours.

Remarks – Results

The actual concentrations of the test substance in WAFs were measured periodically at 0, 24, 48, and 96 hours within the 96-h test period. However, median lethal loading rate (LL50) and no observed effect loading rate (NOEL) values were calculated based on the nominal loading rates as the toxicity cannot be attributed to a single component or mixture of components but to the test substance as a whole.

All validity criteria for the test were satisfied. All the exposure treatments (loading rates) were observed to be clear and colourless. The 96-hour LL50 was calculated by trimmed Spearman-Karber method.

CONCLUSION The analogue and, by inference, the notified chemical are toxic to fish.

TEST FACILITY Harlan (2012a)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Analogue

METHOD OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Static Test

Species *Daphnia magna*

Exposure Period 48 hours

Auxiliary Solvent Not reported

Water Hardness 250 mg $CaCO_3$/L

Analytical Monitoring Gas Chromatography (GC) Analysis

Remarks – Method

The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported.

The daphnia ecotoxicity test was conducted in Water Accommodated Fractions (WAFs) of the analogue chemical as it is a complex mixture and

has low water solubility. WAFs of nominal loading rates were prepared by stirring the test substance in water by using a magnetic stirrer for 23 hours followed by a 1-hour settlement period. WAF treatment solutions were separated from mixtures by siphoning. Due to the light sensitive nature of the test substance, all WAF preparations were performed under laboratory safety lighting and/or shielded from the light. The chemical analysis results indicated that the test substance was stable over the test period.

RESULTS

Nominal Concentration (WAF;mg/L)	*Number of D. magna*	*Cumulative % Immobilised*	
		24 h	*48 h*
Control	40	0	0
10	40	0	5
18	40	15	15
32	40	100	100
56	40	100	100
100	40	100	100

EL50 21 mg/L at 48 hours
NOEL 10 mg/L at 48 hours
Remarks – Results The actual concentrations of the test substance in WAFs were measured periodically at 0, 24 and 48 hours within the 48-h test period. However, EL50 and NOEL values were calculated based on the nominal loading rates as the toxicity cannot be attributed to a single component or mixture of components but to the test substance as a whole.

All validity criteria for the test were satisfied. All the exposure treatments (loading rates) were observed to be clear and colourless. The 48-hour EL_{50} was calculated by the maximum-likelihood probit method using the ToxCalc software.

CONCLUSION The analogue and, by inference, the notified chemical are harmful to aquatic invertebrates.

TEST FACILITY Harlan (2012b)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Analogue

METHOD OECD TG 201 Alga, Growth Inhibition Test.
Species *Pseudokirchneriella subcapitata*
Exposure Period 96 hours
Concentration Range Nominal: 6.25, 12.5, 25, 50, and 100 mg/L
Auxiliary Solvent Not reported
Water Hardness Not reported
Analytical Monitoring Gas Chromatography (GC) Analysis
Remarks – Method The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported. The test was conducted for 96 hours, but 72-hours endpoints were presented as standard for algae test.

The algae ecotoxicity test was conducted in Water Accommodated Fractions (WAFs) of the analogue chemical as it is a complex mixture and has low water solubility. WAFs of nominal loading rates were prepared by stirring the test substance in water by using a magnetic stirrer for 23 hours followed by a 1-hour settlement period. WAF treatment solutions were separated from mixtures by siphoning. Due to the light sensitive nature of the test substance, all WAF preparations were performed under laboratory

safety lighting and/or shielded from the light. The chemical analysis results indicated that the loss of test substance was due to adsorption to the algal cells presents in the test.

RESULTS

Biomass (72 h)		*Growth (72 h)*	
E_yL50 (mg/L)	*NOE_yL (mg/L)*	*E_rL50 (mg/L)*	*NOE_rL (mg/L)*
26	6.25	73	12.5

Remarks – Results

The actual concentrations of the test substance in WAFs were measured periodically at 0, 24, 48, 72, and 96 hours within the 96-h test period. However, E_rL50 and NOE_rL values were calculated based on the nominal loading rates as the toxicity cannot be attributed to a single component or mixture of components but to the test substance as a whole.

All validity criteria for the test were satisfied. All the exposure treatments (loading rates) were observed to be clear and colourless. The E_rL50 and E_yL50 were calculated using one way analysis of variance for homogeneity of variance by Bartlett's test. The NOE_rC and NOE_yC values were calculated by using Dennett's multiple comparison procedure.

CONCLUSION

The analogue and, by inference, the notified chemical are harmful to algae.

TEST FACILITY

Harlan (2012c)

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