File No: LTD/1843

August 2015

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Butanal, 4-(heptyloxy)-3-methyl

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (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:

Street Address:	Level 7, 260 Elizabeth Street, SURRY HILLS NSW 2010, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX:	+ 61 2 8577 8888
Website:	www.nicnas.gov.au

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/1843	International Flavours and Fragrances (Australia) Pty Ltd	Butanal, 4-(heptyloxy)-3-methyl-	Yes	≤ 1 tonne per annum	Fragrance ingredient

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 of 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*
Skin corrosion/irritation	H315 – Causes skin irritation
Sensitisation, skin	H317 – May cause an allergic skin reaction

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:

R43: May cause sensitisation by skin contact

The environmental hazard classification according to the *Globally Harmonised System of 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 aquatic toxicity (category 2)	H401 Toxic to aquatic life
Chronic aquatic toxicity (category 3)	H412 Harmful 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.

Based on the available information, when used at ≤ 0.18% in deodorants, ≤ 0.35% in fine fragrances, ≤ 0.5% in other cosmetics and household products, and ≤ 0.2% in air care 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 and the reported 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:
  - Skin corrosion/irritation (Category 2): H315 – Causes skin irritation
  - Sensitisation, skin (Category 1): H317 – May cause an allergic skin reaction

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.

- 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 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

- 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 processes:
  - Enclosed, automated processes, where possible
  - Exhaust ventilation

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

- 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 processes:
  - Coveralls, impervious gloves, goggles

  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 of 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

- Where reuse or recycling are not appropriate, dispose of the notified chemical in an environmentally sound manner in accordance with relevant Commonwealth, state, territory and local government legislation.

Storage

- The handling and storage of the notified chemical should be in accordance with the Safe Work Australia Code of Practice for *Managing Risks of Hazardous Chemicals in the Workplace* (SWA, 2012) or relevant State or Territory Code of Practice.

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 notified chemical exceeds or intended to exceed ≤ 0.18% in deodorants, ≤ 0.35% in fine fragrances, ≤ 0.5% in other cosmetics and household products, and ≤ 0.2% in air care products;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a fragrance ingredient, 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(S)
International Flavours and Fragrances (Australia) Pty Ltd (ABN: 77 004 269 658)
310 Frankston-Dandenong Road
Dandenong VIC 3175

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

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

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed for flammability.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
United States (2015)
Canada (2015)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Starfresh

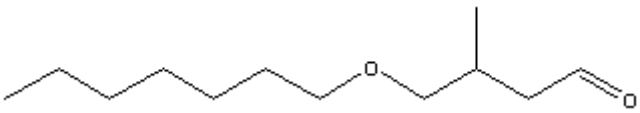
CAS NUMBER
1093653-57-6

CHEMICAL NAME
Butanal, 4-heptyloxy)-3-methyl-

OTHER NAME(S)
4-(heptyloxyl)-3-methylbutanal
TM 09-217
FRET 06-0154

MOLECULAR FORMULA
$C_{12}H_{24}O_2$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
200.32 Da

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

3. COMPOSITION

DEGREE OF PURITY
> 95%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

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: clear liquid

Property	Value	Data Source/Justification
Freezing Point	< -25 °C	Measured
Boiling Point	238 °C at 101.3 kPa	Measured
Relative Density	0.87	Measured
Vapour Pressure	0.091 kPa at 25 °C	Measured
Water Solubility	9.6 x 10^{-2} g/L at 25 °C	Measured
Hydrolysis as a Function of pH	t½ > 1 year at 25 °C (pH 4, 7 & 9)	Measured
Partition Coefficient (n-octanol/water)	log Pow = 4.8 at 20 °C	Measured
Surface Tension	48 mN/m at 20 °C	Measured. The notified chemical is surface active
Adsorption/Desorption	log K_{oc} = 3.4 at 25 °C	Measured
Dissociation Constant	Not determined	No dissociable functionality
Flash Point	112 °C at 101.6 kPa	Measured
Flammability	Not determined	Not expected to be flammable based on flash point
Autoignition Temperature	196 °C	Measured
Explosive Properties	Not expected to be explosive	Based on chemical structure
Oxidising Properties	Not expected to be oxidising	Based on chemical structure

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 of 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. The notified chemical will be imported as a component of fragrance oil at ≤ 10% concentration.

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
Melbourne

IDENTITY OF MANUFACTURER/RECIPIENTS
International Flavours and Fragrances (Australia) Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemical (at ≤ 10% concentration) will be imported into Australia typically in 205 L polypropylene-lined steel drums and transported by road to the notifier's facility. The end-use products (containing the notified chemical at ≤ 0.5% concentration) will be packaged in containers suitable for retail sale.

USE
The notified chemical will be used as a fragrance ingredient in a variety of cosmetic and household products (at proposed usage concentrations of ≤ 0.18% in deodorants, ≤ 0.35% in fine fragrances, ≤ 0.5% in other cosmetics and household products, and ≤ 0.2% in air care products).

OPERATION DESCRIPTION
No manufacturing, processing, reformulation or repackaging of the notified chemical will occur at the notifier's facility. Imported products containing the notified chemical (at ≤ 10% concentration) will be stored at this facility until transported to customer facilities for reformulation into consumer products.

Reformulation
The procedures for incorporating the fragrance oil containing the notified chemical (at ≤ 10% concentration) into end-use products will likely vary depending on the nature of the formulated products and may involve both automated and manual transfer steps. However, in general it is expected that the reformulation processes will involve blending operations that will be highly automated and use closed systems with adequate ventilation, followed by automated filling of the reformulated products into containers of various sizes.

End use
Household products
Household products containing the notified chemical (at ≤ 0.5% concentration) may be used by consumers and professional workers (such as cleaners). The products may be used in either closed systems with episodes of controlled exposure (for example automatic washing machine cycles), or open manual processes including rolling, brushing, spraying and dipping, using a cloth, sponge, mop or brush and followed by wiping. In some cases, the household product will be diluted with water prior to application.

Cosmetic products
The finished cosmetic products containing the notified chemical (at ≤ 0.5% concentration) will be used by consumers and professionals (such as beauticians and hairdressers). Depending on the nature of the products, application of products could be by hand, spray or through the use of an applicator.

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 warehouse workers	None	Incidental exposure only
Plant operators – Mixing compounding	4	250
Plant operators – Drum handling	1	250
Plant operators – Mixing cleaning/washing	2	250
Plant operators – Equipment cleaning/washing	2	250
Plant operators – Quality control	1	250

EXPOSURE DETAILS

Transport and storage

Transport and storage workers may come into contact with the notified chemical as a component of fragrance oils (at ≤ 10% concentrations) only in the event of accidental rupture of the drum containers.

At the notifier's facility, the primary work activity undertaken by transport and warehouse workers will include handling, loading and off-loading of drums containing fragrance oils with the notified chemical at up to 10% concentration. Exposures of these workers will be limited to situations involving product sampling for quality control or, in the event of a discharge, cleaning up from a spill or leaking drum. If such an event occurs, workers may mainly be exposed through dermal and ocular contact. Inhalation exposure to the notified chemical is not expected based on the low vapour pressure of the chemical at room temperature. The notifier states that such exposures will be minimised through the use of personal protective equipment (PPE) including protective coveralls, chemical resistant gloves and safety glasses.

Formulation of end products

During reformulation, dermal, ocular and perhaps inhalation exposure of workers to the notified chemical (at ≤ 10% concentration) may occur during weighing and transfer stages, blending, quality control analysis, packaging of materials and cleaning and maintenance of equipment. The notifier states that exposure is expected to be minimised through the use of adequate local ventilation and self-contained breathing apparatus if required, and through the use of PPE such as coveralls, goggles and impervious gloves.

Beauty care and cleaning professionals

Exposure to the notified chemical in end-use products (at ≤ 0.5% concentration) may occur in professions where the services provided involve the application of cosmetic products to clients (e.g. hair dressers, workers in beauty salons) or the use of household products in the cleaning industry. The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible. Such professionals may use PPE to minimise repeated exposure, but the use is not always expected. However, the notifier states that good hygiene practices are expected to be in place. If appropriate PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using the finished products containing the notified chemical.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemical through the use of a wide range of cosmetic and household products (at ≤ 0.5% concentration in individual products). The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible, particularly if the products are applied by spray.

Data on typical use patterns of product categories in which the notified chemical may be used are shown in the following tables (SCCS, 2012; Cadby *et al.*, 2002; ACI, 2010; Loretz *et al.,* 2006). 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, a dermal absorption (DA) of 100% was assumed for the notified chemical (ECHA, 2014). For the inhalation exposure assessment, a 2-zone approach was used (Steiling *et al*., 2014; Rothe *et al*., 2011; Earnest, Jr, 2009). An adult inhalation rate of 20 m^3/day (enHealth, 2012) was used and it was conservatively assumed that the fraction of the notified chemical inhaled is 50%, with the reminder ending up, as intended, on the hair. A lifetime average female body weight (BW) of 64 kg (enHealth, 2012) was used for calculation purposes.

Cosmetic products (dermal exposure)

Product type	**Amount** (mg/day)	**C** (%)	**Retention Factor (RF)** (unitless)	**Daily systemic exposure** (mg/kg bw/day)
Body lotion	7820	0.5	1	0.6109
Face cream	1540	0.5	1	0.1203
Hand cream	2160	0.5	1	0.1688
Fine fragrances	750	0.35	1	0.0410
Deodorant (non-spray)	1500	0.18	1	0.0422
Shampoo	10460	0.5	0.01	0.0082
Conditioner	3920	0.5	0.01	0.0031
Shower gel	18670	0.5	0.01	0.0146
Hand wash soap	20000	0.5	0.01	0.0156
Hair styling products	4000	0.5	0.1	0.0313
Total				**1.0559**

C = concentration of the notified chemical; RF = retention factor.
Daily systemic exposure = (Amount × C × RF × DA/BW

Household Products (Indirect dermal exposure – from wearing clothes)

Product type	Amount (g/use)	C (%)	Product Retained (PR) (%)	Percent Transfer (PT) (%)	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	230	0.5	0.95	10	0.0171
Fabric softener	90	0.5	0.95	10	0.0067
Total					**0.0238**

Daily systemic exposure = (Amount × C × PR × PT × DA)/BW

Household products (Direct dermal exposure)

Product type	Frequency (use/day)	C (%)	Contact Area (cm^2)	Product Usage (g/cm^3)	Film Thickness (cm)	Time Scale Factor (unitless)	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	1.43	0.5	1980	0.01	0.01	0.007	0.0002
Dishwashing liquid	3	0.5	1980	0.009	0.01	0.03	0.0013
All-purpose cleaner	1	0.5	1980	1	0.01	0.007	0.0108
Total							**0.0122**

Daily systemic exposure = Frequency × C × Contact Area × Product Usage × Film Thickness on skin × Time Scale Factor × DA/ BW

Aerosol products (Inhalation exposure)

Product type	Amount (g/day)	C (%)	Inhalation Rate (m^3/day)	Exposure Duration (Zone 1) (min)	Exposure Duration (Zone 2) (min)	Fraction Inhaled (%)	Volume (Zone 1) (m^3)	Volume (Zone 2) (m^3)	Daily systemic exposure (mg/kg bw/day)
Hairspray	9.89	0.5	20	1	20	50	1	10	0.0161

Daily systemic exposure = [(Amount × C × Inhalation Rate × Fraction Inhaled × 0.1) / BW × 1440)] × [Exposure Duration (Zone 1)/Volume (Zone 1) + Exposure Duration (Zone 2)/Volume (Zone 2)]

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above tables that contain the notified chemical. This would result in a combined internal dose of 1.108 mg/kg bw/day. It is acknowledged that inhalation exposure to the notified chemical from use of other cosmetic and household products may occur. However, it is considered that the combination of the conservative (screening level) hair spray inhalation exposure assessment parameters, 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 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 are summarised in the following table. For full details of the studies, refer to 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
Rat, acute inhalation toxicity	LC50 > 5 mg/L/4 hour; low toxicity
Skin corrosion (*in vitro*) - EpiDerm™ Reconstructed Human Epidermis Model	non-corrosive
Skin irritation (*in vitro*) - EpiSkin™ Reconstructed Human Epidermis Model	irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
Human, skin sensitisation – RIPT	no evidence of sensitisation at 2.5%
Rat, repeat dose oral toxicity – 28 days.	NOAEL 368 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic

Genotoxicity – *in vitro* mammalian chromosome aberration test in human lymphocytes	genotoxic
Genotoxicity – *in vitro* mammalian cell gene mutation test in mouse lymphoma L5178Y cells	genotoxic
Genotoxicity – *in vitro* micronucleus test in human lymphocytes	non genotoxic
Genotoxicity – *in vivo* mammalian bone marrow chromosome aberration test	non genotoxic
Genotoxicity – *in vitro* BlueScreen HC assay	non genotoxic

Toxicokinetics.
No toxicokinetic data on the notified chemical were submitted.

Dermal absorption is expected to be limited given the low water solubility (9.6 x 10^{-2} g/L at 25 °C) and high lipophilicity (log Kow = 4.8) of the notified chemical limiting penetration of the hydrophilic epidermis. Given the low molecular weight (200.32 Da) of the notified chemical absorption across the gastrointestinal and respiratory tract may occur.

Acute toxicity.
The notified chemical is of low acute oral, dermal and inhalation toxicity based on studies conducted in rats.

Irritation.
The notified chemical was determined to be non-corrosive under the conditions of an *in vitro* skin (EpiDerm™ Reconstructed Human Epidermis Model) corrosion study and irritating under the conditions of an *in vitro* skin (EpiSkin™ Reconstructed Human Epidermis Model) irritation study.

The notified chemical was a slight eye irritant in rabbits. Conjunctival redness was observed in all three rabbits on initial exposure reducing to very slight conjunctival redness in two animals over 24 hours. All animals had fully recovered 48 hours after exposure. The eye irritation effects were not at a level to warrant hazard classification.

Skin sensitisation.
The notified chemical was found to be a skin sensitiser in mice (Local Lymph Node Assay; stimulation indices (SI) of 4.6, 8.7 and 11.3 at 25, 50 and 100% concentrations, respectively). Based on these results an EC3 value of 19.1% was determined based on the recommendations of ICCVAM (2009).

The sensitising potential of the notified chemical was also tested in a human repeat insult patch test (HRIPT; 104 subjects completing the study). The notified chemical was not a skin sensitiser when tested at 2.5% concentration under the conditions of the study.

Repeated dose toxicity.
In a 28-day repeated dose oral dietary study in rats the No Observed (Adverse) Effect Level (NO(A)EL) was established as 368 mg/kg bw/day based on reduced sperm production in males at the highest dose tested (1061 mg/kg bw/day). Test substance related effects were also observed in the kidney and liver in both sexes; however, as the liver and kidney findings were considered non-adverse by the study authors, the NOAEL for females was considered to be 1150 mg/kg bw/day (the highest dose tested).

Mutagenicity/Genotoxicity.
The notified chemical gave a positive result in an *in vitro* mammalian chromosome aberration test in human lymphocytes and in an *in vitro* mammalian cell gene mutation test in mouse lymphoma L5178Y cells. In the chromosome aberration test a positive response only occurred at the high dose without metabolic activation whereas in the cell gene mutation test a positive response occurred with metabolic activation and a dose response was observed. However, the notified chemical was negative in an *in vivo* mammalian bone marrow chromosome aberration test. A reduction in the mitotic index at all tested doses provides evidence that the notified chemical had reached the bone marrow. The notified chemical was also negative in a bacterial reverse mutation assay, in an *in vitro* micronucleus test in human lymphocytes and in an *in vitro* BlueScreen HC assay.

Overall, based on the weight of evidence, the notified chemical is not expected to be genotoxic.

Health hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System of 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*
Skin corrosion/irritation	H315 – Causes skin irritation
Sensitisation, skin	H317 – May cause an allergic skin reaction

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(s):

R43: May cause sensitisation by skin contact

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Transport and Storage
Workers may experience dermal and accidental ocular exposure to the notified chemical (at ≤ 10% concentration) where the fragrance oils are sampled for quality control purposes or in the event of a discharge via spill or drum leakage. The use of PPE (e.g. impervious gloves, goggles, coveralls, hard hats and respiratory protection, if necessary) should minimise the potential for exposure. Provided adequate control measures and safe work practices are in place to minimise worker exposure, including PPE, the risk to workers from the notified chemical is not considered to be unreasonable.

Reformulation
Exposure of workers to the notified chemical (at ≤ 10% concentration) may occur during blending operations. The notified chemical is considered to be a skin sensitiser. In addition, harmful effects following inhalation and/or repeated exposure to the notified chemical are possible. Therefore, caution should be exercised when handling the notified chemical during reformulation processes.

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

End-use
Cleaners and beauty care professionals will handle the notified chemical at up to 0.5% concentration, similar to public use. Therefore the risk to workers who regularly use products containing the notified chemical is expected to be of a similar or lesser extent than that experience by members of the public who use such products ion a regular basis. For details of the public health risk assessment see Section 6.3.2.

6.3.2. Public Health
Members of the public may experience repeated exposure to the notified chemical through the use of cosmetic and household products (containing the notified chemical at ≤ 0.5% in individual products). The main route of exposure is expected to be dermal with some potential for accidental ocular or oral exposure.

Irritation
The notified chemical is slightly irritating to eyes. Given the low proposed use concentration (≤ 0.5%) irritation effects are not expected.

Skin sensitisation
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 cosmetic products may be estimated (SCCS, 2012 and Cadby *et al,* 2002).

Following consideration of the available data on skin sensitisation (and the study details/results of these studies) and application of appropriate safety factors, an Acceptable Exposure level (AEL) of 13.85 µg/cm^2 was derived

(using the EC3 value of 19.1%, which was obtained in an LLNA study on the notified chemical). In this instance, the factors employed included an interspecies factor (3), intraspecies factor (10), a matrix factor (3.16) and a use and time factor (3.16), giving an overall safety factor of approximately 300.

Product Type	Proposed maximum usage concentration (%)	CEL Chemical (µg/cm^2)	AEL Chemical (µg/cm^2)
Deodorant	0.18	13.50	13.85
Fine fragrances	0.35	13.13	13.85
Other cosmetic products (using face cream as worst case scenario)	0.5	13.63	13.85

As the AEL > CEL, the risk to the public of the induction of sensitisation that is associated with the use of the notified chemical in deodorants at ≤ 0.18%, fine fragrances at ≤ 0.35% and other cosmetic products (using face cream as a worst case scenario) at ≤ 0.5% is not considered to be unreasonable.

Based on the lower expected exposure level from use of household products (≤ 0.5% notified chemical), by inference, the risk of induction of sensitisation associated with the use of these products is also not considered to be unreasonable. 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.

Repeat dose toxicity
The potential systemic exposure to the public from the use of the notified chemical in cosmetics and household products was estimated to be 1.108 mg/kg bw/day (see Section 6.1.2). Using a NOAEL of 368 mg/kg bw/day, which was derived from a 28 day repeated dose oral dietary toxicity study on the notified chemical, the margin of exposure (MOE) was estimated to be 332. A MOE value greater than or equal to 100 is considered acceptable to account for intra- and inter-species differences.

Therefore, based on the information available, the risk to the public associated with use of the notified chemical at ≤ 0.18% in deodorants, ≤ 0.35% in fine fragrances, ≤ 0.5% in other cosmetics and household products, and ≤ 0.2% in air care 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 be imported as a neat chemical or component of fragrance preparations for local reformulation into a variety of consumer products (cosmetics and household products). Accidental spills during transport or reformulation are expected to be collected with inert material and disposed of to landfill. Import containers will either be recycled or disposed of through an approved waste management facility. It is expected that most sites will have closed, automated mixing and dosing equipment. The residues in import containers may be ≤ 1% of the import volume. The rinsate from the empty containers is expected to be sent to an on-site waste water plant or to the sewer system.

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 domestic products, which will be either washed off the hair and skin of consumers, or disposed of following cleaning activities.

RELEASE OF CHEMICAL FROM DISPOSAL
It is estimated that a maximum of 1% of the consumer products 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 before potential release to surface waters on a nationwide basis. The notified chemical is ultimately biodegradable and, based on its calculated adsorption coefficient (log Koc = 3.36), partitioning to sludge is expected. The notified chemical is not likely to bioaccumulate based on its calculated low bioconcentration factor (BCF < 100). In surface waters, the notified chemical is expected to disperse and eventually degrade through biotic and abiotic processes to form water and oxides of carbon.

The half-life of the notified chemical in air is calculated to be < 2 hours based on reactions with hydroxyl radicals (AOPWIN v1.92, US EPA, 2011). Therefore, in the event of release to atmosphere, the notified chemical is not expected to persist in the atmospheric compartment.

A proportion of notified chemical may be applied to land when effluent is used for irrigation or when sewage sludge is used for soil remediation, or disposed of to landfill. Notified chemical residues in landfill, soil and sludge are expected to have slight mobility based on its water solubility and its calculated soil adsorption coefficient (log K_{oc} = 3.36). In the soil compartments, the notified chemical is expected to degrade through biotic and abiotic processes 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 is 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	200.0	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 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 0.606 µg/L may potentially result in a soil concentration of approximately 4.04 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

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	96 h LC50 = 1.87 mg/L	Toxic to fish
Daphnia Toxicity	48 h EC50 = 1.8 mg/L	Toxic to aquatic invertebrates
Algal Toxicity	72 h EC50 = 3.22 mg/L	Toxic to algae

Under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009) the notified chemical is considered to be toxic to fish, aquatic invertebrates and algae and is formally classified as 'Acute Category 2: Toxic to aquatic life'. On the basis of the acute toxicity and the lack of ready biodegradability, the notified chemical is classified as 'Chronic Category 3: May cause long lasting harmful effects to aquatic life'.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) has been calculated based on the endpoint of the most sensitive species (daphnia, EC50 = 1.8 mg/L). An assessment factor of 100 was used as acute toxicity values from three trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50(Daphnia)	1.8	mg/L
Assessment Factor	100	
PNEC:	18	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River:	0.61	18	0.033
Q - Ocean:	0.06	18	0.0033

The risk quotient for discharge of the notified chemical to the aquatic environment indicates that the notified chemical is unlikely to reach ecotoxicologically significant concentrations based on its annual importation quantity. The notified chemical has a low potential for bioaccumulation. Therefore, on the basis of the PEC/PNEC ratio, maximum annual importation 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 < -25 °C

Method OECD TG 102 Melting Point/Melting Range.
Remarks Test substance did not solidify as the temperature decreased. Substance remained as a liquid at - 25 °C.
Test Facility Huntingdon (2014a)

Boiling Point 238 °C at 101.3 kPa

Method OECD TG 103 Boiling Point.
Remarks Determined according to the Siwoloboff method.
Test Facility Huntingdon (2014a)

Relative Density 0.87

Method OECD TG 109 Density of Liquids and Solids.
Remarks Density measured using Pycnometer.
Test Facility Huntingdon (2014a)

Vapour Pressure 0.091 kPa at 25 °C

Method OECD TG 104 Vapour Pressure.
Remarks Measured using a static vapour pressure apparatus
Test Facility Huntingdon (2013)

Water Solubility 9.6×10^{-2} g/L at 25 °C

Method OECD TG 105 Water Solubility.
EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks Flask Method
Test Facility Huntingdon (2010a)

Hydrolysis as a Function of pH t½ > 1 year at 25 °C (pH 4, 7 & 9)

Method OECD TG 111 Hydrolysis as a Function of pH.
EC Council Regulation No 440/2008 C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*T (°C)*	*t½*
4	25	> 1 year
7	25	> 1 year
9	25	> 1 year

Remarks The preliminary study on the notified chemical showed that at each of pH 4, 7 and 9 and 50 ± 0.5 ºC, less than 10% hydrolysis had occurred after 5 days, equivalent to a half-life of greater than 1 year under environmental conditions (25 °C). No further testing was considered necessary. Based on this the notified chemical was determined to be hydrolytically stable.
Test Facility Huntingdon (2014b)

Partition Coefficient (n-octanol/water) log Pow = 4.8 at 25 °C

Method OECD TG 117 Partition Coefficient (n-octanol/water).
EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks HPLC Method
Test Facility Huntingdon (2010a)

Surface Tension 48 mN/m at 20 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions.
Remarks	Concentration: 90% saturated aqueous solution
Test Facility	Huntingdon (2014a)

Adsorption/Desorption log K_{oc} = 3.4 at 25 °C

Method	OECD TG 121 Estimation of the Adsorption Coefficient.
Remarks	Using HPLC Method
Test Facility	Huntingdon (2014c)

Flash Point 112 °C at 101.6 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks	Determined using Pensky-Martens Closed Cup Flash Point apparatus
Test Facility	Huntingdon (2014a)

Autoignition Temperature 196 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Test Facility	Huntingdon (2014a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method. EC Council Regulation No 440/2008 B.1 tris Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Crl:CD (SD)
Vehicle	Corn oil
Remarks - Method	No protocol deviations. GLP compliance.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 F	300	1/3
2	3 F	300	0/3
3	3 F	2000	0/3
4	3 F	2000	0/3

LD50	> 2000 mg/kg bw
Signs of Toxicity	One animal dosed at 300 mg/kg died on Day 5. This death was not attributed to the test substance, but to an error during intubation at dosing based on macroscopic observations at necropsy (including perforation to the oesophagus at diaphragm level and gaseous distension of the GI tract). No other deaths were recorded. No clinical signs were observed in surviving animals in the low dose groups. Animals in the high dose groups (2000 mg/kg) exhibited piloerection (6/6), underactive behaviour (5/6), hunched posture (4/6), loose faeces (3/), irregular breathing (1/6), and elevated gait (1/6) one hour after exposure with all animals recovering by Day 3 of the observation period.
Effects in Organs	Pallor of kidneys was recorded in 2 animals exposed at 300 mg/kg (both in group 2). No other abnormalities recorded in any of the surviving animals.
Remarks - Results	All surviving animals (11/12) achieved satisfactory weight gains.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Huntingdon (2014d)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity. EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/ Crl:CD (SD)
Vehicle	None
Type of dressing	Semi-occlusive
Remarks - Method	No protocol deviations. GLP compliance.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 M, 5 F	2000	0/10

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Very slight to well-defined erythema noted in 1/5 M and 4/5 F with full recovery by Day 9 of the observation period. Eschar/scab formation was recorded in 1/5 F on Day 7 with full recovery by Day 13.
Signs of Toxicity - Systemic	No treatment related signs of systemic toxicity were noted in any of the animals over the study period.
Effects in Organs	No treatment related abnormalities were noted in any of the animals at termination.
Remarks - Results	Body weight loss was recorded for 1/5 F on Day 8. Low body weight gains were recorded in 1/5 F on Day 8 and 2/5 F on Day 15. All remaining animals made satisfactory body weight gains throughout the study.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	Huntingdon (2014e)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 436 Acute Inhalation Toxicity – Acute Toxic Class Method
Species/Strain	Rat/ Crl:CD (SD)
Vehicle	None
Method of Exposure	Oro-nasal exposure
Exposure Period	4 hours
Physical Form	Liquid aerosol
Particle Size	MMAD 3.6 µm
Remarks - Method	No significant protocol deviations. GLP compliance.

RESULTS

Group	*Number and Sex of Animals*	*Concentration mg/L*		*Mortality*
		Nominal	*Actual*	
1	3 M, 3 F	5	5.25	0/6

LC50	> 5 mg/L/4 hours
Signs of Toxicity	There were no unscheduled deaths. All animals exhibited slow breathing and closed eyelids during exposure with 1 F struggling during dosing. These signs were not noted immediately after exposure. Immediately following exposure, all animals exhibited chin rubbing with 1 F exhibiting partially closed eyelids. Recovery from these effects was observed 1 hr post-exposure. Wet fur noted in 1 M (during, immediately after and up to 1 hr after exposure) and 1 F (immediately and up to 1 hr after exposure) was considered by the authors to be related to the method of restraint rather than the test substance. All animals were considered clinically normal 2 hr after exposure.
Effects in Organs	No treatment related signs of systemic toxicity were noted in any of the animals over the study period. The observation of pale areas in the lungs of 1 F was considered by the authors to be consistent with commonly seen background macroscopic changes in this species of rat.
Remarks - Results	Body weight loss on the day following the 4 hour exposure was observed in all animals. The authors attributed this to the removal of food and water during the exposure period rather than the test substance. All animals showed recovery from the body weight loss at the next weighing occasion

and mean body weights in all animals increased for the remaining observation period.

CONCLUSION The notified chemical is of low toxicity via inhalation.

TEST FACILITY Huntingdon (2014f)

B.4. Irritation – skin (in vitro)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 431 In vitro Skin Corrosion - Human Skin Model Test
EpiDerm™ Reconstructed Human Epidermis Model
Vehicle Water
Remarks - Method No significant protocol deviations. GLP compliance.

Positive (8.0 N potassium hydroxide) and negative (purified water) controls were run concurrently with test items.

As MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) was reduced by the test substance, freeze killed tissues (no metabolic activity) were included in the assay together with the live tissues as a control.

RESULTS

Test material	*Exposure time (min)*	*Mean OD_{540} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	3	1.759	100.0	7.3
	60	2.194	100.0	15.1
Test substance	3	2.123	120.7	9.2
	60	2.288	104.3	4.4
Positive control	3	0.205	11.7	0.4
	60	0.043	2.0	0.2

OD = optical density; SD = standard deviation

Remarks - Results Positive and negative controls performed as expected.

CONCLUSION The notified chemical was non-corrosive to the skin under the conditions of the test.

TEST FACILITY Huntingdon (2014g)

B.5. Irritation – skin (in vitro)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 439 In vitro Skin Irritation: Reconstructed Human *Epidermis* Test Method
EpiSkin™ Reconstituted Human Epidermis Model
Vehicle Water
Remarks - Method No significant protocol deviations. GLP compliance

Positive (5% sodium dodecyl sulphate) and negative (Dulbecco's phosphate buffered saline) controls were run concurrently with test items.

RESULTS

Test material	*Mean OD_{540} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*

Negative control	0.832	100.0	3.2
Test substance	0.217	26.1	3.2
Positive control	0.131	15.7	3.7

OD = optical density; SD = standard deviation

Remarks - Results Positive and negative controls performed as expected.

CONCLUSION The notified chemical was irritating to the skin under the conditions of the test.

TEST FACILITY Huntingdon (2014h)

B.6. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Council Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation).
Species/Strain Rabbit/New Zealand White
Number of Animals 3
Observation Period 72 hours
Remarks - Method No deviations from the protocol. GLP Compliance.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0	0.3	0.3	1	< 48 hr	0
Conjunctiva: chemosis	0	0	0	0	-	0
Conjunctiva: discharge	0	0	0	0	-	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

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

Remarks - Results Irritation was observed on exposure to the notified chemical with animals exhibiting slight conjunctival redness (3/3), very slight chemosis (2/3) and slight to moderate discharge (3/3) at the 1 hr observation. Very slight conjunctival redness was observed in 2/3 animals at the 24 hr observation. All animals had recovered at the 48 hr observation.

CONCLUSION The notified chemical is slightly irritating.

TEST FACILITY Huntingdon (2014i)

B.7. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)
Species/Strain Mouse/CBA/Ca
Vehicle Acetone:olive oil (4:1 v/v)
Preliminary study Yes
Positive control Hexyl cinnamic aldehyde (HCA). Not conducted in parallel with the test substance but had been conducted previously in the test laboratory using contemporaneous studies.
Remarks - Method No protocol deviations. GLP Compliance.

^{3}H-methyl Thymidine (^{3}HTdR) used to determine cellular proliferation..

RESULTS

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	4 F	526.38	-
25	4 F	2435.31	4.6
50	4 F	4584.13	8.7
100	4 F	5974.06	11.3

Remarks - Results

There were no unscheduled deaths. A loss in bodyweight was recorded for 1 animal in the high dose group, and no change in body weight was recorded for 1 animal in each of the low- and high-dose groups. All remaining animals (13/16) exhibited satisfactory weight gain over the course of the study.

Greasy fur on the cranial region was observed in all animals which resolved in 15/16 animals by Day 6.

No signs of irritation were observed during the study.

An EC3 of 19.1% was calculated for the notified chemical.

CONCLUSION

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

TEST FACILITY

Huntingdon (2010b)

B.8. Skin sensitisation – human volunteers

TEST SUBSTANCE

Notified chemical (2.5% concentration)

METHOD

Repeated insult patch test with challenge

Study Design

Induction Procedure: Patches containing 0.2 mL test substance were applied 3 times per week (Monday, Wednesday and Friday) for a total of 9 applications. Patches were removed by the applicants after 24 h and graded after an additional 24 h (or 48 h for patches applied on Friday).
Rest Period: 14 days
Challenge Procedure: A patch was applied to a naïve site. Patches were removed by a laboratory technician after 24 h. Sites were graded 24, 48 and 72 h post-patch removal.

Study Group

81 F, 31 M; age range 18 - 68 years

Vehicle

Alcohol SD39C:DEP (25:75)

Remarks - Method

Occluded. The test substance was spread on a 3.63 cm^2 patch and allowed to evaporate for 30 – 90 minutes prior to patch application.`

RESULTS

Remarks - Results

104/112 subjects completed the study. Eight subjects who discontinued the study were deemed by the authors to do so for reasons unrelated to the test material. Eight subjects withdrew voluntarily, four withdrew prior to starting the induction readings, three withdrew following the second (2) and fourth (1) induction readings and one subject withdrew prior to the challenge procedure. One subject completed the study, but was absent for the application of the final induction and the reading at 48 hr post-challenge.

No adverse responses were noted during the induction procedure or at challenge.

CONCLUSION	The notified chemical at 2.5% concentration was non-sensitising under the conditions of the test.
TEST FACILITY	CRL (2010)

B.9. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain	Rats/Crl:CD(SD)
Route of Administration	Oral – diet
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 2 weeks
Vehicle	Rat and Mouse No. 1 Maintenance Diet
Remarks - Method	No significant protocol deviations GLP Compliant

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration mg/kg/day*		*Mortality*
		Nominal	*Actual*	
control	5 M, 5 F	0	0	0/10
low dose	5 M, 5 F	35	38 (M), 43 (F)	0/10
mid dose	5 M, 5 F	350	368 (M), 397 (F)	0/10
high dose	5 M, 5 F	1000	1061 (M), 1150 (F)	0/10
control recovery	5 M, 5 F	0	0	0/10
high dose recovery	5 M, 5 F	1000	38 (M), 43 (F)	0/10

Mortality and Time to Death

There were no unscheduled deaths

Clinical Observations

Mean forelimb (males and females) and hindlimb (males) grip strength was reduced in high-dose groups. All animals exposed to the test substance showed reduced rearing and cage floor activity. The study authors did not consider the effects to be treatment related as no dose-response relationship was apparent and the differences observed were not statistically significant,.

Animals in the low- and mid-dose groups gained body weight as expected. Females in the high-dose group showed significantly reduced body weight over the course of the study. Males in the high-dose group exhibited reduced body weight during the first week, but then gained weight over the course of the study. However, their overall weight at the end of the study was reduced compared to controls. During the recovery period, animals in the high-dose group showed body weight gains similar or above those of the control animals. A slight reduction in food intake was recorded for males in the high-dose group. Males in this group showed improved food intake during the recovery period.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Males in the high-dose group exhibited a statistically significant increase in mean total leucocyte counts (with associated increases in neutrophil, lymphocyte, eosinophil, basophil and monocyte counts) as well as reduced concentrations of triglycerides. A slightly higher albumin/globulin ratio was observed in females in the high-dose group although not in the equivalent recovery group. These effects were considered to be recoverable from by the study authors as males and females in the high-dose recovery group did not exhibit similar effects.

A dose-response relationship was observed in the slight reduction of albumin/globulin ratios in treated males. These reduced levels were also observed in males in the high-dose recovery group.

Effects including increased sodium, calcium and phosphorous levels (all treated males), increased alkaline

phosphatase, alanine amino-transferase, aspartate aminotransferase and haemoglobin concentration (high-dose males), reduced concentrations of bile acid and bilirubin (treated females), and lower haemoglobin and mean cell haemoglobin concentrations, prothrombin time and activated partial thromblastin time (high-dose females) were not considered to be treatment related by the study authors based on the absence of dose-response relationships, variation between the sexes or could be attributable to normal biological variation.

Reduced urinary pH was observed in high-dose females. Males in all treatment groups exhibited reduced urinary volume, total creatine, chloride, sodium, potassium and protein. Small amounts of ketones were also present. Males in the high-dose group also exhibited reduced urinary pH and increased specific gravity. Males in the high-dose recovery group did not exhibit the same effects except for reduced levels of protein. Other effects were not considered to be treatment related by the study authors based on the absence of dose-response relationships, variation between the sexes or the effects could be attributed to normal biological variation.

Lower sperm and significantly lower spermatid numbers and reduced beat cross frequency (BCF) values were observed in high-dose males. During the recovery period there was evidence of recovery from these effects, however the BCF remained statistically reduced. Changes in the percent of motile, progressively motile sperm, percentage normal morphology and motion parameters were observed in high-dose males. However these changes were attributed to a single animal with immotile sperm and the study authors considered that these effects were not attributable to the test chemical.

Effects in Organs

Significant increases in kidney (mid- and high-dose) and liver weights (high-dose) were recorded in females. Males in the high-dose group exhibited significant increases in liver and thymus weight as well as increases in kidney and spleen weights. Both males and females in the high-dose recovery group exhibited increased kidney weights and increased liver weights were exhibited by males only. All other organ weights showed weights similar to controls.

Macroscopic examination did not indicate any treatment related effects on animals in the low-, mid- and high-dose groups or high-dose recovery group. One male in the high-dose recovery group exhibited a mass in the left epididymis. The epididymis was used for sperm analysis and the mass was not examined. This animal was not the one exhibiting immotile sperm.

Minimal to slight centrilobular hypertrophy in the liver was observed in females in the high-dose group but was absent in high-dose recovery females. Mid- and high-dose females exhibited an increased incidence and severity of tubular basophilia in the kidneys, although no dose-response relationship was evident. Tubular basophilia was also observed in the kidneys of high-dose recovery females, but at minimal severity.

A cyst at necropsy was observed in the kidney of a male in the low-dose group. Males in the high-dose group exhibited a slight increase in the incidence and severity of plasmacytosis in the mandibular lymph node. No other lymphatic changes were observed and the effect was not considered to be treatment related by the study authors.

Remarks – Results

The liver and kidney were most affected by the test substance in males and females, however the effects were considered non-adverse by the study authors. Reduced sperm production was also observed in males exposed to high-doses of the test substance.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 368 mg/kg bw/day in this study, based on reduced sperm production.

TEST FACILITY	Huntingdon (2015a)

B.10. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.

	Test 1: Plate incorporation procedure Test 2: Pre-incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2*uvr*A (pKM101)
Metabolic Activation System	S9 fraction from phenobarbital/5,6-benzoflavone induced rat liver
Concentration Range in Main Test	Test 1: a) With metabolic activation: 5, 15, 50, 150, 500, 1500, 5000 µg/plate b) Without metabolic activation: 5, 15, 50, 150, 500, 1500, 5000 µg/plate Test 2: a) With metabolic activation: 50, 150, 500, 1500*, 5000 µg/plate b) Without metabolic activation: 50, 150, 500, 1500*, 5000 µg/plate * maximum concentration tested for TA1537 based on results from Test 1.
Vehicle	DMSO
Remarks - Method	No deviations from protocol. GLP Compliance. No preliminary toxicity test performed. Positive controls: with metabolic activation: 2-Aminoanthracene [TA100, TA1535, WP2*uvr*A (pKM101)], Benzo[a]pyrene (TA98, TA1537); without metabolic activation: Sodium azide (TA100, TA1535), 9-Aminoacridine (TA1537), 2-Nitrofluorene (TA98), 4-Nitroquinoline-1-oxide [WP2*uvr*A (pKM101)].

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	NA	≥ 1,500	> 5,000	negative
Test 2	NA	≥ 1,500	> 5,000	negative
Present				
Test 1	NA	≥ 1,500	> 5,000	negative
Test 2	NA	≥ 1,500	> 5,000	negative

Remarks - Results	In both tests, toxicity was observed in all strains at 5000 µg/plate and in TA1537 at 1,500 µg/plate (maximum concentration tested for this strain in test 2). No substantial increases in the number of revertant colonies was recorded for any of the strains in the presence or absence of metabolic activation in either Test 1 or Test 2. Positive and negative controls performed as expected in both tests.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Huntingdon (2010c)

B.11. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 fraction from phenobarbital/5,6-benzoflavone induced rat liver
Vehicle	Ethanol

Remarks - Method
No deviations from protocol.
GLP Compliance.

Positive controls: with metabolic activation: Cyclophosphamide; without metabolic activation: Mitomycin C.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	20*, 30, 40*, 50*, 100, 125, 150, 175, 200, 225, 250, 275, 300	3	18
Test 2	5, 10*, 20, 30*, 40, 50, 60*, 70, 80, 90, 100	21	21
Present			
Test 1	25, 50, 65, 80, 95*, 110*, 120*, 130, 150	3	18
Test 2	50*, 100, 120, 140*, 160, 180*, 200, 220, 240, 260	3	18

*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	≥ 33.62	≥ 50	> 300	negative
Test 2		≥ 60	> 100	positive
Present				
Test 1	≥ 93.39	≥ 120	> 150	negative
Test 2		≥ 180	> 260	negative

Remarks - Results
In Test 1, the test substance did not cause a statistically significant increase in the proportion of cells with chromosomal aberrations at any concentration in the presence or absence of metabolic activation.

In Test 2, in the absence of metabolic activation, a statistically significant increase in the proportion of cells with chromosomal aberrations was observed at a concentration of 60 µg/mL only. In the presence of metabolic activation the test substance did not cause a statistically significant increase in the proportion of cells with chromosomal aberrations at any concentration.

Positive and negative controls performed as expected in both tests.

CONCLUSION
The notified chemical is clastogenic to human lymphocytes treated in vitro under the conditions of the test.

TEST FACILITY
Huntingdon (2010d)

B.12. Genotoxicity – in vitro

TEST SUBSTANCE
Notified chemical

METHOD
OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
Species/Strain
Mouse
Cell Type/Cell Line
Lymphoma L5178Y
Metabolic Activation System
S9 fraction from phenobarbital/5,6-benzoflavone induced rat liver
Vehicle
Ethanol
Remarks - Method
No deviations from the protocol.
GLP Compliant.

Positive controls: with metabolic activation: Benzo[a]pyrene (BaP);

without metabolic activation: Methyl methanesulphonate (MMS).

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	5, 20*, 30*, 35*, 40*, 45, 50	3	48
Test 2	2.5, 5*, 7.5, 10*, 12.5, 15*, 20*, 25*	24	48
Present			
Test 1	30*, 60, 70*, 80, 90, 100*, 110, 120*, 130	3	48

*Cultures selected for mutation frequency.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 62.55	≥ 40	> 50	negative
Test 2	≥ 15.64	≥ 20	> 25	negative
Present				
Test 1	≥ 125.11	≥ 120	> 130	positive

Remarks - Results

In Test 1, in the presence of metabolic activation, increases in the mean mutant frequency at 100 and 120 μg/mL was observed and there was evidence of a dose-response relationship. The increases in mean mutant frequency were predominantly due to an increase in small and large colony formation. In the absence of metabolic activation, no increases in mean mutant frequencies were observed at any test concentration.

In Test 2, there were no increases in the mean mutant frequencies of any test concentrations.

The notified chemical was observed to have mutagenic potential following metabolic activation at concentrations greater than 100 μg/mL. This potential was not observed in the absence of metabolic activation.

Positive and negative controls performed as expected in all tests.

CONCLUSION

The notified chemical is clastogenic to mouse lymphoma L5178Y cells treated in vitro under the conditions of the test.

TEST FACILITY

Huntingdon (2011)

B.13. Genotoxicity – in vitro

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 487 In vitro Mammalian Cell Micronucleus Test.

Species/Strain: Human

Cell Type/Cell Line: Lymphocytes

Metabolic Activation System: S9 fraction from phenobarbital/5,6-benzoflavone induced rat liver

Vehicle: Ethanol

Remarks - Method: GLP Compliant

Positive controls: with metabolic activation: Cyclophosphamide (exposure concentrations 5 and 10 μg/mL); without metabolic activation: Mitomycin C (exposure concentrations 0.05 and 0.075 μg/mL); Colchicine (exposure concentrations 0.005, 0.01, 0.02, 0.03 and 0.04 μg/mL).

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	10*, 60*, 75*, 80, 82.5, 85, 87.5	3	20
Test 2	10*,20, 25, 30*, 35, 40, 45*, 50	20	-
Present			
Test 1	50*, 120*, 130, 140*, 142.5	3	20

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:* *Cytotoxicity in Main Test*	*Genotoxic Effect*
Absent		
Test 1	≥ 75	negative
Test 2	≥ 45	negative
Present		
Test 1	≥ 140	negative

Remarks - Results

In both Test 1 and Test 2, in the presence or absence of metabolic activation, the test substance did not induce any statistically significant increases in the number of binucleate cells containing micronuclei and a dose-response relationship was not observed. All data was within the historical range expected. Positive and negative controls performed as expected.

CONCLUSION

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

TEST FACILITY

Huntingdon (2012)

B.14. Genotoxicity – in vitro

TEST SUBSTANCE

Notified chemical

METHOD

Species/Strain: Human

Cell Type/Cell Line: Lymphoblastoid TK 6 (GLuc-T01) incorporated with *Gaussia* luciferase (GLuc)

Metabolic Activation System: S9 fraction from 1% (v/v) Aroclor-1254 induced rat liver

Vehicle: DMSO (1% w/v)

Remarks - Method

Cytotoxicity of the test substance to cells is measured by lysis of the cells and addition of a fluorescent DNA binding stain. The resulting fluorescence is proportional to cell proliferation, which is lowered by toxic analytes.

Genotoxicity of the test substance is evaluated by the induction of GLuc expression where exposure to a genotoxic compound increases expression of GLuc. The expression of GLuc is quantified at the assay endpoint by the detection of luminescence generated for the reaction of GLuc with a coelenterazine substrate, added to the microplate wells just before measurement. Luminescence is proportional to the activity of the cell's DNA repair system which is increased by genotoxic analytes. Luminescence is normalised to the fluorescence signal to correct for variation in cell yield caused by cytotoxicity.

Each dilution of test substance is combined with an equal volume of specialised growth medium containing BlueScreen HC cells. Tests are performed in duplicate within a single microplate assay.

In the absence of metabolic activation, microplates were covered with a

breathable membrane and incubated at 37 °C (5% CO_2, 95% humidity) for 48 hours. The assay plates are then analysed using a microplate reader that measures the fluorescence and flash luminescence for the cells and solutions in the microplate wells.

In the presence of metabolic activation, microplates are covered with a breathable membrane and incubated at 37 ^{0}C (5% CO_2, 95% humidity) for 3 hours. Cells are then washed in phosphate buffered saline, harvested by centrifugation and then allowed to recover in Recovery Medium at 37 °C (5% CO_2, 95% humidity) for 45 hours.

In the absence of metabolic activation, a compound is considered cytotoxic when the relative cell density is reduced to less than 80% (compared to the vehicle control). Genotoxicity is evaluated by induction of GLuc expression. The statistically defined threshold for a positive result (for this protocol) is 1.8 (80% induction over the baseline for vehicle control).

In the presence of metabolic activation, a compound is considered cytotoxic when the relative cell density is reduced to less than 80% (compared to the vehicle control) at one or more test concentrations. Genotoxicity is evaluated by the induction of GLuc expression. The statistically defined threshold for a positive result (for this protocol) is 1.5 (50% induction over the baseline for vehicle control).

Positive control: with metabolic activation: Cyclophosphamide (at 5 µg/mL and 25 µg/mL concentrations); without metabolic activation: 4-Nitroquinoline 1-oxide (4-NQO) (at 0.125 µg/mL and 0.5 µg/mL concentrations).

A vehicle control was run in the absence of metabolic activation and media and S9 controls were run in the presence of metabolic activation.

Metabolic Activation	*Test Substance Concentration (µM)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	39.1, 78.1, 156, 313, 625, 1250, 2500, 5000	48	-
Present			
Test 1	39.1, 78.1, 156, 313, 625, 1250, 2500, 5000	3	45

RESULTS

Metabolic Activation	*Test Substance Concentration (µM) Resulting in:*	
	Cytotoxicity in Main Test	*Genotoxic Effect*
Absent		
Test 1	positive	negative
Present		
Test 1	positive	negative

Remarks - Results

The test substance did not induce a genotoxic response in the presence or absence of metabolic activation.

All positive and negative controls performed as expected in the presence and absence of metabolic activation.

CONCLUSION

The notified chemical was not genotoxic to human lymphocytes treated in vitro under the conditions of the test.

TEST FACILITY

Gentronix (2012)

B.15. Genotoxicity – in vivo

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 475 Mammalian Bone Marrow Chromosome Aberration Test.
Species/Strain	Mouse/ICR
Route of Administration	Intraperitoneal injection
Vehicle	Corn oil
Remarks - Method	No significant protocol deviations. GLP Compliant. Range-finding study was performed with no mortality or severe signs of toxicity observed at the highest dose (2000 mg/kg) in either males or females. Male animals used in the main study. Colchicine (4 mL/kg) was used to arrest the cells in metaphase ~ 3 hours prior to bone marrow collection. Femoral bone marrow harvested at 18 ± 0.5 and 42 ± 0.5 hours post-dose. Positive control: Cyclophosphamide monohydrate (50 mg/kg). Positive and negative (vehicle) controls were run concurrently.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	5 M	-	18
II (vehicle control)	5 M	-	42
III (low dose)	5 M	500	18
IV (mid dose)	5 M	1000	18
V (high dose)	5 M	2000	18
VI (high dose)	5 M	2000	42
VII (positive control, CP)	5 M	50	18

CP=cyclophosphamide.

RESULTS	
Doses Producing Toxicity	> 2000 mg/kg.
Genotoxic Effects	No statistically significant increases were noted in the number of cells with structural aberrations in any of the test groups. A reduction in mean mitotic index of 45%, 26% and 21% compared to the vehicle control was observed in the 500, 1000 and 2000 mg/kg treatment groups at 18 hours post-dose. A 53% reduction in the mitotic index was observed in the 2000 mg/kg treatment group at 42 hours post-dose.
Remarks - Results	There were no unscheduled deaths. No adverse effects were observed in the low-dose group. Piloerection was observed in animals dosed with ≥ 1000 mg/kg. Lethargy was also noted in the high-dose group.
CONCLUSION	The notified chemical was not clastogenic under the conditions of this in vivo mammalian bone marrow chromosome aberration test.
TEST FACILITY	BioReliance (2013)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 310 B Ready Biodegradability: CO2 Evolution Test in sealed vessel Test. .
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	Acetone
Analytical Monitoring	Total Inorganic Carbon (TIC)
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. A stock solution of the test substance was prepared in acetone by weighing (nominally) 69.5 mg into a 50 mL volumetric flask before being made up to volume with solvent to give a nominal concentration of I mgC/mL. Aliquots (I mL) of the solvent stock were then added to the respective vials and the acetone evaporated in a gentle stream of nitrogen depositing the test substance on the walls of the vessels. The vials were left to stand for at least one hour before being re-flushed with nitrogen to remove any traces of solvent. The final, nominal test substance concentration was 10 mgC/L.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	17.5	7	75.9
14	46.8	14	88.8
21	62	21	87.8
28	62.2	28	85.7

Remarks - Results	The validity criteria for the test were met. The toxicity control attained 87.3% degradation by day 7 of the study thereby confirming that the notified chemical was not toxic to the sewage treatment micro-organisms used in the study. Mean production of CO_2 by mixtures containing the notified chemical was equivalent to 10.0% of the theoretical maximum after approximately 4 days, 46.8% after 14 days and 62.0 % by Day 21. Substances are considered to be readily biodegradable in this test if CO_2 production is equal to or greater than 60% of the theoretical value within ten days of the level achieving 10%. Therefore, notified chemical was not considered to be readily biodegradable, however it was considered to be ultimately biodegradable under the conditions of OECD Guideline 310.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	Huntingdon (2010e)

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
Species	Chinese Rare Minnow (*Gobiocypris rarus*)

Exposure Period	96 hour
Auxiliary Solvent	None
Water Hardness	165 mg $CaCO_3$/L
Analytical Monitoring	UPLC-MS (Ultra performance liquid chromatography – mass spectrum)
Remarks – Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. A nominal concentration of 100 mg/L of the test substance was prepared and stirring the mixture (100 mg test substance in 1 litre of water) in the dark for 72 h at 500 rpm followed by filtration using a 0.45 µm nitrocellulose membrane to make a saturated solution stock solution. The test solutions were prepared by dilution of the stock solution with test water.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*			
Nominal	*Actual*		*24 h*	*48 h*	*72 h*	*96 h*
Control		7	0	0	0	0
0.96	0.62	7	0	0	0	0
1.7	1.1	7	0	0	0	0
3.1	2.24	7	0	2	5	5
5.6	3.73	7	2	7	7	7
10	6.71	7	7	7	7	7

LC50	1.87 mg/L at 96 hours.
Remarks – Results	The validity criteria for the test were met. The study end-points are based on geometric mean of measured concentrations, as the measured values were outside the acceptable range of 80- 120% of nominal concentrations.
CONCLUSION	The notified chemical is toxic to fish
TEST FACILITY	BSAL (2013)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test - Semi Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	Not given
Analytical Monitoring	GC-MS
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. The test substance (53.8 µL) was added to dilution medium in a volumetric flask (2 L) and made up to volume with mixing to give a stock solution with a nominal concentration of 23.4 mg/L. From this test solutions with nominal concentrations of 10.6, 4.84, 2.20 and 1.00 mg/L were made.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control		20	0	0
1.00	0.282	20	0	0
2.20	0.930	20	0	2

4.84	2.78	20	6	16
10.6	6.22	20	20	20
23.4	18.4	20	20	20

LC50	1.8 mg/L at 48 hours
NOEC (or LOEC)	0.93 mg/L at 48 hours
Remarks - Results	The validity criteria for the test were met. The study end-points are based on geometric mean of measured concentrations, as the measured values were outside the acceptable range of 80- 120% of nominal concentrations.
CONCLUSION	The notified chemical is toxic to aquatic invertebrates
TEST FACILITY	|Huntingdon (2015b)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 0.0954, 0.977, 3.13 and 10.0 mg/L Actual: 0.0466, 0.0732, 0.470, 1.68 and 9.021 mg/L
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	GC-MS
Remarks - Method	The test substance (57.5 µL) was added to dilution medium in a volumetric flask (1 L) and made up to volume with mixing to give a stock solution with a nominal concentration of 50 mg/L. From this test solutions with nominal concentrations of 10.0, 3.13, 0.977 and 0.0954 mg/L were made.

RESULTS

Biomass		*Growth*	
EyC50 *mg/L at 72 h*	*NOEC* *mg/L*	*ErC50* *mg/L at 72 h*	*NOEC* *mg/L*
1.82	0.470	3.22	0.470

Remarks - Results	The validity criteria for the test were met. At the start of the test, the measured levels of test substance in samples of the test cultures ranged between 112 and 182% of their nominal values. After 72 hours, the measured levels had decreased to below the limit of detection at nominally 0.305 mg/L and below to 65%. Therefore, the study end-points are based on geometric mean of measured concentrations.
CONCLUSION	The notified chemical is toxic to algae
TEST FACILITY	Huntingdon (2015c)

BIBLIOGRAPHY

ACI (2010) Consumer Product Ingredient Safety, Exposure and Risk Screening methods for Consumer Product Ingredients, 2nd Edition, American Cleaning Institute, Washington DC.

Api *et al* (2008) Api, A.M., Basketter, D.A., Cadby, P.A., Cano, M.F., Ellis, G., Gerberick, G.F., Griem, P., McNamee, P.M., Ryan, C.A., Safford, R.; Dermal Sensitisation Quantitative Risk Assessment (QRA) for Fragrance Ingredients, Regulatory Toxicology and Pharmacology 52 (2008) 3-23.

BioReliance (2013) Bone Marrow Chromosome Aberration Test in Mice Following Intraperitoneal Injection (IP) of TM 09-217c (Study No. AD39TS.108M.BTL, November, 2013). Maryland, USA, BioReliance (Unpublished report submitted by the notifier).

BSAL (2013) Acute toxicity test of TM 09-217 to Chinese Rare Minnow (*Gobiocypris rarus*) (R1212080629A December 2013). Shanghai, Bioassay and Safety Assessment Lab, P R China (Unpublished report submitted by the notifier).

Cadby *et al.* (2002) Cadby, P.A., Troy, W.R., Vey, M.G.; Consumer exposure to fragrance: Providing estimates for safety evaluation, Regulatory Toxicology and Pharmacology 36 (2002) 246-252.

CRL (2010) Repeated Insult Patch Test (Study No. CRL51710-1, August, 2010). New jersey, USA, Clinical Research Laboratories Inc (Unpublished report submitted by the notifier).

Earnest, C.W., Jr. (2009) A Two-Zone Model to Predict Inhalation Exposure to Toxic Chemicals in Cleaning Products, MSCEng thesis, The University of Texas at Austin

ECHA (2014) Guidance on information requirements and chemical safety assessment, Chapter R.7c: Endpoint specific guidance, p172, 2014.

enHealth (2012) Australian Exposure Factor Guide, companion document to: Environmental Health Risk Assessment: Guidelines for assessing human health risks from environmental hazards, EnHealth, Commonwealth of Australia.

Gentronix (2012) Report on the Testing of 8 compounds in the BlueScreen HC Assay (-/+ S9 Metabolic Activation) for IFF R&D (July, 2012). Manchester, UK Gentronix (Unpublished report submitted by the notifier).

Huntingdon (2010a) TM 09-217 Physiochemical Properties (Study No. JMN0008, May, 2010). Suffolk, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2010b) TM 09-217: Assessment of Skin Sensitisation Potential using the Local Lymph Node Assay in the Mouse (Pooled treatment group approach) (Study No. JMN0012, June, 2010). Cambridgeshire, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2010c) TM 09-217 Bacterial Reverse Mutation Test (Study No. JMN0010, August, 2010). Suffolk, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2010d) TM 09-217 *In Vitro* Mammalian Chromosome Aberration Test In Human Lymphocytes (Study No. JMN0011, November, 2010). Suffolk, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2010e) TM 09-217 Assessment of Ready Biodegradability Test (Study No. JMN0009, October, 2010). Suffolk, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2011) TM 09-217: *In Vitro* Mutation Test Using Mouse Lymphoma L5178Y Cells (Study No. JMN0032, December, 2011). Suffolk, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2012) TM 09-217 *In Vitro* Micronucleus Test in Cultured Human Lymphocytes (Study No. JMN0033, February, 2012). Suffolk, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2013) TM 09-217 Vapour Pressure (Study No. JMN0040, October, 2013). Suffolk, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2014a) TM 09-217 Physiochemical Properties (Study No. JMN0043, August, 2014). Suffolk, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2014b) TM 09-217 Hydrolysis (Study No. JMN0054, August, 2014). Suffolk, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2014c) TM 09-217 Soil Adsorption (Study No. JMN0055, October, 2013). Suffolk, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2014d) TM 09-217: Acute Oral Toxicity to the Rat (Acute Toxic Class Method) (Study No. JMN0044, August, 2014). Cambridgeshire, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2014e) TM 09-217: Acute Dermal Toxicity to the Rat (Study No. JMN0050, August, 2014). Cambridgeshire, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2014f) TM 09-217: Acute (Four Hour) Inhalation Study in Rats (Study No. JMN0058, October, 2014). Cambridgeshire, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2014g) IFF TM 09-217 In Vitro Skin Corrosivity Test Using the EpiDerm™ Human Skin Model (Study No. JMN0045, August, 2014). Cambridgeshire, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2014h) IFF TM 09-217 EpiSkin™ Skin Irritation Test 15 min – 42 hours (Study No. JMN0046, September, 2014). Cambridgeshire, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2014i) TM 09-217: Eye Irritation to the Rabbit (Study No. JMN0052, October, 2014). Cambridgeshire, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2015a) TM 09-217: Toxicity Study by Dietary Administration to Sprague-Dawley Rats for 4 Weeks Followed by a 2 Week Recovery Period (Study No. JMN0056, January, 2015). Suffolk, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2015b) TM 09-217: Acute Toxicity to Daphnia Magna (Study No. JMN0048, March, 2015). Suffolk, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

Huntingdon (2015c) TM 09-217: Algal Growth Inhibition Assay (Study No. JMN0049, April, 2015). Suffolk, UK, Huntingdon Life Sciences (Unpublished report submitted by the notifier).

ICCVAM (2009) Updated ICCVAM-Recommended Protocol: The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products: Appendix http://ntp.niehs.nih.gov/iccvam/docs/immunotox_docs/llna-pot/2-appb.pdf (last accessed 16 July 2015).

Loretz *et al.* (2006) Loretz, L., Api, A.M., Barraj, L., Burdick, J., Davis, D.A., Dressler, W., Gilberti, E., Jarrett, G., Mann, S., Pan, Y.H.L., Re, T., Renskers, K., Scrafford, C., Vater, S.; Exposure data for personal care products: Hairspray, perfume, liquid foundation, shampoo, body wash and solid antiperspirant, Food and Chemical Toxicology 44 (2006) 2008-2018.

NOHSC (2004) Approved Criteria for Classifying Hazardous Substances, 3rd edition [NOHSC:1008(2004)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

RIVM (2010) Observations of the Methodology for Quantitative Risk Assessment of Dermal Allergens, Report 320015003/2010, National Institute of Public Health and the Environment, Netherlands

Rothe *et al.* (2006) Rothe, H., Fautz, R., Gerber, E., Neumann, L., Rettinger, K., Schuh, W., Gronewold, C.; Special aspects of cosmetic spray evaluations: Principles on inhalation risk assessment, Toxicology Letters 205 (2011) 97-104.

SCCS (2012) The SCCS' Notes of Guidance for the Testing of Cosmetic Substances and their Safety Evaluation (8th revision) European Commission – Scientific Committee on Consumer Safety

Steiling *et al.* (2014) Steiling, W., Bascompta, M., Carthew, P., Catalano, G., Corea, N., D'Haese, A., Jackson, P., Kromidas, L., Meurice, P., Rothe, H., Singal, M..; Principle considerations for the risk assessment of sprayed consumer products, Toxicology Letters 227 (2014) 41-49.

SWA (2012) Code of Practice: Managing Risks of Hazardous Chemicals in the Workplace, Safe Work Australia, http://www.safeworkaustralia.gov.au/sites/swa/about/publications/pages/managing-risks-of-hazardous-chemicals-in-the-workplace.

United Nations (2009) Globally Harmonised System of Classification and Labelling of Chemicals (GHS), 3rd revised edition. United Nations Economic Commission for Europe (UN/ECE), <http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html >.

US EPA (2011) Ecological Structure Activity Relationship toxicological Structural activity v1.11.United States Environmental Protection Agency. Washington, DC, USA.