File No: LTD/1851

November 2015

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

2-Butenoic acid, 1-ethyl-2-methylpropyl ester, (2E)-

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

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/1851	International Flavours and Fragrances (Australia) Pty Ltd	2-Butenoic acid, 1-ethyl-2-methylpropyl ester, (2E)-	Yes	≤ 1 tonne per annum	Fragrance ingredient

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, 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.

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 3)	H402 - Harmful to aquatic life

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:

R38: Irritating to skin

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 for the proposed uses and concentrations, 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

CONTROL MEASURES

Occupational Health and Safety

- 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 eyes and skin

- 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:

- Eye protection
- Coveralls, impervious gloves

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

- A copy of the (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.

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 exceeds or is intended to exceed 2.5% in fine fragrances, 0.2% in deodorants, 0.1% in hair care products, 0.1% in leave-on cosmetic products, 1% in rinse-off cosmetics, 20% in non-spray air fresheners and candle products, or 0.5% in other 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, or is likely to change significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

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

(Material) Safety Data Sheet
The (M)SDS of the notified chemical and product containing the notified chemical provided by the notifier were 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 as follows: Particle Size, Flammability, Dissociation Constant, Hydrolysis as a Function of pH, and Adsorption/Desorption.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
US TSCA (2014)
Canada DSL (2015)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Cosmofruit

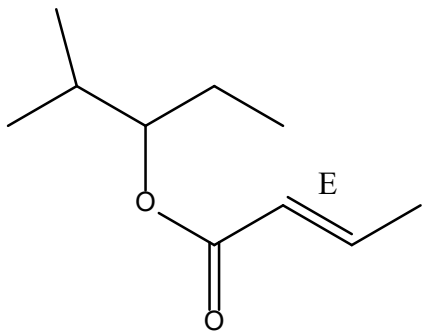
CAS NUMBER
1370711-06-0

CHEMICAL NAME
2-Butenoic acid, 1-ethyl-2-methylpropyl ester, (2E)-

OTHER NAME(S)
2-Methylpentan-3-yl (2E)-but-2-enoate

MOLECULAR FORMULA
$C_{10}H_{18}O_2$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
170 Da

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

3. COMPOSITION

DEGREE OF PURITY
> 95% (typical 99%)

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
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: Clear liquid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	< -20 °C	Measured
Boiling Point	197 ± 1 °C at 100.2 kPa	Measured
Density	885 kg/m^3 at 20.0 °C	Measured
Vapour Pressure	1.07x10^{-3} kPa at 25 °C	Measured
Water Solubility	0.114 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Kb $t_{1/2}$ at pH8 = 23.55 yr pH7 = 235.5 yr	Calculated (EPISuite HYDROWIN v2.00, US EPA, 2011)
Partition Coefficient (n-octanol/water)	log Pow = 3.75 at 20 °C	Measured
Adsorption/Desorption	log K_{oc} = 2.9	Calculated (EPISuite KOCWIN v2.00, US EPA, 2011)
Dissociation Constant	Not determined	No dissociable functionality
Surface Tension	69.4 mN/m at 20 °C	Measured
Particle Size	Not determined	The notified Chemical is a liquid.
Flash Point	72.28 °C at 100.1 kPa	Measured
Flammability limits	Not determined	-
Autoignition Temperature	300 ± 5 °C	Measured
Explosive Properties	Not explosive	The notified chemical does not contain structural groups that would imply explosive properties.
Oxidising Properties	Not oxidising	The notified chemical does not contain structural groups that would imply oxidising 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 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 be imported into Australia as a component of compounded fragrances at concentrations up to 20%.

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 will be imported into Australia as a component in a finished fragrance oils in polypropylene-lined steel drums which will be transported from the docks by roads to the notifier facility. The imported formulated fragrance will be stored until provided to customers for incorporation into cosmetic, personal care and household products. The finished final consumer products will be packaged and transported primarily by road to retail stores.

USE
The notified chemical will be used as a fragrance ingredient and incorporated into a variety of cosmetics, soaps, detergents, cleaners and other household and consumer products.

The concentrations of the notified chemical in the final finished products will vary as in the following table:

Product type	**Proposed maximum use concentration (%)**
Fine Fragrances	2.5
Leave-on cosmetics including baby products	0.1
Deodorant	0.2
Hair care products	0.1
Wash-off personal care products including baby products	1
Non-spray air freshener and candle products	20
Household and other consumer products	0.5

OPERATION DESCRIPTION
The notified chemical will not be manufactured in Australia. It will be imported into Australia in finished fragrance oils. No reformulation, processing or repackaging will occur at the notifier facility.

At the customer facilities, the procedures for incorporating the notified chemical into end-use products will likely vary depending on the nature of the formulated products. The fragrance oils containing the notified chemical at up to 20% concentration will mainly be blended with other ingredients in the manufacture of soaps, detergents, cleaners and other household products in addition to the cosmetic and personal care products.

The blending, filling and packaging processes are expected to be highly automated and closed systems with adequate ventilation are also anticipated to be used. During the formulation process, samples of the notified chemical and the finished cosmetic products will be taken for quality control testing.

The concentration of the notified chemical in the final products will vary and depend on the level of the ingredient in the finished fragrance oil and the level of the fragrance oil in the final consumer products.

6. HUMAN HEALTH IMPLICATIONS

6.1.1. Exposure Assessment

6.1.2. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and warehouse workers	Unknown	Incidental exposure only
Plant operators (blending, compounding)	4	250
Plant operators (drum handling)	1	250
Plant operators (drum cleaning/washing)	2	250
Plant operators (equipment cleaning/washing)	2	250
Plant operators (quality control)	1	250
Professional users (e.g. hair and beauty salon workers)	Not specified	Not specified

EXPOSURE DETAILS

Transport and storage
Transport and warehouse workers may come into contact with the notified chemical at up to 20% concentration, which is contained in drums or at up to 2.5% in end-use product containers, except in the event of accidental rupture of the drums or spills or leakage of the end-use product containers. Such exposure will be minimised through the use of personal protective equipment (PPE) including safety glasses, chemical resistant gloves, and protective overalls.

Reformulation
At the customer facilities, the formulation process will typically involve blending the fragrance oils with other ingredients and packaging of the finished consumer products which are expected to be automated and in enclosed systems. Workers involved in blending, dispensing, handling drums, cleaning, maintaining the equipment and product sampling for quality control may experience dermal, ocular and inhalation exposure to the notified chemical during these operations. Workers are expected to use personal protective equipment (PPE) including safety glasses, chemical resistant gloves, and protective overalls during formulation process. Adequate local ventilation and a self-contained breathing apparatus will be also used in the workplace if required. The production process will be in compliance with Good Manufacturing Practices.

End-use
Exposure to the notified chemical in end-use products (at ≤ 2.5% concentration) may occur in professions where the services provided involve the application of cosmetic and personal care products to clients (e.g. hairdressers, workers in beauty salons). The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible. Such professionals may use some PPE to minimise repeated exposure, but this is not expected to occur in all workplaces. However, good hygiene practices are expected to be in place. 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
The notified chemical is intended to be used in a variety of cosmetics, personal care, soaps, detergents, cleaners and other household and consumer products.

Public exposure to the notified chemical is expected to be widespread and frequent through daily use of personal care products containing the notified chemical at varying concentrations up to 2.5% and at up to 20% in non-spray air freshener and or candle products. Exposure to the notified chemical will vary depending on individual use patterns. The principal route of exposure will be dermal. Ocular and inhalation exposure may also occur. Incidental ingestion from the facial use of cosmetic products containing the notified chemical is also possible.

Data on typical use patterns of cosmetic product categories in which the notified chemical may be used are shown in the following 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. A dermal absorption (DA) of 100% was assumed for the notified chemical. For the inhalation exposure assessment, a 2-zone approach was used (Steiling et al., 2014; Rothe et al., 2011; Earnest, C.W, 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%, which accounts for a number of other exposure considerations (e.g., the amount ending up on the hair, as intended). A lifetime average female body weight (BW) of 64 kg (enHealth, 2012) was used for calculation purposes. It is considered that the inhalation calculations for hair spray are also sufficient to account for other cosmetic products that may be applied by spray.

Cosmetic products (dermal exposure):

Product type x	Amount (mg/day)	C (%)	RF	Daily systemic exposure (mg/kg bw/day)
Body lotion	7820	0.1	1	0.122
Face cream	1540	0.1	1	0.024
Hand cream	2160	0.1	1	0.034
Deodorant (non-spray)	1500	0.2	1	0.047
Fine fragrances	750	2.5	1	0.293
Hair styling products	4000	0.1	0.1	0.006
Shower gel	18670	1	0.01	0.029
Hand wash soap	20000	1	0.01	0.031
Shampoo	10460	1	0.01	0.016
Hair conditioner	3920	1	0.01	0.01
Total				**0.612**

C = concentration; RF = retention factor.
Daily exposure = Amount × C × RF; Daily systemic exposure = daily exposure × 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 (Zone2) (min)	Fraction Inhaled (%)	Volume (Zone 1) (m^3)	Volume (Zone 2) (m^3)	Daily systemic exposure (mg/kg bw/day)
Air fresheners (non-spray)	9.89	20	20	1	20	50	1	10	0.6439

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 1.2919 mg/kg bw/day.

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 > 5.42 mg/L/4 hour; low toxicity
Skin corrosion (EpiSkin)	non-corrosive
Skin irritation (EpiSkin)	non-irritating (not classifiable)
Rabbit, skin irritation	moderately irritating
Rabbit, eye irritation	slightly irritating
Eye irritation (BCOP)	not corrosive or severely irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Rat, repeat dose oral (diet) toxicity – 28 days.	NOAEL: 215 mg/kg bw/day (males) NOAEL: 1110 mg/kg bw/day (females)
Genotoxicity – Bluescreen HC	non genotoxic
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* Chromosome aberration	non genotoxic

Toxicokinetics, metabolism and distribution.
Based on the low molecular weight (170 Da), water solubility (0.114 g/L) and measured partition coefficient (log Pow = 3.75) of the notified chemical, dermal absorption and passive diffusion across the gastrointestinal (GI) tract are expected to occur. The notified chemical may also be absorbed across the respiratory tract.

Acute toxicity.
The notified chemical is of low acute toxicity by oral, dermal and inhalation routes.

Irritation and sensitisation.
Based on *in vivo* and *in vitro* tests, the notified chemical is a moderate skin irritant and slight eye irritant. Based on the skin irritation test in rabbits, the notified chemical would be classified under the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)* as a skin irritant Category 3. This category is not adopted in Australia.

In a local lymph node assay the notified chemical was tested at up to 50% and showed no evidence of skin sensitisation.

Repeated dose toxicity.
A NOAEL of 215 mg/kg bw/day in males was determined from a 28 day repeated dose toxicity test in rats, based on effects in the prostate, seminal vesicles and coagulating gland at 1011 mg/kg bw day. Other effects in the kidneys of male rats at both 215 and 1011 mg/kg bw/day were attributed to male rat nephropathy syndrome, and not applicable to humans. The NOAEL for females in the study was set at 1110 mg/kg bw/day, the highest dose tested.

Mutagenicity/Genotoxicity.
All provided genotoxicity tests on the notified chemical (bacteria reverse mutation, *in vitro* chromosome aberration and bluescreen HC test) showed negative results.

Health hazard classification
Based on the available information, 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.

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:

R38: Irritating to skin

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the available information the notified chemical has potential for skin and eye irritation, and showed adverse effects after repeated exposure in a study on rats. Workers may experience dermal, ocular and inhalation exposure to the notified chemical (at ≤ 2.5% concentration) during reformulation processes, including during transfers of the chemical, sampling and quality control processes, cleaning and/or maintenance of the equipment.

Control measures such as the use of enclosed automated processes and PPE (safety glasses with shields, gloves, apron or coverall), and adequate ventilation are expected to be used in place to minimise worker exposure during formulation processes.

Based on the use of measures used to mitigate exposure, the risk to workers from transport/storage and reformulation of the notified chemical is not considered to be unreasonable.

End-use
Workers involved in professions where the services provided involve the application of cosmetic products containing the notified chemical to clients (e.g., hairdressers and beauty salon workers) may be exposed to the notified chemical.

Such professionals may use PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. The risk to such workers is expected to be of a similar or lesser extent than that experienced by consumers who regularly use the various cosmetic products containing the notified chemical. For details of the public health risk assessment see Section 6.3.2.

6.3.2. Public Health
Members of the public will experience widespread and frequent exposure to the notified chemical through daily use of cosmetic products containing it at up to 2.5% and at up to 20% in non-spray air freshener and candle products.

The potential for skin and eye irritation is expected to be significantly reduced at the proposed concentrations to be used in cosmetic products. The potential systemic exposure to the public from the use of the notified chemical in cosmetic products was estimated to be 1.2919 mg/kg bw/day. Using a NO(A)EL of 215 mg/kg bw/day, which was derived from a repeated dose toxicity study on the notified chemical, the margin of exposure (MOE) was estimated to be 166. A MOE value greater than or equal to 100 is considered acceptable to account for intra- and inter-species differences, therefore, the MOE is considered to be acceptable. The risk is also acceptable for the use of baby products.

Based on the available toxicity data, the notified chemical is not considered to pose an unreasonable risk to public health under the proposed uses.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported as a component of 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 readily biodegradable (72% after 28 days). The notified chemical is not likely to bioaccumulate based on its calculated low bioconcentration factor (BCF = 138). 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 < 5 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} = 2.9). 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*
Acute Toxicity		
Fish Toxicity	96 h LC50 = 25.52 mg/L	Harmful to fish
Daphnia Toxicity	48 h EC50 = 13 mg/L NOEC = 5.8 mg/L	Harmful aquatic invertebrates
Algal Toxicity	72 h EC50 = 20 mg/L NOEC = 2.1 mg/L	Harmful 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 3: Harmful to aquatic life'. On the basis of the chronic toxicity and the ready biodegradability, the notified chemical is not classified for long-term hazard.

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 (algae, NOEC = 2.1 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		
NOEC (Algae).	2.1	mg/L
Assessment Factor	100	
PNEC:	21.0	µg/L

7.3. Environmental Risk Assessment

The Risk Quotients (Q = PEC/PNEC) for the discharge scenario have been calculated to be < 1 for the river and ocean compartments.

Risk Assessment	***PEC µg/L***	***PNEC µg/L***	***Q***
Q - River:	0.61	21	0.029
Q - Ocean:	0.06	21	0.003

As a result of its use pattern, the majority of the total annual import volume is expected to be released to the sewer. In sewage treatment plants the notified chemical is expected to sorb to sludge and/or biodegrade. 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

Melting Point/Freezing Point < -20 °C

Method	OECD TG 102 Melting Point/Melting Range.
Remarks	The test substance did not freeze in a dry ice/acetone mixture which kept at a temperature of -20 °C
Test Facility	Harlan (2013a)

Boiling Point 197 ± 1 °C at 100.2 kPa

Method	OECD TG 103 Boiling Point. EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks	DSC method (Differential Scanning Calorimetry).
Test Facility	Harlan (2014a)

Density 885 kg/m^3 at 20.0 ± 0.5 °C

Method	OECD TG 109 Density of Liquids and Solids. EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks	Pycnometer method.
Test Facility	Harlan (2014a)

Vapour Pressure $1.07x10^{-3}$ kPa at 25 °C

Method	OECD TG 104 Vapour Pressure. EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks	Vapour pressure Balance method.
Test Facility	Harlan (2014)

Water Solubility 0.114 g/L at 20 °C

Method	OECD TG 105 Water Solubility.
Remarks	Flask Method. All individual water solubility results were within ± 15% of the mean; thus, satisfying the criteria specified in the guideline.
Test Facility	Harlan (2013a)

Partition Coefficient (n-octanol/water) log Pow = 3.75 at 20 °C

Method	OECD TG 117 Partition Coefficient (n-octanol/water).
Remarks	HPLC Method.
Test Facility	Harlan (2013a)

Flash Point 72.28 °C at 100.1 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks	Closed cup method.
Test Facility	Harlan (2014)

Autoignition Temperature 300 ± 5 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks	A Carbolite flask heater was used.
Test Facility	Harlan (2014)

Explosive Properties

Method	EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks	Predicted negative based on the chemical structure of the test substance.

Test Facility Harlan (2014)

Oxidizing Properties

Method EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids).
Remarks Predicted negative based on the chemical structure of the test substance.
Test Facility Harlan (2014)

Surface Tension 69.4 mN/m at 20 °C

Method OECD TG 115 Surface Tension of Aqueous Solutions.
EC Council Regulation No 440/2008 A.5 Surface Tension.
Remarks Concentration: 90% saturated aqueous solution
Test Facility Harlan (2014a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified Chemical (97.9%)
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/Female Wistar
Vehicle	DMS
Remarks - Method	GLP Compliance. No significant protocol deviations.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity	Hunched posture and ataxia were noted in the second group of animals treated at 2000 mg/kg bw.
Effects in Organs	No abnormalities were observed at necropsy.
Remarks - Results	All animals survived until the scheduled termination and showed gains in bodyweight over the study period.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Harlan (2014e)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified Chemical (97.9%)
METHOD	OECD TG 402 Acute Dermal Toxicity. EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/Wistar
Vehicle	None
Type of dressing	Semi-occlusive.
Remarks - Method	Limit test GLP Compliance. No significant protocol deviations.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Slight erythema was noted on day 1 at the test sites of one male and all female rats.
Signs of Toxicity - Systemic	There were no treatment related signs of systemic toxicity noted in any of the animals over the study period.
Effects in Organs	No abnormalities were observed at necropsy.
Remarks - Results	All animals survived until the scheduled termination and showed gains in

bodyweight over the study period.

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

TEST FACILITY Harlan (2014f)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE Notified Chemical (97.9%)

METHOD OECD TG 403 Acute Inhalation Toxicity (2009).
EC Council Regulation No 440/2008, 93/21/EEC B.2 Acute Toxicity (Inhalation).

Species/Strain	Rat Rcc Han /WIST
Vehicle	None
Method of Exposure	Oro-nasal exposure.
Exposure Period	4 hours
Physical Form	Liquid aerosol.
Particle Size	0.43 to 9.6 μm
Remarks - Method	GLP Compliance. Mean Mass Aerodynamic Diameter (MMAD) was 3.89 μm and fraction < 4 μm was 51.4%. No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Concentration <mg/L>*		*Mortality*
		Nominal	*Actual*	
1	5M, 5F	12.1	5.42	0

LC50	> 5.42 mg/L/4 hours
Signs of Toxicity	Increased respiratory rate, hunched posture, pilo-erection and wet fur were observed, and were considered by the study authors to be common and not test-material related. Animals recovered and appeared normal from Days 3 to 6 post-exposure.
Effects in Organs	No abnormalities were observed at necropsy.
Remarks - Results	All animals showed body weight losses or no body weight gains one day after dosing. All male animals showed reasonable body weight gains during the recovery period. This recovery was slower in female animals and two females had slight body weight losses in the second week of the recovery period. In general the body weights of females 14 days after dosing was close to the pre-dosing weight.

CONCLUSION The notified chemical is of low toxicity via inhalation.

TEST FACILITY Harlan (2014g)

B.4. Irritation – skin (in vitro)

TEST SUBSTANCE Notified Chemical (97.9%)

METHOD OECD TG 431 In vitro Skin Corrosion - Human Skin Model Test
EC Council Regulation No 440/2008 B.40 Bis. In vitro Skin Corrosion - Human Skin Model Test
EpiSkin™ Reconstituted Human Epidermis Model

Vehicle	None
Remarks - Method	GLP Compliance. No significant protocol deviations. The test substance (50 μL) was applied to the tissues in duplicate for

exposure period of 3, 60 and 240 minutes prior to treatment with MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide).
Negative control used was 0.9% sodium chloride and the positive control was glacial acetic acid.
The optical densities were determined at 562 nm.
The study authors used the criterion in the Episkin INVITTOX No 118 protocol (relative mean tissue viability ≥ 35% with 240 minutes treatment time) to determine if materials are non-corrosive.

RESULTS

Test material	*Mean OD_{562} of duplicate tissues (240 minutes)*	*Relative mean Viability (%)*
Negative control	1.106	100
Test substance	1.463	132.3
Positive control	0.079	7.1

Remarks - Results
The relative mean viabilities of the test item treated tissues were:
240 minutes exposure: 132.3%
60 minutes exposure: 113.8%
3 minutes exposure: 115.1%
It was demonstrated that the test substance did not directly reduce MTT. The positive and negative controls met the criteria set by the test laboratory, confirming the validity of the test system.

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

TEST FACILITY
Harlan (2014h)

B.5. Irritation – skin (in vitro)

TEST SUBSTANCE
Notified chemical (97.9%)

METHOD
OECD TG 439 In vitro Skin Irritation: Reconstructed Human Epidermis Test Method (2010)
EpiSkin™ Reconstituted Human Epidermis Model

Vehicle
None

Remarks - Method
The test substance (10 µL) was applied to the tissues in triplicate. Following 15 minute exposure periods, the tissues were rinsed and then incubated at 37 °C for approximately 42 hours, prior to treatment with MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

Positive (sodium dodecyl sulphate; 5%) and negative (phosphate buffered saline) controls were run in parallel with the test substance.

The optical densities were determined at 562 nm.

RESULTS

Test Material	*Mean OD_{562} of triplicate tissues*	*± SD of OD_{562}*	*Relative mean viability (%)*	*± SD of relative mean viability (%)*
Negative Control	0.857	0.043	100*	5.0
Positive Control	0.044	0.010	5.2	1.1
Test Substance	0.704	0.009	82.2	1.1

OD = optical density; SD = standard deviation
*The mean viability of the negative control tissues is set as 100%.

Remarks - Results
The relative mean viability of the test substance treated tissues was 82.2% after a 15-minute exposure period and 42 hours post-exposure incubation period.

It was confirmed that the test substance does not directly reduce MTT.
The positive and negative controls gave satisfactory results, confirming the validity of the test system.
A mean tissue viability of > 50% is considered as non-irritating (below the level for classification).

CONCLUSION The notified chemical was considered to be below the level for classification for skin irritation under the conditions of the test.

TEST FACILITY Harlan (2014i)

B.6. Irritation – skin

TEST SUBSTANCE Notified chemical (97.9%)

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion (2002).
EC Council Regulation No 440/2008 B.4 Acute Toxicity (Skin Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3M
Vehicle	None
Observation Period	14 days
Type of Dressing	Semi-occlusive.
Remarks - Method	There were no deviations from protocol.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	2	2	1.7	2	< 14 d	0
Oedema	2	1	1	2	< 7 d	0

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

Remarks - Results The erythema extended approximately 10 mm around all treated skin sites at the 24 and 48 h observations. Well-defined erythema and slight oedema were noted at all treated skin sites at the 72-hour observation. Very slight erythema and slight desquamation were noted in all treated animals at the day 7 observation.

CONCLUSION The notified chemical is irritating to the skin.

TEST FACILITY Harlan (2014j)

B.7. Irritation – eye

TEST SUBSTANCE Notified chemical (97.9%)

METHOD OECD TG 405 Acute Eye Irritation/Corrosion (2012).
EC Council Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3F
Observation Period	7 days
Remarks - Method	There were no deviations from protocol.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0.67	1	0.67	2	< 7 d	0
Conjunctiva: chemosis	0	0.67	0.33	2	< 72 h	0
Conjunctiva: discharge	0	0	0	2	< 24 h	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	1	< 24 h	0

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

Remarks - Results: Two treated eyes appeared normal at the 72 hour observation and one treated eye appeared normal at the day 7 observation.

CONCLUSION: The notified chemical is slightly irritating to the eye.

TEST FACILITY: Harlan (2014k)

B.8. Irritation – eye (in vitro)

TEST SUBSTANCE: Notified chemical (97.9%)

METHOD: Compatible with OECD TG 437 Bovine Corneal Opacity and Permeability Assay (2009).

Vehicle: Deionised water

Remarks - Method: Assessment of the potential test substance to cause ocular irritancy potential to the isolated bovine corneas.
Two tests were performed as the prediction was not clearly identified in all corneas in test one.
0.9% w/v sodium chloride solution was used as negative control and ethanol as positive control.
The test method does not evaluate iridial or conjunctival effects.

RESULTS

Test material	*Mean opacities of triplicate tissues (SD)*	*Mean permeabilities of triplicate tissues*	*IVIS*
Vehicle control	0.3	0.019	0.6
*Test substance**	6.3	0.051	7.1
*Positive control**	21.3	1.267	40.3

IVIS = in vitro irritancy score
*Corrected for background values

Remarks - Results: Post-treatment the corneal epithelium appeared cloudy in the positive control group, and clear in the negative control and test groups. Results from the two test method endpoints, opacity and permeability, were combined in an empirically derived formula to generate an In Vitro Irritancy Score (IVIS) for each group. The positive and negative control results were within acceptable limits set by the study authors. Standard deviation values were not calculated. The IVIS of the test group was below 55, the criterion for defining it as a severe irritant/corrosive. The IVIS value of 7.1 suggests that it has some irritancy potential.

CONCLUSION: The test substance is not an ocular corrosive or severe irritant under the conditions of the test.

TEST FACILITY: Harlan (2014l)

B.9. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Notified chemical (99.0%)
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay (2010) EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)
Species/Strain	Mouse/ CBA/Ca
Vehicle	Acetone/olive oil 4:1
Preliminary study	Yes
Positive control	Conducted in parallel with the test substance using α-Hexylcinnamaldehyde, tech., 85%, at a concentration of 25% v/v in acetone/olive oil 4:1.
Remarks - Method	No significant protocol deviations. A preliminary toxicity study (using two mice) was performed with the test substance at 50% or 25% v/v in acetone/olive oil 4:1, and was used to select the concentrations for the main test. No mortality, signs of local skin irritation, increase in ear thickness > 25% or systemic toxicity were noted.

RESULTS

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response Mean DPM/lymph node(SD)*	*Stimulation Index (results)*
0 (vehicle control)	5F	3657.61 (±881.17)	NA
Test Substance			
10	5F	3074.95 (±1309.14)	0.84
25	5F	4122.52 (±1082.76)	1.13
50	5F	6843.39 (±3925.81)	1.87
Positive Control	5F	37284.28 (±9277.46)	10.19

Sd: Standard Deviation

Remarks - Results	There were no mortality and no signs of systemic toxicity were noted in the test or control animals during the test. The stimulation index of the test substance did not exceed 3 at any of the tested concentrations. The positive control performed as expected, verifying the sensitivity of the test.
CONCLUSION	There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	Harlan (2013b)

B.10. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical (97.9%)
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rats/Wistar Han™:RccHan™:WIST
Route of Administration	Oral – diet
Exposure Information	Total exposure days: 28 days Dose regimen: dietary concentrations of 0, 500, 3500 and 15000 ppm (equivalent to a mean achieved dosage of 26.4, 215.1 and 1010.7 mg/kg bw/day for males and 28.5, 254.3 and 1110.3 mg/kg bw/day for females respectively) 7/7 days per week.

Two recovery groups, each of five males and five females, were treated with the high dose (15000 ppm) or basal laboratory diet for twenty-eight consecutive days and then maintained without treatment for a further fourteen days.

Remarks - Method

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration*		*Mortality*
		Males	*Females*	
control	5M, 5F	0	0	0
recovery control	5M, 5F	0	0	
low dose	5M, 5F	500 ppm (26.4 mg/kg bw/day)	500 ppm (28.5 mg/kg bw/day)	0
mid dose	5M, 5F	3500 ppm (215 mg/kg bw/day)	3500 ppm (254.3 mg/kg bw/day)	0
high dose	5M, 5F	15,000 ppm (1010.7 mg/kg bw/day)	15,000 ppm (1110.3 mg/kg bw/day)	0
recovery high dose	5M, 5F	15,000 ppm (1010.7 mg/kg bw/day)	15,000 ppm (1110.3 mg/kg bw/day)	0

Mortality and Time to Death

No unscheduled mortality was observed in any treatment group.

Clinical Observations

There were no clinical signs of toxicity noted. There were no changes in the behavioural parameters functional performance or sensory reactivity that were considered treatment related.

Body weight gains for high dose male and female rats were statistically significant different from controls for the first week of treatment and continued to be slightly lower in males of this group during the test period.

High dose animals had slightly lower food consumption compared to controls. This occurred throughout the treatment period for males and only in the first week for females.

No effects on water intake were observed in any group during the period of the treatment.

Main phase and recovery female rats showed evidence of oestrous cycle, except that one recovery control female was acyclic.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There were no changes considered to be toxicologically significant in any of the haematological, blood chemistry or urinalytical parameters measured.

Effects in Organs

No macroscopic abnormalities were detected for any treated female rats. In the high dose group, male rats had a statistically significant reduction in prostate and seminal vesicle weight at the end of the treatment period and two rats in this group showed small prostate and seminal vesicles at necropsy. This was associated with minimal to moderate degree hypoplastic acinar epithelium in the prostate, seminal vesicles and coagulating gland in three high dose male rats. These effects were considered by the study authors to be adverse, although not accompanied by effects in the testis/epididymis.

Other changes in organ weights included increases in liver weight in both male and female high dose animals, with liver weight in high dose recovery females still significantly high, and a reduction in thyroid weight in high dose females. These changes were not associated with histopathological effects.

Microscopic alterations were noted in the kidneys (slight degree glandular casts in two male rats, slight increased incidence in corticomedullary tubular basophilia and increased severity to moderate cortical hyaline droplets were present at in all high dose male rats. Cortical hyaline droplets were also present at increased severity up to moderate in all mid dose male rats. .

Following the fourteen day treatment-free recovery phase the following treatment related findings were recorded in high dose male rats. Granular casts at minimal degree were noted in one instance. Corticomedullary tubular basophilia remained at slightly increased incidence and severity (up to slight) in four animals. Cortical hyaline droplets were present at minimal to moderate severity in all 5 male rats.

Remarks – Results
The effects in the kidneys of male rats were considered by the study authors to be male rat nephropathy syndrome, which is a species and sex specific condition in rats and therefore normally does not represent a risk to humans.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 3500 ppm for males (equivalent to 215.1 mg/kg bw/day) and 15000 ppm for females (equivalent to 1110.3 mg/kg bw/day) in this study, based on the microscopic findings (prostate, seminal vesicles and coagulating glands and the associated changes in the weight of these organs).

TEST FACILITY	Harlan (2015)

B.11. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	BlueScreen HC Assay
Cell Type/Cell Line	Genetically modified strain of cultured human lymphoblastoid TK6 cells (GLuc-T01; GLuc reporter system is reported to exploit the proper regulation of the GADD45a gene, which mediates the adaptive response to genotoxic stress)
Metabolic Activation System	S9 fraction from Aroclor-1254 induced rat liver.
Concentration Range in Test	a) With metabolic activation (used as the positive control): 625-10,000 µM (~151- 1,781 µg/mL) b) Without metabolic activation: 625-10,000 µM (~151- 1,781 µg/mL)
Vehicle	DMSO and water
Remarks - Method	Utilised a 96-well microplate format, testing the test substance with the vehicle and positive controls, over 8 dilutions. - BlueScreen HC assay (without metabolic activation): The microplates containing the test substance and medium were covered with a breathable membrane and incubated at 37 °C with 5% CO_2 and 95% humidity for 48 hours. The plates are then assessed using fluorescence measurements to determine cell density, then using flash luminescence to determine genotoxicity. - BlueScreen HC S9 assay (with metabolic activation): wells containing the test substance, S9 fraction and medium were incubated (as above) for 3 hours, then washed, harvested and allowed to recover in medium for 45 hours at 37 °C with 5% CO2 and 95% humidity. The plates were then assessed similar to that above. Reduced (≤ 80%) cell density compared to untreated cells (vehicle control) was used to provide a measure of cytotoxicity of the test substance. Increased (1.8 fold without metabolic activation; 1.5 fold with metabolic activation) induction of GLuc expression relative to the vehicle control was used to provide a measure of genotoxicity of the test substance. Where a positive result was obtained, the Lowest Effective Concentration (LEC; µM) was determined. Vehicle and positive (without metabolic activation: 4-nitroquinoline-1-oxide (4-NQO) at 0.125 and 0.5 µg/mL; with metabolic activation: cyclophosphamide at 5 and 25 µg/mL) controls were used in parallel with the test substance.

RESULTS

Metabolic Activation	*Cytotoxicity*	*LEC (µM)*	*Genotoxic Effect*	*LEC (µM)*
Absent	negative	-	negative	-
Present	negative	-	negative	-

*LEC = Lowest Effective Concentration for a positive result

Remarks - Results: In the presence and absence of metabolic activation, the test substance was negative for genotoxicity at up to 10,000 µM in the standard BlueScreen HC assay. Intra-assay quality control checks passed test criteria following the standard protocol. No cytotoxicity was observed.

CONCLUSION: The notified chemical was considered by the study authors to be not genotoxic under the conditions of the test.

TEST FACILITY: Gentronix Limited (2012)

B.12. Genotoxicity – bacteria

TEST SUBSTANCE: Notified chemical (97.9%)

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

Species/Strain: *S. typhimurium*: TA1535, TA1537, TA98, TA100, *E. coli*: WP2uvrA
Metabolic Activation System: S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver.
Concentration Range in Main Test: a) With metabolic activation: 5 – 5,000 µg/plate
b) Without metabolic activation: 5 – 5,000 µg/plate
Vehicle: Dimethyl sulphoxide
Remarks - Method: No significant protocol deviations.
GLP Compliance.

A preliminary test was conducted using TA100 and WP2uvrA in the presence and absence of metabolic activation between 0.15 – 5,000 µg/plate, in order to choose concentrations for the main tests.

Vehicle and positive controls were used in parallel with the test material.
Positive controls: i) without S9: N-ethyl-N'-nitro-N-nitrosoguanidine (used as the positive control for the tester strains: WP2uvrA-, TA100, TA1535), 9-aminoacridine (TA1537) and 4-nitroquinoline-1-oxide (TA98); ii) with S9: 2-aminoanthracene (TA100, TA1535, TA1537, WP2uvrA-) and benzo(a)pyrene (TA98).

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 1,500 (TA100)	≥ 1,500	≥ 5,000	negative
Test 2	≥ 5,000 (WP2uvrA)	≥ 500	≥ 5,000	negative
Present				
Test 1	≥ 1,500 (TA100)	≥ 1,500	≥ 5,000	negative
Test 2	≥ 5,000 (WP2uvrA)	≥ 500	≥ 5,000	negative

Remarks - Results: The test material in test 1 caused a visible reduction in the growth of the bacterial background lawns of all tester strains (except TA98), at ≥ 1,500 µg/plate both in the presence and absence of metabolic activation.
The test material in test 2 induced a stronger toxic response (all strains) with weakened bacterial background lawns initially noted from 500

µg/plate in both the absence and presence of S9-mix. A test item precipitate (globular in appearance) was noted at 5000 µg/plate.

No significant increases in the frequency of revertant colonies were recorded for any of the strains of bacteria, at any dose level either with or without metabolic activation or exposure method.

Both vehicle and positive controls produced satisfactory responses, thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

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

TEST FACILITY Harlan (2013c)

B.13. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical (97.9%)

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Cell Type/Cell Line Human Peripheral Lymphocytes
Metabolic Activation System Phenobarbitone/β-naphthoflavone induced rat liver (S9 homogenate)
Vehicle Dimethyl sulfoxide
Remarks - Method No significant protocol deviations, except that no analyses were carried out to confirm homogeneity, concentration or stability.
Mytomycin C (MMC) and Cyclophosphamide (CP) were used as positive controls in the absence and presence of metabolic activation respectively.
The doses selected for the study were based on the outcomes of a preliminary study (cytotoxicity and/or the presence of precipitate).

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 13.28*, 26.56*, 53.13*, 106.25*, 212.5, 425, 850 and 1700, MMC 0.4*	4	20
Test 2	0*, 12.5, 25, 50*, 75*, 100*, 200, MMC 0.2*	24	24
Present			
Test 1	0*, 13.28, 26.56*, 53.13*, 106.25*, 212.5, 425, 850 and 1700, CP 5*	4	20
Test 2	0*, 12.5, 25*, 50*, 100*, 150, 200, CP 5*	4	20

*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	≥26.56	≥106.25	≥425	negative
Test 2		≥100	Not present	negative
Present				
Test 1	≥212.5	≥106.25	≥850	negative
Test 2		≥100	Not present	negative

Remarks - Results There was dose related inhibition of the mitotic index, with 65% and 37% inhibition at 106.25 µg/mL without and with metabolic activation respectively. The test item did not induce any statistically significant increases in the frequency of cells with aberrations, in either of two experiments, using a dose range that was limited by the test item induced

	toxicity. No statistically significant increases in polyploidy cells were observed. The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.
CONCLUSION	The notified chemical was not clastogenic to human peripheral blood lymphocytes treated in vitro under the conditions of the test.
TEST FACILITY	Harlan (2014m)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test.
Inoculum	Activated Sludge
Exposure Period	28 days
Auxiliary Solvent	None reported
Analytical Monitoring	Biological oxygen demand (BOD)
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. A test concentration of 100 mg/L was selected for use in the study following the recommendations of the Test Guideline. Control solutions with inoculum and the reference item, aniline, together with a toxicity control were used for validation purposes.

RESULTS

Test substance		*aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
1	0	1	0
7	9	7	37
14	55	14	58
21	68	21	72
28	72	28	75

Remarks - Results	The validity criteria for the test were met. The test item attained 72% biodegradation after 28 days and satisfied the 10-Day window validation criterion, whereby 60% biodegradation must be attained within 10 days of the biodegradation exceeding 10%.
CONCLUSION	The notified chemical is readily biodegradable.
TEST FACILITY	Harlan (2014b)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified Chemical
METHOD	HJ/T 155-2004 The Guidelines of Good Laboratory Practices on Chemical Testing. MEP. China – Semi static.
Species	Zebrafish (*Brachydanio rerio*)
Exposure Period	96 hours
Auxiliary Solvent	None reported
Water Hardness	45 mg $CaCO_3$/L
Analytical Monitoring	HPLC-MS
Remarks – Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. The table below shows the cumulative death values for three test samples (7 fish in each sample).

RESULTS

Concentration mg/L	*Number of Fish per sample*	*Cumulative death*			
Nominal		*24 h*	*48 h*	*72 h*	*96 h*
0	7	0	0	0	0

15	7	0	0	0	0
18	7	0	0	0	0
21.6	7	0	0	1	1
25.9	7	12	14	14	16
31.1	7	21	21	21	21

LC50 24.524 mg/L at 96 hours.

Remarks – Results The validity criteria for the test were met. The LC50 (96h) of the notified chemical to zebrafish was 24.524 mg/L which was calculated as nominal concentrations, and the 95% confidence limit was 23.500-25.533 mg/L under the current test conditions.

CONCLUSION The notified chemical is harmful to fish

TEST FACILITY Suzhou (2013)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified Chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - Static.

Species *Daphnia magna*
Exposure Period 48 hours
Auxiliary Solvent None reported
Water Hardness 250 mg $CaCO_3$/L
Analytical Monitoring GC-MS
Remarks - Method Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

RESULTS

Concentration mg/L	*Number of D. magna*	*Number Immobilised*	
Nominal		*24 h*	*48 h*
Control	10	0	0
0.1	10	1	1
1.0	10	0	0
10.0	10	0	0
100.0	10	10	10

LC50 13 mg/L at 48 hours
NOEC 5.8 mg/L at 48 hours

Remarks - Results The validity criteria for the test were met.

CONCLUSION The notified chemical is harmful to aquatic invertebrates.

TEST FACILITY Harlan (2014c)

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: 0.1, 1.0, 10.0 and 100.0 mg/L
Actual: 1.0, 3.2, 10.0, 32.0 mg/L
Auxiliary Solvent None reported

Water Hardness	Not reported.
Analytical Monitoring	Gas Chromatography
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. Due to the potential volatile nature of the test item, testing was conducted in completely filled, stoppered test vessels in order to minimise possible losses due to volatilisation. In order to prevent inhibition of growth due to the restriction of gaseous exchange, additional sodium bicarbonate was added to the culture medium to provide a source of carbon dioxide for algal growth.

RESULTS

Biomass		*Growth*	
EyC50	*NOEC*	*ErC50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
8.4 mg/L (95% confidence limits 6.8 - 10 mg/L)	2.1	20 mg/L (95% confidence limits 14 - 28 mg/L)	2.1

Remarks - Results	The validity criteria for the test were met.

CONCLUSION The notified chemical is harmful to algae

TEST FACILITY Harlan (2014d)

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