File No: STD/1562

October 2015

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

2-Heptanol, 3,6-dimethyl-

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:

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1562	Kao Australia Pty Ltd	2-Heptanol, 3,6-dimethyl-	Yes	≤ 10 tonne/s 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 Sensitisation (Category 1B)	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 (Category 3)	H402 - Harmful to aquatic life

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 ≤ 0.01% in cosmetics and household 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 Sensitisation (Category 1B): 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.

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 physical containment, collection and subsequent safe disposal.

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory

obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

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

(1) Under Section 64(1) of the Act; if
- the notified chemical exceeds or is intended to exceed 0.01% in cosmetics and household products.

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from 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)
Kao Australia Pty Ltd (ABN: 059 054 708 299)
Level 1
19 – 23 Prospect Street
Box Hill VIC 3128

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: analytical data, degree of purity, impurities, use details, and import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: all physical-chemical endpoints.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None.

NOTIFICATION IN OTHER COUNTRIES
European Union (REACH, 2015)
US (TSCA, 2015)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
TERPIROSA

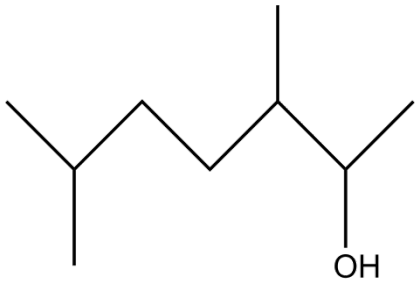
CAS NUMBER
1247790-47-1

CHEMICAL NAME
2-Heptanol, 3,6-dimethyl-

OTHER NAME(S)
3,6-Dimethyl-2-heptanol
3,6-dimethylheptan-2-ol

MOLECULAR FORMULA
$C_9H_{20}O$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
144.3 Da

ANALYTICAL DATA
Reference NMR and IR, spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Colourless, transparent liquid with aromatic odour.

Property	Value	Data Source/Justification
Melting Point/Freezing Point	< -20 °C	Measured
Boiling Point	186 °C at 102.4 kPa	Measured
Density	823 kg/m^3 at 20 °C	Measured
Vapour Pressure	0.063 kPa at 25 °C	(M)SDS
Water Solubility	0.557 g/L at 20 ± 0.5 °C	Measured.
Hydrolysis as a Function of pH	$t_{½}$ > 1 year at 25 °C (pH 4, 7 and 9)	Measured
Partition Coefficient (n-octanol/water)	log Pow = 3.47	Measured. Expected to partition to the interface between octanol and water, based on its surfactant properties
Surface Tension	40.7 mN/m at 21.8 °C	Measured
Adsorption/Desorption	log K_{oc} = 2.54	Measured. Expected to partition to phase boundaries based on its surfactant properties.
Dissociation Constant	Not determined	Does not contain dissociable functionality
Flash Point	77 °C	(M)SDS
Autoignition Temperature	276 °C	(M)SDS
Explosive Properties	Not determined	Not expected to be explosive based on chemical structure
Oxidising Properties	Not determined	Not expected to be an oxidiser 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 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*
Flammable Liquids (Category 4)	H227 – Combustible liquid

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as the raw material (> 90% concentration), or as a component of a fragrance ingredient (at < 5% concentration) for formulation of cosmetic and household products. The notified chemical will also be imported as a component of finished cosmetic products (at ≤ 0.01% concentration).

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 10	< 10	< 10	< 10	< 10

PORT OF ENTRY
Brisbane, Melbourne, Sydney and Perth

IDENTITY OF MANUFACTURER/RECIPIENTS
Kao Australia Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemical will be transported in 30 L or 200 L drums (when imported as the raw material at > 90% concentration), or in 1 L aluminium flasks or 205 L drums (when imported as a component of a fragrance ingredient at < 5% concentration). The notified chemical may also be imported as a component of end use products (at ≤ 0.01% concentration) which will be packaged in various types of containers suitable for retail sale.

USE
The notified chemical will be used as an ingredient in cosmetic and household products at concentrations up to 0.01%.

OPERATION DESCRIPTION
The notified chemical will be imported its raw form or as a component of a fragrance ingredient (at < 5% concentration) for formulation of cosmetic and household products, or as a component of finished cosmetic products (at ≤ 0.01% concentration) which will be sold to the public in the same form in which they are imported.

Reformulation
When reformulated, the notified chemical (at > 90% concentration), or as a component of a fragrance ingredient (containing the notified chemical at < 5% concentration) will be blended into end-use consumer products at customer sites. Procedures will vary depending on the nature of the cosmetic or household product being formulated. Both manual and automated steps will likely be involved. For example, a sample of the notified chemical will be taken manually for QA purposes, while automated processes may include mixing and filling of end-use containers with products.

End-use
Finished products containing the notified chemical at ≤ 0.01% concentration will be used by the public and may also be used by professionals such as workers in beauty salons. Depending on the nature of the product, these could be applied by hand or by using 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	1	24
Reformulation workers	8	24
Retail workers	0.2 - 2	200

EXPOSURE DETAILS
Transport, storage and retail workers may come into contact with the notified chemical at > 90% concentration, as a component of a fragrance ingredient (at < 5% concentration) or at ≤ 0.01% concentration in cosmetics and household products only in the event of accidental rupture of packages.

Reformulation
During reformulation into cosmetics and household products, dermal, ocular and inhalation exposure of workers to the notified chemical at > 90% concentration may occur. Exposure is expected to be minimised through the use of exhaust ventilation and/or automated/enclosed systems as well as through the use of personal protective equipment (PPE) such as coveralls, eye protection, impervious gloves and respiratory protection (as appropriate).

End-use
Exposure to the notified chemical in end-use products at ≤ 0.01% concentration may occur in professions where the services provided involve the application of cosmetic products to clients (e.g. workers in beauty salons) or where laundry services are provided. The principal route of exposure will be dermal, while ocular 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. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical.

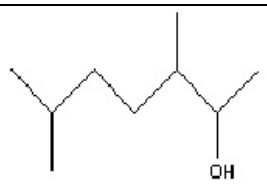
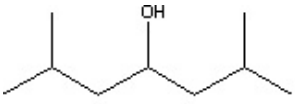
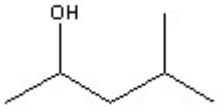
6.1.2. Public Exposure
There will be widespread and repeated exposure of the public to the notified chemical at ≤ 0.01% concentration through the use of cosmetics and household products. The principal route of exposure will be dermal. Accidental ocular and oral exposure (from the use of facial products) is also possible. Inhalation exposure is not expected based on the use pattern and the low vapour pressure of the notified chemical.

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*
Skin irritation (in vitro) - EpiSkin™ reconstituted human epidermis model	non-irritating
Eye irritation (in vitro) – Bovine Corneal Opacity and Permeability test	non-corrosive or severely irritating
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation at 92.7%
Rat, repeat dose oral toxicity – 90 days.	NOAEL 40 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian cell micronucleus test	non genotoxic
Genotoxicity – in vitro mammalian cell gene mutation test	non genotoxic
Rat, reproductive and developmental toxicity	NOAEL 1000 mg/kg bw/day

Toxicity information is also available on two analogues of the notified chemical. A comparison of the structural and physicochemical properties of the analogue chemicals with the notified chemical is provided below.

	Notified Chemical	**Analogue 1**	**Analogue 2**
Chemical Name	3,6-Dimethyl-2-heptanol	2,6-Dimethyl-4-heptanol	4-methyl-2-pentanol
CAS Number	1247790-47-1	108-82-7	108-11-2
Structural Formula			
Molecular Weight	144.3 Da	144.3 Da	102.2 Da
Water Solubility	0.557 g/L at 20 °C	0.614 g/L (Belsito *et al*., 2010)	13.8 g/L (Belsito *et al*., 2010)
Partition Coefficient (Log Pow)	log Pow = 3.47	3.08 (Belsito *et al*., 2010)	1.68 (Belsito *et al*., 2010)

Toxicokinetics, metabolism and distribution.
No toxicokinetic data on the notified chemical were submitted. For dermal absorption, molecular weights below 100 Da, water solubility between 100-10,000 mg/L and Log Pow values between 1 and 4 are favourable for absorption (ECHA, 2014). Therefore, the dermal absorption of the notified chemical is expected to be high. Similar branched chain saturated alcohols have been shown to be absorbed through the skin, gastrointestinal tract and the respiratory tract (Belsito *et al*., 2010).

Acute toxicity.
There were no acute toxicity studies provided on the notified chemical. In repeated dose oral toxicity studies the notified chemical was found to be of low toxicity at doses up to 1,000 mg/kg bw/day, and hence acute toxicity at doses up to 2,000 mg/kg bw is not expected. Additionally analogues 1 and 2 were found to have low acute oral and dermal toxicity (McGinty *et al*., 2010a and McGinty *et al*., 2010b). However, when exposed to analogue 2, 5/6 rats died within 14 days after 8 hour exposure at 2,000 ppm and in a separate experiment in mice, sleepiness and anaesthesia were observed following exposure to saturated air for 4 – 15 hour, with mortality recorded at 10 hours (6/10 mice) and 15 hours (8/10 mice) (McGinty *et al*., 2010b). Analogue 1 was reported to cause rats to die after 8 hours exposure to a saturated vapour, but mice exposed to a saturated vapour for 12 hours did not die (McGinty *et al*., 2010a). Full study reports for the analogue acute toxicity studies were not provided.

Irritation.
The notified chemical is not expected to be a skin irritant based on an *in vitro* (EpiSkin™ reconstituted human epidermis) model. Analogues 1 and 2 were shown to be slight skin irritants when tested on rabbits (McGinty *et al*., 2010a and McGinty *et al*., 2010b).

The notified chemical is not expected to be corrosive or highly irritating to the eye based on an *in vitro* Bovine Corneal Opacity and Permeability test. However, moderate to severe eye irritation was observed in rabbits following exposure to undiluted samples of analogues 1 and 2 (McGinty *et al*., 2010a and McGinty *et al*., 2010b).

Vapours of analogue 1 were reported to cause eye irritation at 5 ppm and nose and throat irritation at 10 ppm (McGinty *et al*., 2010a).

Overall based on the available information, the notified chemical is not expected to be irritating to the skin but may be irritating to the eye.

Sensitisation.
The notified chemical was found to be a skin sensitiser in mice (Local Lymph Node Assay; stimulation indices of 2.4, 2.9 and 3.1 at 25, 50 and 92.7% concentrations, respectively). Based on the results of this study, the EC_3 value was 71.4%.

Repeated dose toxicity.
A 90 day (including 4 week recovery control and high-dose groups), repeated dose oral study was conducted on rats at doses of 40, 200 and 1,000 mg/kg bw/day.

A NOAEL of 1,000 mg/kg bw/day was established based on an absence of toxicologically relevant adverse effects at all doses.

Mutagenicity/Genotoxicity.
The notified chemical was found to be non-mutagenic in a bacterial reverse mutation assay and non-genotoxic in an *in vitro* mammalian cell micronucleus test (human lymphocytes) and an *in vitro* mammalian cell gene mutation test (Chinese Hamster cells).

Reproductive and developmental toxicity.
A reproductive and developmental toxicity study found no adverse effects on reproductive ability (including delivery and lactation). No treatment related effects were observed on the number of live offspring (or number delivered), viability, sex ratios and body weights following exposure to the test substance.

Males in the high-dose group exhibited an increase in hyaline droplet in the proximal tubules and/or regeneration with hypercellularity of tubules in the kidneys. However, these histopathological findings are considered to be species specific and were not considered by the study authors when determining the NOAEL of 1,000 mg/kg bw/day.

Phototoxicity
Branched chain saturated alcohols such as the notified chemical are not expected to produce phototoxic or photoallergic responses (Belsito *et al*., 2010).

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 Sensitisation (Category 1B)	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

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Reformulation
Based on the available information for the notified chemical and analogous branched chain saturated alcohols, the notified chemical is a skin sensitiser and may be irritating to eyes. The main route of exposure is expected to be dermal with some potential for accidental ocular or oral exposure. Therefore, caution should be exercised when handling the notified chemical during reformulation and quality control processes. The use of exhaust ventilation and/or automated/enclosed systems as well the use of personal protective equipment (PPE) such as coveralls, eye protection, impervious gloves and respiratory protection (as appropriate) should minimise the potential for exposure. Therefore, provided that adequate control measures are in place to minimise worker exposure, the risk to workers from use of the notified chemical is not considered to be unreasonable.

End-use
Workers involved in professions where cosmetic and/or household services are provided (*e.g.*, beauticians, hospitality and laundry workers) may be exposed to the notified chemical at concentrations of ≤ 0.01%. If PPE is used, the risk to these workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical (for details of the public health risk assessment, see Section 6.3.2).

6.3.2. Public Health
Cosmetic and household products containing the notified chemical will be available to the public at ≤ 0.01% concentration. The main route of exposure is expected to be dermal with some potential for accidental ocular or oral exposure.

Local effects
The notified chemical is not expected to be irritating at the proposed concentrations.

A significant risk associated with use of the notified chemical is its potential to cause sensitisation by skin contact. 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). Using a worst case scenario example for all of the proposed products that may contain the notified chemical, the Consumer Exposure Level (CEL) is estimated to be 0.27 μg/cm^2 (Cadby *et al*., 2002).

When tested in an LLNA study the EC_3 value was 71.4%. Consideration of the study details and application of appropriate safety factors allowed the derivation of an Acceptable Exposure Level (AEL) of 49.15 μg/cm^2. In this instance, the factors employed included an interspecies factor (3), intraspecies factor (10), a matrix factor (3.16), a use and time factor (3.16), and a database factor (1), giving an overall safety factor of 300.

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 cosmetic products at ≤ 0.01% concentration is not considered to be unreasonable. Based on

the generally lower expected exposure level from household products (containing ≤ 0.01% 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 the aggregate exposure has not been conducted.

Systemic effects
The potential for the notified chemical to induce systemic toxicity is expected to be low based on the absence of toxicologically relevant adverse effects at doses up to 1,000 mg/kg bw/day in a 90 day repeated dose study.
Therefore, based on the information available, the risk to the public associated with the use of the notified chemical at ≤ 0.01% in cosmetics and household products is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

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

During reformulation processes, limited release of the notified chemical is expected from cleaning of equipment as washings will be reused. A total of up to 0.2% of the import volume is estimated to be generated as waste from residues in empty containers and spills during reformulation. Empty containers containing the notified chemical will either be recycled or disposed of through an approved waste management facility.

RELEASE OF CHEMICAL FROM USE

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

RELEASE OF CHEMICAL FROM DISPOSAL

It is estimated that a small amount of the 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

For the details of the environmental fate study please refer to Appendix C. The notified chemical is readily biodegradable based on a biodegradation study of the notified chemical. The notified chemical is hydrolytically stable at pH 4, 7 and 9.

The majority of the notified chemical is expected to be released to Sewage Treatment Plants (STPs) via domestic wastewater. Based on its ready biodegradability, the notified chemical is expected to be largely degraded by sewage treatment processes. The notified chemical is expected to partition to phase boundaries as it is surface active. Therefore, the notified chemical in sewage released to STPs is expected to partition to sludge. Notified chemical remaining in treated sewage effluents is likely to be released to surface waters or applied to land when used for irrigation. Notified chemical in sewage sludge is anticipated be disposed of to landfill or applied to land when sludge is used for soil remediation. Based on its surface active property, the notified chemical is not expected to bioaccumulate. The notified chemical is expected to degrade in STPs, surface waters, soils and landfill due to its ready biodegradability to form water and oxides of carbon

The notified chemical is not expected to be significantly volatile and is not likely to volatilise to air during use or STP processes. The half-life of the notified chemical in air is calculated to be 12 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.

7.1.3. Predicted Environmental Concentration (PEC)

The notified chemical will be released to sewers following its use in cosmetic and household products. Therefore, under a worst case scenario, it is assumed that 100% of the total import volume of the notified chemical will be discharged into sewers nationwide over 365 days per year. Assuming no removal of the notified chemical in the sewage treatment processes for the worst case scenario, the resultant Predicted Environmental Concentration (PEC) in sewage effluent on a nationwide basis is estimated as follows:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	10,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	10,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	27.40	kg/day
Water use	200	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:	6.06	µg/L
PEC - Ocean:	0.61	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 6.1 µg/L may potentially result in a soil concentration of approximately 40.4 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 201.9 µg /kg and 403.9 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 the studies of the analogue can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Daphnia Toxicity	EL50 = 13 mg/L	Harmful to aquatic invertebrates
Algal Toxicity	ErL50 = 15 mg/L	Harmful to algae

On the basis of the acute toxicity data, the notified polymer is considered harmful to aquatic invertebrates and algae. Therefore, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009), the notified polymer is formally classified as Acute Category 3; Harmful to aquatic life. Based on the acute toxicity and ready biodegradability, the notified chemical has not been formally classified for long term hazard under the GHS.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) has been calculated from the acute daphnia toxicity of the notified chemical and an assessment factor of 1000 as measured acute endpoints are available for only two trophic levels.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Invertebrates).	13	mg/L
Assessment Factor	1000.00	
PNEC:	13	µg/L

7.3. Environmental Risk Assessment

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

Risk Assessment	*PEC µg/L*	*PNEC µg/L*	*Q*
Q - River:	6.06	13	0.47
Q - Ocean:	0.61	13	0.047

The risk quotient for discharge of treated effluents containing the notified chemical to the aquatic environment indicates that the notified chemical is unlikely to reach ecotoxicologically significant concentrations based on its annual importation quantity. The notified chemical is expected to have 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 household products, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Freezing Point < -20 °C

Method OECD TG 102 Melting Point/Melting Range.
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks Crystallizing point method. Test substance did not freeze.
Test Facility Harlan (2013a)

Boiling Point 186 °C at 102.4 kPa

Method OECD TG 103 Boiling Point.
EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks Differential scanning calorimetry method.
Test Facility Harlan (2013a)

Density 826 kg/m^3 at 20 ± 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 (2013a)

Water Solubility 0.557 g/L at 20 ± 0.5 °C

Method OECD TG 105 Water Solubility.
Commission Directive 92/69/EEC A.6 Water Solubility.
Remarks Flask Method.
Test Facility Harlan (2013a)

Hydrolysis as a Function of pH

Method OECD TG 111 Hydrolysis as a Function of pH.
Commission Directive 92/69/EEC C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

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

Remarks The estimated half-life of the test item at 25 for pH 4,7 and 9 has been shown to be greater than 1 year.
Test Facility Harlan (2013a)

Partition Coefficient (n-octanol/water) Log Pow = 3.47

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

Surface Tension 40.7 mN/m at 21.8 ± 0.5 °C

Method OECD TG 115 Surface Tension of Aqueous Solutions.
EC Council Regulation No 440/2008 A.5 Surface Tension.
Remarks Ring method. Concentration: 90%. Considered to be a surface active material. (< 60 mN/m)
Test Facility Harlan (2013a)

Adsorption/Desorption Log K_{oc} = 2.54

Method	OECD TG 121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)
Remarks	HPLC method
Test Facility	Harlan (2013a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. 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: None.

Remarks - Method: The test substance (10 µL) was applied to the tissues in triplicate. Following a 15 minute exposure period, the tissues were rinsed and incubated at ~37 °C in fresh medium for 42 hours. The tissues were then treated with 0.3 mg/mL MTT and incubated at ~37 °C for 3 hours. Following extraction, the optical densities were determined (540 nm).

The study authors indicated that a preliminary test had been conducted, which indicated that the test substance does not directly reduce MTT.

Positive and negative controls were run in triplicate and concurrently with the test substance:
- Negative control (NC): Phosphate Buffered Saline Dulbecco's with Ca^{++} and Mg^{++}
- Positive control (PC): Sodium Dodecyl Sulphate 5% w/v aqueous solution.

RESULTS

Test material	*Mean OD_{540} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	0.763 ± 0.016	100	2.1
Test substance	0.562 ± 0.006	73.6	0.9
Positive control	0.062 ± 0.007	8.1	0.9

OD = optical density; SD = standard deviation

Remarks - Results: Positive and negative controls performed as expected.

The relative mean viability of tissues exposed to the test substance was 73.6% after a 15 min exposure period.

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

TEST FACILITY: Harlan (2013b)

B.2. Irritation – eye (in vitro)

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants

Vehicle: None.

Remarks - Method: Concurrent positive (ethanol) and negative (0.9% w/v sodium chloride solution) controls were run.

Controls and test substance were run in triplicate.

Following exposure to sodium fluorescein, 360 µL of medium (representing each cornea) was added to a 96-well plate and the optical density at 492 nm was measured (OD_{492}).

RESULTS

Test material	*Mean opacities of triplicate tissues (SD)*	*Mean permeabilities of triplicate tissues (SD)*	*IVIS (SD)*
Vehicle control	-	0.129 (0.025)	8.9(1.9)
*Test substance**	9.7 (1.5)	0.342 (0.2)	14.8 (4.4)
*Positive control**	20 (2)	0.787 (0.145)	31.8 (3.8)

SD = Standard deviation; IVIS = in vitro irritancy score
*Corrected for background values

Remarks - Results The positive and negative controls performed as expected.

Corneas treated with test substance were clear after exposure to the notified chemical, but were cloudy after incubation with sodium fluorescein. Negative control corneas were clear after exposure and incubation steps, while positive control corneas were cloudy after exposure and incubation.

CONCLUSION The notified chemical was not corrosive or a severe eye irritant under the conditions of the test.

TEST FACILITY Harlan (2013c)

B.3. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain Mouse/CBA/J[SPF]
Vehicle Acetone:olive oil (4:1, v/v)
Preliminary study Yes
Positive control 25% α-Hexylcinnamaldehyde.
Positive control was conducted in parallel with the test substance.
Remarks - Method All test animals were female.
Pre-screening test was performed and the undiluted substance was kept as the highest concentration as it was not expected to induce an adverse response.
Test concentrations of 25% and 50% were corrected for purity.
The lymph nodes from each ear for each treatment group were pooled together for analysis.

RESULTS

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response (DPM/animal)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	4 female	760.6	-
25%	4 female	1792.1	2.4
50%	4 female	2239.0	2.9
92.7% (undiluted)	4 female	2358.2	3.1
Positive Control			
25%	4 female	4029.4	5.3

EC3 71.4%
Remarks - Results No adverse clinical signs were observed and erythema or eschar formation was not observed on the application sites.
No evident increase in ear thickness was observed during the sensitising period and no significant body weight changes were observed during the

	sensitisation period. A dose-response relationship was observed between increasing lymph node weight and tests substance concentration. Lymph node weight in the positive control group was also higher than that in the negative control group. A dose-response relationship was also observed between increasing dose and S.I index.
CONCLUSION	There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	BSRC (2012)

B.4. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents.
Species/Strain	Rat/Crl:CD(SD) [SPF]
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 90 days Dose regimen: 7 days per week Post-exposure observation period: 4 weeks
Vehicle	Corn oil
Remarks - Method	Concentrations used were corrected for purity. No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10 M, 10 F	-	0/20
low dose	10 M, 10 F	40	0/20
mid dose	10 M, 10 F	200	0/20
high dose	10 M, 10 F	1000	0/20
control recovery	10 M, 10 F	-	0/20
high dose recovery	10 M, 10 F	1000	0/20

Mortality and Time to Death

No animals died during the course of the study.

Clinical Observations

The study authors attributed slight and transient salivation in animals in the mid- and high-dose groups as due to the aromatic odour of the test substance. Any other effects were observed sporadically or did not exhibit a dose-response relationship.

Daily food consumption in males in the high-dose group was often significantly higher (weeks 3, 4, 5, 6, 8, 9, 10 and 13). Females in the high-dose group exhibited significantly increased food consumption in week 13. No significant differences were observed in food consumption in animals in the recovery groups. There were no significant effects on body weight observed in animals in any of the control or exposure groups.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Males in the high-dose group exhibited significantly lower haematocrit (↓3.3%), haemoglobin (↓2.6%) and mean corpuscular volume (↓2.9%), while the platelet (↑15.6%) and monocyte count (↑30%) were significantly higher. Males in the low-, mid- and high-dose groups exhibited lower ratios of unstained cells (↓40%, 33% and 33% respectively). Males in the low-dose group also exhibited a significantly higher ration of basophils (↑100%), although there was no statistically significant increase in the higher dose groups. These effects were not observed in females in the low-, mid- and high-dose groups, or in any of the animals exposed to a high-dose in the recovery group.

Prothrombin time (↑35.7%) and activated partial thromboplastin time (↑16.6%) were significantly increased in males in the high-dose. Fibrinogen was significantly increased in males in the mid-dose (↑11.8%) and high-dose (↑15%) groups and also in females in the high-dose-recovery group (↑13.7%). With the exception of the fibrinogen increase in females, these effects were not observed in females or males in the high-dose recovery group.

Males and females in the high-dose group exhibited higher total protein (↑8.5% male; ↑12.5% female), total cholesterol (↑40.3% male; ↑38.2% female) and calcium (↑4.9% female only) levels, while chloride (↓2.4% male; ↓2.2%) and glucose (↓15.1% female only) were lower. γ-Glutamyl transpeptidase (↑66.7%) was also higher in males in the high-dose group. Lower potassium (↓8.0%) levels (high-dose group), increased levels of total protein (↑5.7%) (low-dose) and sodium (↑1.2%) (mid-dose) were also observed in females. Females in the recovery group did not exhibit any adverse changes while males in this group exhibited significantly higher levels of triglyceride (↑50.8%) and lower levels of creatinine (↓10%).

Albumin (↓4.5% low-dose; ↓3.8% mid-dose; ↓4.7% high dose; ↓3.9% recovery) and albumin/globulin ratios (↓8.7% low-dose; ↓7.8% mid-dose; ↓8.7% high-dose; ↓7.4% recovery) were significantly lower in all males exposed to the test substance, including those males in the high-dose recovery group, with no significant change in albumin concentration. Females in the high-dose recovery group exhibited significantly lower albumin (↓4.9%) and albumin/globulin (↓11.4%) ratios.

Significantly higher concentrations of α_1-globulin (↑18.2% males in high-dose group, ↑8.2% males and ↑9.7% females in high-dose-recovery groups), α_2-globulin (↑17.8% males and ↑25% females in high-dose group) and β-globulin (↑9.4% males and ↑12.5% females in high-dose group) were observed.

Urinary volume was increased across males and females in the low-, mid- and high-dose groups, with a statistically significant increase recorded in the high-dose group (↑199% male; ↑62.4% female). Urinary potassium concentration was lower in males in the low- (↓40.7%) and high-dose (↓48.6%) groups. The total excretion of potassium (↑46.6%) sodium (↑131%) and chloride (↑77.4%) were increased in males in the high-dose group. No significant changes were recorded in the high-dose-recovery animals.

Effects in Organs

There were no treatment related ophthalmological findings.

Absolute liver weight was higher in males and females in the low- and mid-dose groups, and significantly higher in the high-dose (↑47.4% male; ↑37.7% female) groups. Relative liver weight was significantly higher in mid- (↑9.4% males only) and high-dose (↑38.6% males and ↑34.9% females) groups. Enlarged livers were observed in 9/10 males and 1/10 females in the high-dose group.

Absolute kidney weight was significantly increased in males in the low- (↑12.5%), mid- (↑11.5%) and high-dose (↑36.8%) groups, while the relative weight was increased in the mid- (↑8.5%) and high-dose (↑29%) groups. Increased hyaline droplets and regenerative tubule with hypercellularity were observed in males in the low-, mid- and high-dose groups. Males in the control (3/10) and high-dose (3/10) recovery groups also exhibited regenerative tubule with hypercellularity. Granular casts in the renal tubular lumen in the cortico-medullary junction were observed in 1/10 males in the high-dose group and 3/10 animals in the high-dose recovery group. An increase in lipid droplets in the zona fasiculata of the adrenal cortex was observed in males in the control (1/10 animals), low- (1/10 animals), mid- (3/10 animals), high- (3/10 animals) and high-dose recovery (3/10 animals) groups.

Similar kidney and adrenal effects were not recorded in females exposed to the test substance in any of the groups.

Absolute heart (↑11.8%) and spleen (↑19.4%) weights were significantly higher in males in the high-dose group. Absolute and relative weights of the kidney (↑12.7% absolute; ↑10.4% relative) and adrenals (↑27.3% absolute; ↑25% relative) were significantly increased in females in the high-dose group. No significant changes in organ weights or gross necropsy were observed in animals in the high-dose recovery group.

Remarks – Results

Kidney and liver weights were increased in male and female animals when exposed to the test substance. Liver enlargement was also observed in male animals in the high-dose group. However, the changes were not present in high dose recovery animals and were not supported by adverse histopathology observations.

The authors considered that the morphological features observed in the male kidneys were consistent with α_2-microglobulin nephropathy. While the lesions observed are considered adverse, the neuropathy had disappeared or decreased after the 4 week recovery period. In addition, α_2-microglobulin is a rat specific protein and this type of neuropathy is not expected to occur in humans.

The observed clinical chemistry, haematology and urinary effects were not supported by adverse histopathology observations. Changes observed were not considered adverse by the authors as they were either slight or within the laboratory's historical data, were sporadic, did not show a dose-dependent trend or are often observed in this strain of rat.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1,000 mg/kg bw/day in this study, based on an absence of toxicologically relevant adverse effects at all doses.

TEST FACILITY BSRC (2013a)

B.5. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
Pre incubation procedure

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2*uvr*A
Metabolic Activation System	S9 fraction from phenobarbital and 5,6-benzoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 10, 20, 39, 78, 156, 313 µg/plate b) Without metabolic activation: 10, 20, 39, 78, 156, 313 µg/plate
Vehicle	Dimethyl sulfoxide
Remarks - Method	A preliminary toxicity study was performed at the concentrations 1.2, 4.9, 20, 78, 313, 1250, and 5000 µg/plate in the presence and absence of metabolic activation. Precipitates were not observed in the presence or absence of metabolic activation. Positive controls: a) in the absence of metabolic activation: 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (TA100, WP2*uvr*A, TA98), Sodium azide (TA1535), 2-methoxy-6-chloro-9-[3-(2-chloroethyl) aminopropylamino] acridine.2HCl (TA1537); b) in the presence of metabolic activation: Benzo[*a*]pyrene (TA100, TA98, TA1537), 1-Aminoanthracene (TA1535, WP2*uvr*A). Positive and vehicle controls were run concurrently with the test substance. Concentrations used were corrected for purity.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 313	≥ 313	> 313	negative
Test 2		≥ 313	> 313	negative
Present				
Test 1	≥ 313	≥ 313	> 313	negative
Test 2		≥ 313	> 313	negative

Remarks - Results Visible reduction in the growth of the bacterial background lawn was observed in all tester strains, with and without metabolic activation.

No biologically relevant increases in the number of revertant colonies were recorded for any of the tester strains, in the presence or absence of metabolic activation.

Positive and negative controls performed as expected, 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 BML (2012)

B.6. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
Species/Strain Chinese Hamster
Cell Type/Cell Line V79
Metabolic Activation System S9 fraction from phenobarbital and 5,6-benzoflavone induced rat liver
Vehicle Dimethyl sulphoxide
Remarks - Method A preliminary toxicity study was performed over a concentration range of 12.5 and 1600 µg/mL in the presence (4 hr exposure) and absence (4 hr and 24 hr exposure) of metabolic activation. After 4 hr of exposure, cytotoxicity was observed at ≥ 400 µg/mL (absence of metabolic activation) and ≥ 800 µg/mL (presence of metabolic activation). After 24 hr of exposure, cytotoxicity was observed at ≥ 800 µg/mL (presence of metabolic activation). Phase separation was observed at 800 and 1600 µg/mL after 4 and 24 hr exposure periods in the presence or absence of metabolic activation.

Excessive cytotoxicity was reported in test 1 at ≥ 100 µg/mL (absence of metabolic activation) and in test 2 at ≥ 400 µg/mL (presence of metabolic activation). Based on this, the dose range of the two experiments were adjusted and the experiment repeated.

Positive controls: a) in the absence of metabolic activation: ethylmethane sulfonate; b) in the presence of metabolic activation: 7,12-dimethylbenz(a)anthracene.

Positive and vehicle controls were run concurrently.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	6.3*, 12.5*, 25*, 50*, 100, 150, 200, 300	4	7 days	8 days
Test 2	25*, 50*, 100*, 200*, 400, 600	24	7 days	8 days
Present				
Test 1	50, 100*, 200*, 400*, 600*, 800*	4	7 days	8 days
Test 2	12.5*, 25*, 50*, 100*, 200*, 400, 800, 1200	4	7 days	8 days

*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	≥ 400	≥ 100	> 300	negative

Test 2	≥ 800	≥ 400	> 600	negative
Present				
Test 1	≥ 800	> 800	≥ 800	negative
Test 2		≥ 400	≥ 800	negative

Remarks - Results

Precipitates were not observed in the presence or absence of metabolic activation, however phase separation was observed in the presence of metabolic activation at concentrations of 800 µg/mL or greater.

The induction threshold exceeded the threshold for a negative result in test 1 at 12.5 µg/mL (4 hr exposure in the absence of metabolic activation) and at 600 µg/mL (4 hr exposure in the presence of metabolic activation). However, as these values were not reproducible, did not show a dose-dependent response and were within the historical range of vehicle controls, the study authors considered the results as biologically irrelevant.

No statistically significant or dose-dependent increase in mutant frequency was observed.

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

CONCLUSION

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

TEST FACILITY

Harlan CCR (2013a)

B.7. 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 and 5,6-benzoflavone induced rat liver

Vehicle: Dimethyl sulfoxide (0.5%)

Remarks - Method

A preliminary cytotoxicity test was performed. No precipitation was observed. Concentration range met the requirements for cytogenetic evaluation and the authors included the test in the results for the main study (designated Test 1A in table).

Positive controls: a) in the absence of metabolic activation: mitomycin C and demecolcin; b) in the presence of metabolic activation: cyclophosphamide.

Test 2A in the presence of metabolic activation was repeated due to a positive effect in the solvent control. The repeated tests results were included in the report as Test 2B.

Positive and vehicle controls were run concurrently.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1A	10.1, 17.6, 30.8, 54.0, 94.4, 165.3, 289.2*, 506.1*, 885.7*, 1550.0	4 h	20 h	40 h
Test 1B	125*, 250*, 500*, 600, 700, 750, 800, 850, 900, 1000, 1100*, 1200*, 1550*	4 h	20 h	40 h
Test 2A	10.1, 17.6, 30.8, 54.0, 94.4*, 165.3*, 289.2*, 506.1, 885.7, 1550.0	20 h	20 h	40 h

Present				
Test 1A	10.1, 17.6, 30.8, 54.0, 94.4, 165.3, 289.2*, 506.1*, 885.7*, 1550.0	4 h	20 h	40 h
Test 2B	125, 250*, 500*, 600*, 700, 750, 800, 850, 900, 1000, 1100, 1200, 1550	4 h	20 h	40 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1A	> 1550	> 1550	> 1550	negative
Test 1B		> 1550	> 1550	negative
Test 2A		> 1550	> 1550	negative
Present				
Test 1A	> 1550	> 1550	> 1550	negative
Test 2B		> 600	> 1550	negative

Remarks - Results

Precipitates were not observed in the presence or absence of metabolic activation. Phase separation was observed in test 1A at ≥ 506.1 μg/mL (presence and absence of metabolic activation), in test 1B at ≥ 500 μg/mL (absence of metabolic activation) and in test 2A at ≥ 500 μg/mL and test 2B ≥ 600 μg/mL (absence of metabolic activation).

Cytotoxicity was not observed in test 1A (presence and absence of metabolic activation) or test 1B (absence of metabolic activation). A cytotoxic response was observed in test 2A (absence of metabolic activation) at 506.1 μg/mL. However a dose-response relationship was not observed at higher concentrations.

Neither a statistically significant or biologically relevant increase in the number of micronucleated cells was observed after treatment with the test substance.

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

CONCLUSION

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

TEST FACILITY

Harlan CCR (2013b)

B.8. Developmental toxicity

TEST SUBSTANCE

Notified Chemical

METHOD

OECD TG 421 Reproduction/Developmental Toxicity Screening Test.

Species/Strain

Rat/Crl:CD(SD) [SPF]

Route of Administration

Oral – gavage

Exposure Information

Exposure days:
Males: 42 consecutive days (14 days prior to mating, 14-day mating period, 14 days post mating period).
Females: 41 – 45 consecutive days (14 days prior to mating, up to 14-days until copulation, until day 3 of lactation after parturition including gestation period for copulated females).

Vehicle

Corn oil

Remarks - Method

Two preliminary studies were performed to set the tested dose levels. A 14-day repeated dose study supplied doses of 30, 100, 300 and

1000 mg/kg bw/day of the test substance to rats. An increase in liver weight in males and females (1000 mg/kg bw/day) was the only obvious treatment effect noted by the authors. A teratogenicity study also found no treatment related effects for dams and embryo-foetal development in a 1000 mg/kg bw/day dose group.

One female in the control group did not copulate. Three females did not deliver naturally (one female in each of the control, mid- and high-dose groups) and were determined to be non-pregnant by the authors as no stained implantation sites were observed in their uteri.

Histopathology was performed on control and high-dose group animals as well as the non-pregnant female (control), a mated male (mid-dose group) and two females (one each from the low- and high-dose groups) and their offspring who exhibited macroscopic abnormal organs/tissues.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	12 M, 12 F	0	0/24
low-dose	12 M, 12 F	100	0/24
mid-dose	12 M, 12 F	300	0/24
high-dose	12 M, 12 F	1000	0/24

Mortality and Time to Death

There were no unscheduled deaths.

Effects on Parental Animals

There were no treatment-related effects on the clinical signs, body weights, food consumption or organ weights.

Males in the high-dose group exhibited an increase in hyaline droplet in the proximal tubules and/or regeneration with hypercellularity of tubules in the kidneys. These histopathological findings are considered to be species specific and were not considered by the authors when determining the no-observed-adverse-effect level (NOAEL).

The ratio of pachytene spermatocytes and round spermatids to Sertoli cells was significantly decreased in the mid-dose group and all the test-substance-treated groups respectively. The decrease in the ratio of pachytene spermatocytes in males in the mid-dose group was considered not to be toxicologically significant because the change was not in a dose-related fashion. In addition, the study authors observed that there was no histopathology to support this as toxicologically significant.

Effects on Dams

In the control group females, endometritis caused by retained placenta in the uterus (dam) and nephroblastoma in the kidney (female who did not copulate) were observed.

An irregular estrous cycle was observed in 4/60 animals (1/12 control, 2/12 mid-dose, 1/12 high-dose). However, there was no significant difference in the incidences of irregular estrous cycles among the groups. Mean estrous cycle days were 4.0 (control and mid-dose groups) and 4.3 (low- and high-dose groups) and no statistical difference was observed between the control and exposure groups.

The gestation length, numbers of corpora lutea and implantation sites and total numbers of live offspring delivered were comparable among the control group and all of the test substance treated groups. There were no significant differences in the gestation index, implantation index, delivery index, live birth index, viability index on day 4 of lactation or sex ratio between the control and exposure groups. 1 cannibalized offspring was observed on Day 0 of lactation in the mid-dose group.

Effects on Newborns

No significant differences in body weights of males and females (control and exposure groups). Malformations

observed in two females [no tail (low-dose) and dilated renal pelvis (high-dose)] were considered by the study authors to be spontaneous (single incidences) and not an effect of exposure to the test substance.

Of the offspring that died, abnormal findings were observed in 2 males [persistent left umbilical artery and elongate innominate (control) and thymic remnant in neck (high-dose)] that died during lactation. These lesions were determined to be unrelated to cause of death by the study authors.

Remarks - Results

At mating, 1 pair failed to copulate in the control group and the copulation index was 91.7%. All pairs in the exposed groups copulated successfully. Infertility was observed in 1 female in the control (fertility index 90.9%), mid- and high-dose groups (fertility index of 91.7% in both) each. There were no significant differences in female fertility indices among the groups.

Other changes observed were not considered adverse by the study authors as they were either slight or within the laboratory's historical data, were sporadic, did not show a dose-dependent trend or are often observed in this strain of rat.

There were no adverse effects on reproductive ability (including delivery and lactation). Observation of offspring revealed no effects on the number of live offspring (or number delivered), viability, sex ratios and body weights following exposure to the test substance.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1,000 mg/kg bw/day in this study, based on an absence of adverse effects attributable to the test substance.

TEST FACILITY BSRC (2013b)

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 sewage sludge.
Exposure Period	28 days.
Auxiliary Solvent	Not reported.
Analytical Monitoring	Biological Oxygen Demand (BOD)
Remarks - Method	The test was conducted according to the guidelines above using good laboratory practice (GLP). No significant deviations from the test guidelines were reported.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	8.0	3	67
14	76.5	14	77
28	91.0	28	76

Remarks - Results

All validity criteria for the test were satisfied. The reference compound, aniline, reached the 60% pass level by day 7 indicating the suitability of the inoculum. The toxicity control attained 69% biodegradation within 14 days showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the test substance after the cultivation period was 91% within 28 days and satisfied the 10-day window validation criterion. Therefore, the test substance can be classified as readily biodegradable according to the OECD (301 F) guideline.

CONCLUSION	The notified chemical is readily biodegradable
TEST FACILITY	Harlan (2013d)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Static Test
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Not reported
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	Gas Chromatography (GC) Analysis
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP). No significant deviations from the test guidelines were reported.

The daphnia ecotoxicity test was conducted in Water Accommodated Fractions (WAFs) of the notified chemical as it has low water solubility.

A stock solution was prepared by dispersing a pre-measured amount of the

test substance in a culture medium by stirring at approximately 1500 rpm for 24 hours. Any undissolved test item was removed by filtration. The stock solution was used as the highest treatment concentration. Predetermined volumes of stock solution were used to prepare the remaining treatments.

RESULTS

Concentration		*Number of D. magna*	*Cumulative % Immobilised*	
Nominal (mg/L)	*Mean Measured (mg/L)*		*24 h*	*48 h*
Control	Control	20	0	0
1.0	0.4	20	0	0
3.2	1.7	20	0	0
10	6.8	20	0	0
32	26.0	20	100	100
100	90.0	20	100	100

EL50	13 (6.8 – 26) mg/L at 48 hours
NOEL	6.8 mg/L at 48 hours
Remarks - Results	All validity criteria for the test were satisfied. The 48-hour EL50 was calculated from mean measured concentrations.

CONCLUSION The notified chemical is harmful to aquatic invertebrates

TEST FACILITY Harlan (2013e)

C.2.2. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD	OECD TG 201 Alga, Growth Inhibition Test
Species	*Pseudokirchneriella subcapitatus*
Exposure Period	72 hours
Concentration Range	Nominal: 0.1, 1.0, 10 and 100 mg/L Measured: 5.3, 11, 23, 42 and 91 mg/L
Auxiliary Solvent	Not reported
Water Hardness	Not reported
Analytical Monitoring	Gas Chromatography (GC) Analysis
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported. The algae toxicity test was conducted in Water Accommodated Fractions (WAFs) of the notified chemical as it has low water solubility. A stock solution was prepared by dispersing a pre-measured amount of the test substance in a culture medium by stirring at approximately 1500 rpm for 24 hours. Any undissolved test item was removed by filtration. The stock solution was used as the highest treatment concentration. Predetermined volumes of stock solution were used to prepare the remaining treatments.

RESULTS

Biomass (72 h)		*Growth (72 h)*	
E_yL50 (mg/L)(Confidence intervals)	*NOE_yL (mg/L)*	*E_rL50 (mg/L) (Confidence intervals)*	*NOE_rL (mg/L)*
30 (27 – 33)	5.3	15 (14 – 17)	5.3

Remarks - Results	All validity criteria for the test were satisfied. The endpoints were calculated based on the 0-hour measured test concentration. SAS, statistical analysis, was used to calculate the endpoints.
CONCLUSION	The notified chemical is harmful to algae
TEST FACILITY	Harlan (2013f)

BIBLIOGRAPHY

Api AM, Basketter DA, Cadby PA, Cano MF, Ellis G, Gerberick GF, Griem P, McNamee PM, Ryan CA and Safford R (2008) Dermal Sensitisation Quantitative Risk Assessment (QRA) for Fragrance Ingredients, Regulatory Toxicology and Pharmacology, 52:3-23.

Belsito, D., Bickers, D., Bruze, M., Calow, P., Greim, H.M Hanifin, J.M., Rogers, A.E., Saurat, J.H., Sipes, I.G., Tagami, H., The RIFM Expert Panel. (2010) A safety assessment of branched chain saturated alcohols when used as fragrance ingredients. Food and Chemical Toxicology 48: S1-S46.

BML (2012) Mutagenicity Study of 3,6-dimethylheptan-2-ol with the Bacterial Reverse Mutation Assay (Study No. 16842, November, 2012). Tokyo, Japan, BML, INC, General Laboratory (Unpublished report submitted by the notifier).

BSRC (2012) Mouse Local Lymph Node Assay of 3,6-dimethylheptan-2-ol (Study No. E413 (474-112), December, 2012). Shizuoka, Japan, Public Interest Incorporated Foundation Biosafety Research Center (BSRC) (Unpublished report submitted by the notifier).

BSRC (2013a) 90-Day Repeated-Oral Dose Toxicity Study of 3,6-dimethylheptan-2-ol in Rats (Study No. E410 (474-110), August, 2013). Shizuoka, Japan, Public Interest Incorporated Foundation Biosafety Research Center (BSRC) (Unpublished report submitted by the notifier).

BSRC (2013b) Reproduction/Developmental Toxicity Screening Test of 3,6-dimethylheptan-2-ol in Rats (Study No. E408 (474-108), June, 2013). Shizuoka, Japan, Public Interest Incorporated Foundation Biosafety Research Center (BSRC) (Unpublished report submitted by the notifier).

Cadby, PA, Troy, WR and Vey, MGH (2002) Consumer Exposure to Fragrance Ingredients: Providing Estimates for Safety Evaluation. Regulatory Toxicology and Pharmacology, 36:246-52.

ECHA (2014) Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7c: Endpoint specific guidance, November 2014, version 2.0. European Chemicals Agency, http://echa.europa.eu/documents/10162/13632/information_requirements_r7c_en.pdf.

Harlan (2013a) 3,6-dimethylheptan-2-ol: Determination of General Physico-Chemical Properties (Study No. 41300965, September, 2013). Shardlow, United Kingdom, Harlan Laboratories Ltd. (Unpublished report submitted by the notifier).

Harlan (2013b) 3,6-dimethylheptan-2-ol: Determination of Skin Irritation Potential using the EPISKIN™ Reconstructed Human Epidermis Model (Study No. 41300980, September, 2013). Shardlow, United Kingdom, Harlan Laboratories Ltd. (Unpublished report submitted by the notifier).

Harlan (2013c) 3,6-dimethylheptan-2-ol: Determination of Eye Irritation Potential using the Bovine Corneal Opacity and Permeability (BCOP) Assay (Study No. 41300982, August, 2013). Shardlow, United Kingdom, Harlan Laboratories Ltd. (Unpublished report submitted by the notifier).

Harlan (2013d) 3,6-dimethylheptan-2-ol: Assessment of Ready Biodegradability; Manometric Respirometry Test (Project No. 41300987, October 2013). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2013e) 3,6-dimethylheptan-2-ol: Daphnia sp., 48-Hour Acute Immobilization test (Study No. 41300983, September 2013). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2013f) 3,6-dimethylheptan-2-ol: Algal Growth Inhibition Test (Project No. 41300984, October 2013). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan CCR (2013a) 3,6-dimethylheptan-2-ol: Gene Mutation Assay in Chinese hamster V79 Cells *In Vitro* (V79/HPRT) (Study No. 1544602, July, 2013). Rossdorf, Germany, Harlan Cytotest Cell Research GmbH (Unpublished report submitted by the notifier).

Harlan CCR (2013b) 3,6-dimethylheptan-2-ol: *In vitro* Micronucleus Test in Human Lymphocytes (Study No. 1544601, September, 2013). Rossdorf, Germany, Harlan Cytotest Cell Research GmbH (Unpublished report submitted by the notifier).

McGinty D., Scognamiglio J., Letizia C.S. and Api A.M. (2010a) Fragrance material review on 2,6-dimethyl-4-heptanol. Food and Chemical Toxicology, **48**:S89-S92.

McGinty D., Scognamiglio J., Letizia C.S. and Api A.M. (2010b) Fragrance material review on 2,6-dimethyl-4-heptanol. Food and Chemical Toxicology, **48**:S55-S59.

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

NTC (National Transport Commission) 2007 Australian Code for the Transport of Dangerous Goods by Road and Rail (ADG code), 7th Edition, Commonwealth of Australia

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.

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 >.