File No: LTD/1738 and LTD/1764

April 2015

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Cyclohexanepropanal, 4-(2-methylpropyl)-, *cis*- (LTD/1738)
Cyclohexanepropanal, 4-(2-methylpropyl)-, *trans*- (LTD/1764)

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
LTD/1738 LTD/1764	International Flavours & Fragrances (Australia) Pty Ltd	Cyclohexanepropanal, 4-(2-methylpropyl)-, *cis*- (LTD/1738) Cyclohexanepropanal, 4-(2-methylpropyl)-, *trans*- (LTD/1764)	Yes	≤ 1 tonne per annum (combined introduction volume)	Fragrance ingredients

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemicals are 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 1)	H317 – May cause an allergic skin reaction

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

R38: Irritating to skin
R43: May cause sensitisation by skin contact

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

Hazard classification	*Hazard statement*
Acute (Category 1)	H400 – Very toxic to aquatic life
Chronic (Category 1)	H410 – Very toxic to aquatic life with long lasting effects

Human health risk assessment
Under the conditions of the occupational settings described, the notified chemicals are not considered to pose an unreasonable risk to the health of workers.

When used in the proposed manner, the notified chemicals are 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 chemicals are not expected to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemicals should be classified as follows:

- Skin sensitisation (Category 1): H317 – May cause an allergic skin reaction

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

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

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 chemicals during reformulation processes:
  - Enclosed, automated processes, where possible
  - Ventilation system including local 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 chemicals during reformulation processes:
  - Avoid contact with 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 chemicals during reformulation processes:
  - Coveralls, impervious gloves

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

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

- If products and mixtures containing the notified chemicals are classified as hazardous to health in accordance with the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Disposal

- Where reuse or recycling are not available or practical, dispose of the chemicals 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 chemicals should be handled by containment, physical 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 chemicals 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 chemicals, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemicals are 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 for the notified chemicals;
- the combined concentration of the notified chemicals exceeds or is intended to exceed 0.5% in individual cosmetic or household products;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemicals has changed from fragrance ingredients, or is likely to change significantly;
- the amount of the chemicals being introduced has increased, or is likely to increase, significantly;
- the chemicals have begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemicals on occupational health and safety, public health, or the environment.

(Material) Safety Data Sheet
The (M)SDS of the notified chemicals provided by the notifier was reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
International Flavours and Fragrances (Australia) Pty Ltd. (ABN: 77 004 269 658)
310 Frankston-Dandenong Rd
Dandenong VIC 3175

NOTIFICATION CATEGORY
LTD/1738: Limited-small volume: Chemical other than polymer (1 tonne or less per year).
LTD/1764: Limited-small volume (Reduced fee notification) – Chemical other than polymer (1 tonne or less per year) - Chemical is being notified at the same time as a similar chemical.

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: dissociation constant, hydrolysis as a function of pH.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None.

NOTIFICATION IN OTHER COUNTRIES
US, Japan.

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
STARFLEUR (mixture of the notified chemicals)

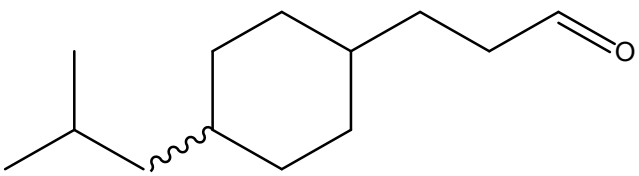
CAS NUMBER
LTD/1738: 1315250-65-7 (*cis*-isomer)
LTD/1764: 1315250-67-9 (*trans*-isomer)

CHEMICAL NAME
LTD/1738: Cyclohexanepropanal, 4-(2-methylpropyl)-, *cis*-
LTD/1764: Cyclohexanepropanal, 4-(2-methylpropyl)-, *trans*-

OTHER NAME(S)
FRET 08-0318 (mixture of the notified chemicals)
TM 10-202 (mixture of the notified chemicals)
13-216-01 (mixture of the notified chemicals)

MOLECULAR FORMULA
$C_{13}H_{24}0$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
196.34 Da

ANALYTICAL DATA

Reference NMR, IR, UV, GC, GC-MS spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 90% (mixture of the isomers, *cis*- (LTD/1738; 60-67%) and *trans*- (LTD/1764; 27-33%), which are not isolated).

IDENTIFIED IMPURITIES/RESIDUAL MONOMERS
Chemical Name Cyclohexane, 1-[3-(1-methylethoxy)propyl]-4-(2-methylpropyl)-
CAS No. - *Weight %* ~3%

Chemical Name Cyclohexane, 1-(3,3-dimethoxypropyl)-4-(2-methylpropyl)-
CAS No. - *Weight %* ~2%

ADDITIVES/ADJUVANTS
None.

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Colourless liquid*

Property	Value	Data Source/Justification
Melting Point/Freezing Point*	< -20 °C	Measured
Boiling Point*	265 ± 1.0 °C at 101.9 kPa	Measured
Density*	891 kg/m^3 at 20 ± 0.5 °C	Measured
Vapour Pressure*	2.5 x 10^{-3} kPa at 25 °C	Measured
Water Solubility*	Not determined	Measured but the value cannot be established
Hydrolysis as a Function of pH	Not determined	The notified chemicals are expected to hydrolyse readily in the environment based on the ecotoxicity study for *Daphnia*
Partition Coefficient* (n-octanol/water)	log Pow = 5.2 – 5.4	Measured
Surface Tension*	70.7 mN/m at 21.0 ± 0.5 °C	Measured
Adsorption/Desorption	log K_{oc} = 3.4	Calculated using KOCWIN v2.0 (US EPA, 2011)
Dissociation Constant	Not determined	The notified chemicals do not contain dissociable functional groups
Flash Point*	121 ± 2.0 °C at 101.3 kPa	Measured
Autoignition Temperature*	218 ± 5.0 °C	Measured
Explosive Properties	Predicted negative	Contain no functional groups that would imply explosive properties.
Oxidising Properties	Predicted negative	Contain no functional groups that would imply oxidative properties.

*mixture of the notified chemicals

DISCUSSION OF PROPERTIES
For full details of tests on physical and chemical properties, refer to Appendix A.

Reactivity
The notified chemicals are expected to be stable under normal conditions of use. Direct sources of heat and contact with strong acids, alkali or oxidising agents should be avoided.

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemicals will not be manufactured within Australia. The notified chemicals will be imported as components of fragrance oils at a combined concentration of ≤ 5% or as components of finished products (combined concentration ≤ 0.5%).

MAXIMUM COMBINED INTRODUCTION VOLUME OF NOTIFIED CHEMICALS (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 & Fragrances (Australia) Pty Ltd.

TRANSPORTATION AND PACKAGING
The notified chemicals (at a combined concentration of ≤ 5%) will be imported as components of fragrance oils in polypropylene-lined steel drums or as components of finished products in containers suitable for retail sale. The imported and finished products containing the notified chemicals will be transported primarily by road.

USE
The notified chemicals will be used as fragrance ingredients in a variety of cosmetic and household products (proposed combined concentration of ≤ 0.5%).

OPERATION DESCRIPTION
The notified chemicals will not be manufactured within Australia. No reformulating or repackaging of the notified chemicals will occur at the notifier facility. The fragrance oil containing the notified chemicals will be stored at this facility until it is sold and shipped to customer facilities.

Reformulation
The procedures for incorporating the notified chemicals (at ≤ 5% concentration) into end-use products will likely vary depending on the nature of the formulated products and may involve both automated and manual transfer steps. However, in general, it is expected that for the reformulation process, the notified chemicals will be weighed and added to the mixing tank where they will be blended with additional additives to form the finished cosmetic and household products. This will be followed by automated filling of the reformulated products into containers of various sizes. THE BLENDING OPERATIONS ARE EXPECTED TO BE HIGHLY AUTOMATED AND USE CLOSED SYSTEMS AND/OR ADEQUATE VENTILATION. During the formulation process, samples of the notified chemicals and the finished cosmetic products will be taken for quality control testing.

Household products.
Household products containing the notified chemicals (≤ 0.5% concentration) may be used by consumers and professional workers (such as cleaners). The products may be used in either closed systems with episodes of controlled exposure, for example automatic washing machines, or open processes and manually by rolling, brushing, spraying and dipping.

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

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration*	*Exposure Frequency*

	(hours/day)	*(days/year)*
Transport and Warehouse workers	Unknown	Unknown
Plant operators - Mixing/blending	4	250
Plant operators - Drum handling	1	250
Plant operators - Drum cleaning/washing	2	250
Plant operators – Equipment maintenance	2	250
Quality control workers	1	250
End users (professionals)	Not specified	Not specified

EXPOSURE DETAILS

Transport and storage

Transport and storage workers may come into contact with the notified chemicals (at a combined concentration of ≤ 5%) only in the event of accidental rupture of the drum containers.

At the notifier facility, the primary work activity undertaken by transport and warehouse workers will include the handling, loading and off-loading of drums containing fragrance oils formulated with the notified chemicals at ≤ 5% concentration. Exposures of these workers will be limited to situations involving products sampling for quality control or, in the event of a discharge, clean up from a spill or leaking drum. If such an event occurs, a worker may be exposed through dermal or ocular contact. The notifier states that such exposures will be minimised to the extent possible through the use of personal protective equipment (PPE) including protective overalls, chemical resistant gloves and safety glasses.

Reformulation

During reformulation, dermal, ocular and perhaps inhalation exposure of workers to the notified chemicals (at a combined concentration of ≤ 5%) may occur during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. Exposure is expected to be minimised through the use of mechanical ventilation, local exhaust ventilation and/or enclosed systems, and through the use of PPE such as coveralls, goggles and impervious gloves.

End-use

Exposure to the notified chemicals in end-use products (at a combined concentration of ≤ 0.5%) may occur in professions where the services provided involve the application of cosmetic products to clients (e.g. hair dressers, workers in beauty salons) or the use of household products in the cleaning industry. The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible. Such professionals may use some PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemicals.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemicals (at a combined concentration of ≤ 0.5%) through the use of a wide range of cosmetic and household products. The principal routes of exposure will be dermal, while ocular and inhalation exposures (e.g. through the use of spray products) are also possible.

Data on typical use patterns of cosmetic and household cleaning product categories in which the notified chemical may be used are shown in the following table (SCCS, 2012; Cadby *et al*., 2002; SDA, 2005). For the purposes of the exposure assessment via the dermal route, Australian use patterns for the various product categories are assumed to be similar to those in Europe. In the absence of dermal absorption data, a dermal absorption of 100% was assumed for the notified chemical. For the inhalation exposure assessment (European Commission, 2003; SDA, 2005), an adult inhalation rate of 23 m^3/day (enHealth, 2004) was used and it was assumed that the bioavailability of the notified chemical via the inhalation route is 100%. An adult bodyweight of 60 kg was used for calculation purposes.

- Cosmetic products (Dermal exposure):

Product type	**Amount** (mg/day)	**C** (%)	**RF** (unitless)	**Daily systemic exposure** (mg/kg bw/day)
Body lotion	7820	0.5	1	0.6517
Face cream	1540	0.5	1	0.1283
Hand cream	2160	0.5	1	0.1800

Fine fragrances	750	0.5	1	0.0625
Deodorant spray	1430	0.5	1	0.1192
Shampoo	10460	0.5	0.01	0.0087
Conditioner	3920	0.5	0.01	0.0033
Shower gel	18670	0.5	0.01	0.0156
Hand soap	20000	0.5	0.01	0.0167
Hair styling products	4000	0.5	0.1	0.0333
Total				1.2192

C = concentration (%); RF = retention factor.
Daily systemic exposure = Amount x C x RF x dermal absorption /body weight

- 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	0.0182
Fabric softener	90	0.5	0.95	10	0.0071
Total					0.0253

Daily systemic exposure = Amount x C x PR x PT x dermal absorption /body weight

- Household products (Direct dermal exposure):

Product type	**Frequency** (use/day)	**C** (%)	**Contact Area** (cm^2)	**Product Use C** (g/cm^3)	**Film Thickness** (cm)	**Time Scale Factor**	**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	0.0116
Total							0.0131

Daily systemic exposure = Frequency x C x Contact area x Product Use Concentration x Film Thickness on skin x Time Scale Factor x dermal absorption /body weight

- Cosmetic products (Inhalation exposure):

Product type	**Frequency** (use/day)	**Amount** (g/use)	**C** (%)	**Inhalation rate** (m^3/day)	**Exposure duration** (mins)	**Airspace volume** (m^3)	**Daily systemic exposure** (mg/kg bw/day)
Hairspray	2	10	0.5	23	15	2	0.1997

C = concentration.
Daily systemic exposure = Frequency x Amount x C x Inhalation rate x Exposure duration x bioavailability via the inhalation route/(Airspace volume x body weight)

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 1.4572 mg/kg bw/day. It is acknowledged that inhalation exposure to the notified chemical from use of other cosmetic and household products (in addition to hair spray) may occur. However, it is considered that the combination of the conservative hair spray inhalation exposure assessment parameters, in particular assuming an airspace volume of 2 m^3, and the aggregate exposure from use of the dermally applied products, which assumes a conservative 100% absorption rate, is sufficiently protective to cover additional inhalation exposure to the notified chemical from use of other spray cosmetic and household products with lower exposure factors (e.g. air fresheners).

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the mixture of the notified chemicals 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 > 2,000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Skin corrosion (in vitro) EPISKIN™ model	non-corrosive
Skin irritation (in vitro) SkinEthic Reconstituted Human Corneal Epithelium Model	non-irritating
Rabbit, skin irritation	irritating
Eye irritation (in vitro) SkinEthic Reconstituted Human Corneal Epithelium Model	non-irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
Human, skin sensitisation – RIPT (5%)	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOEL = 38.2 mg/kg bw/day (males) 42.7 mg/kg bw/day (females) NO(A)EL = 1,137.8 mg/kg bw/day (males) 1,239.7 mg/kg bw/day (females)
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosome aberration	non genotoxic

Toxicokinetics, metabolism and distribution.
Based on the partition coefficient (log P_{ow} = 5.2 – 5.4) and the low molecular weight (196.34 Da) of the notified chemicals, passive diffusion across the gastrointestinal (GI) tract and dermal absorption are expected to occur (although the extent of absorption may be limited). The notified chemical may also be absorbed across the respiratory tract.

Acute toxicity.
The mixture of notified chemicals was found to have low acute toxicity via the oral and dermal routes in studies in rats.

No acute inhalation toxicity data were provided for the notified chemicals.

Irritation.
Two in vitro dermal studies were conducted using reconstructed human epidermis models (EpiSkin). These studies indicated that the mixture of notified chemicals was non-corrosive and non-irritating.

A skin irritation study in rabbits was also performed. Well-defined erythema and very slight oedema was noted in all animals. All effects had resolved by the end of the study period. The skin irritant effects in this study warranted classification of the chemicals according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), but not according to the GHS, as adopted in Australia.

An in vitro eye irritation study was also conducted using a reconstituted human corneal epithelium model (SkinEthic), which indicated that the notified chemicals were non-irritating to the eyes after ten minutes exposure. When tested in rabbits, slight to moderate conjunctival irritation, chemosis and discharge were noted in all treated eyes for varying durations of effect up to the day 14 observations. The notified chemicals were therefore deemed slightly irritating to the eyes, however the effects did not warrant classification of the chemicals as eye irritants.

Sensitisation.
The mixture of notified chemicals was found to be a skin sensitiser in mice (Local Lymph Node Assay; stimulation indices of 1.77, 2.92 and 4.68 at 25, 50 and 100% concentrations, respectively). The EC_3 value was calculated to be 52%. This EC_3 value places the sensitisation potential of the notified chemical in the weak potency range (Basketters *et. al*, 2003).

The sensitising potential of the notified chemicals was also tested in a human repeat insult patch test (HRIPT) at 5% combined concentration (with 105 subjects completing the study), with the mixture of notified chemicals

considered by the study authors not to be a skin sensitiser. However, barely perceptible erythema was seen in 2 female subjects during the challenge phase. In the first subject, it was noted at patch removal only. In the second subject, it was noted 48 and 72 hours following patch removal.

Repeated dose toxicity.
An oral (dietary) repeated dose toxicity study on the mixture of notified chemicals was conducted with rats, in which the test substance was administered at 500, 3,500 and 15,000 ppm (equating to mean achieved doses of 38.2, 269.1 and 1,137.8 mg/kg bw/day for males and 42.7, 313.2 and 1,239.7 mg/kg bw/day for females) for 28 consecutive days, with a 14 day recovery period for high dose animals.

A range of clinical and laboratory observations were noted, including, for example, reduction in mean body weight gains, reduced food consumption and efficiency and blood chemistry parameter changes. At necropsy, observed effects included weight variations and microscopic histopathological abnormalities of the liver (including an increased incidence of single cell hepatocyte necrosis) and kidneys, seen in various animals of both sexes at the mid to high dose levels.

The effects in the kidneys were not considered relevant to human exposure and the microscopic liver changes and associated blood chemistry changes were considered by the study authors to represent adaptive changes and were not considered to represent serious damage to the health of the test animals. Therefore, the No Observed Effect Level (NOEL) was established by the study authors as 500 ppm (equivalent to a mean achieved dose of 38.2 mg/kg bw/day in males and 42.7 mg/kg bw/day in females) in this study. The No Observed (Adverse) Effect Level (NO(A)EL) was established by the study authors as 15,000 ppm (equivalent to a mean achieved dose of 1,137.8 mg/kg bw/day in males and 1,239.7 mg/kg bw/day in females) in this study.

Mutagenicity/Genotoxicity.
The mixture of notified chemicals was not mutagenic in a bacterial reverse mutation study and non-clastogenic in an in vitro mammalian chromosome aberration test.

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

Hazard classification	***Hazard statement***
Skin sensitisation (Category 1)	H317 – May cause an allergic skin reaction

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

R38: Irritating to skin
R43: May cause sensitisation by skin contact

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Reformulation (and quality control processes)
Workers may experience dermal and accidental ocular and perhaps inhalation exposure to the notified chemicals (at a combined concentration of ≤ 5%) during reformulation processes (and during sampling and quality control processes at storage sites). The notified chemical is considered to be a skin irritant and a skin sensitiser. Therefore, caution should be exercised when handling the notified chemical during reformulation and quality control processes.

The use of enclosed, automated processes and PPE (e.g. impervious gloves, coveralls) 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 chemicals is not considered to be unreasonable.

End-use
Cleaners, hair and beauty care professionals will handle the notified chemicals at a combined concentration of ≤ 0.5%. Such professionals may use PPE to minimise repeated exposure, and good hygiene practices are

expected to be in place. 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 chemicals on a regular basis (for details of the public health risk assessment, see Section 6.3.2.).

6.3.2. Public Health

Sensitisation and skin irritation
While the notified chemicals are considered to be skin irritants, irritation effects are not expected from use of the notified chemicals at the proposed concentration. The main risk associated with use of the notified chemicals at a combined concentration of ≤ 0.5% in cosmetic and household products, is its potential to cause sensitisation by skin contact.

Methods for the quantitative risk assessment of dermal sensitisation have been proposed and been the subject of significant discussion (see for example, Api *et al.*, 2008 and RIVM, 2010). Using fine fragrances (containing the notified chemicals at a combined concentration of ≤ 0.5%) as an example product that may contain the notified chemicals, as a worst case scenario, the Consumer Exposure Level (CEL) is estimated to be 18.75 μg/cm^2 (Cadby et. al, 2002, SCCS, 2012). Following consideration of the available data on skin sensitisation (and the responses in these studies), and application of appropriate safety factors, an Acceptable Exposure Level (AEL) of 38.61 μg/cm^2 was derived (using the EC3 value of 52%, which was obtained in the LLNA study on the mixture of notified chemicals). In this instance, the factors employed included an interspecies factor (3), intraspecies factor (10), a matrix factor (3.16), database factor (1) and a use and time factor (3.16), 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 fine fragrances (a worst case example of a leave-on cosmetic product) with a combined concentration of ≤ 0.5% of the notified chemicals, is not considered to be unreasonable. Based on the lower expected exposure level from use of other leave-on and rinse-off cosmetic products and household products, by inference, the risk of induction of sensitisation associated with the use of the other product types (also containing a combined concentration of ≤ 0.5% of the notified chemicals) is not considered to be unreasonable. It is acknowledged that consumers may be exposed to multiple products containing the notified chemicals, and a quantitative assessment based on the aggregate exposure has not been conducted.

Repeat dose toxicity
The repeat dose toxicity potential of the notified chemicals was estimated by calculation of the margin of exposure (MoE) using the worst case exposure scenario from use of multiple products of 1.4572 mg/kg bw/day (see Section 6.1.2). A NO(A)EL of 1,137.8 mg/kg bw/day was also used, based on the results seen in the 28-day repeated dose toxicity study on the notified chemicals. A MoE value ≥ 300 is considered acceptable to account for intra- and inter-species differences and the duration of the study, noting also the uncertainty on the significance of effects observed in both sexes at the mid to high dose levels. Using the abovementioned NO(A)EL, a MoE of 781 was estimated, which is considered to be acceptable.

Therefore, based on the information available, the risk to the public associated with the use of the notified chemicals at a combined concentration of ≤ 0.5% 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 chemicals will be imported as components of fragrance oils or as components of finished products for retail sale. No significant release of the notified chemicals is anticipated from distribution/transportation to customer sites except in the event of an accident. Accidental spills of the notified chemicals will be collected and disposed of to landfill.

Releases of the chemicals from reformulation are anticipated to be low. Any wash waters resulting from the blending/cleaning operations are likely to be discharged to an onsite wastewater treatment plant and/or a local sewage treatment plant.

RELEASE OF CHEMICAL FROM USE

It is expected that most of the imported notified chemicals will be washed down to sewers after use nationwide.

RELEASE OF CHEMICAL FROM DISPOSAL

It is expected that all spilled notified chemicals and clean up absorbent will be collected and placed in sealed containers for disposal to landfill.

7.1.2. Environmental Fate

The notified chemicals are not readily, but showed inherent, primary biodegradability based on the provided studies. For the details of the environmental fate studies please refer to Appendix C. The notified chemicals are also expected to hydrolyse readily into carboxylic acid form of the chemicals. It is expected to be bioaccumulative based on the reported log P_{OW} of 5.2-5.4. However, the carboxylic acid form is not expected to be bioaccumulative. It is predicted to have a low BCF value based on a measured BCF value of 2 for a close analogue chemical (Naphthenic acids, C8-C20, 1-ring; CAS No. 1338-24-5). Available literature also indicates that compounds with carboxylic acids are expected to have low bioaccumulative potential (Van Den Berg, et al., 1995; Schuurmann, et al., 1995). Therefore, the bioaccumulation potential of the notified chemicals is not considered to be a concern given they readily hydrolyse. The half-life of the notified chemicals in air is calculated to be 2.98 hours based on reactions with hydroxyl radicals (AOPWIN v1.92; US EPA, 2011). Therefore, in the event of release to atmosphere, the notified chemicals are not expected to persist in the atmospheric compartment.

Most of the notified chemicals will be released to the sewer after use and directed to sewage treatment plants (STPs) nationwide. A small amount of the notified chemicals may be sent to landfill as collected spills or container residues. In STPs, the majority of the notified chemicals are expected to be removed from the water column, via adsorption to sediment sludge, based on their high log P_{OW} (5.2-5.4) and sent to landfill. In landfill or water, the notified chemicals are expected to undergo biotic or abiotic degradation processes, forming water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has been calculated assuming, for the worst case scenarios, entire release of the notified chemicals to the sewer system, and no removal of the notified chemicals from sewage treatment plants (STP).

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	1,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	2.74	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	22.613	million

Removal within STP	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 chemicals in this volume are 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 0.61 µg/L may potentially result in a soil concentration of approximately 4.0 µg/kg. Assuming accumulation of the notified chemicals in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemicals in the applied soil in 5 and 10 years may be approximately 20.2 µg/kg and 40.4 µg/kg, respectively. In addition, the hydrolysis is expected to significantly decrease the expected concentration of the notified chemicals in soil.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on the notified chemicals are summarised in the table below. The endpoints for the *Daphnia* and alga represent the mixture of the notified chemicals and hydrolysis degradates (oxidation product) in carboxylic acid form of the notified chemicals. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 > 0.33 mg/L	Potentially very toxic to fish*
Daphnia Toxicity	48 h EC50 = 0.84 mg/L	Very toxic to *Daphnia*
Algal Toxicity	72 E_rC50 = 18 mg/L	Harmful to alga
	72 h NOEC = 4.5 mg/L	

* Uncertain data needs to be taken with caution, see Appendix C for details.

Based on the above toxicity data for *Daphnia*, the notified chemicals are considered to be very toxic to aquatic organisms. Under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009) the notified chemicals are very toxic to aquatic organisms and are formally classified as 'Acute Category 1: Very toxic to aquatic life'. The notified chemicals are not readily biodegradable. However, they are expected to readily hydrolyse. On the basis of the acute toxicity, the partition coefficient, and hydrolysis, the notified chemicals are classified as 'Chronic Category 1: Very toxic to aquatic life with long lasting effects'.

7.2.1. Predicted No-Effect Concentration

The toxicity data provided by the notifier for fish is not considered to be reliable for risk assessment purposes. ECOSAR (US EPA, 2011) estimates for the toxicities of the notified chemicals to aquatic organisms indicate that *Daphnia* is more acutely sensitive than fish and alga. The predicted no-effect concentration (PNEC) was calculated using the available endpoint for *Daphnia* as shown below. A conservative safety factor of 1000 was used as acute toxicity values from only two trophic levels (*Daphnia* and alga) are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (*Daphnia*)	0.84	mg/L
Assessment Factor	1000	
PNEC:	0.84	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.61	0.84	0.73
Q - Ocean	0.06	0.84	0.07

The risk quotient (RQ = PEC/PNEC) for discharge of treated effluents containing the notified chemicals to the aquatic environment indicates that the notified chemicals are unlikely to reach ecotoxicologically significant

concentrations based on the annual importation quantity. Therefore, on the basis of the PEC/PNEC ratio and the assessed use pattern, the notified chemicals are not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

All studies were conducted using a mixture of the notified chemicals.

Melting Point/Freezing Point < -20 °C

Method	OECD TG 102 Melting Point/Melting Range.
Remarks	Determined using a crystallization point procedure using an aliquot of the test substance in an acetone/dry ice bath.
Test Facility	Harlan (2013a)

Boiling Point 265 ± 1.0 °C at 101.9 kPa

Method	OECD TG 103 Boiling Point.
Remarks	Determined by differential scanning calorimetry. Aliquots of the test substance were placed in crucibles heated from 20 °C to 450 °C at 20 °C per minute.
Test Facility	Harlan (2013a)

Density 891 kg/m^3 at 20 ± 0.5 °C

Method	OECD TG 109 Density of Liquids and Solids.
Remarks	Determined using a pycnometer method.
Test Facility	Harlan (2013a)

Vapour Pressure 2.5 x 10^{-3} kPa at 25 °C

Method	OECD TG 104 Vapour Pressure.
Remarks	Determined using the vapour pressure balance method.
Test Facility	Harlan (2013b)

Water Solubility Not determined

Method	OECD TG 105 Water Solubility.
Remarks	Flask Method. The mixtures of the notified chemicals with distilled water at 40 mg/L were shaken at approximately 30 °C for 19 hours. After standing at 20 °C for a period of not less than 24 hours, the mixtures were centrifuged at 10,000 rpm for 30 minutes and then filtered through a 0.2 µm Nylon membrane filter. The concentration of the sample solutions was determined by gas chromatography to be 1.44 × 10^{-4} g/L at 20 °C. This was supposed to be the water solubility. It is noted in the *Daphnia* and algal studies that the notified chemicals may readily hydrolyse into a carboxylic acid form of the chemicals. Therefore, the low concentration detected 43 hours after the mixture preparation may be due to hydrolysis and may not represent the actual water solubility of the notified chemical.
Test Facility	Harlan (2011a)

Partition Coefficient (n-octanol/water) log Pow = 5.2 – 5.4

Method	OECD TG 117 Partition Coefficient (n-octanol/water).
Remarks	HPLC Method. As the test item was considered to contain no ionisable functional groups, testing was carried out around neutral pH with the test item in a non-ionised form. The partition coefficient of the mixture of notified chemicals was determined to be in the range of 1.57 x 10^5 to 2.49 x 10^5, or the log P_{OW} is in the range of 5.20 to 5.40.
Test Facility	Harlan (2011a)

Surface Tension 70.7 mN/m at 21.0 ± 0.5 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions.
Remarks	Concentration: 90% saturated aqueous solutions of test substance. Determined using a tensiometer, following the ring method.

The test substance was not considered to be surface active.
Test Facility Harlan (2013a)

Flash Point 121 ± 2.0 °C at 101.3 kPa

Method EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks Determined using a closed cup equilibrium method.
Test Facility Harlan (2013c)

Autoignition Temperature 218 ± 5.0 °C

Method EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks Determined by heating aliquots of the test substance with a flask heater and observing for any ignition.
Test Facility Harlan (2013c)

Explosive Properties Predicted negative

Method EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks Observation of functional groups that would imply explosive properties.
Test Facility Harlan (2013c)

Oxidizing Properties Predicted negative

Method EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids).
Remarks Observation of functional groups that would imply oxidizing properties.
Test Facility Harlan (2013c)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

All studies were conducted using a mixture of the notified chemicals.

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemicals (isomers present at: *trans*- 32.1% and *cis*- 60.3%)
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/ Wistar (RccHan™:WIST)
Vehicle	Arachis oil BP (300 mg/kg bw) or none (2,000 mg/kg bw).
Remarks - Method	No significant protocol deviations. GLP Compliance.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3F	300	0/3
2	3F	2,000	0/3
3	3F	2,000	0/3

LD50	> 2,000 mg/kg bw
Signs of Toxicity	Clinical observations seen in animals treated at 2,000 mg/kg bw included piloerection (3/6 animals) and hunched posture (6/6 animals). One of these animals was seen to additionally exhibit ptosis, ataxia, lethargy and laboured, noisy and gasping respiration. This animal was still showing signs at the end of the observation period. Signs of toxicity were observed in all other animals from 1 hour to 4 hours post dosing only. No signs of systemic toxicity were noted in any animals treated at 300 mg/kg bw.
Effects in Organs	No macroscopic abnormalities were seen at necropsy in any of the test animals.
Remarks - Results	All test animals showed body weight gains during the study period, except for one animal treated at 2,000 mg/kg bw, which showed weight loss during the first week, but gained weight during the second week of observation. This was the same animal which showed additional signs of toxicity throughout the study period.
CONCLUSION	The mixture of notified chemicals is of low toxicity via the oral route.
TEST FACILITY	Harlan (2013d)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemicals (isomers present at: *trans*- 32.1% and *cis*- 60.3%)
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test.
Species/Strain	Rat/Wistar (RccHan™:WIST)
Vehicle	None.
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations. GLP Compliance.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 per sex	2,000	0/10

LD50	> 2,000 mg/kg bw
Signs of Toxicity - Local	Signs of dermal irritation were seen in 4/5 female test animals only. Well defined to very slight (barely perceptible) erythema was noted in the 4 females from day 2 to day 6. Very slight (barely perceptible) oedema was also seen in the 4 females (day 2 only). Slight desquamation and/or small superficial scattered scabs and crust formation were also noted in the animals from day 2 to day 9. All signs had resolved by day 10 of the study.
Signs of Toxicity - Systemic	There were no signs of systemic toxicity noted in the test animals.
Effects in Organs	No macroscopic abnormalities were detected at necropsy.
Remarks - Results	All test animals showed body weight gains during the study period.

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

TEST FACILITY Harlan (2014a)

B.3. Corrosion – skin (in vitro)

TEST SUBSTANCE Notified chemicals (isomers present at: *trans*- 32.1% and *cis*- 60.3%)

METHOD OECD TG 431 In vitro Skin Corrosion – Reconstructed Human Epidermis (RHE) Test Method

Vehicle	None.
Remarks - Method	EPISKIN™ In Vitro Reconstructed Human Epidermis (RHE) Model. No significant protocol deviations. GLP Compliance.

In the pre-test, the test substance was shown to directly reduce MTT. Therefore, the main test was performed in parallel on viable and water-killed tissues (true viability values are presented for the test substance in the results table below).

For the main test, the test substance (50 µL) was applied to the tissues in duplicate. Following exposure periods of 3 minutes (test 1), 1 hour (test 2) and 4 hours (test 3), all at room temperature, the tissues were rinsed, treated with 2.0 mL of MTT solution (0.3 mg/mL) and then incubated at 37 °C for 3 hours.

Positive and negative controls were run in parallel with the test substance:
Negative control (NC): 0.9% sodium chloride solution
Positive control (PC): Glacial acetic acid

RESULTS

RESULTS

Test material	*Test 1 (3 minute exposure period)*		*Test 2 (1 hour exposure period)*		*Test 3 (4 hour exposure period)*	
	Mean OD_{562} of duplicate tissues	*Relative mean viability (%)*	*Mean OD_{562} of duplicate tissues*	*Relative mean viability (%)*	*Mean OD_{562} of duplicate tissues*	*Relative mean viability (%)*
Negative control	-	-	-	-	0.818	100*
Test substance	0.855	104.5	0.761	93.0	0.822	100.5
Positive control	-	--	-	-	0.027	3.3

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

Remarks - Results	The positive and negative controls gave satisfactory results, confirming the validities of the test systems.
CONCLUSION	The mixture of notified chemicals was non-corrosive to the skin under the conditions of the test.
TEST FACILITY	Harlan (2014b)

B.4. Irritation – skin (in vitro)

TEST SUBSTANCE	Notified chemicals (isomers present at: *trans*- 32.1% and *cis*- 60.3%)
METHOD	OECD TG 439 In vitro Skin Irritation – Reconstructed Human Epidermis Test Method
Vehicle	None.
Remarks - Method	EPISKIN^{TM} Reconstructed Human Epidermis Model. No significant protocol deviations. GLP Compliance. In the pre-test, the test substance was shown to directly reduce MTT. Therefore, the main test was performed in parallel on viable and water-killed tissues. For the skin irritation test, the test substance (10 µL) was applied to the tissues in triplicate. Following an exposure period of 15 minutes at room temperature, the tissues were rinsed and then incubated in fresh medium at 37 °C for ~42 hours. The tissues were then treated with MTT and incubated at 37 °C for 3 hours. Positive and negative controls were run in parallel with the test substance: Negative control (NC): Phosphate Buffered Saline Dulbecco's (PBS) with Ca^{++} and Mg^{++} Positive control (PC): sodium dodecyl sulphate (SDS) 5% w/v

RESULTS

Irritation test

Test material	*Mean OD_{562} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	0.863	100.0*	9.8
Test substance	0.733	85.0	6.3
Positive control	0.069	8.0	1.0

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

Remarks - Results	The study authors considered that the results of this test showed no degree of interference due to direct reduction of MTT. It was hence considered unnecessary to use the results of the water-killed tissues. The positive and negative controls gave satisfactory results, confirming the validities of the test systems.
CONCLUSION	The mixture of notified chemicals was non-irritating to the skin under the conditions of the test.
TEST FACILITY	Harlan (2014c)

B.5. Irritation – skin

TEST SUBSTANCE	Notified chemicals (isomers present at: *trans*- 32.1% and *cis*- 60.3%)

METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White (Hsdlf:NZW)
Number of Animals	3 M
Vehicle	None.
Observation Period	14 days.
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations. GLP Compliance.

RESULTS

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

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

Remarks - Results	Well-defined erythema and very slight oedema were seen in all 3 animals at the 24, 48 and 72 hour observations. Erythema had dissipated to very slight by the day 7 observations. Loss of elasticity was noted in all 3 animals at the 72 hour observations only. In addition, moderate desquamation was noted in 2 animals on day 7 (1 of these animals showing glossy skin) and crust formation (which prevented the evaluation of erythema and oedema) was noted in the third animal. All effects were resolved by the day 14 observation. All animals showed weight gains during the study period.
CONCLUSION	The mixture of notified chemicals is irritating to the skin.
TEST FACILITY	Harlan (2014d)

B.6. Irritation – eye (in vitro)

TEST SUBSTANCE	Notified chemicals (isomers present at: *trans*- 32.1% and *cis*- 60.3%)
METHOD	Determination of Ocular Irritation Potential Using the SkinEthic Reconstituted Human Corneal Epithelium Model
Vehicle	None.
Remarks - Method	GLP Compliance. The test substance (30 µL) was applied to the tissues in triplicate. Following 10 minute exposure periods, the tissues were rinsed and then treated with MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 0.5 mg/mL; incubation period of 3 hours at 37 °C]. Following extraction, the optical densities were determined (562 nm). A positive (sodium dodecyl sulphate (SDS) in sterile water at 2% w/v) and negative control (Solution A supplied by SkinEthic) were run in parallel with the test substance. The test substance was considered by the study authors to be an irritant if the relative mean tissue viability was ≤ 60%. The study authors indicated that a preliminary test had been conducted, which indicated that the test substance directly reduces MTT, therefore a MTT viability assay was performed in parallel on viable and freeze-killed

tissues.

RESULTS

Test material	*Mean OD_{562} of triplicate tissues*	*Relative mean viability (%)*
Negative control	1.016	100*
Test substance	1.021	100.5
Positive control	0.183	18.0

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

Remarks - Results

The study authors considered that the results of this test showed a negligible degree of interference due to direct reduction of MTT. It was hence considered unnecessary to use the results of the freeze-killed tissues.

The relative mean viability of the test substance treated tissues after a 10-minute exposure period was 100.5%.

The positive and negative controls gave satisfactory results, confirming the validities of the test systems.

CONCLUSION

The mixture of notified chemicals was considered to be non-irritating to the eye under the conditions of the test.

TEST FACILITY

Harlan (2013e)

B.7. Irritation – eye

TEST SUBSTANCE

Notified chemicals (isomers present at: *trans*- 32.1% and *cis*- 60.3%)

METHOD

OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain: Rabbit/New Zealand White (Hsdlf:NZW)
Number of Animals: 3 M
Observation Period: 14 days
Remarks - Method: GLP Compliance.

Prior to the test substance administration (~1 hour), buprenorphine (0.01 mg/kg) was administered by subcutaneous injection. In addition, ~5 minutes prior to administration, tetracaine hydrochloride (0.5%; 2 drops) was applied to each eye. Following test substance administration (~8 hours) buprenorphine (0.01 mg/kg) and meloxicam (0.5 mg/kg) were administered to provide continuous systemic analgesia (~12 hours).

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	2	1.67	1.33	2	< 14 days	0
Conjunctiva: chemosis	1	1.67	1	2	< 14 days	0
Conjunctiva: discharge	1	0.67	1	2	< 14 days	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	1	< 24 hours	0

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

Remarks - Results

Moderate conjunctival irritation was noted in all treated eyes 1 hour after treatment. This degree of reaction (conjunctival redness) continued in 2 treated eyes at the 24 and 48 hour observations (and in one of these, at the 72 hour observation), with only slight conjunctival irritation effects

recorded thereafter. Conjunctival chemosis and discharge were seen in all 3 treated eyes, varying in severity and persistence between the animals up to day 14.

All animals showed weight gains during the study period.

CONCLUSION The mixture of notified chemicals is slightly irritating to the eye.

TEST FACILITY Harlan (2014e)

B.8. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Notified chemicals (93.2%, individual isomer concentrations not specified)

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain Mouse/ CBA/CaOlaHsd
Vehicle Acetone/olive oil (AOO; 4:1)
Remarks - Method No significant protocol deviations.
GLP Compliance.

A preliminary toxicity study was performed with the undiluted test substance and used to select the concentrations for the main test. No signs of toxicity were noted in this test.

A concurrent positive control study was not run, but had been previously conducted in the test laboratory (α-Hexylcinnamaldehyde, as a 25% v/v dilution in AOO).

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/animal)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	1,149.36 (± 630.41)	-
25	2,033.61 (± 935.56)	1.77
50	3,357.72 (± 730.21)	2.92
100	5,383.10 (± 1,755.99)	4.68

Remarks - Results No signs of systemic toxicity were noted in the test or control animals.

An EC-3 of 52% was calculated for the notified chemicals.

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

TEST FACILITY Harlan (2011b)

B.9. Skin sensitisation – human volunteers

TEST SUBSTANCE Notified chemicals (5% w/w; individual isomer concentrations not specified)

METHOD Repeated insult patch test with challenge.
Study Design Induction Procedure: Patches containing 0.2 mL test substance were applied 3 times per week (Monday, Wednesday and Friday) for a total of 9 applications. Patches were removed by the applicants after 24 h and graded after an additional 24 h (or 48 h for patches applied on Friday).
Rest Period: approximately 2 weeks
Challenge Procedure: A patch was applied to a naïve site. Patches were

	removed by technicians after 24 h. Sites were graded 24 and 48 h post-patch removal.
Study Group	95 F, 18 M; age range 19 to 69 years
Vehicle	EtOH:DEP (1:3)
Remarks - Method	Occluded. The test substance was spread on a 3.63 cm^2 patch, and allowed to evaporate for 30-90 minutes prior to patch application. A panel of 113 healthy human subjects (devoid of any physical or dermatological conditions) was amassed.

RESULTS

Remarks - Results	105/113 subjects completed the study. The 8 subjects who discontinued were deemed by the study authors to do so for reasons unrelated to the test material. Prior to the day 1 induction, 2 subjects were discontinued. For 4 subjects, discontinuation occurred in the induction phase (1-4 induction observations recorded). Another subject was absent from the 24 hour challenge patch removal and subsequently discontinued. The final subject was discontinued prior to the final challenge observation. None of the discontinued subjects showed clinical signs prior to discontinuation. A male subject showed barely perceptible erythema and dryness at the 6th induction observation. At the 7th induction observation, well defined erythema was observed in this subject and subsequently the application site was changed for this subject. No responses were noted at challenge in this subject. Barely perceptible erythema was seen in 2 female subjects during the challenge phase. In the first subject it was noted at patch removal only. In the second subject, it was noted 48 and 72 hours post-patch removal. No responses were evident in the remaining test subjects during either the induction or challenge phases.
CONCLUSION	The mixture of notified chemicals was deemed by the study authors to be non-sensitising under the conditions of the test.
TEST FACILITY	CRL (2013)

B.1. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical (92.4%, individual isomer concentrations not specified)
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain	Rat/Wistar Han™:RccHan™:WIST.
Route of Administration	Oral – dietary
Exposure Information	Total exposure days: 28 days Post-exposure observation period: 14 days Dose regimen: 7 days per week
Remarks - Method	No significant protocol deviations. GLP Compliance. The mean achieved doses were 38.2, 269.1 and 1137.8 mg/kg bw/day for males and 42.7, 313.2 and 1239.7 mg/kg bw/day for females.

RESULTS

Group	*Number and Sex of Animals*	*Dose ppm bw/day*	*Mortality*
control	5 per sex	0	0/10
low dose	5 per sex	500	0/10
mid dose	5 per sex	3,500	0/10

high dose	5 per sex	15,000	0/10
control recovery	5 per sex	0	0/10
high dose recovery	5 per sex	15,000	0/10

Mortality and Time to Death

There were no unscheduled deaths during the study period.

Clinical Observations

No toxicologically significant changes were noted in behaviour or results in sensory reactivity tests, for animals of either sex, dosed at any level, throughout the study period. Clinical observations were restricted to the observation of diuresis in 2 high dose females on day 18 of the study only. While there were statistically significant variations in functional performance in fore and/or hind limb grip strength assessment seen in all female dose groups and high dose males, the study authors did not deem any differences attributable to a neurotoxic effect of the treatment due to a lack of true dose response relationship and/or supporting clinical observations.

Statistically significant effects on mean body weight gains were noted in both sexes at the highest dose level. During the first week of treatment, group mean body weight gains for both sexes of the high dose groups were reduced compared to controls. This resolved in week 2 for females and week 3 in males.

Various effects on water consumption, food efficiency and consumption were noted at observation points throughout the study period, particularly in high dose animals (the study authors considered that there was an initial reluctance to eat the dietary formulation).

No effects were seen in the estrous cycles of the female animals during the study period.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There were some statistically significant effects seen in haematological parameters:

Treatment (ppm)	Males	Females
15,000	↓ haemoglobin, haematocrit, total leukocyte count, neutrophil count, eosinophil count and platelet count.	↑ prothrombin time ↓ haemogloblin, haematocrit, activated partial thromboplastin time
3,500	↓ haemoglobin, haematocrit, total leukocyte count, neutrophil count and eosinophil count	↑ prothrombin time
500	↓ haemoglobin and haematocrit.	-

As many of the individual animal values for the haematological parameters fell inside the normal range for rats of the strain and age used and/or due to the absence of a true dose-response relationship or any associated changes, the study authors did not deem any of these results to be of toxicological significance. No statistically significant effects on haematological parameters were noted following recovery.

There were also several statistically significant effects on blood chemistry parameters seen in all dose groups at the end of the treatment period:

Treatment (ppm)	Males	Females
15,000	↑ albumin/globulin ratio, alkaline phosphatase, chloride ↓ total protein, albumin, triglycerides, cholesterol, calcium, bilirubin	↑ albumin/globulin ratio, potassium, aspartate aminotransferase, bile acid, urea, alkaline phosphatase ↓ cholesterol, calcium, total protein, albumin
3,500	↓ total protein, albumin, bilirubin	↑ alkaline phosphatase ↓ total protein, albumin
500	↓ total protein, bilirubin	-

High dose recovery group females continued to show statistically significant increases in alkaline phosphatase and decreases in cholesterol levels at the end of the recovery period. As the majority of the individual animal blood chemistry values fell outside the normal range for rats of the strain and age used and/or due to the associated histopathological changes seen, the study authors considered that a relationship to treatment could not be excluded. The intergroup differences were considered by the study authors to be most likely associated

with altered metabolism as result of adaptive liver changes.

There were no toxicologically significant effects detected on the urinalytical parameters measured.

Effects in Organs

Macroscopic necropsy findings did not indicate any adverse effect of treatment. A low dose female animal had a malformed uterus and cervix with off-white fluid in the vagina and a recovery control group female was noted to have reddened mandibular lymph nodes. No other macroscopic abnormalities were noted in any other test animals.

There were no effects seen on sperm concentration, motility, morphological assessment or homogenisation-resistant spermatid count during the treatment period. While high dose recovery group males showed a statistically significant reduction in sperm concentration and motility values at the end of the study, these observations were not considered by the study authors to be toxicologically significant due to the absence of a similar effect in non-recovery males and/or correlating histopathological changes.

Various statistically significant effects were seen on organ weights:

Treatment (ppm)	Males	Females
15,000	↑ liver (absolute & relative), kidney (absolute & relative), brain (absolute & relative), ↓ thyroid/parathyroid (absolute & relative).	↑ liver (absolute & relative), ↓ pituitary (absolute & relative),
3,500	↑ liver (absolute & relative); ↓ thyroid/parathyroid (absolute & relative).	↑ liver (absolute & relative),
500	↓ thyroid/parathyroid (absolute & relative).	-

High dose recovery group females also showed an increase in liver weights (absolute and relative), decrease in thyroid/parathyroid weight (absolute and relative) and a decrease in pituitary weights (absolute and relative) at the end of the recovery period. The intergroup differences in thyroid, brain and pituitary weights were considered by the study authors to be of no toxicological significance due to the individual values falling within the expected range for the strain and age of rat, the absence of a true dose-response relationship and/or associated histopathological findings.

Some microscopic histopathological abnormalities were detected in the liver and kidney of various animals. Diffuse hypertrophy of the liver (characterised by homogenous eosinophilic cytoplasm) was detected in animals of both sexes in the mid and high dose groups. An increased incidence of single cell hepatocyte necrosis (of minimal severity) was also evident in the mid and high dose animals. While not present in the control animals, it occurred at a similar incidence in the low dose group animals to control and high dose recovery group animals and so was considered by the study authors to be an incidental finding at this dose level, with complete recovery of the liver evident. As there was considered to have been a complete recovery after the treatment free period, the study authors did not consider the effects in the liver to be adverse.

Hyaline droplets were evident in treated and recovery males. However, this observation is male rat-specific and is not relevant to human exposure to the test substances.

Remarks – Results

Over the course of the study period, treatment related effects were evident in animals of both sexes, at the mid and high dose levels. The microscopic liver changes and associated blood chemistry changes were considered by the study authors to represent adaptive changes and were not considered to represent serious damage to the health of the test animals.

CONCLUSION

The effects in the kidneys were not considered relevant to human exposure and the microscopic liver changes and associated blood chemistry changes were considered by the study authors to represent adaptive changes and were not considered to represent serious damage to the health of the test animals. Therefore, the No Observed Effect Level (NOEL) was established by the study authors as 500 ppm (equivalent to a mean achieved dose of 38.2 mg/kg bw/day in males and 42.7 mg/kg bw/day in females) in this study. The No Observed (Adverse)

Effect Level (NO(A)EL) was established by the study authors as 15,000 ppm (equivalent to a mean achieved dose of 1,137.8 mg/kg bw/day in males and 1,239.7 mg/kg bw/day in females) in this study.

TEST FACILITY Harlan (2014f)

B.2. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemicals (93.2%, individual isomer concentrations not specified)

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

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: 1.5 - 5000 µg/plate
b) Without metabolic activation: 0.015 – 5,000 µg/plate

Vehicle Acetone

Remarks - Method No significant protocol deviations.
GLP Compliance.

A preliminary toxicity test (0 – 5,000 µg/plate) was performed to determine the toxicity of the test material (TA100 and WP2uvrA$^-$ only) in the presence and absence of metabolic activation. The test substance was found to be toxic to the TA100 tester strain at and above 50 µg/plate with metabolic activation and 500 µg/plate with metabolic activation. The test substance was non-toxic to the WP2uvra tester strain.

Tests 1 (range-finding test using direct plate incorporated method) and 2 (pre-incubated) were conducted on separate days using fresh cultures and test substance solutions. The concentration range for Test 1 was 5 – 5,000 or 0.5 – 500 µg/plate for the *Salmonella* strains, with and without S9 fraction, respectively and 50 – 5,000 µg/plate for WP2uvrA. The concentration range for Test 2 was amended for each strain (all within 0.015 - 5000 µg/plate) based on the results of Test 1.

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	≥ 50	≥ 50	≥ 5,000	negative
Test 2		≥ 5	≥ 5,000	negative
Present				
Test 1	≥ 500	≥ 500	≥ 5,000	negative
Test 2		≥ 150	≥ 5,000	negative

Remarks - Results No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, at any dose level of the test substance, with or without metabolic activation.

The positive controls gave satisfactory responses confirming the validity

of the test system.

CONCLUSION The notified chemicals were not mutagenic to bacteria under the conditions of the test.

TEST FACILITY Harlan (2010)

B.3. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemicals (93.2%, individual isomer concentrations not specified)

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.

Species/Strain Human.

Cell Type/Cell Line Lymphocytes.

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

Vehicle Acetone

Remarks - Method No significant protocol deviations.
GLP Compliance.

A preliminary toxicity study was performed (4 hours exposure, with and without activation followed by a 20 hours recovery period, and a continuous 24 hours exposure period without activation) at concentrations 0 – 1961.8 µg/mL. Haemolysis was noted in cultures without metabolic activation at ≥ 7.66 µg/mL. Precipitation of a cloudy nature was seen in cultures at ≥ 61.31 µg/mL, and of a greasy/oily nature at ≥ 122.61 µg/mL.

Vehicle and positive controls were used in parallel with the test material. They included mitomycin C (dissolved in Minimal Essential Medium) without metabolic activation and cyclophosphamide (dissolved in DMSO) with metabolic activation.

The S9 fraction was used in Test 1 at 2% final concentration and Test 2 at 1% final concentration.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 2.5*, 5*, 10*, 15*, 20, 30	4 h	24 h
Test 2	0*, 2.5, 5*, 10*, 20*, 30, 40	24 h	24h
Present			
Test 1	0*, 10*, 20*, 30*, 40*, 50, 60	4 h	24 h
Test 2	0,* 5, 10*, 20*, 30*, 40, 50	4 h	24 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 15.33	≥ 15	> 15	negative
Test 2	≥ 30.65	> 20	> 20	negative
Present				
Test 1	≥ 61.31	≥ 40	> 40	negative
Test 2		≥ 30	> 30	negative

Remarks - Results The maximum dose levels selected for metaphase analysis was based on growth inhibition (mitotic index) of ~50%, or an acceptable level of

	toxicity (excessive toxicity noted at higher doses). No toxicologically significant increases in the number of cells with aberrations or polyploidy cells were noted at any dose level, with or without metabolic activation, in either of the two experiments. The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.
CONCLUSION	The mixture of notified chemicals was not clastogenic to human lymphocytes treated in vitro under the conditions of the test.
TEST FACILITY	Harlan (2011c)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemicals
METHOD	OECD TG 310 Ready Biodegradability-CO_2 in sealed vessels (Headspace Test).
Inoculum	Sewage sludge.
Exposure Period	28 days.
Auxiliary Solvent	Not applied.
Analytical Monitoring	The degradation of the test item was assessed by the determination of carbon dioxide produced.
Remarks - Method	No significant protocol deviations. GLP Compliance. The test was conducted at a concentration of 20 mg C/L. Control solutions with inoculum and the reference item sodium benzoate (20 mg C/L), together with a toxicity control (40 mg C/L) were used for validation purposes.

RESULTS

Test substance		*<Reference Substance>*	
Day	*% Degradation*	*Day*	*% Degradation*
2	0	2	53
10	2	10	76
28	11	28	76

Remarks - Results	All the test validity criteria were met. The toxicity control reached 34% degradation by 28 day, indicating the test substance is not toxic to the micro-organisms. The test results in the table above indicate that the notified chemicals are not readily biodegradable.
CONCLUSION	The notified chemicals are not readily biodegradable
TEST FACILITY	Harlan (2011d)

C.1.2. Inherent biodegradability

TEST SUBSTANCE	Notified chemicals
METHOD	OECD Guideline 302C: Inherent Biodegradability: Modified MITI Test (II) (1981); SEPA, P.R. China the Guidelines for the Testing of Chemical No. 302C: Inherent Biodegradability: Modified MITI Test II (2004)
Inoculum	Activated sludge.
Exposure Period	28 days.
Auxiliary Solvent	Not applied.
Analytical Monitoring	The inherent biodegradability was determined by measuring the biochemical oxygen demand (BOD).
Remarks – Method	No significant protocol deviations. GLP Compliance. The test item was adsorbed onto a piece of glass fibre filter and then directly added to test vessels at a nominal level of 32 mg/L in triplicate. In addition, tests for abiotic control, reference control, and blank controls were also conducted.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*[*]	*Day*	*% Degradation*
4	0.3	4	6.1
14	5.3	7	61.8
28	30	28	86.2

* Average value for triplicate samples

Remarks – Results	All the test validity criteria were met for the OECD test guideline. Based on the calculation for BOD, the notified chemicals reached an average degradation degree of 30% by day 28. Based on this, they are considered to exhibit inherent, primary biodegradability.
CONCLUSION	The notified chemicals have inherent, primary biodegradability
TEST FACILITY	Safety Evaluation Centre (2012)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemicals
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-static test. SEPA, P.R. China the Guidelines for the Testing of Chemical No. 203" Fish, Acute Toxicity Test" (May 2004)..
Species	Zebra fish.
Exposure Period	96 hours.
Auxiliary Solvent	Acetone.
Water Hardness	Not reported.
Analytical Monitoring	The test concentration was determined by gas chromatography.
Remarks – Method	No significant protocol deviations. GLP Compliance. Following a range-finding test, the definitive test was conducted as a limited test at 0.5 mg/L. The test medium was renewed every 24 hours. Acetone was used for preparation of stock solution at 5 mg/mL. A solvent control test was conducted.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Solvent control	Not applicable	7	0	0	0	0	0
0.5	0.33	7	0	0	0	0	0

LC50	> 0.33 mg/L at 96 hours
NOEC	0.33 mg/L at 96 hours
Remarks – Results	All the test validity criteria were met for the OECD test guideline. The notified chemicals showed no signs of effects at the level of 0.33 mg/L. Based on the test outcome, the notified chemicals are considered to be potentially very toxic to fish. It is noted in the *Daphnia* study below that a concentration of 14 mg/L for the notified chemicals was prepared at 0 hour. Therefore, this test outcome should be treated with caution.
CONCLUSION	The notified chemicals may be potentially very toxic to fish on an acute basis

TEST FACILITY Safety Evaluation Centre (2013)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemicals

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test- Semi-static test
Species *Daphnia magna.*
Exposure Period 48 hours.
Auxiliary Solvent Not applied.
Water Hardness 250 mg $CaCO_3$/L
Analytical Monitoring Test concentrations for the notified chemicals and the hydrolysis degradate were analysed using gas chromatography.
Remarks - Method No significant protocol deviations.
GLP Compliance.

Following a preliminary range-finding test, the definitive test was performed at concentrations of 10, 18, 32, 56 and 100% (v/v) saturated solution for 48 hours at 20 °C. Filtered water accommodated fractions (WAFs) were used for the test. The test item solutions were prepared by stirring an excess (50 mg/L) of test item in test water for 24 hours. After the stirring period any undissolved test item was removed by filtration (0.2 µm Sartorius Sartopore filter, first approximate 1 litre discarded in order to pre-condition the filter) to produce a 100% (v/v) saturated solution of the test item. This saturated solution was then further diluted to provide the remaining test concentrations.
The EC50 values were calculated by the maximum-likelihood probit method using the ToxCalc computer software package. Probit analysis was used where two or more partial responses to exposure were shown.

RESULTS

Concentration		*Number of D. magna*	*Number Immobilised*	
Nominal (% v/v saturated solution)	*Actual (mg/L)*		*24 h*	*48 h*
Control	Control	20	0	0
10	0.51	20	1	1
18	1.4	20	16	19
32	3.3	20	20	20
56	6.3	20	20	20
100	14	20	20	20

EC50 0.84 mg/L at 48 hours
NOEC 0.51 mg/L at 48 hours
Remarks - Results All the test validity criteria were met for the OECD test guideline.
Chemical analysis of samples taken from the range-finding test indicated that in addition to notified chemicals, there was a significant amount of an hydrolysis degradate (oxidation product) in carboxylic acid form of the notified chemicals. Test organisms were therefore exposed to a mixture of both notified chemicals and the hydrolysis degradate. A decline in measured concentrations was observed through the test period. Therefore, the endpoints were expressed based on the geometric test concentrations. The notified chemicals (together with hydrolysis degradate) are considered to be very toxic to *Daphnia* based on the test outcome.

CONCLUSION The notified chemicals are very toxic to *Daphnia* on an acute basis

TEST FACILITY Harlan (2014g)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemicals
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Pseudokirchneriella subcapitata.*
Exposure Period	72 hours.
Concentration Range	Nominal: 1.0%, 3.2%, 10%, 32%, and 100% v/v saturated solution Actual: 0.18, 0.75, 4.5, 11, and 36 mg/L (time-weighted mean measured)
Auxiliary Solvent	Not reported.
Water Hardness	Not reported.
Analytical Monitoring	Test concentrations for the notified chemicals and the hydrolysis degradate were analysed using gas chromatography.
Remarks - Method	No significant protocol deviations. GLP Compliance. Following a preliminary range-finding test, the definitive test was performed at concentrations of 1.0, 3.2, 10, 32 and 100% (v/v) saturated solution for 72 hours at 24°C. Filtered water accommodated fractions (WAFs) were used for the test. The test item solutions were prepared by stirring an excess (50 mg/L) of test item in test water for 24 hours. After the stirring period any undissolved test item was removed by filtration (0.2 µm Sartorius Sartopore filter, first approximate 1 litre discarded in order to pre-condition the filter) to produce a 100% (v/v) saturated solution of the test item. This saturated solution was then further diluted to provide the remaining test concentrations. One way analysis of variance incorporating Bartlett's test for homogeneity of variance (Sokal and Rohlf, 1981) and Dunnett's multiple comparison procedure for comparing several treatments with a control (Dunnett, 1955) was carried out on the growth rate and yield data after 72 hours to determine any statistically significant differences between the test and control groups. All statistical analyses were performed using the SAS computer software package (SAS, 1999 - 2001).

RESULTS

Biomass		*Growth*	
E_bC50	*NOEC*	*E_rC50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
11	4.5	18	4.5

Remarks - Results	All the test validity criteria were met for the OECD test guideline. Chemical analysis indicated that in addition to notified chemicals, there was a significant amount of an hydrolysis degradate (oxidation product) in carboxylic acid form of the notified chemicals. Test organisms were therefore exposed to a mixture of both notified chemicals and the hydrolysis degradate. A decline in measured concentrations was observed through the test period. Therefore, the endpoints were expressed based on the time-weighted mean measured test concentrations. The notified chemicals (together with hydrolysis degradate) is considered to be harmful to alga based on the test outcome.
CONCLUSION	The notified chemicals are harmful to alga on an acute basis
TEST FACILITY	Harlan (2014h)

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