File No: LTD/1773

January 2015

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Benzeneacetonitrile, α-butylidene-, (Z)-

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
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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/1773	Firmenich Limited	Benzeneacetonitrile, α-butylidene-, (Z)-	Yes	≤ 1 tonne per annum	Fragrance ingredient

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard classification	*Hazard statement*
Acute Oral Toxicity (Category 4)	H302 – Harmful if swallowed
Skin Sensitisation (Category 1)	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:
R22: Harmful if swallowed
R43: May cause sensitisation by skin contact

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 information available, when used at ≤ 0.22% in fine fragrances, ≤ 0.1% in other leave-on cosmetic products and ≤ 1% in rinse-off cosmetic 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, maximum annual importation volume and assessed use pattern the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Acute Oral Toxicity (Category 4): H302 – Harmful if swallowed
  - Skin Sensitisation (Category 1): H317 – May cause an allergic skin reaction

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

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

Health Surveillance

- As the notified chemical is a skin 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
  - 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 chemical 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 chemical during reformulation processes:
  - Impervious gloves, coveralls

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

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

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

Public Health

- The following measures should be taken by formulators to minimise public exposure to the notified chemical:
  - The notified chemical should only be used at ≤ 0.22% in fine fragrances, ≤ 0.1% in other leave-on cosmetic products and ≤ 1% in rinse-off cosmetic and household products.

Disposal

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

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by containment, 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 chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the

notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

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

(1) Under Section 64(1) of the Act; if
- the importation volume exceeds one tonne per annum notified chemical;
- the concentration of the notified chemical exceeds or is intended to exceed 0.22% in fine fragrances, 0.1% in other leave-on cosmetic products and 1% in rinse-off cosmetic or household products;

or

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

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

(Material) Safety Data Sheet
The (M)SDS of the notified chemical 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)
Firmenich Limited (ABN: 86 002 964 794)
73 Kenneth Rd
BALGOWLAH, NSW 2093

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

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

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: absorption/desorption, dissociation constant, particle size, flammability limits, reactivity, explosive and oxidising properties.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None.

NOTIFICATION IN OTHER COUNTRIES
China (2010), EU (2008), Philippines (2008), Switzerland (2008), USA (2011)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Salicynile

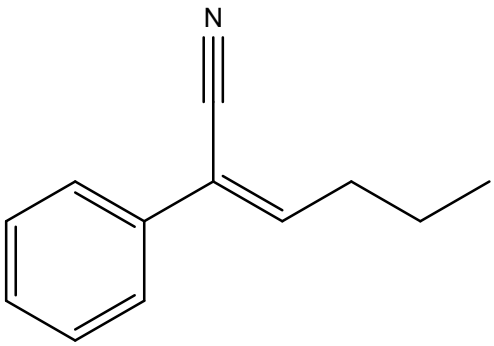
CHEMICAL NAME
Benzeneacetonitrile, α-butylidene-, (Z)-

OTHER NAME(S)
(2Z)-2-phenyl-2-hexenenitrile
Benzeneacetonitrile, α-butylidene-, (αZ)-

CAS NUMBER
130786-09-3

MOLECULAR FORMULA
$C_{12}H_{13}N$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
171.24 Da

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

3. COMPOSITION

DEGREE OF PURITY
> 90%

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 ± 0.5 °C	Measured
Boiling Point	270 ± 2 °C at 97.8 kPa	Measured
Density	971 kg/m^3 at 20 ± 0.5 °C	Measured
Vapour Pressure	5.9 x 10^{-4} kPa at 25 °C	Measured
Water Solubility	0.019 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	t½ > 1 year at 25 °C (pH 4 – 9)	Measured
Partition Coefficient (n-octanol/water)	log Pow = 3.7 at 20 °C	Measured
Adsorption/Desorption	log K_{oc} = 3.36	Calculated (using KOCWIN v2.00; US EPA, 2009)
Dissociation Constant	Not determined	Not readily dissociable functionality
Flash Point	127 ± 2 °C at 101.3 kPa	Measured
Autoignition Temperature	392 ± 5 °C	Measured
Explosive Properties	Predicted negative	Contains no functional groups that would imply explosive properties.
Oxidising Properties	Predicted negative	Contains no functional groups that would imply oxidising properties.

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

Reactivity
The notified chemical is expected to be stable under normal conditions of use.

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. The notified chemical will be imported into Australia at 100% concentration, as well as a component of compounded fragrance formulations (at concentrations ≤ 10%) and various formulated end-use cosmetic and household products (at concentrations ≤ 1%).

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1

PORT OF ENTRY
Sydney, by wharf or airport.

IDENTITY OF MANUFACTURER/RECIPIENTS
Firmenich Limited.

TRANSPORTATION AND PACKAGING
The notified chemical (at ≤ 100% concentration) will be imported into Australia in lacquered drums of sizes ranging from 5 kg up to 180 kg. The end-use products (≤ 1% notified chemical) will be packaged in typical consumer-sized containers suitable for retail sale.

The notified chemical will be transported from the port of entry by road to the notifier's warehouse facilities for storage in its original packaging until transportation to the customer site. Alternatively, the notified chemical and products containing it will be shipped directly from the port of entry to the customer site.

USE
The notified chemical will be used as a fragrance component in a variety of cosmetic and household products. The content in the final consumer products will vary, with the following proposed usage concentrations: fine fragrances (≤ 0.22%), other leave-on cosmetic products (≤ 0.1%) and rinse-off cosmetic and household products (≤ 1%).

OPERATION DESCRIPTION
No manufacturing, processing, reformulating or repackaging of the notified chemical will occur at the notifier's facility. The imported products containing the notified chemical will be stored at this facility until they are transported to customer facilities (in original importation packaging).

At the customer facilities, the procedures for incorporating the imported fragrance preparations (containing ≤ 100% notified chemical) into end-use products will likely vary depending on the nature of the cosmetic and household products formulated, and may involve both automated and manual transfer steps. However, in general, it is expected that the reformulation processes will involve blending operations that will be highly automated and occur in a fully enclosed environment, followed by automated filling of the reformulated products into containers of various sizes.

Household products
Household products containing the notified chemical (≤ 1% concentration) may be used by consumers and professional workers. The products may be used in either closed systems with episodes of controlled exposure, for example automatic washing machines, or open processes and manually applied by rolling, brushing, spraying and dipping, using a cloth, sponge, mop or brush and followed by wiping. In some cases the household product will be diluted with water prior to application.

Cosmetics
The finished cosmetic products containing the notified chemical at ≤ 1% concentration will be used by consumers and professionals (such as beauticians and hairdressers). Depending on the nature of the product, application of products 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 (hours/day)*	*Exposure Frequency (days/year)*
Transport workers	unspecified	unspecified
Mixer	4	2
Drum Handling	4	2
Drum Cleaning	4	2
Maintenance	4	2
Quality Control	0.5	1
Packaging	4	2
Hairdressers/beauticians/cleaners	unspecified	unspecified

EXPOSURE DETAILS

Transport and storage

Transport and storage workers may come into contact with the notified chemical, at 100% concentration or as a component of the imported fragrance preparations (≤ 10% concentration) or end-use products (≤ 1% concentration), only in the event of accidental rupture of 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 the notified chemical at ≤ 100% concentration. Exposures of these workers will be limited to situations of an accidental discharge, spill or leaking drum, requiring clean up. If such an event occurs, a worker may be exposed through dermal or ocular contact. The notifier states that such exposures will be minimised through the use of personal protective equipment (PPE) including protective clothing, chemical resistant gloves and eye protection.

Formulation of end products

During reformulation, dermal, ocular and perhaps inhalation exposure of workers to the notified chemical (at ≤ 100% concentration) may occur during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. The notifier states that exposure is expected to be minimised through the use of mechanical ventilation and/or enclosed systems, and through the use of PPE such as protective clothing, eye protection, impervious gloves and respiratory protection (if appropriate).

Beauty care and cleaning professionals

Exposure to the notified chemical (at ≤ 1% concentration) in end-use products 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 chemical.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemical through the use of the cosmetic and household products (≤ 1% concentration in individual products). The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible, particularly if products are applied by spray.

A combined internal dose of 0.5151 mg/kg bw/day was estimated using data on typical use patterns of cosmetic and household cleaning product categories in which the notified chemical may be used (SCCS, 2012; Cadby *et al.*, 2002; SDA, 2005; specific use details of the notified chemical are considered as exempt information). This estimation assumed a worst case scenario and is for a person who is a simultaneous user of a selection of cosmetic and household products that may contain 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*
Rat, acute oral toxicity	LD50 300 - 2,000 mg/kg bw; harmful
Rat, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
Human, skin sensitisation – RIPT (2.5%)	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL > 600 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosome aberration test.	non genotoxic
Genotoxicity – in vivo mammalian erythrocyte micronucleus test	non genotoxic

Toxicokinetics, metabolism and distribution.
Based on the water solubility (0.019 g/L at 20 ºC), partition coefficient (log P_{ow} = 3.7) and the low molecular weight (< 500 Da) of the notified chemical, passive diffusion across the gastrointestinal (GI) tract and dermal absorption are possible. The notified chemical may also be absorbed across the respiratory tract.

Acute toxicity.
The notified chemical was found to be harmful in an acute oral toxicity study in rats. Clinical symptoms including hunched posture, lethargy, pilo-eretion, ataxia, tiptoe gait, dehydration, hypothermia, decreased respiratory rate and laboured respiration were noted in the single animal treated at 2000 mg/kg bw. This animal was sacrificed after 1 day of treatment for humane reasons. Changes noted at necropsy in this animal included abnormally red lungs and haemorrhage of the gastric mucosa, non-glandular region of the stomach and intestines. No signs of toxicity or abnormalities at necropsy were observed in the animals dosed at 300 mg/kg bw.

The notified chemical was found to have low acute dermal toxicity in rats. No signs of systemic toxicity were noted in any animal. Erythema was noted in animals at ≤ 12 days following patch removal. In addition, slight scaling was noted in 5/5 males and 4/5 females from day 8 until the end of the observation period (14 days).

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

Irritation.
In an acute dermal irritation study in rabbits, a single 4-hour, semi-occluded application of the notified chemical resulted in very slight erythema in two animals at the 48 and 72 hour observations, with slight desquamation seen at the treatment site of one of these animals at the day 7 observation (end of the observation period). Based on the effects observed in this study and the local effects observed in the acute dermal toxicity study (discussed above), the hazard statement "AUH066 – Repeated exposure may cause skin dryness or cracking" is relevant. However, as the notified chemical is a skin sensitiser, the recommended occupational control measures also address those relevant for the AUH066 hazard.

In an acute ocular irritation in rabbits, a single instillation of the notified chemical resulted in iridial inflammation and/or moderate conjunctival irritation in the treated eyes. However, all animals appeared normal by the 72 hour observations. The effects in this study did not warrant classification of the chemical as an eye irritant.

Sensitisation.
The notified chemical was a skin sensitiser in mice (Local Lymph Node Assay; tested at 1, 5, 10, 20 and 40% concentration, with 20 and 40% concentration test substance producing stimulation indices of 3.7 and 10.8, respectively). The EC_3 value was calculated to be 10.1%.

In a human repeat insult patch test (HRIPT) completed on 109 subjects, the notified chemical (at 2.5% concentration) was determined by the study authors to not induce skin sensitisation. However, it is noted that faint, minimal erythema was evident in 4 subjects during the challenge phase (in 2 subjects at 24 hours post-patch removal, 1 subject at 24-72 hours post-patch removal and 1 subject 48-72 hours post-patch removal), with no responses observed in these subjects during the induction phase.

Repeated dose toxicity
In a 28 day repeat dose study by oral gavage, rats were administered the notified chemical at 60, 200 and 600 mg/kg bw/day (with a 2-week recovery group also included for animals treated at 600 mg/kg bw/day). A diverse range of clinical observations, haematological, biochemical and urinalysis findings were observed in test animals of both sexes across the dosed groups (many with statistical significance), for example, statistically significant decreases in mean body weights were noted in animals treated at 600 mg/kg bw/day at either the end of the treatment period (males) or beginning of the recovery period (females), with the weights increasing to expected levels during the recovery period.

Changes in organ weights were noted in all dose groups tested at the end of the treatment period, including elevated mean absolute and relative liver weights (statistically significant in animals treated at 200 and 600 mg/kg bw/day and in males treated at 60 mg/kg bw/day), elevated absolute and relative kidney weights (statistically significant in all treated groups, except relative weight in males treated at 60 mg/kg bw/day). Microscopic findings seen in animals of both sexes in the mid and high dose groups included centrilobular

hypertrophy, increased haematopoiesis of the spleen and tubular hypertrophy of the kidneys (not observed in females of the mid dose group).

While many of the effects seen in this study were statistically significant and/or appeared to have a dose-dependent distribution, overall, the study authors considered the findings to be reversible and/or not adverse. Therefore, the No Observed (Adverse) Effect Level (NO(A)EL) was established by the study authors as > 600 mg/kg bw/day in this study, based on the absence of adverse effects at the highest dose tested.

Mutagenicity/Genotoxicity.
The notified chemical was not mutagenic in a bacterial reverse mutation studies, non-clastogenic in an in vitro mammalian chromosome aberration test and non-genotoxic in a mammalian erythrocyte micronucleus test.

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

Hazard classification	*Hazard statement*
Acute Toxicity (Category 4)	H302 – Harmful if swallowed
Skin Sensitisation (Category 1)	H317 – May cause an allergic skin reaction

In Australia, additional non-GHS hazard statements apply (see *Guidance on the Classification of Hazardous Chemicals Under the WHS Regulations* for further information; SWA, 2012). These include hazard statement "AUH066 – Repeated exposure may cause skin dryness or cracking".

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

R22: Harmful if swallowed
R43: May cause sensitisation by skin contact

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Reformulation
Exposure of workers to the notified chemical (at ≤ 100% concentration) may occur during blending operations. While the notified chemical is considered to be harmful to human health via the oral route, ingestion is unlikely under the occupational settings described. The notified chemical has the potential to cause skin irritation and is considered to be a skin sensitiser. In addition, harmful effects following inhalation exposure to the notified chemical cannot be ruled out. Therefore, caution should be exercised when handling the notified chemical during reformulation processes.

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

End-use
Cleaners and beauty care professionals will handle the notified chemical at ≤ 1% concentration, similar to public use. Therefore, the risk to workers who regularly use products containing the notified chemical is expected to be of a similar or lesser extent than that experienced by members of the public who use such products on a regular basis. For details of the public health risk assessment see Section 6.3.2.

6.3.2. Public Health

Sensitisation
The notified chemical is considered to have the potential to cause skin sensitisation. 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 a fine fragrance (containing 0.22 % notified chemical) as an

example product that may contain the notified chemical, as a worst case scenario, the Consumer Exposure Level (CEL) is estimated to be ~ 8.2 µg/cm^2 (Cadby *et al.*, 2002).

Following consideration of the available data on skin sensitisation (and the study details/results of these studies) and application of appropriate safety factors, an Acceptable Exposure Level (AEL) of ~ 8.2 µg/cm2 was derived (using the EC3 value of 10.1%, which was obtained in an LLNA study on the notified chemical). In this instance, the factors employed included an interspecies factor (3), intraspecies factor (10), a matrix factor (3.16) and a use and time factor (3.16), giving an overall safety factor of ~ 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) at ≤ 0.22% concentration is not considered to be unreasonable. Based on the lower expected exposure level from use of other leave-on cosmetic products (≤ 0.1% notified chemical) and rinse-off cosmetic and household products (≤ 1% 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.

Irritation
The notified chemical has the potential to cause slight irritation to eyes and skin. Skin and eye irritation effects are not expected from use of the notified chemical at the proposed concentrations in cosmetic and household products.

Repeated-dose toxicity
Members of the public may experience repeated exposure to the notified chemical (at ≤ 1% concentration) through the use of the cosmetic and household products.

The repeat dose toxicity potential was estimated by calculation of the margin of exposure (MoE) of the notified chemical using the worst case exposure scenario from use of multiple products of 0.5151 mg/kg bw/day (see Section 6.1.2). While an NOAEL of > 600 mg/kg bw/day was established by the study authors in a 28-day repeated dose toxicity study on the notified chemical, it is noted that many effects were seen in treated animals, including effects that were statistically significant and/or appeared to have a dose-response relationship, but overall, the findings were considered by the study authors to be reversible and/or not adverse. A MoE value ≥ 100 is considered acceptable to account for intra- and inter-species differences. Even with use of the low-dose concentration level (60 mg/kg bw/day) in the repeat dose toxicity study in the calculation of the MoE, a value of > 100 (109) is estimated.

Therefore, based on the information available, the risk to the public associated with the use of the notified chemical at ≤ 0.22% in fine fragrances, ≤ 0.1% in other leave-on cosmetic products and ≤ 1% in rinse-off cosmetic products 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 be imported at 100% concentration or as a component of fragrance preparations for local reformulation into a variety of consumer products. Release during reformulation in Australia is expected to arise from spills (0.1%), formulation equipment cleaning (no release estimate as cleaning water will be recycled) and residues in import containers (0.1%). Accidental spills during transport or reformulation are expected to be collected with inert material and disposed of to landfill. Import containers will either be recycled or disposed of through an approved waste management facility. Therefore, up to 0.2% or up to 2 kg of the import volume is estimated to be released to landfill as a result of reformulation in Australia.

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

RELEASE OF CHEMICAL FROM DISPOSAL

It is estimated that a maximum of 3% of the consumer products containing the notified chemical will remain in end-use containers. These will be disposed of through domestic garbage disposal and will enter landfill or be recycled.

7.1.2. Environmental Fate

Following its use in Australia, the majority of the notified chemical is expected to enter the sewer system before potential release to surface waters on a nationwide basis. The notified chemical is not readily biodegradable and, based on its calculated adsorption coefficient (log Koc = 3.36), partial partitioning to sludge is expected. As only partial adsorption to sludge is expected, release to surface waters may occur. The notified chemical is not likely to bioaccumulate due to its n-octanol/water partition coefficient (log Pow = 3.7). In surface waters, the notified chemical is expected to disperse and degrade through biotic and abiotic processes to form water and oxides of carbon and nitrogen.

The notified chemical is slightly volatile from water (log H = 0.726 Pa/m^3/mol; European Commission, 2003) and may slowly volatilise to air during use or sewage treatment. The half-life of the notified chemical in air is calculated to be 7 h, based on reactions with hydroxyl radicals (AOPWIN v1.92; US EPA, 2009). Therefore, the notified chemical is not expected to persist in the air compartment.

A proportion of notified chemical may be applied to land when effluent is used for irrigation or when sewage sludge is used for soil remediation, or disposed of to landfill. Notified chemical residues in landfill, soil and sludge are expected to have slight mobility based on its predicted soil adsorption coefficient (log K_{oc} = 3.36) and is expected to eventually degrade to form water and oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

The following predicted environmental concentrations (PEC) have been calculated assuming that all of the imported quantity of the notified chemical is released to sewer and up to 32% (12% volatilisation and 20% adsorption to sludge) is removed from waste water by sewage treatment processes (Simple Treat European Commission, 2003) before discharge to surface waters on a nationwide basis.

Insert the PEC if possible and describe assumptions used, otherwise say no aquatic release, etc.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	1,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	2.74	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	32%	Mitigation
Daily effluent production:	4,523	mL
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.41	µg/L
PEC - Ocean:	0.04	µg/L

Partitioning to biosolids in STPs Australia-wide may result in an average biosolids concentration of 1.212 mg/kg (dry wt). Biosolids are applied to agricultural soils, with an assumed average rate of 10 t/ha/year. Assuming a soil bulk density of 1,500 kg/m^3 and a soil-mixing zone of 10 cm, the concentration of the notified chemical may approximate 0.008 mg/kg in applied soil. This assumes that degradation of the notified chemical occurs in the soil within 1 year from application. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated biosolids application, the concentration of notified chemical in the applied soil in 5 and 10 years may approximate 0.04 mg/kg and 0.08 mg/kg, respectively.

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1,000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1,500 kg/m^3). Using these assumptions, irrigation with a

concentration of 0.412 µg/L may potentially result in a soil concentration of approximately 2.746 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 13.73 µg/kg and 27.46 µg/kg, respectively.

7.2. Environmental Effects Assessment

No ecotoxicity data were submitted. By using ECOSAR (US EPA, 2011), the following acute toxicity data have been predicted for the notified chemical.

Endpoint	*Result*	*Assessment Conclusion*
Fish toxicity	96 h LC50 = 0.61 mg/L	Very toxic to fish
Daphnia toxicity	48 h EC50 = 0.45 mg/L	Very toxic to aquatic invertebrates
Algal toxicity	96 h EC50 = 0.26 mg/L	Very toxic to alga

The notified chemical is considered to be very toxic based on the above predicted endpoints. These data are for risk assessment purposes only. Modelled data are not used for the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009). Therefore, the notified chemical has not been formally classified under GHS.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) has been calculated from the estimated acute algal toxicity of the notified chemical and an assessment factor of 500. A more conservative assessment factor of 500 is appropriate, in this case, as although acute endpoints for three trophic levels are available as a general indication of potential toxicity, these endpoints are modelled estimates from a classed-based model which has a low number of chemicals in the training set.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment	
Acute Algal Toxicity	0.26 mg/L
Assessment Factor	500
PNEC:	0.52 µg/L

7.3. Environmental Risk Assessment

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

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.41	0.52	0.78
Q - Ocean	0.04	0.52	0.078

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 in surface waters based on its maximum annual importation quantity. Whilst the notified chemical may be persistent in the environment due to its hydrolytic stability, and a lack of ready biodegrdability, the notified chemical has a low potential for bioaccumulation. On the basis of the PEC/PNEC ratio, maximum annual importation volume and the assessed use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point < - 20 ± 0.5 °C

Method OECD TG 102 Melting Point/Melting Range.
Method A1 of Commission Directive 92/69/EEC.
Remarks Carried out in duplicate using a dry ice/isopropanol bath.
Test Facility Firmenich (2005)

Boiling Point 270 ± 2 °C at 97.8 kPa

Method OECD TG 103 Boiling Point.
Method A2 of Commission Directive 92/69/EEC.
Remarks Determined according to the Siwoloboff method.
Test Facility Firmenich (2005)

Density 971 kg/m^3 at 20 ± 0.5 °C

Method OECD TG 109 Density of Liquids and Solids.
Method A3 of Commission Directive 92/69/EEC.
Remarks Determined using the oscillating density meter method
Test Facility Firmenich (2005)

Vapour Pressure 5.9 x 10^{-4} kPa at 25 °C

Method OECD TG 104 Vapour Pressure.
EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks Determined using the gas saturation method.
Test Facility Harlan (2009a)

Water Solubility 1.9 x 10^{-2} g/L at 20 °C

Method OECD TG 105 Water Solubility.
EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks Flask Method
Test Facility Safepharm (2007a)

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

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

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

Remarks A preliminary test was conducted on the notified chemical at 50°C. The degradation of the notified chemical was found to be < 10% after 5 days at pH 4, 7 & 9. This is equivalent to a half life of > 1 year at 25 °C.
Test Facility Harlan (2009b)

Partition Coefficient (n-octanol/water) log Pow = 3.7 at 20 °C

Method OECD TG 117 Partition Coefficient (n-octanol/water).
EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks HPLC Method
Test Facility Safepharm (2007a)

Flash Point 127 ± 2 °C at 101.3 kPa

Method	Method A9 of Commission Directive 92/69/EEC.
Remarks	Determined using a closed cup equilibrium method.
Test Facility	Firmenich (2005)

Autoignition Temperature 392 ± 5 °C

Method	EC Directive 440/2008/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks	Determined by heating aliquots of the test material in a flask and observing any ignition.
Test Facility	Safepharm (2008)

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. The oxygen balance was calculated to be <-200. The exothermic decomposition energy was determined by DSC, with 2 peaks detected between room temperature and 500 °C (decomposition energy < 500 J/g).
Test Facility	Harlan (2008a)

Oxidizing Properties Predicted negative

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

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure.
EC Council Regulation No 440/2008 B.1 bis Acute toxicity (oral) fixed dose method.

Species/Strain: Rat/ Sprague Dawley
Vehicle: Arachis oil BP
Remarks - Method: No significant protocol deviations.
GLP Compliance.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	1 F	2,000	1/1
2	1 F	300	0/1
3	4 F	300	0/4

LD50: 300 mg/kg bw < LD50 < 2,000 mg/kg bw
Signs of Toxicity: Clinical symptoms noted in the animal dosed at 2,000 mg/kg bw prior to termination on day 1 included hunched posture, lethargy, pilo-eretion, ataxia, tiptoe gait, dehydration, hypothermia, decreased respiratory rate and laboured respiration.
No signs of toxicity were observed in the animals dosed at 300 mg/kg bw.
Effects in Organs: Changes noted at necropsy in the animal treated at 2,000 mg/kg bw included abnormally red lungs and haemorrhage of the gastric mucosa, non-glandular region of the stomach and intestines.
No abnormalities were noted at necropsy for animals dosed at 300 mg/kg bw.
Remarks - Results: The animal dosed at 2,000 mg/kg bw was terminated 1 day after dosing, showing a decrease in body weight. All other animals showed gains in bodyweight over the study period.

CONCLUSION: The notified chemical is harmful via the oral route

TEST FACILITY: Safepharm (2007b)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 402 Acute Dermal Toxicity.
EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal).

Species/Strain: Rat/HanRcc:WIST (SPF)
Vehicle: PEG 300
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	No toxicity was noted in any animal during the day 1 observations. Local signs observed after that point included moderate erythema in all animals on day 2 subsiding to slight erythema up to day 6 (with a female exhibiting erythema up to day 12). Slight scaling was seen in all males and 4 females with onset on day 8 up to the end of the observation period.
Signs of Toxicity - Systemic	No clinical signs were noted in any animal over the test period.
Effects in Organs	No macroscopic findings were seen in any of the animals at necropsy.
Remarks - Results	No deaths occurred during the study. All animals gained weight over the course of the study.

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

TEST FACILITY RCC (2008a)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Directive 2004/73/EC B.4 Acute Toxicity (Skin Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 Males
Vehicle	Water (1:1)
Observation Period	7 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	0.66	0.66	0	1	< 7 days	0
Oedema	0	0	0	0	-	0

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

Remarks - Results	Very slight erythema was noted in 2 animals at the 48 and 72 hour observations. Slight desquamation was seen at the treatment site of 1 of these test animals at the day 7 observation. No oedema was noted.

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY Safepharm (2007c)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 2004/73/EC B.5 Acute Toxicity (Eye Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 Male
Observation Period	72 hours
Remarks - Method	No significant protocol deviations. GLP Compliance.

The animals were treated sequentially. One drop of local anaesthetic (tetracaine hydrochloride) was instilled into both eyes of the third animal

1-2 minutes prior to treatment with the test substance.

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	1	1	1	2	< 72 hours	0
Conjunctiva: chemosis	0.33	0.33	0.33	1	< 48 hours	0
Conjunctiva: discharge	0.33	0.33	0.33	2	< 48 hours	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0.33	0	1	< 48 hours	0

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

Remarks - Results
No corneal effects were noted.
Iridial inflammation was noted in one treated eye 1 hour after treatment and at the 24 hour observation.
Moderate conjunctival irritation was noted in all treated eyes 1 hour after treatment and at the 24 hour observation, with minimal conjunctival irritation noted in all treated eyes at the 48 hour observation. All treated eyes had recovered by the 72 hour observations.

CONCLUSION
The notified chemical is slightly irritating to the eye.

TEST FACILITY
Safepharm (2007d)

B.5. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE
Notified chemical

METHOD
OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)
Species/Strain: Mouse/ CBA/J
Vehicle: Acetone:olive oil (AOO; 4:1)
Positive Control: Isoeugenol (5% in AOO); Hydroxycitronellal (15% and 60% in AOO)
Remarks - Method: No significant protocol deviations.
GLP Compliance.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	29.1	-
1	47.7	1.6 ± 0.2
5	46.0	1.6 ± 0.4
10	79.3	2.7 ± 0.3
20	107.7	3.7 ± 1.0
40	315.0	10.8 ± 2.6
Positive Control		
Isoeugenol 5%	196.7	6.8 ± 1.5
Hyroxycitronellal 15%	61.8	2.1 ± 0.5
Hyroxycitronellal 60%	254.9	8.8 ± 1.9

Remarks - Results
No signs of systemic toxicity were noted in the test or control animals.
The positive controls elicited stimulation indices that were > 3, confirming the validity of the test system.

	The stimulation indices at 20% and 40% concentrations of the test substance were 3.7 and 10.8, respectively. Based on these results, the EC_3 value was calculated to be 10.1%.
CONCLUSION	There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	BRT (2006)

B.6. Skin sensitisation – human volunteers

TEST SUBSTANCE	Notified chemical (2.5%)
METHOD	Repeated insult patch test with challenge
Study Design	Induction Procedure: 25 mm Hill Top chamber (occlusive) patches containing 0.3 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 hours and graded after an additional 24 hours (or 48 hours for patches applied on Friday). Rest Period: ~14 days Challenge Procedure: a patch was applied to a naïve site. Patches were removed by a technician and the sites graded at patch removal and 24, 48, and 72 hours post-patch removal.
Study Group	91 F, 29 M; age range 18 - 68 years
Vehicle	75% Diethyl Phthalate : 25% Ethanol
Remarks - Method	A panel of 120 healthy human subjects (devoid of any physical or dermatological conditions) was amassed. Of these, 109 (82 female and 27 male) test subjects completed the study; 8 subjects reportedly discontinued due to personal reasons (2-9 induction observations and 0-3 challenge observations recorded), 1 subject was discontinued due to a protocol violation (no observations recorded), 1 subject was discontinued due to being hospitalised (6 induction observations recorded) and 1 subject was discontinued due to the medication that she was prescribed (by the consulting dermatologist) for reactions at the last induction reading and during the rest period to another test material on the panel (9 induction observations recorded).
RESULTS	
Remarks - Results	Two test subjects experienced dryness for 1-3 observation periods during the induction phase. Of these, 1 subject and an additional 2 test subjects experienced faint, minimal erythema for 1-5 observation periods during the induction phase. Faint, minimal erythema was also evident in 4 subjects during the challenge phase (in 2 subjects at 24 hours post-patch removal, 1 subject 24-72 hours post-patch removal and in 1 subject 48-72 hours post-patch removal), i.e. with 2 subjects showing signs at the end of the test period. None of these 4 subjects had shown responses to the test substance during the induction phase. These reactions were not considered by the study authors to be evidence of skin sensitisation.
CONCLUSION	The notified chemical was considered by the study authors to be non-sensitising under the conditions of the test.
TEST FACILITY	HRL (2012)

B.7. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical

METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rats/ Wistar SPF
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 2 weeks
Vehicle	PEG 300
Remarks - Method	No significant protocol deviations. GLP Compliance.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 per sex	0	1/10
low dose	5 per sex	60	0/10
mid dose	5 per sex	200	0/10
high dose	5 per sex	600	0/10
control recovery	5 per sex	0	0/10
high dose recovery	5 per sex	600	0/10

REMARKS – RESULTS

Mortality and Time to Death

One control group female spontaneously died on day 24 of the treatment period. This mortality was considered by study authors not to be test item related. There were no other unscheduled deaths during the study.

Clinical Observations

Salivation was noted in all animals in the test groups during the treatment period. Dyspnea was noted in three animals each of the high and mid dose groups. These findings were considered by the study authors to be test-item related, but not of an adverse nature. No changes in functional observational battery, locomotor activity and grip strength were noted in any dose group, when compared to the control animals. No changes in mean absolute and relative food consumption and body weights for the low and mid dose groups were attributed to the test item. For the high dose group, animals showed statistically significantly decreased body weights either at the end of the treatment period (males) or beginning of the recovery period (females), but the weights increased to predicted levels during the recovery period. Relative food consumption was increased in the high dose group during both the treatment and recovery periods. These findings were considered by the study authors to not be adverse.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No haematological effects were seen in animals of the low dose group. Haematological effects (statistically significant) seen in animals of both sexes of the high dose group included elevated mean corpuscular volume, decreased mean corpuscular haemoglobin concentration, decreased haemoglobin distribution width, elevated absolute and relative reticulocytes. Decreased L-reticulocytes and elevated H-reticulocytes were seen only in the males of this group. Elevated prothrombin time and haematocrit, along with decreased partial thromboplastin time were seen in females of this group. The study authors suggested these test item related clinical markers to be indicative of anaemia, but did not consider them to be toxicologically adverse. The mid dose group findings were limited to decreased haemoglobin in male animals, decreased haemoglobin distribution width in females, and elevated absolute and relative reticulocytes in both sexes.

The aforementioned changes were noted during the treatment period, The study authors generalised that the effects observed following recovery occurred at a lower extent than changes following the treatment period. Exceptions to this observation included statistically significant findings in females such as elevated red blood cell count (RBC) with decreased RBC distribution width, decreased mean corpuscular haemoglobin (MCH) and MCH concentrations, elevated mean corpuscular volume in males and elevated haematocrit in both sexes. The study authors correlate these haematological signs with the microscopic observations of increased haematopoiesis of the spleen of several animals of the high and mid dose groups (see below). Overall, the study authors considered the haematology effects to be test substance related, but were of a non-adverse and reversible nature.

No test item related changes in biochemistry markers were noted in animals of the low and mid dose groups during the treatment period. However, after the recovery period, some statistically significant changes were noted in both sexes and groups. Decreased creatinine and phosphorus was seen in low dose group males, elevated urea was observed in low dose group females, and decreased creatinine levels were noted in females of the mid dose group. Statistically significant changes seen in animals of both sexes of the high dose group at the completion of treatment included decreased glucose, decreased creatinine, elevated bilirubin, elevated alanine aminotransferase, elevated potassium and elevated protein. Male specific signs included elevated urea, sodium, calcium, phosphorus and albumin. Female specific signs were a decrease in chloride ions and an increase in cholesterine and triglycerides. Most biochemistry parameters normalised after the recovery period and were considered by the study authors to be test substance related but non-adverse and reversible.

Some changes in urinalysis were noted for animals of all dose groups. The female animals of the low dose group showed elevated leucocytes. The mid dose group findings included elevated leucocytes in both sexes, elevated ketones (in males only) and a turbid amber coloured appearance (females only). High dose group changes included elevated relative density and elevated protein in males only, as well as elevated ketones and urobilinogen and amber coloured urine, seen in both sexes. While urinalysis findings were noted in some instances in a dose-dependent distribution, they were observed to be fully reversible during the recovery period.

Effects in Organs

Changes in organ weights noted in all dose groups tested (males and females) included elevated absolute and relative liver weights (not statistically significant in low dose females), elevated absolute kidney weights and elevated relative kidney weights (not observed in males of the low dose group). As these increases were markedly reduced after the recovery period when compared to measurements following the treatment period and were comparable to control values following the treatment period, the study authors considered these changes to be generally reversible.

No macroscopic findings were noted in the low dose group animals or females of the mid and high dose groups. One male animal each in the mid and high dose groups showed an enlarged liver at necropsy. The study authors did not considered these finding to be adverse as they were not noted after the recovery period.

No microscopic findings were noted in animals of the low dose group. Microscopic findings seen in animals of both sexes in the mid and high dose groups included centrilobular hypertrophy, increased haematopoiesis of the spleen and tubular hypertrophy of the kidneys (not observed in females of the mid dose group). The study authors note that the incidence and severity of these findings showed a dose-dependent distribution, but were fully reversible during the recovery period and therefore were not considered to be adverse effects.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established by the study authors as > 600 mg/kg bw/day in this study, based on the absence of adverse effects at the highest dose tested.

TEST FACILITY Harlan (2009c)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation 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 Preliminary Test	a) With metabolic activation: 0 – 5,000 µg/plate b) Without metabolic activation: 0 – 5,000 µg/plate
Concentration Range in Main Test	a) With metabolic activation: 0 – 5,000 µg/plate b) Without metabolic activation: 0 – 5,000 µg/plate
Vehicle	Dimethyl sulfoxide
Remarks - Method	No significant protocol deviations. GLP Compliance.

Five-six dose levels were used and tests were conducted in triplicate. Based on the results of the preliminary toxicity test, the dose range for strains TA100 and TA1500 in Tests 1 and 2 was 0-1500 µg/plate, whereas it was 0-5000 µg/plate for the remaining strains.

Five positive control tests were conducted in parallel with the test substance. N-ethyl-N'-nitro-N-nitrosoguanidine, 9-Aminoacridine and 4-Nitroquinoline-1-oxide were conducted in the absence of S9-mix. 2-Aminoacridine and Benzo(a)pyrene were conducted with S9-mix.

RESULTS

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

Remarks - Results

The notified chemical caused a visible reduction in the growth of the bacterial background lawn to the strains TA100 and TA1535 from 500 µg/plate and TA1537 at 5,000 µg/plate, with and without metabolic activation (Tests 1 and 2). The notified chemical caused no visible reduction in the growth of the bacterial background lawn to either strains TA98 or WP2uvrA, with and without metabolic activation. No test material precipitate was observed on any of the plates, at any dose, in the presence or absence of S9-mix.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, either with or without metabolic activation.

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

CONCLUSION

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

TEST FACILITY

Safepharm (2006a)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
EC Directive 2000/32/EC B.10 Mutagenicity - 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 hour exposure, with and without activation and 24 hour exposure without activation) at concentrations 6.69 – 1,712 µg/mL.

Vehicle and positive controls (mitomycin C without metabolic activation and cyclophosphamide with metabolic activation) were used in parallel with the test material.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period hours*	*Harvest Time hours*
Absent			
Test 1	0*, 26.75*, 53.5*, 107*, 160.5, 214, 321	4	24
Test 2	0*, 13.38*, 26.75*, 53.5*, 80.25*, 107, 214	24	24
Present			
Test 1	0*, 26.75*, 53.5*, 107*, 160.5, 214, 321	4	24
Test 2	0*, 13.38, 26.75*, 53.5*, 107*, 160.5, 214	4	24

*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	≥ 214	≥ 107	≥ 214	negative
Test 2	≥ 107	≥ 80.25	≥ 214	negative
Present				
Test 1	≥ 214	≥ 107	≥ 214	negative
Test 2		≥ 107	≥ 214	negative

Remarks - Results

In Test 1, the study authors noted the observation of a steep toxicity curve, with sufficient scorable metaphases only present at ≤ 107 µg/mL (in the absence of S9, the mitotic indices were 78 and 0 at 107 and 160.5 µg/mL, respectively, and 70, 92 and 0 at 107, 160.5 and 214 µg/mL, respectively, in the presence of S9). In Test 2, the maximum analysed concentrations corresponded to those associated with > 50% mitotic inhibition.

No toxicologically significant increases in the number of cells with aberrations were noted, with or without metabolic activation. The test material did not induce a statistically significant increase in the number of polyploidy cells at any dose in either test.

The positive and vehicle 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

Safepharm (2006b)

B.10. Genotoxicity – in vivo

TEST SUBSTANCE

Notified chemical (ST 20 C 05)

METHOD

OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
EC Directive 2000/32/EC B.12 Mutagenicity - Mammalian Erythrocyte Micronucleus Test.

Species/Strain: Mice/ Crl:CD-1 (ICR)BR
Route of Administration: Oral – gavage
Vehicle: Arachis oil
Remarks - Method: No significant protocol deviations.
GLP Compliance.

Vehicle and positive controls (cyclophosphamide (CP)) were used in parallel with the test material. A range-finding test was conducted to determine the maximum tolerated dose (MTD) of the test material for use in the main test. Due to premature deaths of animals treated at 2,000 mg/kg bw (2/2 mice killed *in extremis*), 1,000 mg/kg bw was set as the MTD. Based on the range-finding test results suggesting that there was no marked difference in toxicity of the test material between the sexes, only male mice were used for the main test.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	7 M	0	24
II (vehicle control)	7 M	0	48
III (positive control: CP)	5 M	50	24
IV (low dose)	7 M	250	24
V (mid dose)	7 M	500	24
VI (high dose)	7 M	1,000	24
VII (high dose)	7 M	1,000	48

CP=cyclophosphamide.

RESULTS

Doses Producing Toxicity	> 1,000 mg/kg
Genotoxic Effects	No evidence of a significant increase in the incidence of micronucleated polychromatic erythrocytes in treated animals at any dose, compared to the vehicle control groups, were noted.
Remarks - Results	Clinical signs seen in animals (at 250 mg/kg bw and higher) included hunched posture, ptosis, ataxia, lethargy and splayed gait. Although toxicity in the bone marrow was not evident, the observation of clinical signs was taken by the study authors to be indicative of systemic absorption of the test material. There were no premature deaths seen in any of the dose groups. The positive and vehicle controls gave satisfactory responses confirming the validity of the test system

CONCLUSION The notified chemical was not clastogenic under the conditions of this in vivo mammalian erythrocyte micronucleus test.

TEST FACILITY Safepharm (2006c)

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 from a predominantly domestic sewage treatment plant
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biochemical Oxygen Demand (BOD)
Remarks - Method	No significant protocol deviations. GLP Compliance.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
3	0	3	53
15	-1.5	15	87
28	-2.5	28	89

Remarks - Results	All validity criteria were satisfied and no significant deviations to protocol were reported. Examination of the degradation curve for the toxicity control showed that the toxicity control attained 44% degradation by day 14 of the study thereby confirming that the notified chemical was not toxic to the sewage treatment micro-organisms used in the study. After 28 days the toxicity control remained on 44%. The notified chemical attained -2.5% degradation after 28 days. Therefore, the notified chemical cannot be considered as readily biodegradable under the conditions of OECD Guideline 301F.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	IBACON (2006)

C.1.2. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	ISO Guideline 14593: (1999) "Water Quality: evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium-Method by analysis of inorganic carbon in sealed vessels (CO_2 headspace test)'
Inoculum	Activated sewage sludge from a predominantly domestic sewage treatment plant
Exposure Period	27 days
Auxiliary Solvent	None
Analytical Monitoring	Total Organic Carbon (TOC)
Remarks - Method	No significant protocol deviations. GLP Compliance.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*

4	-0.77	4	90.26
18	-1.2	18	99.61
27	0.06	27	73.16

Remarks - Results: All validity criteria were satisfied and no significant deviations to protocol were reported.

Examination of the degradation curve for the toxicity control showed that the toxicity control attained 42.74% degradation by day 18 of the study thereby confirming that the notified chemical was not toxic to the sewage treatment micro-organisms used in the study. After 27 days the toxicity was 44.54%.

The notified chemical attained 0.06% degradation after 27 days. Therefore, the notified chemical cannot be considered as readily biodegradable under the conditions of ISO Guideline 14593 (1999).

CONCLUSION: The notified chemical is not readily biodegradable.

TEST FACILITY: Firmenich (2006)

C.2. Ecotoxicological Investigations

C.2.1. Inhibition of microbial activity

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 209 Activated Sludge, Respiration Inhibition Test.
- Inoculum: Activated sewage sludge from domestic sewage treatment plant
- Exposure Period: 3 hours
- Concentration Range: Nominal: 10, 32, 100, 320 and 1000 mg/L; Actual: Not measured
- Remarks – Method: No significant protocol deviations. GLP Compliance.

RESULTS
- EC50: > 1000 mg/L
- Remarks – Results: All validity criteria were satisfied and no significant deviations to protocol were reported.

CONCLUSION: The notified chemical is not expected to be inhibitory to micro-organisms at concentrations < 1000 mg/L.

TEST FACILITY: RCC (2008b)

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