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June 2018

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

2-Tridecenoic acid, 2-acetyl-4-methyl-, ethyl ester

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 and Energy.

This Public Report is 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

TABLE OF CONTENTS

SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/2023	Givaudan Singapore Pte Ltd	2-Tridecenoic acid, 2-acetyl-4-methyl-, ethyl ester	Yes	< 1 tonne per annum	Fragrance ingredient in laundry fabric softener products

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard classification	*Hazard statement*
Acute toxicity, Category 4	H332 - Harmful if inhaled
Skin sensitisation, Category 1	H317 – May cause an allergic skin reaction

Human health risk assessment
Provided that the recommended controls are being adhered to, under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

When used in the proposed manner, the notified chemical is not considered to pose an unreasonable risk to public health.

Environmental risk assessment
On the basis of the assumed low hazard, 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 toxicity, Category 4: H332 - Harmful if inhaled
  - 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.

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 skin 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
  - Adequate local exhaust ventilation

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure to 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:
  - Coveralls
  - Impervious gloves

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

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

Disposal

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

Storage

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

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by physical containment, collection and subsequent safe disposal.

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

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

(1) Under Section 64(1) of the Act; if
  - the importation volume exceeds one tonne per annum notified chemical;
  - the concentration of the notified chemical exceeds or is intended to exceed 0.5% in laundry fabric softener products;

or

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

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

Safety Data Sheet
The SDS of the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Givaudan Singapore Pte Ltd (ABN: 79 368 011 578)
1 Pioneer Turn
SINGAPORE 627576

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

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

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Philippines (2017)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Scentaurus Clean

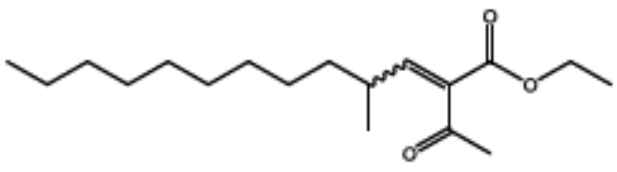
CAS NUMBER
960253-23-0

CHEMICAL NAME
2-Tridecenoic acid, 2-acetyl-4-methyl-, ethyl ester

OTHER NAME(S)
(E/Z)-ethyl 2-acetyl-4-methyltridec-2-enoate
GR-86-6599

MOLECULAR FORMULA
$C_{18}H_{32}O_3$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
296.4 g/mol

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

3. COMPOSITION

DEGREE OF PURITY
> 85% (The notified chemical contains both *E* and *Z* isomers at 1:0.62 molar ratio)

IDENTIFIED IMPURITIES

Chemical Name	Butanoic acid, 3-oxo-, ethyl ester		
CAS No.	141-97-9	*Weight %*	~6%
Hazardous Properties	Not known		

Chemical Name	Undecanal, 2-methyl-		
CAS No.	110-41-8	*Weight %*	~1%
Hazardous Properties	H315 – Causes skin irritation H317 – May cause an allergic skin reaction H400 – Very toxic to aquatic life H410 – Very toxic to aquatic life with long lasting effects		

Chemical Name	Ethyl 2-(1-hydroxyethylidene)-4-methyltridec-3-enoate (tautomer)		
CAS No.	Not known	*Weight %*	~4%
Hazardous Properties	Not known		

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Pale yellow viscous liquid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	< -50°C	Measured
Boiling Point	306 °C at 101.3 kPa	Measured
Density	925 kg/m^3 at 25°C	Measured
Vapour Pressure	2 × 10^{-6} kPa at 20°C	Measured
Water Solubility	4 x 10^{-5} g/L at 20°C	Measured
Hydrolysis as a Function of pH	Not determined	Contains hydrolysable functional group but significant hydrolysis is not expected in the environmental pH range of 4-9
Partition Coefficient (n-octanol/water)	log P_{ow} = 5.3; 5.9 and 6.6	Measured
Adsorption/Desorption	Several peaks corresponding to log K_{oc} 1.0 to 4.9	Measured
Dissociation Constant	Not determined	Not expected to be ionised in the environmental pH range of 4-9
Flash Point	108°C at 101.3 kPa	Measured
Flammability (contact with water)	Not flammable	Based on structure
Autoignition Temperature	325±10°C	Measured
Explosive Properties	Not explosive	Contains no functional groups that imply explosive properties.
Oxidising Properties	Not oxidising	Contains no functional groups that imply oxidative 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.

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. It will be imported only as a component of fragrance compounds, in which its content does not exceed 25%. The fragrance compounds are then used in laundry fabric softener products. The concentration of the notified chemical in the final consumer products will not exceed 0.5%.

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 sea or air)
Perth (by air)

IDENTITY OF MANUFACTURER/RECIPIENTS
Givaudan Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemical within the imported fragrance compounds will be imported into Australia in drums, glass bottles and lacquer-lined containers. The proposed packaging size will be 1, 5, 10, 25, 100, 190 kg.

The finished consumer products containing the notified chemical will be transported, primarily by road, to retail stores or other distribution points.

USE
The notified chemical will be used as a fragrance ingredient in liquid laundry fabric softeners at a concentration of up to 0.5% by weight. In some cases, the fabric softener products will be diluted with water prior to application. The dilution factor, which is often on the product label, depends on the type of the fabric to be treated, loading volume, and the type and method of application. The finished products containing the notified chemical will be used by the public and by professional cleaners.

OPERATION DESCRIPTION
The notified chemical will not be manufactured in Australia. It will be imported as a component of fragrance mixtures, in which its content will not exceed 25%. The fragrance mixture will be blended with other ingredients at customer formulation sites to make fabric softener products for consumer use, containing the notified chemical at up to 0.5%. The formulation process at different sites may vary significantly due to the final product type and dosing equipment. The packaged consumer products will be transported to retail or trade outlets for sale to the public or to professional cleaners.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and Warehouse workers	none	Incidental exposure only
Mixer (plant operators)	4	2

Drum handling	4	2
Drum cleaning/washing	4	2
Maintenance	4	2
Quality control worker	4	2
Packager	4	2
End users (professionals)	1-8	200

EXPOSURE DETAILS

Transport and storage

Transport and warehouse workers will be exposed to the fragrance compounds (containing up to 25% of the notified chemical) only in the event of a spill due to an accident or leaking drum. Workers will wear protective overalls, hard hats, chemical resistant gloves and safety glasses.

Reformulation

At customer facilities (consumer product manufacturers), exposure to the fragrance compounds (containing up to 25% of the notified chemical) or consumer products (containing the notified chemical up to 0.5%) is possible during handling of the drums, cleaning and maintenance of the equipment. Skin, inhalation and eye contact (due to splashing) are likely to be the main routes of exposure. The level of exposure would vary from site to site depending on the level of automation of the formulation process. However, it is anticipated that work practices by consumer product manufacturers will include the use of adequate local ventilation, appropriate PPE, enclosed mixing vessel and filling areas as well as a high degree of process automation to protect workers from exposure.

End-use

Exposure of professional cleaners to the notified chemical in end-use products at ≤ 0.5% concentration may occur where the services provided involve the use of fabric-care products. The principal route of exposure will be dermal, while ocular exposure is also possible. Such professionals may use some PPE to minimise repeated exposure, but this is not expected to occur in all workplaces. However, good hygiene practices are expected to be in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical.

6.1.2. Public Exposure

The finished fabric softener products containing the notified chemical are designated to be sold to consumers. The general public will be repeatedly exposed to low-levels of the notified chemical (< 0.5%) during use of the consumer products, while wearing treated clothes, and potentially during the dilution of products prior to use. Exposure to the notified chemical will vary depending on individual use patterns. The principal route of exposure will be dermal, while incidental ocular exposure is also possible. Significant inhalation exposure is not expected, as the products will not be applied by spray.

Data on typical use patterns of the product in which the notified chemical will be used are shown in the following tables and these are based on information provided in literature (ACI, 2010, RIVM, 2006). Dermal absorption (DA) of 100% was assumed for the notified chemical and an average female body weight of 64 kg (enHealth, 2012) was used for calculation purposes.

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 (as surrogate for fabric softener)	1.43	0.5	1980	0.01	0.01	0.007	0.0002

C = maximum intended concentration of notified chemical

Daily systemic exposure = (Frequency × C × Contact area × Product Use Concentration × Film Thickness on skin × Time Scale Factor × DA)/BW

Indirect dermal exposure - from wearing clothes

Product type	Amount (g/use)	C (%)	Product Retained (PR) (%)	Percent Transfer (PT) (%)	Dermal Absorption (%)	Daily systemic exposure (mg/kg bw/day)
Fabric softener	90	0.5	0.95	10	100	0.0067

C = maximum intended concentration of notified chemical
Daily systemic exposure = (Amount × C × PR × PT × DA)/BW

The scenario estimation using these assumptions is for a person who uses fabric softener products that contain the notified chemical at the maximum intended concentrations specified by the notifier. This would result in a combined internal dose of 0.0069 mg/kg bw/day for the notified chemical.

6.2. Human Health Effects Assessment

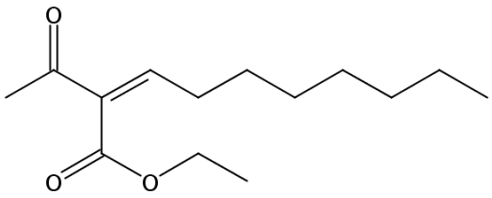
The results from toxicological investigations conducted on the notified chemical are summarised in the following table. For full details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute inhalation toxicity	LC50 = 1-5 mg/L; harmful
Skin irritation (*in vitro*)	non irritating
Eye irritation (*in vitro*)	non irritating
Skin sensitisation (*in vitro*) – DPRA	evidence of sensitisation (low reactivity)
Skin sensitisation (*in vitro*) – KeratinoSens	evidence of sensitisation
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* human micronucleus test	non genotoxic

Additional skin sensitisation data for the notified chemical were also obtained by read-across from an analogue substance listed below.

Analogue

Skin sensitisation data for the notified chemical was obtained from a close analogue 2-Decenoic acid, 2-acetyl-, ethyl ester (CAS no. 64354-15-0) as identified by using the OECD quantitative structure-activity relationship (QSAR) Toolbox 4.2. The analogue is structurally similar to the notified chemical differing only in a three carbon shorter chain length and a lack of a side methyl group attached to the main carbon chain. In this respect the proposed analogue is expected to be more reactive than the notified chemical. Based on the above, the analogue is considered acceptable to estimate the skin sensitisation potency of the notified chemical.



Analogue

Toxicokinetics, metabolism and distribution

No toxicokinetic data on the notified chemical were submitted. For dermal and gastrointestinal absorption, molecular weights below 100 g/mol are favourable for absorption and molecular weights above 500 g/mol do not favour absorption (ECHA, 2014). Dermal uptake is likely to be moderate to high if the water solubility is between 100 – 10,000 mg/L. Dermal uptake through the epidermis is expected if the partition coefficient (log P_{ow}) values are between -1 and 4 (ECHA, 2014). Based on the molecular weight of the notified chemical (296.4 g/mol), low water solubility (4 x 10^{-5} g/L at 20°C) and high lipophilicity (log Pow = 5.3 or higher at 20 °C), passive diffusion across the gastrointestinal tract and percutaneous absorption of the notified chemical are expected to be limited.

Acute toxicity

The notified chemical is of low acute oral toxicity based on a study conducted in rats.

In an acute inhalation toxicity study, the rats were exposed (nose only) to an aerosol of the notified chemical at 5 mg/L. Lethargy, hunched posture, slow breathing, laboured respiration, rales, piloerection and ptosis were seen in all animals up to Day 9 after exposure. Scales were seen on the back of the females between Days 10 and 13. One male died during the course of the study, while 3 males and one female were sacrificed for humane reasons. Macroscopic post-mortem analysis revealed abnormalities of the lungs (pale with many dark red or black foci), stomach and intestines (distended with gas) and thymus (many dark red foci). No abnormalities were seen in the surviving animals. Based on these findings the notified chemical is expected to be harmful via the inhalation route.

Irritation and sensitisation
An *in vitro* skin irritation test in human reconstructed epidermal model (Episkin) and an *in vitro* eye irritation test in bovine corneas (BCOP) were provided for the notified chemical. Based on the findings of these tests, the notified chemical is not expected to be irritating to the skin and eyes.

Sensitisation
A battery of tests consisting of one *in chemico* and one *in vitro* assay were conducted to evaluate the sensitisation potential of the notified chemical. The tests are part of Integrated Approach to Testing and Assessment (IATA) which address two specific events of the Adverse Outcome Pathway (AOP) leading to development of skin sensitisation (OECD, 2012). The tests are thus considered relevant for assessment of the skin sensitisation potential of the notified chemical.

The first key event or molecular initiating event in the AOP for sensitisation is the covalent binding to nucleophilic centres in skin proteins. The *in chemico* Direct Peptide Reactivity Assay (DPRA) measures the interaction of a test substance with cysteine and lysine-containing small synthetic peptides (representing the nucleophilic centres in skin protein). Thus, the assay is proposed to address the molecular initiating event.

The second key event in the AOP for sensitisation is the activation of keratinocytes which leads to upregulation of stress related proteins (cytokines) via transcriptional upregulation of the genes. The Keratinocyte ARE-Reporter Cell Line KeratinoSens Assay measures change in expression of luciferase gene under the transcriptional control of a constitutive promoter fused with an Antioxidant Response Element (ARE) from a gene that is known to be upregulated by contact sensitisers. Hence the assay addresses the second key event in the AOP for skin sensitisation.

The notified chemical showed a positive response in both of the above *in vitro* sensitisation tests, suggesting that the notified chemical is a skin sensitiser.

Given that data for only two out of three tests recommended for *in vitro* evaluation of skin sensitisation were provided (OECD, 2012) and these assays tested positive, additional ways of confirming the skin sensitisation potential of the notified chemical were considered. The quantitative structure-activity relationship (QSAR) Toolbox 4.2 was used for identifying a close analogue for the notified chemical for which *in vivo* skin sensitisation data with potency information was available. This analogue, 2-Decenoic acid, 2-acetyl-, ethyl ester (CAS no. 64354-15-0), was reported to be a skin sensitiser with an EC3 value of 2.6% in a mouse Local Lymph Node Assay (LLNA) (Givaudan, 2005). Based on the available information on the notified chemical and the analogue, the notified chemical is considered to be a skin sensitiser with similar potency.

Mutagenicity/Genotoxicity
The notified chemical was negative in a bacterial reverse mutation assay and in an *in vitro* mammalian micronucleus test in cultured peripheral human lymphocytes.

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

Hazard classification	***Hazard statement***
Acute toxicity, Category 4	H332 - Harmful if inhaled
Skin sensitisation, Category 1	H317 – May cause an allergic skin reaction

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Based on the available toxicological information, the notified chemical is harmful when inhaled and a skin sensitiser. Information on repeated dose toxicity is not available.

Reformulation

During reformulation, workers may be exposed to the notified chemical introduced at ≤ 25% concentration. At this concentration, workers may be at risk of skin sensitisation. According to the notifier, engineering controls such as enclosed and automated processes and local ventilation will be implemented whenever possible. Appropriate PPE (coveralls, impervious gloves, eye protection and respiratory protection) will be used to limit worker exposure. Therefore, provided that control measures are in place to minimise worker exposure, under the occupational settings described, the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

End-use

Workers involved in professional cleaning where the services provided involve use of household products such as fabric softeners may be exposed to the notified chemical at ≤ 0.5% concentration. 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 such workers is expected to be of a similar or lesser extent than that experienced by consumers using the products containing the notified chemical.

6.3.2. Public Health

Members of the public may experience frequent exposure to the notified chemical at ≤ 0.5% concentration through daily use of fabric softener products. The main route of exposure is expected to be dermal, while incidental ocular exposure may also occur. Due to the low concentration of the notified chemical in the product and the method of use, inhalation exposure to high concentrations in air is not expected.

Two *in vitro* skin sensitisation tests, DPRA and KeratinoSens, indicated the notified chemical is a skin sensitiser. Based on the results of an LLNA assay from a close analogue, the notified chemical is expected to have a similar potency for skin sensitisation (EC3 value of 2.6%). Using the fabric softener products that contain the notified chemical at 0.5% concentration as a worst case scenario, the Consumer Exposure Level (CEL) is estimated to be 0.73 μg/cm^2/day. Consideration of available information and application of appropriate safety factors allowed the derivation of an Acceptable Exposure Level (AEL) of 2 μg/cm^2/day. In this instance, the safety factors employed included an interspecies factor (3), interspecies factor (10), a matrix factor (3.16), use/time factor (3.16) and database factor (1), giving an overall safety factor of 300.

As the AEL > CEL, the risk to the public of the induction of skin sensitisation that is associated with direct skin exposure to the fabric softeners is not considered to be unreasonable. However, it is acknowledged that consumers may be indirectly exposed (at a low level) to the notified chemical from wearing treated clothes, and a quantitative assessment based on aggregate exposure has not been conducted.

Overall, based on the information available, the risk to the public associated with the use of the notified chemical at ≤ 0.5% in fabric softener 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 into Australia as a component of fragrance solutions for reformulation into end-use household products. In general, the reformulation processes are expected to involve blending operations that will normally be automated and occur in an enclosed system, followed by automated filling of finished products into end-use containers. Liquid waste from cleaning of reformulation equipment, estimated by the notifier to contain up to 1% of the total import volume of notified chemical, will either be treated onsite or disposed of, to sewers. Release of the notified chemical to the environment in the event of accidental spills or leaks during reformulation, storage and transport is expected to be collected for disposal to landfill in accordance

with local government regulations. Residue notified chemical in empty import containers, estimated by the notifier to account for up to 1% of the total import volume, will either be treated onsite or disposed of, to sewers.

RELEASE OF CHEMICAL FROM USE
The majority of the notified chemical is expected to be released to sewers across Australia as a result of its use in household products, which are released from cleaning activities.

RELEASE OF CHEMICAL FROM DISPOSAL
Residues of the notified chemical in empty end-use containers, estimated by the notifier to account for up to 1% of the total import volume, are likely to either share the fate of the containers and be disposed of to landfill, or be released to the sewer system when the containers are rinsed before recycling through an approved waste management facility.

7.1.2. Environmental Fate

Based on its use as a component of household products, the majority of the notified chemical is expected to be released to sewers, and then to sewage treatment plants (STPs) before potential release to surface waters. Based on its very low water solubility (0.04 mg/L) and high log P_{ow} (log P_{ow} = 5.3-6.6), the majority of the notified chemical is expected to present in the solid phase in STPs. A ready biodegradability study conducted on the notified chemical shows that it is readily biodegradable (62% degradation after 28 days). Therefore, the notified chemical is expected to be removed effectively by biodegradation and adsorption to sludge at STPs, and only a small proportion of the notified chemical may be released to surface waters after STPs. For details of the biodegradability study, refer to Appendix C. The waste sludge containing the notified chemical will be sent to landfill for disposal or agricultural land for remediation. A minor amount of the notified chemical may also be disposed of to landfill as collected spills and empty container residues. The major proportion of the notified chemical (78%) has log $K_{oc} \geq 4.6$ and is expected to have low mobility in soil and landfill. The notified chemical is not expected to significantly bioaccumulate in biota based on its ready biodegradability. In landfill, sludge and water, the notified chemical is expected to undergo degradation by biotic and abiotic processes, eventually forming water and oxides of carbon.

The half-life of the notified chemical in air is calculated to be < 4 h, based on reactions with hydroxyl radicals (US EPA, 2012; calculated using AOPWIN v1.92). Therefore, the notified chemical is not expected to persist in the air compartment.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has been calculated to assume the worst case scenario with 100% release of the notified chemical into sewer systems nationwide over 365 days per annum. It is also assumed under the worst-case scenario that there is no removal of the notified chemical during sewage treatment processes. The resultant PEC in sewage effluent on a nationwide basis is estimated as follows:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	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)	24.386	million
Removal within STP	0	%
Daily effluent production:	4,877	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.56	µg/L
PEC - Ocean:	0.06	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.56 µg/L may potentially result in a soil concentration of approximately 3.74 µg/kg.

7.2. Environmental Effects Assessment

The results from the ecotoxicological investigation conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Daphnia Toxicity	48 h EC50 > 123 µg/L	Not harmful to aquatic invertebrates up to its water solubility limit
Algal Toxicity	72 h EC50 > 22 µg/L	Not harmful to alga up to its water solubility limit

Based on the above ecotoxicological endpoints for the notified chemical, it is not expected to be harmful to aquatic organisms up to its water solubility limit. Therefore, the notified chemical is not formally classified under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) for acute and chronic toxicities (United Nations, 2009).

7.2.1. Predicted No-Effect Concentration

A predicted no-effect concentration (PNEC) for the aquatic compartment has not been calculated as the notified chemical is not considered to be harmful to aquatic organisms up to its water solubility limit.

7.3. Environmental Risk Assessment

A Risk Quotient (PEC/PNEC) has not been calculated as the notified chemical is not considered to be harmful to aquatic organisms up to its water solubility limit.

Based on its assumed low hazard, the notified chemical is not expected to pose an unreasonable risk to the aquatic environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point < -50°C

Method OECD TG 102 Freezing Point/Melting Range

Remarks No freezing of the test substance was observed down to a temperature of -50°C in the preliminary test. No further testing was undertaken.

Test Facility Givaudan (2016a)

Boiling Point 306°C at 101.3 kPa

Method OECD TG 103 Boiling Point

Remarks Method according to Siwoloboff (capillary tube method).

Test Facility Givaudan (2017a)

Density 925 kg/m^3 at 20°C

Method OECD TG 109 Density of Liquids and Solids

Remarks Oscillating densitometer method.

Test Facility Givaudan (2016b)

Vapour Pressure 2×10^{-6} kPa at 20°C

Method OECD TG 104 Vapour Pressure

Remarks Gas saturation method.

Test Facility Givaudan (2017b)

Water Solubility 4×10^{-5} g/L at 20°C

Method OECD TG 105 Water Solubility.
EC Council Regulation No 440/2008 A.6 Water Solubility.
EPA OPPTS 830.7840 Water Solubility.

Remarks Flask Method

Test Facility Givaudan (2016c)

Partition Coefficient (n-octanol/water) log P_{ow} = 5.3; 5.9* and 6.6*

Method OECD TG 117 Partition Coefficient (n-octanol/water).
EC Council Regulation No 440/2008 A.8 Partition Coefficient.
EPA OPPTS 830.7570 Partition Coefficient.

Remarks HPLC Method. The chromatograms show three peaks corresponding to log P_{ow} = 5.3 (21%); 5.9*(52%) and 6.6*(27%), *indicative values, peaks are outside calibration domain.

Test Facility Givaudan (2016d)

Adsorption/Desorption log K_{oc} = 1.0; 4.6; 4.7; 4.7 and 4.9

Method OECD TG 121 Adsorption Coefficient
EC Council Regulation No 440/2008 C.19 Adsorption Coefficient

Remarks HPLC Method. The chromatograms show five peaks corresponding to log K_{oc} = 1.0 (22%); 4.6 (58%) ; 4.7 (4%); 4.7 (3%) and 4.9 (13%).

Test Facility Givaudan (2017c)

Flash Point 108°C at 101.3 kPa

Method EC Council Regulation No 440/2008 A.9 Flash Point

Remarks Pensky-Martens closed cup method. An anticipated flash point was determined in advance of the main test. The result was corrected for atmospheric pressure.

Test Facility Givaudan (2017d)

Autoignition Temperature 325±10°C

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

Remarks The injected volume was 100 µL and the ignition delay was 6 seconds.
Test Facility Givaudan (2016e)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method (2001) EC Council Regulation No 440/2008 B.1 tris Acute Oral Toxicity – Acute Toxic Class Method
Species/Strain	Rat/Wistar strain Crl:WI (Han)
Vehicle	The test item was dosed undiluted as delivered by the sponsor.
Remarks - Method	Toxicity of the test item was tested by stepwise treatment of two groups of 3 female animals. Initially, three females were given a single dose of 2000 mg/kg by oral gavage. The absence or presence of mortality of animals dosed at this step determined the next step. The animals were deprived of food overnight prior to dosing and until 3-4 hours after administration of the test item (Day 1). Observations for signs of mortality/viability, body weight fluctuation and clinical signs were performed at regular intervals for 15 days. At the end of the observation period, all animals were sacrificed by oxygen/carbon dioxide procedure and subjected to necropsy. Two study deviations were noted: change in the minimum level of daily mean relative humidity and the first group of animals were deprived of food overnight at the end of Day 1. The authors stated that the study integrity was not adversely affected by these deviations. The study was carried out according to GLP.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity	No mortality occurred. The mean body weight gain shown by the animals over the study period was considered to be similar to that expected for normal untreated animals of the same sex and strain.
Effects in Organs	No abnormalities were found at macroscopic post mortem examination of the animals.
Remarks - Results	Hunched posture and piloerection were noted for all animals on days 1 and/or 2.
CONCLUSION	The notified chemical is of low acute toxicity via the oral route.
TEST FACILITY	Charles River (2017a)

B.2. Acute toxicity – inhalation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 403 Acute Inhalation Toxicity (2009) EC Council Regulation No 440/2008, 93/21/EEC B.2 Acute Toxicity (Inhalation)
Species/Strain	Rat/Wistar strain Crl:WI (Han)
Vehicle	The test item was dosed undiluted as delivered by the sponsor.

Method of Exposure	Nasal exposure
Exposure Period	4 hours
Physical Form	Liquid aerosol
Particle Size	3 µm
Remarks - Method	The test substance was administered as an aerosol by nose-only inhalation for 4 hours to two groups of rats. Five animals of each sex were exposed in a limit test to a target concentration of the test item of 5 mg/L. Based on these results, the males were identified as most sensitive sex and therefore one additional group of five males was exposed to the next lower target concentration of 1 mg/L. Mortality and clinical signs were observed daily during the observation period of 15 days, and body weights were determined on Days 1, 2, 4, 8 and 15 and at death. Macroscopic examination was performed on the day of death or after terminal sacrifice (Day 15). For the 5 mg/L exposure group, no clinical observations and body weights were recorded on Day 2, but this study deviation did not adversely affect the study integrity. The study was carried out according to GLP.

RESULTS

Group	*Number and Sex of Animals*	*Concentration (mg/L)*		*Mortality*
		Nominal	*Actual*	
1	5F/5M	6.9	5.1	4/5 M 1/5 F
2	5M	1.7	1.2	0/10

LC50	1-5 mg/L/ 4 hours
Signs of Toxicity	At 5 mg/L, one male was found dead and one male and one female were sacrificed for humane reasons on Day 3. Two males were sacrificed between Days 7 and 9. No further mortality occurred in any of the other animals assigned to the limit test. At 5 mg/L, slow respiration was noted during exposure. After exposure, lethargy, hunched posture, slow breathing, laboured respiration, rales, piloerection and ptosis were seen for the animals up to Day 9. Scales were seen on the back of the females between Days 10 and 13. At 1 mg/L, hunched posture and rales were seen for the animals between Days 1 and 4. At 5 mg/L, body weight loss was noted for all surviving animals during the first week post exposure. All animals regained weight during the second week. At 1 mg/L, the body weight gain of animals over the study period was within the range expected for rats of this strain and age.
Effects in Organs	At 5 mg/L, macroscopic post mortem examination of the animals that were found dead or sacrificed for ethical reasons during the study, revealed abnormalities of the lungs (pale with many dark red or black foci), stomach and intestines (distended with gas) and thymus (many dark red foci). No abnormalities were seen in the surviving animals. At 1 mg/L, no abnormalities were found at macroscopic examination of the animals.
Remarks - Results	Incidental findings included advanced autolysis for one male found dead (5 mg/L).

The inhalation LC50 (4h) value of the tested substance in Wistar rats was established to be within the range of 1 – 5 mg/L.

CONCLUSION The notified chemical is harmful via inhalation route.

TEST FACILITY Charles River (2017b)

B.3. Irritation – skin (*in vitro* human 3D epidermal model EPISKIN Small model (EPISKIN-SM™)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method (2015)
EC Council Regulation No 440/2008 B.46. *In vitro* Skin Irritation – Reconstructed Human Epidermis Model Test

Vehicle None.

Remarks - Method The test substance was applied undiluted (25 µl) directly on top of the 3 skin tissues for 15 minutes. After a 42 hour post-incubation period, determination of the cytotoxic (irritancy) effect was performed. Cytotoxicity is expressed as the reduction of mitochondrial dehydrogenase activity measured by formazan production from 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) at the end of the treatment. Viable cells have the ability to enzymatically reduce MTT into blue formazan. Skin irritation is expressed as the remaining cell viability after exposure to the test item. The amount of the extracted formazan was determined spectrophotometrically at 570 nm in duplicates with the TEACAN Infinite® M200 Pro Plate reader.

The test item was checked for colour interference in aqueous solutions and for possible direct MTT reduction by adding the test item to MTT medium. Non-specific MTT reduction by the test item was noted in this preliminary test. Therefore, in addition to the normal procedure, three killed tissues treated with test item and three killed untreated tissues were used for cytotoxicity evaluation with MTT.

Phosphate-buffered saline (PBS) and 5% sodium dodecyl sulfate (SDS) were used as a negative and positive control test substances, respectively. The controls were also performed in triplicates.

There were no deviations from the study protocol. The study was carried out according to GLP.

RESULTS

Test material	*Mean OD_{570} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	0.835 ± 0.033	100	3.9
Test substance	0.824 ± 0.025	99	3.0
Positive control	0.186 ± 0.043	22	5.2

OD = optical density; SD = standard deviation

Remarks - Results The test item reacted with MTT producing non-specific MTT reduction (NSMTT). The NSMTT by the test item was -3.1% of the negative control tissues. Since the % NSMTT was ≤ 0.0, there was no correction applied on the ODs of the test item treated tissues.

The relative mean tissue viability obtained after treatment with the test substance compared to the negative control tissues was 99%. Since the mean relative tissue viability for the test item was above 50% after

treatment, the test substance is considered to be non-irritating.

The positive control had a mean cell viability of 22% after exposure. The absolute mean OD570 (optical density at 570 nm) of the negative control tissues was within the laboratory historical control data range. The standard deviation value of the percentage viability of three tissues treated identically was less than 6%, indicating that the test system functioned properly.

CONCLUSION The test substance is non-irritating to the skin.

TEST FACILITY Charles River (2017c)

B.4. Irritation – eye (*in vitro* Bovine Corneal Opacity and Permeability test - BCOP test)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage

Vehicle None. The test substance was applied undiluted (96.4% purity).

Remarks - Method Seven hundred and fifty µl of either the negative control (PBS), positive control (Ethanol) or test item was applied in triplicates onto the epithelium of the cornea and incubated for 10 minutes at 32°C. After exposure the cornea was thoroughly washed and incubated for 2 hours with fresh medium, followed by opacity and permeability measurement.

One of the negative control eyes was excluded since the final opacity value of 4.2 was outside the historical data range of -2.9 – 3.0. The authors stated that the study integrity was not adversely affected by this deviation.

The study was carried out according to GLP.

RESULTS

Test material	*Mean opacities of triplicate tissues (SD)*	*Mean permeabilities of triplicate tissues (SD)*	*IVIS (SD)*
Negative control	1.1	0.004	1.2
*Test substance**	-0.2	-0.002	-0.2
*Positive control**	19.9	1.690	45.3

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

Remarks - Results The negative control responses for opacity and permeability were less than the upper limits of the laboratory historical range indicating that the negative control did not induce irritancy on the corneas.

The mean *in vitro* irritancy score of the positive control (Ethanol) was 45, which was within two standard deviations of the current historical positive control mean and was therefore concluded that the test system functioned properly.

The test substance did not induce ocular irritation through both endpoints, resulting in a mean *in vitro* irritancy score of -0.2 after 10 minutes of treatment. Since the test item induced an IVIS ≤ 3, no classification is required for eye irritation or serious eye damage.

CONCLUSION The test substance is not classified as an eye irritant.

TEST FACILITY Charles River (2017d)

B.5. Skin sensitisation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 442c *In Chemico* Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA) (2015)
Vehicle	Acetonitrile (25%)
Remarks - Method	The test substance was dissolved in 25% acetonitrile and mixed with a 0.5 mM Cysteine- and Lysine-containing peptides. The final concentration of the test substance in the two mixes was 5 mM and 25 mM, respectively. After 24 h incubation time, peptide depletion induced by the test substance was determined by HPLC-UV coupled with a UV detector (220 nm wavelength). The test was run in triplicates. In each replicate cinnamic aldehyde and vehicle only (acetonitrile) were included as a positive and negative control, respectively.

RESULTS

Sample	*Cysteine Peptide Depletion (% ± SD)*	*Lysine Peptide Depletion (% ± SD)*
Vehicle	2.03*	1.73*
Test Substance	27.8 (± 3.7)	1.3 (± 1.9)
Control – Cinnamic aldehyde	65.9 (± 0.7)	50.7 (± 2.8)

* – normalised to 100%; SD = Standard Deviation

Remarks - Results	The test substance gave 27.8 % depletion of the Cys-peptide and 1.3 % depletion of the Lys-peptide. The average peptide depletion is 14.6 %. This is above the threshold of 6.38% and below 22.62%, and the substance is thus classified to the low reactivity class, rating it as a sensitizer according to the DPRA prediction model. Additional analysis with LC-MS (not a requirement of the OECD guideline) indicated that the test substance indeed formed a covalent adduct with the test peptide and confirmed that significant cysteine dimer formation had not occurred. The positive and negative controls fulfilled all quality criteria confirming the validity of the test.
CONCLUSION	The test substance was considered a skin sensitiser.
TEST FACILITY	Givaudan (2017e)

B.6. Skin sensitisation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 442d *In Vitro* Skin Sensitisation: ARE-Nrf2 Luciferase Test Method (2015)
Vehicle	Dimethylsulfoxide (DMSO)
Remarks - Method	The test substance was dissolved in DMSO (final concentration 1%) and tested according to the standard operating procedure of the KeratinoSens assay at 12 concentrations. Due to the high cytotoxicity of the test item, more narrowly spaced dilutions between 1.38 and 62.5 µM were selected. DMSO and Cinnamic aldehyde (4, 8, 16, 32, and 64 µM) were used as negative and positive controls, respectively. Three independent assays were conducted. Each assay included a set of 4 plates (3 for gene induction, 1 for cytotoxicity assessment).

Maximal induction of luciferase activity was measured at 565 nm (relative light units), while maximal gene induction (cytotoxicity assessment) was measured using absorption values at 570 nm. For Luciferase induction the maximal fold-induction over solvent control (Imax) and the concentration needed to reach an 1.5-, 2- and 3- fold induction (EC1.5, EC2 and EC3) were calculated. For cytotoxicity the IC50 value was extrapolated.

A test substance is predicted to have sensitisation potential if:
- the EC1.5 value is < 1,000 µM in at least 2 of 3 repetitions,
- cellular viability is > 70% at the lowest concentration with a gene induction > 1.5, and
- there is an apparent overall dose response which was similar between repetitions.

The mean values for cell viability and luciferase induction were provided. Individual values from the replicate experiments were not included in the report.

RESULTS

Sample	*Mean EC1.5 (µM)*	*Mean IC50 (µM)*	I_{max}
Test substance	7.95	13.20	1.87
Positive Control	13.67	> 13.67	2.55

EC1.5 - concentration for an induction of luciferase activity 50% above vehicle control
IC50 - concentration leading to 50% cell viability compared to vehicle control
I_{max} *– maximal induction*

Remarks - Results

In all three repetitions, induction of the luciferase above the threshold of 1.5 was noted and in two of them at non-cytotoxic concentrations. According to the prediction model of the KeratinoSens™ assay, the test substance was rated as a sensitizer. This conclusion is also supported by the analysis of the dose-response curve with overall dose-dependent induction of the luciferase reporter gene just below the cytotoxic concentration to be observed.

The positive and vehicle controls were reported to have performed as expected.

CONCLUSION

The substance was considered a skin sensitiser.

TEST FACILITY

Givaudan (2017f)

B.7. 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

Salmonella typhimurium: TA1535, TA1537, TA98, TA100
Escherichia coli: WP2uvrA

Metabolic Activation System

S9 microsomal fraction from Aroclor 1254-induced rat liver

Concentration Range in Main Test

All *Salmonella* and *E.coli* strains
With and without metabolic activation: 52-5000 µg/plate

Vehicle

Dimethyl sulphoxide (DMSO)

Remarks - Method

There were no deviations from the study plan and operating procedures.

A preliminary toxicity test, Test 1 (5.4-5000 µg/plate), was performed in

triplicate in order to determine the toxicity of the test material in the presence and absence of metabolic activation in all tester strains.

Based on the results of Test 1, five doses increasing with approximately half-log steps (52-5000 µg/plate) were selected and tested in triplicates in the main test, Test 2.

Test 1:
All *Salmonella* and *E.coli* strains with or without 5% S9: 5.4, 17, 52, 164, 512, 1600 and 5000 µg/plate.

Test 2:
All *Salmonella* and *E.coli* strains with or without 10% S9: 52, 164, 512, 1600 and 5000 µg/plate

The vehicle control and positive controls were concurrently tested in each strain in the presence and absence of S9-mix.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test 1	*Cytotoxicity in Main Test 2*	*Precipitation*	*Genotoxic Effect*
Absent				
Test	> 5000 for all strains	> 5000 for all strains	≥ 1600 for all strains	Negative
Present				
Test	> 5000 for all strains	> 5000 for all strains	≥ 1600 for all strains	Negative

Remarks - Results

All bacterial strains showed negative responses over the entire concentration range, i.e. there was no significant concentration -related increase in the number of revertants in two independently repeated experiments.

In tester strain TA1537, a fluctuation in the number of revertant colonies below the laboratory historical control data range was observed in Test 1 at 52 µg/plate in the presence of S9-mix and in Test 2 at 1600 µg/plate in the absence of S9-mix. Since no concentration -relationship was observed in both tests, these reductions were not considered to be caused by toxicity of the test item, but rather by an incidental fluctuation in the number of revertant colonies.

In strain TA100, fluctuations in the number of revertant colonies above the laboratory historical control data range was observed in Test 2 in the presence of S9-mix at 164 and 512 µg/plate. Since the increases were less than two-fold (a maximum of 1.1-fold was reached) and no concentration -relationship was observed, these increases were not considered to be biologically relevant.

The negative and strain-specific positive control values were within the laboratory background historical control data ranges indicating that the test conditions were adequate and that the metabolic activation system functioned properly.

CONCLUSION

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

TEST FACILITY

Charles River (2017e)

B.8. Genotoxicity – *in vitro*

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 487 In Vitro Mammalian Cell Micronucleus Test (2014)
Species	Human
Cell Type/Cell Line	Human peripheral lymphocytes
Metabolic Activation System	S9 fraction from phenobarbital/β-naphthoflavone induced rat liver
Vehicle	Dimethyl sulfoxide
Remarks - Method	There were no deviations from the study protocol. The clastogenic potential of the test item was evaluated in two assays. The first assay (Test 1) was a dose finding test to determine the appropriate dose levels for scoring of micronuclei. The test item was tested at up to 50 and 85 µg/mL for a 3 hour exposure time with a 27 hour harvest time in the absence and presence of S9-fraction, respectively. Appropriate toxicity was reached at these dose levels. In the second cytogenetic assay (Test 2), the substance was tested at up to 70 µg/mL for a 24 hour exposure time with a 24 hour harvest time in the absence of S9-mix. Appropriate toxicity was reached at this dose level. The vehicle for the test item (DMSO) was used as a negative control. The positive controls included: <u>Without metabolic activation:</u> Mitomycin C at 0.25 and 0.38 µg/ml for a 3 hour exposure period and 0.15 and 0.23 µg/mL for a 24 hour exposure period. Colchicine at 0.1 µg/ml for a 3 hour exposure period and 0.05 µg/ml for a 24 hour exposure period. <u>With metabolic activation:</u> Cyclophosphamide at 15 and 17.5 µg/mL for a 3 hour exposure period.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	5, 40, 50	3 h	27 h
Test 2	10, 50, 70	24 h	24 h
Present			
Test 1	20, 40, 85	3h	27 h

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*	
	Cytotoxicity in Main Test	*Genotoxic Effect*
Absent		
Test 1	≥55	negative
Test 2	≥80	negative
Present		
Test 1	≥90	negative

Remarks - Results	The test item did not induce a statistically significant and biologically relevant increase in the number of mono- and binucleated cells with

micronuclei in the absence or presence of S9-mix, in either of the two experiments. In the first study there was a significant but slight increase in the number of mononucleated cells with micronuclei in the absence of metabolic activation, however the value was within the 95% historical controls and was not considered biologically relevant.

The positive and negative controls gave satisfactory responses confirming the validity of the test system. Only one positive control value (first study, without metabolic activation) had a smaller than expected increase in numbers of mononucleated cells with micronuclei. This was not considered relevant as the same positive control gave a significant increase at a lower concentration.

CONCLUSION

The notified chemical did not induce micronuclei in cultured human peripheral blood lymphocytes treated in vitro under the conditions of the test.

TEST FACILITY

Charles River (2017f)

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 EC Council Regulation No 440/2008 C.4-D Ready Biodegradability EPA OPPTS 835.3110 Ready Biodegradability
Inoculum	Activated sludge from a local STP
Exposure Period	61 days
Auxiliary Solvent	None
Analytical Monitoring	Theoretical oxygen demand by Oxitop control system
Remarks - Method	No significant deviations from the test guidelines were reported. The test substance was directly added to the test flasks and then diluted with test medium to 20 mg/L. A toxicity control was run.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
3	7	5	62
4	11	7	72
14	41	14	77
21	61	21	80
28	62	28	79
61	74	61	75

Remarks - Results	The percentage degradation of the reference compound, sodium benzoate surpassed the threshold level of 60% within 14 days indicating the suitability of the inoculums. All validity criteria for the test were satisfied. The toxicity control exceeded 25% biodegradation after 14 days showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the test substance was 62% after 28 days and 74% after 61 days. As the test substance is a mixture of isomers, the 10 day window criterion is not applied.
CONCLUSION	The test substance is readily biodegradable.
TEST FACILITY	Givaudan (2017g)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Semi static EC Council Regulation No 440/2008 C.2 Acute Toxicity for Daphnia - Semi static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	Gas chromatography mass spectrometry (GC-MS)
Remarks - Method	No significant deviations from the test guidelines were reported. The test substance was pipetted onto test water surface at a loading rate of 50 mg/L.

Thereafter slow stirring was applied for 48 hours in a closed vessel to reach a maximum concentration of dissolved test item in the test water. After that, stirring was stopped for 24 hours to allow complete phase separation. Then, the lower aqueous was separated from the non-dissolved upper test item phase. This equilibrated aqueous phase with a loading rate of 50 mg/L, containing dissolved test item only, was used as the highest test concentration. The highest test concentration was subsequently diluted with test water to obtain lower test concentrations. Losses of test item by evaporation, were prevented by using sealed vessels according to OECD Guidance Document No. 23 on Aquatic Toxicity Testing of Difficult Substances and Mixtures, 2000. A semi-static test design with a test medium renewal after 24 hours was used. The test substance was measured at the start and the end of the two test medium renewal periods, and the geometric mean values were presented below.

RESULTS

Concentration		*Number of D. magna*	*Number Immobilised after 48 h*
Nominal (mg/L)	*Mean measured (µg/L)*		
Control	<LOQ*	20	0
10	30	20	0
15	45	20	0
22	65	20	0
33	85	20	0
50	123	20	0

*LOQ: limit of quatification of 4.47 µg/L

LC50	> 123 µg/L at 48 hours
Remarks - Results	The dissolved oxygen concentration at the end of the test was ≥ 8.5 mg/L at 21°C (≥ 95%, USGS, 2011) in all test vessels. All validity criteria for the test were satisfied.

CONCLUSION The test substance is not harmful to aquatic invertebrates up to its water solubility limit

TEST FACILITY IES (2017a)

C.2.2. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test
EC Regulation No 2016/266 C.3 Algal Inhibition Test

Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 10, 15, 22, 33, 50 mg/L Mean measured: 6.8, 10.6, 16.1, 16.7, 22 µg/L
Auxiliary Solvent	None
Water Hardness	15 mg $CaCO_3$/L
Analytical Monitoring	Gas chromatography mass spectrometry (GC-MS)
Remarks - Method	No significant deviations from the test guidelines were reported. The test substance was pipetted onto the test water surface at a loading rate of 50 mg/L. Thereafter slow stirring was applied for 48 hours in a closed vessel to reach a maximum concentration of dissolved test item in the test water. After that, stirring was stopped for 24 hours to allow complete phase separation. Then, the lower aqueous was separated from the non-dissolved upper test item phase. This equilibrated aqueous phase with a loading rate of 50 mg/L, containing dissolved test item only, was used as highest test

concentration and considered to represent the aqueous saturation concentration in test media of the test item. The highest test concentration was subsequently diluted with test water to obtain lower test concentrations. Losses of test item by evaporation, were prevented by using sealed vessels according to OECD Guidance Document No. 23 on Aquatic Toxicity Testing of Difficult Substances and Mixtures, 2000. The test substance was measured at the start, after 24 and 48 hours and at the end of the test, and the geometric mean values were presented above.

RESULTS

Biomass		*Growth*	
EC50	*NOEC*	*EC50*	*NOEC*
µg/L at 72 h	*µg/L*	*µg/L at 72 h*	*µg/L*
> 22	22	> 22	16.1

Remarks - Results The mean cell density in the control increased by 150 times. All validity criteria for the test were satisfied.

CONCLUSION The test substance is not harmful to alga up to its water solubility limit

TEST FACILITY IES (2017b)

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