File No: LTD/2066

November 2019

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Acetamide, 2-(4-methylphenoxy)-*N*-1*H*-pyrazol-3-yl-*N*-(2-thienylmethyl)-

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

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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/2066	Firmenich Pty Limited	Acetamide, 2-(4-methylphenoxy)-*N*-1*H*-pyrazol-3-yl-*N*-(2-thienylmethyl)-	Yes	≤ 1 tonne per annum	Fragrance ingredient

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard Classification	*Hazard Statement*
Skin Sensitisation (Category 1)	H317 – May cause an allergic skin reaction
Acute Toxicity (Category 4)	H332 – Harmful if inhaled

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

Hazard Classification	*Hazard Statement*
Chronic Toxicity (Category 2)	H411 – Toxic to aquatic life with long lasting effects

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.

When used at the proposed concentrations in cosmetics and household products, the notified chemical is not considered to pose an unreasonable risk to public health.

Environmental Risk Assessment
Based on the PEC/PNEC ratio the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following 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 skin contact

- 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
  - Respiratory protection if inhalation exposure may occur

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

- 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 notified chemical is imported in powder form;
  - further information becomes available on skin sensitisation potency of the notified chemical;
  - the final use concentration of the notified chemical exceeds:
    - in oral care products 0.015%,
    - in cosmetics: 0.01% in body lotion, 0.002% in face and hand cream, 0.0013% in fine fragrances, 0.0007% in deodorants, 0.07% in shampoo, 0.18% in conditioner, 0.47% in shower gel, 0.02% in hand wash soap, and 0.013% in hair styling products,
    - in household products 0.1%,

- in air fresheners (electric and spray) 0.2%.

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 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 product containing 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)
Firmenich Pty Limited (ABN: 86 002 964 794)
73 Kenneth Road
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 exempt from publication include: other names, analytical data, degree of purity, identity of impurities and additives/adjuvants.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Schedule data requirements are not varied.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
China (2018)
EU (2018)
USA (2019)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Acetamide, 2-(4-methylphenoxy)-N-1H-pyrazol-3-yl-N-(2-thienylmethyl)-

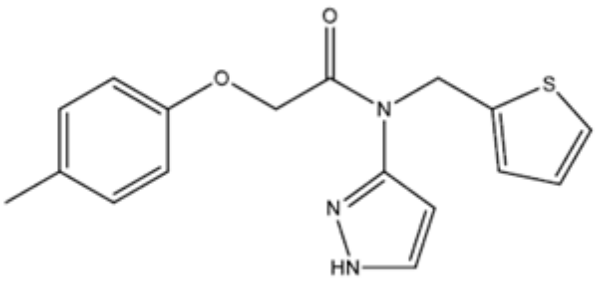
CAS NUMBER
1374760-95-8

CHEMICAL NAME
Acetamide, 2-(4-methylphenoxy)-*N*-1*H*-pyrazol-3-yl-*N*-(2-thienylmethyl)-

OTHER NAME(S)
2-(4-Methylphenoxy)-*N*-1*H*-pyrazol-3-yl-*N*-(2-thienylmethyl) acetamide
2-(4-Methylphenoxy)-*N*-1*H*-pyrazol-5-yl-*N*-(2-thienylmethyl) acetamide
S2227

MOLECULAR FORMULA
$C_{17}H_{17}N_3O_2S$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
327.4 g/mol

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

3. COMPOSITION

DEGREE OF PURITY
> 90 %

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Beige to white free flowing powder

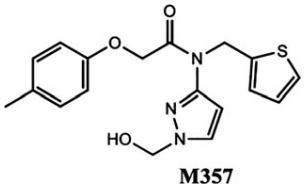
Property	*Value*	*Data Source/Justification*
Melting Point/Freezing Point	117.6 °C	Measured
Boiling Point	> 280 °C at 101.3 kPa	Measured (The test item decomposed before boiling occurred).
Density	380 kg/m^3	Measured
Vapour Pressure	1×10^{-6} kPa at 20 °C	Measured
Water Solubility	13.2 mg/L at 20 °C	Measured
Hydrolysis as a Function of pH	t1/2 >28 days at 40 °C (at pH 4, 7 & 9)	Measured
Partition Coefficient (n-octanol/water)	log Pow = 2.4 at 22.7 °C	Measured
Adsorption/Desorption	log K_{oc} = 2.45 Soil and 2.50 Sewage Sludge at 23.1 °C	Measured
Dissociation Constant	-	Not measured. The 1*H*-pyrazol moiety is a weak base (pKb 11.5) but will not significantly associate in environmentally relevant conditions (pH 4-9).
Particle Size	Mean particle size is about 6 µm	Data extracted from the powder density test. As introduced and used in Australia, the notified chemical is not separated from solution.
Flash Point	Not determined	Solid at room temperature.
Solid Flammability	Not flammable	Measured
Autoignition Temperature	Not determined	As it is not flammable the notified chemical is not expected to autoignite at a low temperature.
Explosive Properties	Not determined	Measured (The DSC for explosive potential was inconclusive. However, the notified chemical contains no functional groups that would imply explosive properties.)
Oxidising Properties	Not determined	Not expected to have 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. EFSA (2018) discussed the stability of the notified chemical and noted that the dry powder form is stable even after heating at 110 °C for 24 hours. The amide function of the notified chemical in aqueous buffers could be partially hydrolysed to secondary amine (M179) and carboxylic acid (M166) as shown below. The percentage of the notified chemical remaining after 24 hours at 100 °C was 90.1%, 97.2% and 83.5% at pH 2.8, 4.0 and 7.1, respectively.

Notified chemical (Amide) M166 (Acid) M179 (Amine)

EFSA (2018) also reported that in a photostability test using a Q-Sun Xenon Test Chamber, the major phototransformation product of the notified chemical (in buffers at pH 2.8 and 4.0) was amide M357 (below).



M357

Physical Hazard Classification

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

5. INTRODUCTION AND USE INFORMATION

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

The notified chemical will be imported into Australia as a component in a fragrance formula (at a concentration < 10%) for incorporation in consumer products ranging from cosmetics to household products. The notified chemical will not be imported in a powder form.

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

IDENTITY OF MANUFACTURER/RECIPIENTS

Firmenich Pty Limited

TRANSPORTATION AND PACKAGING

The notified chemical will be imported into Australia in a fragrance formula (at a concentration < 10%) in lacquered drums of 180 (typical size), 100, 50, 25, 10 or 5 kg in size and transported by road from the port of entry to the notifier's warehouse facilities or to reformulation sites. End-use products (at ≤ 0.2% concentration) will be packaged in containers suitable for retail sale.

USE

The notified chemical will be used as a fragrance component in a variety of cosmetic, oral care and household cleaning products at various allowable use concentrations (refer to the concentrations in section 6.3.2. Table), and in liquid electric and aerosol air fresheners at ≤ 0.2% concentration.

OPERATION DESCRIPTION

Reformulation

The procedures for incorporating the imported preparations (at < 10% concentration) into end-use products (at up to 0.2% concentration) will likely vary depending on the nature of the cosmetic, personal care and household cleaning products formulated, and may involve both automated and manual transfer steps. It is expected that the reformulation processes will involve blending operations that will be highly automated and occur in a fully

enclosed/contained environment, followed by automated filling (using sealed delivery systems) of the reformulated end-use products into containers of various sizes.

End-use
The end-use products containing the notified chemical at various allowable use concentrations (refer to table in Section 6.3.2.) may be used by consumers and professionals such as hairdressers, workers in beauty salons or cleaners. Depending on the nature of the product, these could be applied in a number of ways, such as by hand, using an applicator or sprayed.

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	unknown	unknown
Mixing	4	2
Drum handling	4	2
Drum cleaning/washing	4	2
Maintenance	4	2
Quality control	0.5	1
Packaging	4	2
Professional end users	not specified	not specified

EXPOSURE DETAILS

Transport and storage
Transport and storage workers may come into contact with the notified chemical at < 10% concentration as a component of the imported preparations, only in the event of accidental rupture of containers.

Formulation of end use products
During reformulation, dermal, ocular and potentially inhalation exposure of workers to the notified chemical may occur when weighing and transferring, equipment preparation, blending, quality control analysis and cleaning and maintenance of equipment. Exposure is expected to be minimised through the use of local exhaust ventilation and enclosed and automated systems and through the use of personal protective equipment (PPE) such as gloves, respiratory protection, eye protection and protective clothing.

End-use beauty care and cleaning professionals
Exposure to the notified chemical in end-use products (at up to 0.2% concentration) may occur in professions where the services provided involve the application of cosmetic and personal care products to clients (e.g. hairdressers, workers in beauty salons) or in the cleaning industry. Such professionals may use 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 a variety of cosmetic, oral care and household products at various use concentrations (refer to tables in Section 6.3.2.). The principal route of exposure will be dermal and oral, while ocular and inhalation exposure is also possible, particularly where products are applied by spray.

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*
Acute oral toxicity – rat	LD50 > 2000 mg/kg bw; low toxicity
Acute dermal toxicity – rat	LD50 > 2000 mg/kg bw; low toxicity
Acute inhalation toxicity – rat	LC50 3.34 - 4.8 mg/L/4 h for males and > 4.8 mg/L/4 h for females; harmful
Skin irritation – rabbit	non-irritating
Eye irritation – *in vitro* Human Cornea Model Test	non-irritating
Skin sensitisation – HRIPT (100 ppm)	no evidence of sensitisation
Skin sensitisation – HRIPT (30 ppm)	evidence of sensitisation
Skin sensitisation – *in chemico* DPRA test	positive
Skin sensitisation – *in vitro* Keratinosens test	positive
Repeat dose Oral-Gavage toxicity – rat, 90 days	NOAEL = 100 mg/kg bw/day
Repeat dose inhalation toxicity – rat, 28 days	NOEC = 6.7 mg/m^3 /day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* chromosome aberration assay	non genotoxic
Genotoxicity – *In Vivo* Mouse Bone Marrow Micronucleus Assay	non genotoxic

Toxicokinetics, Metabolism and Distribution
Based on the molecular weight of the notified chemical (< 500 g/mol), there is potential for the chemical to cross biological membranes (ECHA, 2017).

EFSA (2018) summarised several studies carried out on the notified chemical to evaluate absorption, distribution, metabolism and elimination, including *in vitro* profiling for hepatic phase I metabolism, *ex vivo* plasma stability in six species, and *in vivo* single dose kinetic and metabolic study in rats, dogs and mice. The notified chemical can be absorbed after oral administration and is rapidly hydrolysed to the corresponding carboxylic acid (M166) and secondary amine (M179). These are identified as the two major metabolites *in vivo,* and their formation leads to low systemic exposure to the parent amide. Other phase I metabolic products were also identified.

EFSA (2018) evaluated the possibility that the hydrolysis of the notified chemical into the amine M179 (which may occur in the lumen of the GI tract, the intestinal wall or the liver) may lead to the formation of nitrosamines. EFSA estimated that the concentration of the nitrosamine formed in the stomach from swallowing an amount of 1,800 µg/person per day is 0.03×10^{-15} µg/kg bw/day, and stated that this is far below the threshold of toxicological concern (TTC) for substances that are expected to be genotoxic carcinogens (0.0025 µg/kg bw/day). Therefore, they concluded that there was not a safety concern, based on the expected levels of acute exposure via food.

A conservative estimate (using 100% absorption), combining the exposure of exposure from use of personal care, oral care and household products is calculated as 46 µg/kg bw/day (section 6.3.2). Formation of high amounts of endogenous nitrosamines is not expected from use of the chemical at low concentrations in cosmetics and household products.

In vitro receptor and cytochrome P450 interaction profiling
The notified chemical is reported to be a potent activator of the transient receptor potential ion channel TRPM8, and to demonstrate long-lasting cooling effects in sensory testing (Karanewsky et al 2015). *In vitro* tests were carried out to assess whether it might interact with enzymes or receptors, with consequent adverse or unexpected effects. No significant interaction with drug receptors was seen in a screening study of 67 receptors. The notified chemical did not significantly inhibit the hERG ion channel current at a concentration of 10µM. When used on a panel of CYP enzymes using pooled human liver microsomes and CYP-specific substrates, there was significant inhibition of CYP2C19 and CYP3A4, confirmed in a further study at 1.3 and 15 µM respectively (Karanewsky et al 2015). EFSA (2018) considered that the substance would not be expected to interact with CYP enzymes at the estimated levels of dietary exposure.

Significant internal exposure is not expected from use of the chemical in oral care products, cosmetics and household products (see tables in section 6.3.2.). However, accidental ingestion is possible from oral care products use at 0.015%. Considering the low concentrations in cosmetics and oral care products (up to 0.015%) significant plasma concentrations are not expected to interact with CYP enzymes.

Acute Toxicity
The notified chemical was found to have low acute toxicity in rats via the oral and dermal routes. It is harmful via the inhalation route in powder form.

Irritation and Sensitisation
The notified chemical in powder form was not irritating to the skin of rabbits, and was concluded not irritating to eyes in powder form in an *in vitro* test.

The notified chemical was positive in an *in chemico* study (DPRA) and an *in vitro* cell based assay (Keratinosens) for skin sensitisation, representing the first and second key events in the Adverse Outcome Pathway (AOP) leading to development of skin sensitisation. Based on the available data, the chemical is considered to be a skin sensitiser. Two human repeat insult (HRIPT) test on the notified chemical at low concentrations (30 ppm and 100 ppm) were also available with negative results (except for 1/112 at 30 ppm). The higher concentration (100 ppm) with negative results can be used in order to estimate acceptable concentrations of use that will not be sensitising.

Repeated Dose Toxicity
In a 28-day repeated dose inhalation toxicity study in rats (5/sex/dose), the notified chemical was administered daily by nose only at dose levels (0, 0.6, 2.3, and 6.7 mg/m^3 in a solution of ethyl lactate). Interpretation of the study was hindered by significant dose related microscopic changes in the nasal areas and larynx of treated animals, which were attributed to the solvent ethyl lactate. Although the magnitude of effects in test animals was no greater than in the controls, these effects raise some uncertainty. The No Observed Effect Concentration NOEC was established as 6.7 mg/m^3 for the notified chemical in this study, based on the lack of test substance-related findings in any evaluated endpoint.

In a 90-day repeated dose oral-gavage toxicity study in rats (20/sex/dose), the animals were administered the notified chemical at 0, 10, 30, and 100 mg/kg bw/day. No test substance-related mortality, and no macroscopic or microscopic findings or toxicologically significant organ weight changes were reported. The No Observed Adverse Effect Level (NOAEL) was established as 100 mg/kg bw/day in this study.

Developmental Toxicity
Karanewsky et al (2015) reported on a developmental study in rats, which was conducted in accordance with OECD TG 414. The notified chemical was administered orally by gavage in 1% methyl cellulose at dose levels of 0, 125, 300, or 1000 mg/kg bw/day from gestation Days 6 through 20. All females survived to the scheduled necropsy on gestation Day 21. One female in the 1000 mg/kg bw/day group was non-gravid. No test substance related soft tissue or skeletal malformations or variations were observed at any dose level. The soft tissue developmental variation of renal papilla (not developed and/or distended ureters) was noted in 13 (3), 13 (4), 4 (2), and 26 (5) foetuses (litters) in the control, 125, 300, and 1000 mg/kg bw/day groups respectively. Other soft tissue developmental variations observed in the test substance-treated groups consisted of a major blood vessel variation and haemorrhagic ring around the iris. Intrauterine growth and survival were unaffected. Mean numbers of corpora lutea and implantation sites and the mean litter proportions of pre-implantation loss were similar in all groups. The established NOAEL for maternal toxicity and embryo/foetal development was 1000 mg/kg bw/day based on the lack of adverse maternal toxicity or effects on intrauterine growth and survival and foetal morphology at any dosage level.

Mutagenicity/Genotoxicity
The notified chemical was non-mutagenic in a bacterial reverse mutation assay and in an *in vitro* chromosome aberration assay and it was not clastogenic in an *in vivo* mouse bone marrow micronucleus assay.

The metabolite amine M179 was also tested for mutagenicity/genotoxicity and reported by Karanewsky (2015). The metabolite was negative in a reverse mutation study using *S. typhimurium* and *E. coli* strains in the plate incorporation method, both in the presence and absence of metabolic activation. In a chromosome aberration study similar to OECD TG 473, it produced a statistically significant and dose dependant increase in structural aberrations after 4 h exposure in the absence of metabolic activation. It was negative in an *in vivo* micronucleus study in mice and also not-DNA damaging in the liver of mice in an *in vivo* alkaline comet assay.

Overall the notified chemical and its amine metabolite are not considered to be mutagenic or genotoxic.

Phototoxicity
EFSA (2018) reported that the notified chemical was not phototoxic in an *in vitro* study with Balb/c 3T3 cells, according to the INVITTOX 3T3 NRU Phototoxicity test guideline.

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

Hazard Classification	*Hazard Statement*
Skin Sensitisation (Category 1)	H317 – May cause an allergic skin reaction
Acute Toxicity (Category 4)	H332 – Harmful if inhaled

6.3. Human Health Risk Characterisation
The notified chemical is a skin sensitiser. It could cause harmful effects if inhaled at high concentrations (the LC50 = 3.34 - 4.8 mg/L/4hours for males and > 4.8 mg/L/4hours for females).

6.3.1. Occupational Health and Safety

Reformulation
Workers may experience dermal and accidental ocular and perhaps inhalation exposure to the notified chemical (at < 10% concentration) during formulation processes. This exposure may occur during handling of the drums, cleaning and/or maintenance of the equipment. The use of enclosed, automated processes and PPE (impervious gloves, coveralls and respiratory protection) should minimise the potential for exposure. Therefore, provided that adequate control measures are in place to minimise worker exposure, including the use of automated processes and PPE, the risk to workers from use of the notified chemical is not considered to be unreasonable.

End-use
Workers involved in professions where the services provided involve the use of household products in the cleaning industry or application of cosmetic products to clients (e.g. cleaners, beauty salon workers), may be exposed to the notified chemical. Hairdressers may also be repetitively exposed to the notified chemical in their application of shampoo and hairspray to salon clients. Such professionals may use PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. Therefore, the risk to these workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical on a regular basis (for details of the public health risk assessment, see Section 6.3.2.).

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.

6.3.2. Public Health
Members of the public will experience widespread and frequent exposure to the notified chemical at various use concentration (refer to tables in Section 6.3.2.) through daily use of cosmetic, oral care and household products. The main routes of exposure are expected to be dermal and oral, while ocular and inhalational exposures are also possible, particularly if products are applied by spray.

The notified chemical is acutely harmful if inhaled at high levels. Inhalation exposure is most likely to occur from use of spray products e.g. aerosol air fresheners. However concentrations in air from use of spray products are expected to be low and not to pose an acute inhalation risk.

The notified chemical is also a skin sensitiser. Using the negative results in a human repeat insult patch test (HRIPT) carried out at 100 ppm, an allowable concentration of use (AEL) was calculated for cosmetic and household products with dermal exposure. The AEL for oral care products was determined on the basis of the allowable concentration in chewing gum set by EFSA (EFSA, 2018). At these use concentrations (listed in the tables below) the risk of skin sensitisation induction is not expected.

The highest systemic exposure estimation is for a person who is a simultaneous user of all products listed in the tables below that contain the notified chemical. Using the allowable use concentrations for products with dermal exposure and 0.2% concentration in air fresheners for inhalation, this would result in a combined internal dose of 0.0460 mg/kg bw/day (0.0311 + 0.0006 + 0.0052 + 0.0091 = 0.0460 mg/kg bw/day). It is acknowledged that inhalation exposure to the notified chemical from use of other cosmetic and household products may occur. However, it is considered that the combination of the conservative air freshener inhalation exposure assessment parameters, and the aggregate exposure from use of the dermally applied products, which assumes a conservative

100% absorption rate, is sufficiently protective to cover additional inhalation exposure to the notified chemical from use of other spray products.

Using the NOAEL of 100 mg/kg bw/day derived from a 90 day repeated dose oral toxicity study on the notified chemical and an estimated maximum exposure of 0.0460 mg/kg bw/day, the margin of exposure (MOE) for systemic effects was estimated to be > 100 which is generally considered to be acceptable, taking into account intra- and inter-species differences.

Cosmetic/personal care products

Product type	*Amount (mg/day)*	*Retention Factor (RF)*	*AEL* (µg/cm²/day)*	*Allowable use conc. ** (%)*	*Daily systemic exposure (mg/kg bw/day)*
Body lotion	7820	1.000	0.0500	0.0100	0.0122
Face cream	1540	1.000	0.0500	0.0018	0.0004
Hand cream	2160	1.000	0.0500	0.0020	0.0007
Fine fragrances	750	1.000	0.0500	0.0013	0.0002
Deodorant non-spray	1500	1.000	0.0500	0.0007	0.0002
Shampoo	10460	0.010	0.0500	0.0688	0.0011
Conditioner	3920	0.010	0.0500	0.1837	0.0011
Shower gel	18670	0.010	0.0500	0.4687	0.0137
Hand wash soap	20000	0.010	0.0500	0.0215	0.0007
Hair styling products	4000	0.100	0.0500	0.0126	0.0008
					Total: 0.0311

* Allowable Exposure level based on skin sensitisation potential
** Based on skin sensitisation

Household products (Direct dermal exposure)

Product type	*Use Frequency (per day)*	*Use Frequency (calc) (per day)*	*Daily Amount (mg/day)*	*AEL* (µg/cm²/day)*	*Allowable conc.** (%)*	*Daily systemic exposure (mg/kg bw/day)*
Dishwashing liquid	1.1700	1.17	11.7	0.0500	0.0923	0.0002
Cleaning liquid	0.2800	1	10	0.0500	0.1075	0.0002
Laundry liquid	1.0000	1	10	0.05	0.1075	0.0002
						Total: 0.0006

Exposure area = 215 cm², product used amount of 10 mg/use
* Allowable Exposure level based on skin sensitisation potential
** Based on skin sensitisation

Oral exposure

Product Type	*Relative daily amount applied (mg/kg bw/day)*	*Retention Factor*	*Calculated relative daily exposure (mg/kg bw/day)*	*Concentration (%)*	*Daily systemic exposure (mg/kg bw/day)*
Toothpaste (adult)	43.29	0.05	2.16	0.015*	0.0003
Mouthwash	325.40	0.10	32.54	0.015*	0.0049
					Total: 0.0052

* Based on allowable levels in chewing gum (EFSA, 2018)

Inhalation Exposure

Product type	*Amount (g/day)*	*Conc. (%)*	*Inhalation Rate (m³/day)*	*Exposure Duration (min)*	*Volume (m³)*	*Daily systemic exposure (mg/kg bw/day)*
Air freshener	7.5	0.2	9.0	30	10	0.0091
						Total: 0.0091

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

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported as a component of fragrance preparations for local reformulation into a variety of cosmetics and household products. Release during reformulation is expected to arise from spills (0.1%), and residues in import containers (0.1%). No release is expected from equipment cleaning as the wash water will be re-used in reformulated products. Accidental spills during transport or reformulation are expected to be collected with inert material and disposed of to landfill. Import containers will either be recycled or disposed of through an approved waste management facility. Therefore, up to 0.2% (0.2 kg per annum) 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 domestic products, which are either washed off the hair and skin of consumers, or disposed of following cleaning activities.

RELEASE OF CHEMICAL FROM DISPOSAL

It is estimated that a maximum of 3% of the consumer products containing the notified chemical will remain in end-use containers. These containers will be disposed of through domestic garbage disposal and will enter landfill or be recycled. The washings from the recycling process are expected to be sent to the sewer.

7.1.2. Environmental Fate

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 based on the provided test report. For the details of the environmental fate studies refer to Appendix C. The study has also indicated that the notified chemical is hydrolytically stable. Based on the log Pow (2.4), there is an indication that the notified chemical will not bioaccumulate.

Most of the notified chemical will be released to the sewer after use and directed to sewage treatment plants (STPs) nationwide. A small amount of the notified chemical may be sent to landfill (3%) as collected spills or container residues. In STPs, the majority of the notified chemical is expected to be removed from the water column via adsorption to sludge sediment given the hydrophobic structure and the measured log Koc of 2.4 and eventually be sent to landfill. In landfill or water, the notified chemical is expected to undergo biotic or abiotic degradation processes, forming water and oxides of carbon, nitrogen and sulphur.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has been calculated assuming a worst case scenario of 100% release of the notified chemical into sewer systems nationwide and no removal from STPs.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1000	kg/year
Proportion expected to be released to sewer	100.0	%
Annual quantity of chemical released to sewer	1000.000	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)	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/m3). Using these assumptions, irrigation with a concentration of 0.562 µg/L may potentially result in a soil concentration of approximately 3.745×10^{-3} mg/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 1.873×10^{-2} mg/kg and 3.745×10^{-2} mg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	EC50 > 13.1 mg/L (WAF)*	Not harmful to fish up to the water solubility limit
Daphnia Toxicity	EC50 = 2.18 mg/L (WAF)*	Toxic to Daphnia
Algal Toxicity	EC50 > 6.49 mg/L^ (WAF)*	Not harmful to the maximum level tested.
Respiration Inhibition Activated Sludge	EC50 >1000 mg/L	Not inhibitory to microbial activity up to the limit of its water solubility.

* Water accommodated fraction

^ Maximum achievable concentration under test conditions.

Based on the above ecotoxicological endpoints for the notified chemical, it is expected to be acutely toxic to daphnia and is not biodegradable. Therefore, the notified chemical is formally classified under the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS) (United Nations, 2009) for chronic toxicities.

7.2.1. Predicted No-Effect Concentration

The predicted no-effects concentration (PNEC) has been calculated from the most sensitive endpoint for daphnia. A safety factor of 100 was used, as acute endpoints for three trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment	
EC50 (*Daphnia*)	2.18 mg/L
Assessment Factor	100.00
Mitigation Factor	1.00
PNEC:	21.80 µg/L

7.3. Environmental Risk Assessment

The Risk Quotient (Q = PEC/PNEC) has been calculated based on the predicted PEC and PNEC.

Risk Assessment	PEC (µg/L)	PNEC (µg/L)	Q
Q – River	0.56	21.80	**0.026**
Q – Ocean	0.06	21.80	**0.003**

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. The notified chemical is not readily biodegradable. The notified chemical has low potential to bioaccumulate as it is not expected to be significantly bioavailable in the aquatic environment due to its low water solubility.

On the basis of the PEC/PNEC ratios calculated using the maximum annual importation volume and the assessed use pattern in cosmetics and household products, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 117.6 °C

Method	OECD TG 102 Melting Point/Melting Range EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature
Remarks	Differential scanning calorimetry (DSC).
Test Facility	Siemens AG (2018a)

Boiling Point > 280 °C at 101.3 kPa

Method	OECD TG 103 Boiling Point EC Council Regulation No 440/2008 A.2 Boiling Temperature
Remarks	The boiling test of the test item could not be determined as the test item decomposed before boiling could occur at approximately 280 °C (at 101.3 kPa).
Test Facility	Siemens AG (2018a)

Density 380 kg/m^3

Method	OECD TG 109 Density of Liquids and Solids EC Council Regulation No 440/2008 A.3 Relative Density
Remarks	Air comparison pycnometer
Test Facility	Firmenich (2014)

Vapour Pressure 1×10^{-6} kPa at 20 °C

Method	OECD TG 104 Vapour Pressure EC Council Regulation No 440/2008 A.4 Vapour Pressure
Remarks	Effusion method
Test Facility	Siemens AG (2018b)

Water Solubility 13.2 mg/L at 20 °C, pH 6.5

Method	OECD TG 105 Water Solubility
Remarks	Flask Method
Test Facility	NL (2017a)

Hydrolysis as a Function of pH

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)*	*$t_{½}$ Days*
4	40	>28
7	40	>28
9	40	>28

Remarks	Hydrolytically stable in water.
Test Facility	Firmenich SA

Partition Coefficient (n-octanol/water) log Pow = 2.4 at 22.7 °C

Method	OECD TG 117 Partition Coefficient (n-octanol/water).
Remarks	HPLC Method
Test Facility	NL (2017b)

Adsorption/Desorption – main test log K_{oc} = 2.45 Soil and 2.50 Sewage Sludge at 23.1 °C

Method	OECD TG 121 Estimation of the adsorption Coefficient (Koc) on soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC) EC Council Regulation No. 440/2008
Remarks	HPLC Method
Test Facility	NL (2017c)

Solid Flammability Not flammable

Method	2004/10/EU of February 11, 2004 as published on February 20, 2004 Official Journal of the European Union L50/44. Test method for readily combustible solids. Flammability (Solids)
Remarks	The test item could not be ignited with a flame. The test item melted.
Test Facility	Siemens AG (2018c)

Explosive Properties Not determined

Method	OECD 113 Screening test for thermal stability and stability in air (1981).
Remarks	A differential scanning calorimetry (DSC) under nitrogen method was performed. The test item and reference (aluminium oxide) were heated up to 500 °C (final temperature) in nitrogen in closed crucibles at a constant heating rate of 3 K/min. The quantity of absorbed or released heat was measured. The test item showed an endothermal effect between 110 and 135 °C. and an exothermal effect between 250 and 470 °C with a maximum energy release of 591 J/g. The heat of decomposition was above the energy threshold value of 500 J/g. Therefore explosive potential cannot be totally excluded. The DSC screening test for explosive potential was inconclusive based on the UN Recommendations on the Transport of Dangerous Goods Manual of Tests and Criteria Appendix 6. However, None of the test item functional groups is listed in the literature (Bretherick's Handbook of Reactive Chemical Hazards 5th Edition) to be relevant for explosive properties. Further testing in series 2 are needed as suggested by the test author for a definite conclusion.
Test Facility	Siemens AG (2018d)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute Oral Toxicity – Rat, Fixed Dose

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure (2001) EC Council Regulation No 440/2008 B.1 bis Acute toxicity (oral) fixed dose method
Species/Strain	Rat/ Wistar (RccHan:WIST)
Vehicle	Arachis oil BP
Remarks – Method	No significant protocol deviations

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity	No signs of systemic toxicity were observed.
Effects in Organs	No abnormalities were observed at necropsy. One animal from group 3 showed gain in body weight during the first week but no gain during the second week.
Remarks – Results	No deaths were observed.
CONCLUSION	The notified chemical is of low acute toxicity via the oral route.
TEST FACILITY	Envigo (2018a)

B.2. Acute Dermal Toxicity – Rat

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/Wistar (RccHan:WIST)
Vehicle	Moistened with Arachis oil BP
Type of dressing	Semi-occlusive.
Remarks – Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	5 M	2,000	0/5
2	5 F	2,000	0/5

LD50	> 2000 mg/kg bw
Signs of Toxicity – Local	No signs of dermal irritation were observed.
Signs of Toxicity – Systemic	No signs of systemic toxicity were observed.
Effects in Organs	No abnormalities were observed at necropsy. Three females showed body weight loss during the first week but body weight gain was as expected during the second week.
Remarks – Results	No deaths were observed.
CONCLUSION	The notified chemical is of low acute toxicity via the dermal route.
TEST FACILITY	Envigo (2018b)

B.3. Acute Inhalation Toxicity – Rat

TEST SUBSTANCE	Notified chemical/DMSO 50/50 w/w
METHOD	OECD TG 403 Acute Inhalation Toxicity EC Council Regulation No 440/2008, 93/21/EEC B.2 Acute Toxicity (Inhalation)
Species/Strain	Rat/ Wistar (RccHan:WIST)
Vehicle	Dimethyl sulphoxide
Method of Exposure	Nasal exposure
Exposure Period	4 hours
Physical Form	aerosol (particulate).
Particle Size	1-4 μm
Remarks – Method	No significant protocol deviations. Males only were used in the later groups, as the most susceptible sex. Concentrations of the notified chemical in the test chamber atmosphere were determined through measuring the amount of solid material in the samples taken of the atmosphere.

RESULTS

Group	*Number and Sex of Animals*	*Concentration (mg/L)*		*Mortality*
		Nominal	*Actual*	
Sighting	1 M, 1 F	5	4.77	1/1 (M), 0/1 (F)
1	5 M, 5 F	5	4.8	3/5 (M), 0/5 (F)
2	5 M	1	0.95	0/5
3	5 M	3.5	3.34	1/5

LC50	Males 3.34 - 4.8 mg/L/4hours Female > 4.8 mg/L/4hours
Signs of Toxicity	Sighting study: Common abnormalities such as decreased respiratory rate and wet fur were observed during the study. The male died after 130 minutes exposure. The female exhibited laboured respiration, gasping respiration, lethargy, hunched posture, pilo-erection and areas of red/brown staining around the snout and recovered to appear normal on Day 4 post-exposure. The female showed body weight loss on Day 1 post-exposure and from Days 1 to 3 post-exposure and no gain in body weight from Days 3 to 7 post exposures. Main Test: Common abnormalities such as decreased respiratory rate, laboured respiration, hunched posture, pilo-erection and wet fur were observed during the study. The animals exhibited body weight losses on day 1 and/or days 1 to 3 post exposure. Expected body weight gains for most of the animals were noted throughout the remainder of the recovery period.
Effects in Organs	Macroscopic abnormalities were noted at necropsy in three animals of group 1 that died on the day of exposure (haemorrhagic and/or pale lung). Dark patches on the lungs were noted in all animals of group 3. No macroscopic abnormalities in group 2 animals were noted at necropsy.
Remarks – Results	On the basis of the results, the study author stated that the test item meets the criteria for classification in accordance with GHS as Category 4 for acute inhalation toxicity.
CONCLUSION	The notified chemical is acutely harmful by the inhalation route.
TEST FACILITY	Envigo (2018c)

B.4. Skin Irritation – Rabbit

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion EC Council Regulation No 440/2008 B.4 Acute Toxicity (Skin Irritation)
Species/Strain	Rabbit/New Zealand White (Hsdlf:NZW)
Number of Animals	2
Vehicle	Moistened with distilled water
Observation Period	1, 24, 48 and 72 hours
Type of Dressing	Semi-occlusive
Remarks – Method	No significant protocol deviations
Remarks – Results	No evidence of skin irritation was noted during the study. Body weight gain was as expected.
CONCLUSION	The notified chemical is not irritating to the skin.
TEST FACILITY	Envigo (2018d)

B.5. Eye Irritation – *In Vitro* Human Cornea Model Test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 492 Human Cornea Model Test (EpiOcular Eye Irritation Test)
Vehicle	Tissues were wetted with DPBS (Dulbecco's Phosphate-Buffered Saline) before application of the test substance
Remarks – Method	The test is stated as suitable to identify chemicals not classified for eye irritation or serious eye damage under GHS. The test consists of a topical exposure of the neat test item to a human reconstructed cornea model followed by a cell viability test. Cell viability is measured by dehydrogenase conversion of MTT [(3-4,5-dimethyl thiazole 2-yl) 2,5-diphenyl-tetrazoliumbromide], present in cell mitochondria, into a blue formazan salt that is quantitatively measured after extraction from tissues. The percentage reduction of cell viability in comparison to the untreated negative controls is used to predict eye irritation potential. The particle size of the test substance was not stated, and there was no information whether it was ground before use. There was one Deviation to Study Plan: The test item was only pre incubated in water for 1 hour instead of 3 hours. This deviation has no impact on the outcome of the study since the supplier protocol recommends "at least 1 hour". Negative control deionised water and positive control methyl acetate were respectively applied to each of duplicate EpiOcular™ tissue for 6 hours.

RESULTS

Treatment Group	*Mean OD (570 nm) of Treatment Group*	*Mean Rel. Viability [%]*
Negative Control	1.583	100.0
Positive Control	0.448	28.3
Test Item	1.645	103.9

Remarks – Results	The acceptability criteria for the study related to the values for positive and negative controls, and the variability between tissues were all met. The notified chemical was not coloured intensively, did not dye water or isopropanol, and was not a direct MTT reducer.

No irritating effects were observed following incubation with the test item. The mean relative absorbance value of the test item, corresponding to the cell viability, increased to 103.9% (threshold for irritancy: ≤ 60%), indicating the test item was not an eye irritant.

Treatment with the positive control induced a decrease in the mean relative absorbance compared with the negative control to 28.3%, thus the validity of the test system is ensured.

CONCLUSION The notified chemical did not demonstrate eye irritation potential under the conditions of the test (no classification required according the Test Guideline).

TEST FACILITY Envigo (2018e)

B.6. Skin Sensitisation – *In Chemico* DPRA Test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 442c *In Chemico* Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA) (2015)

Vehicle Acetonitrile

Remarks – Method The present test was performed (in Feb. 2014) prior to the OECD adoption of the 442C TG method (in Feb. 2015). No significant deviations from the later adopted OECD test guideline were reported.

The test item was dissolved in acetonitrile at 100 mM. Cinnamaldehyde was used as positive control and solvent reference controls were used in parallel. Incubation of a diluted solution of cysteine or lysine with the test item (at the ratios 1:10 cysteine: test item and 1:50 lysine: test item) was conducted for 24 hours. At the end of the incubation, the concentrations of residual peptides were evaluated by HPLC with ultra-violet detection at 220 nm. It is not known whether the protocol included a check for the presence of cysteine dimers, which is part of the OECD Guideline.

The acceptance criteria of the samples for the calibration curve, and vehicle and positive controls were satisfied. The study was therefore considered to be valid.

RESULTS

Sample	*Cysteine Peptide Depletion (% ± SD)*	*Lysine Peptide Depletion (% ± SD)*
Vehicle Control	0	0
Test Substance	100	0
Positive Control	100	62.54

SD = Standard Deviation

Remarks – Results The mean depletion value was:

- 100% for the cysteine peptide since the cysteine concentrations measured in each sample were below the limit of quantification after depletion (< 0.100 mM),
- for the lysine peptide, two out of three individual depletion values were found negative. The mean depletion value was therefore set to 0%.

The mean percentage of cysteine and lysine depletions was calculated to be 50%. Accordingly, the test item was considered to be highly reactive.

However, it is not known whether the test protocol included detection of the presence of cysteine dimers, which could potentially lead to an over estimation of peptide depletion.

CONCLUSION The test substance was considered to have high reactivity for peptide depletion under the conditions of the test, showing positive results in the first key event (molecular initiating) of the adverse outcome pathway (AOP) for skin sensitisation as defined in the test guideline.

TEST FACILITY CitoxLab (2014)

B.7. Skin Sensitisation – *In Vitro* ARE-Reporter Cell Line Keratinosens Test

TEST SUBSTANCE Notified chemical

METHOD OECD TG draft 442d *In Vitro* Skin Sensitisation: ARE-Nrf2 Luciferase Test Method (2015)

Remarks - Method The experimental design of this study consists of three definitive assays to determine average maximal induction of gene activity (I_{max}), the concentration for average maximal induction of gene activity (CI_{max}), the average concentration inducing gene activity > 50% above the solvent control values (ECI.5 value), and the average concentration leading to 50% cytotoxicity (IC_{50}) for each test substance. The induction of luciferase is evaluated to determine sensitisation potential of the test substance using the ATP luminescence endpoint to calculate the I_{max}, CI_{max}, and the ECI.5 value and the average concentration leading to 50% cytotoxicity (IC_{50}) for each sample. The notified chemical was tested at 12 concentrations ranging from 2000 to 0.975 µM.

Dimethyl sulphoxide (DMSO) was used as the vehicle control. Cinnamic aldehyde was diluted in DMSO was used as the positive control.

A test substance is considered to have sensitisation potential if:
1) The ECl.5 value falls below 1000 µM (or 200 µg/mL) in at least 2 of 3 repetitions;
2) At the lowest concentration with a gene induction above 1.5, cellular viability should be greater than 70%; and
3) An apparent overall dose response should be similar between repetitions.

Cytoxicity was also evaluated by the uptake of neutral red dye (Borenfreund & Puerner, 1985) by the KeratinoSens cells. The amount of reduced MTT or neutral red dye (NRU) are measured by spectrophotometry.

RESULTS

Sample	*EC1.5 Value (µM)*	*Mean IC_{50} (µM) MTT*	*Mean IC_{50} (µM) NRU*	*Maximal Induction (I_{max})*	*Maximal Gene Induction conc. (µM) (CI_{max})*	*Potential Sensitiser*
Test substance	21.20	186.30	180.93	2.53	125	Yes
Positive Control	11.27	> 64*	> 64*	5.61	64	Yes

* When an EC 1.5 or IC_{50} value was not obtained, the results were presented as greater than the highest dose tested.

Remarks - Results The MTT and NRU viability results were similar for the test substance.

The ECI.5 concentration of the neutral red indicated a significant induction of gene activity (50% above solvent controls) and an IC50 concentration (50% viability compared to solvent controls), both demonstrating that the test substance has a keratinocyte activating potential.

	According to the reduced prediction model, the test substance was predicted to be a skin sensitiser.
CONCLUSION	The test substance was considered to be a skin sensitiser.
TEST FACILITY	Institute for In Vitro Sciences, Inc. (2014)

B.8. Skin Sensitisation – Human Volunteers (30 ppm)

TEST SUBSTANCE	Notified chemical at 30 ppm
METHOD	Repeated insult patch test with challenge
Study Design	Induction procedure: Patches containing 0.2 g of the test substance were used semi-occlusively and applied to the left side of the back. Patches were removed by the test subjects after 24 hours (or 48 hours). Sites were graded at 24 and 48 hour post-patch removal. Rest period: 14 days Challenge procedure: A patch was used semi-occlusively and 0.2 g of the test substance was applied to the right side of the back. Patches were removed by the test facility technician after 24 hours. Sites were re-evaluated at 48, 72 and 96 hours after patch removal.
Study Group	74 females, 38 males age range 18 - 70 years (112/120 subjects completed the test. One subject was discontinued due to a protocol violation and seven subjects discontinued due to personal reasons. No subject discontinued due to test material reaction).
Vehicle	The solvent used for dilution was not specified.
Remarks – Method	Semi-occluded. The additional solvents named as PG and (DEP/EtOH) were not fully identified in the test report.
RESULTS	
Remarks – Results	During the induction phase, approximately 20% of the subjects exhibited faint, and minimal erythema and 1-level reactions. During the Challenge, one subject exhibited a 1-level reaction at 3/4 observation times plus an oedema reaction at 2/4 observation times. Approximately 45% of the subjects exhibited faint, and minimal erythema and 1-level reactions. Dryness was observed in some subjects but not considered as an adverse reaction. A re-challenge was performed on the subject who had showed the most adverse effect on challenge, using additional test materials. The subject had a 1-level reaction with oedema to the original test material (30 ppm, solvent not known) and to a solution of 0.9% saline and PG. There was no reaction to 0.9% saline, the notified chemical in DEP/EtOH, or to DEP/EtOH.
CONCLUSION	The test substance at 30 ppm was sensitising under the conditions of the test.
TEST FACILITY	HRL (2013)

B.9. Skin Sensitisation – Human Volunteers (100 ppm)

TEST SUBSTANCE	Notified chemical at 100 ppm
METHOD	Repeated insult patch test with challenge

Study Design	Induction procedure: Patches containing 0.2 g of the test substance were used semi-occlusively and applied to the left side of the back. Patches were removed by the test subjects after 24 hours (or 48 hours). Sites were graded at 24 and 48 hour post-patch removal. Rest period: 14 days Challenge procedure: A patch was used semi-occlusively and 0.2 g of the test substance was applied to the right side of the back. Patches were removed by the test facility technician after 24 hours. Sites were re-evaluated at 48, 72, and 96 hours after patch removal.
Study Group	73 females, 37 males age range 21 - 70 years (110/118 subjects completed the test. Eight subjects discontinued due to personal reasons. No subject discontinued due to test material reaction),
Vehicle	The solvent used for dilution to 100 ppm was not specified.
Remarks – Method	Semi-occluded.
RESULTS	
Remarks – Results	During the induction phase, one subject exhibited a low-level faint, and minimal erythema reaction. During the Challenge, no reactions were observed.
CONCLUSION	The test substance at 100 ppm was not sensitising under the conditions of the test.
TEST FACILITY	HRL (2014)

B.10. Repeat Dose Oral Gavage Toxicity – Rats

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 417 Toxicokinetics / Repeated Dose 90-day Oral Toxicity Study in Rats
Species/Strain	Rat/ Crl:CD Sprague Dawley
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 90, 91 &/or 92 days Dose regimen: 7 days per week Post-exposure observation period: None
Vehicle	Methylcellulose (1% in Reverse Osmosis Deionized Water)
Remarks – Method	The aim of the study was to determine the repeated dose oral toxicity (similar to TG 408), and toxicokinetic characterisation of the test substance (according to TG 417). Toxicokinetic parameters were estimated using WinNonlin pharmacokinetic software. The carboxylic acid metabolite of the notified chemical (S5353) was used as a surrogate for the notified chemical in the toxicokinetic evaluation. All parameters were generated from concentrations in plasma from Days 1, 44 and 90. Parameters were estimated using nominal sampling times relative to the start of each dose administered concentrations determined using the linear trapezoidal method (AUC(0-t)). Haematology and clinical chemistry parameters were evaluated at the end of Week 1 and Week 6, as well as at the end of the study. Minor protocol deviations included animals dosed outside the two hours range from previous day dose, animal room temperature and humidity were outside the specified range in one or two days, animal replacement within 3 days of study start (replaced animals received a sufficient number

of doses). These deviations according to the study author did not impact the overall quality or integrity of the study or the interpretation of the study results or conclusion.

RESULTS

Group	*Number and Sex of Animals (Main Study)*	*Number and Sex of Animals (Toxicokinetic Study)*	*Dose (mg/kg bw/day)*	*Mortality*
Control	20M, 20F	3M, 3F	0	0/40
Low Dose	20M, 20F	6M, 6F	10	0/40
Mid Dose	20M, 20F	6M, 6F	30	0/40
High Dose	20M, 20F	6M, 6F	100	0/40

Mortality and Time to Death

No unscheduled deaths occurred during the study. All animals assigned to the study survived to the scheduled necropsy.

Clinical Observations

No test substance-related clinical observations or changes in mean body weight, body weight gain, food consumption, functional observational battery assessments and ophthalmic findings were observed compared to the controls during the study. Sporadic thin fur, dry tail skin and broken teeth (control and group 4 male animal and group 3 female animal) non-treatment related were occasionally and infrequently observed. Laboured breathing and abnormal breathing sounds were observed only during day 63 in males at high dose and was considered by the author most likely to be related to accidental aspiration during the gavage procedure.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No test substance-related clinical signs or changes in ophthalmic examinations, haematology parameters, coagulation parameters, clinical chemistry parameters, macroscopic or microscopic evaluation or urinalysis data were observed during the course of the study. There was an increased incidence of variations in red blood cell morphology at the higher dose (100 mg/kg bw/day) at the end of Week 1 only, which were not evident at week 6 or at the end of the study (day 91-93).

Effects in Organs

No test substance-related changes in absolute or relative organ weights, or microscopic findings were observed at the end of the study. Mean body and brain weights were statistically significantly reduced from the controls but were not dose related and were considered by the study author to be incidental or to be related to difference of animals' sexual maturity. There were slight increases in mean lung and liver weights at the higher dose but not statistically significant compared to control. In the gross observations, there was one small testis in each of the low and mid dose group males, both associated with degeneration of the seminiferous epithelium and low weight. A small thyroid gland was reported in one high dose male, and a small thymus gland in another high dose male. None of these gross observations were considered by the study author to be test item related.

Toxicokinetics

Time to maximum plasma concentration of the carboxylic acid metabolite of the notified chemical (S5353) (Tmax) was 2 or 3 h post-dose and followed by a mono-exponential decline, with a half-life of 1.43 to 4.41 h. Plasma levels increased in a less than dose-proportionate way, for doses between 10 and 100 mg/kg bw/day. Plasma levels at Day 1 and Day 90 were similar for females, but levels in males at Day 90 were approximately half of the levels at Day 1. Higher systemic levels in females than males was observed on Day 44 and Day 90.

Remarks – Results

No test substance-related mortality or systemic toxicity was observed at up to 100 mg/kg bw/day.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established by the study authors as 100 mg/kg bw/day.

TEST FACILITY Charles River (2014)

B.11. Repeat Dose Inhalation Toxicity – Rats

TEST SUBSTANCE	Notified chemical at 9.2% concentration in Ethyl lactate
METHOD	OECD TG 412 Repeated Dose Inhalation Toxicity: 28-day Study
Species/Strain	Rats/ Crl:CD(SD)
Route of Administration	Inhalation – nose only exposure
Exposure Information	Total exposure days: 4 weeks Dose regimen: 5 days per week Duration of exposure (inhalation): 6 hours/day
Vehicle	Ethyl lactate
Physical Form	Liquid aerosol
Particle Size	Approximately 1 μm (Mean MMAD)
Remarks – Method	Dose levels were chosen on the basis of a preliminary 5-day study, and are reported in the table below as the concentrations of a solution containing 9.2% of the notified chemical, and as concentrations of the notified chemical. The level of ethyl lactate vapour for the vehicle control group in the main study (250 ppm) was chosen to match the level of ethyl lactate in the high dose test group. Bronchoalveolar lavage was performed at necropsy, and bronchiolar lavage fluid was tested for lactate dehydrogenase, total protein, alkaline phosphatase and cytology. A number of deviations from the protocol related to the conduct of the study were identified but were not considered by the study authors to have impacted the overall integrity of the study or the interpretation of the study results and conclusions.

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration (of 9.2% solution) (mg/m³)*		*Dose/Concentration (as notified Chemical) (mg/m³)*	*Mortality*
		Nominal	*Actual*		
Vehicle Control (ethyl lactate at 250 ppm)	5 M, 5 F	0	0	0	0/10
Concurrent Control (humidified filtered air)	5 M, 5 F	0	0	0	0/10
Low Dose	5 M, 5 F	5	6.1	0.6	0/10
Mid Dose	5 M, 5 F	25	25	2.3	0/10
High Dose	5 M, 5 F	75	73	6.7	0/10

Mortality and Time to Death

There were no unscheduled deaths.

Clinical Observations

There were no test substance-related clinical observations. All clinical observations in animals in the treated groups were similar to the animals in control groups.

Body weights, food consumption, functional observational battery, including home cage observations, open field observations, sensory observations, neuromuscular observations, physiological observations, and motor activity were not affected by the test substance administration.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There were no test substance-related effects identified for haematology, coagulation, serum chemistry, urinalysis, or bronchoalveolar lavage fluid, or macroscopic or microscopic observations.

Effects in Organs

No macroscopic changes related to dosing were found. Increased mean heart, kidney and liver weights in high dose females were attributed to a low mean weight of those organs in control animals, and low mean testes weights in low dose males was related to one animal out of 5 in the group with low tubule weight. The study author did not consider this as test substance related.

Substantial microscopic changes in the nasal areas and larynx of treated animals were attributed to the vehicle ethyl lactate, which is known to have similar effects, and also demonstrated in the vehicle control group in this study. These effects included mucous cell hyperplasia, transitional epithelial hyperplasia with mucous cell metaplasia, respiratory epithelial hyperplasia, olfactory epithelial degeneration, and mixed cell inflammation observed in the control animals and in males and females at 0.6, 2.3 and 6.7 mg/m^3. Vehicle control groups showed mixed cell infiltrate in the larynx. The effects were most marked in the control and highest dose animals. Nasal levels III (ventral septum and adjacent meatus) and IV (nasopharyngeal duct) were most affected. No other substance-related histological changes were observed at the scheduled necropsy.

Lesions noted in the liver (granulomatous, pyogranulomatous, chronic inflammation, fibrosis, adhesion), glandular stomach (granulomatous inflammation), and jejunum, ileum, and cecum (fibrosis) were attributed by the study authors to the radiotelemetry device used in the study.

Remarks – Results

Histopathological effects attributed to the vehicle were not considered by the study authors as an adverse effect of the notified chemical.

CONCLUSION

The No Observed Effect Concentration (NOEC) was established by the study authors as 75 mg/m^3/day (equivalent to 6.7 mg/m^3/day of the notified chemical).

TEST FACILITY	Charles River (2017)

B.12. Genotoxicity – Bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test Plate incorporation and Pre incubation procedures
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100, *Escherichia coli*: WP2uvrA
Metabolic Activation System	S9 fraction of liver homogenate from male Sprague-Dawley rats treated with Aroclor 1254.
Concentration Range in Main Test	a) With metabolic activation: 0, 0.063, 0.13, 0.25, 0.50 and 1.0 mg/plate b) Without metabolic activation: 0, 0.063, 0.13, 0.25, 0.50 and 1.0 mg/plate
Vehicle	Dimethyl sulfoxide (DMSO)
Remarks – Method	Doses for the main study were chosen on the basis of solubility in the test system. No significant protocol deviations. Negative control was DMSO Positive controls for experiments without S9 were: aqueous solutions of sodium azide (NaAz), DMSO solutions of 2-nitrofluorene (2-NF), methylmethanesulfonate (MMS), and 9-aminoacridine (9-AA) Positive controls for experiments with S9 were: benzo[α]pyrene (B[α]P), and 2- aminoanthracene (2-AMA) dissolved in DMSO and cyclophosphamide monohydrate (CP) in water.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation (Preincubation)*	*Genotoxic Effect*
Absent	> 2.5		> 0.16	Negative
Test 1 (Incorporation)		> 1	≥ 0.25	Negative
Test 2 (Preincubation)		> 1	≥ 1	Negative
Present	> 2.5		> 0.16	Negative
Test 1 (Incorporation)		> 1	≥ 0.25	Negative
Test 2 (Preincubation)		> 1	≥ 1	Negative

Remarks – Results

Plate incorporation test: with or without metabolic activation, the test substance did not produced any statistically significant increases ($p > 0.01$) in colony counts with the negative controls.

Preincubation test: the test was considered to confirm the negative results of the plate incorporation test.

In the preincubation test, the concentrations of the test substance investigated were identical to the plate incorporation test with or without metabolic activation.

Cytotoxicity was similar to the plate incorporation test when compared to the concurrent negative controls with one exception. For TA1537 without S9, the colony counts were slightly reduced at the highest concentration of 1.0 mg/plate. In the preincubation test with TA1535 without metabolic activation, there were two slight (1.25 and 1.44-fold), but statistically significant increases ($p < 0.01$) in colony counts at 0.13 and 1.0 mg/plate over the concurrent negative control. Despite these increases, the results were within the historical control data range and a dose-response was not observed. Therefore the preincubation test confirmed the negative results of the plate incorporation test.

The negative controls for each tester strain were within the historical negative control data. All concurrent positive controls induced at least a 3.3-fold increase in colony counts per plate when compared to the corresponding negative controls and were at levels similar to the historical positive control data.

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

TEST FACILITY Nucro Technics (2013a)

B.13. Genotoxicity – *In Vitro* Chromosome Aberration Assay

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test (1997) Commission Regulation (EC) No 440/2008; B.10 *In vitro* Mammalian Chromosome Aberration Test

Cell Type/Cell Line Human lymphocytes

Metabolic Activation System Fraction of rat Liver homogenate treated with Phenobarbital-5, 6-benzoflavone

Vehicle Dimethyl Sulfoxide (DMSO)

Remarks - Method

No significant protocol deviations.

Dosages were based on a preliminary test, in which high cytotoxicity was identified from 31 to 310 µg/mL, based on relative cell growth. In the main test, cultures of human lymphocytes were exposed to varying concentrations of the test substance under three different conditions: 1) a 3-hour exposure in the absence of S9 metabolic activation; 2) a 3-hour exposure in the presence of S9 and 3) a 20-hour exposure in the absence of S9 activation. Cells were harvested at approximately 20 hours

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 35, 58*, 97*, 160*, 270	3 h	20 h
Test 2	0*, 23*, 39*, 65*, 110 and 180	20 h	20 h
Present			
Test 1	0*, 1.3*, 2.5*, 5.0*, 10, 20, 40, 80 and 160	3h	20 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 270	≥ 160	≥ 270	Negative
Test 2		> 39	-	Negative
Present				
Test 1		≥ 40	-	Negative

Remarks - Results

The test substance did not induce any statistically significant increases in the frequency of cells with aberrations or in the numbers of polyploid cells at any concentration level in any of the three concentrations, either in the absence or presence of metabolic activation. Low levels of chromosome aberrations were observed at all concentrations, including the solvent control.

In the preliminary test, test substance precipitate was observed starting at about 0.25 mg/mL up to the highest exposure concentration resulting in a range of slight to extreme precipitate. The amount of precipitate and the concentration where it was observed varied slightly depending on the condition tested.

In the main study, slight test substance precipitate was observed only at the highest concentration of 270 µg/mL. The pH and osmolality of all treated cultures were well within the normal physiological ranges.

All conditions were tested at the limit of test article cytotoxicity evaluated by Relative Cell Growth (RCG) and Relative Mitotic Index (RMI) levels.

The concurrent positive and negative controls produced satisfactory responses, thus confirming the validity of the test.

CONCLUSION

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

TEST FACILITY

Nucro Technics (2013b)

B.14. Genotoxicity – *In Vivo* Mouse Bone Marrow Micronucleus Assay

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 474 Mammalian Erythrocyte Micronucleus Test
Species/Strain	Mouse/ Crl:CD-1 (ICR)
Route of Administration	Oral –gavage
Vehicle	1% methylcellulose in deionized water
Remarks – Method	The authors reported minor protocol deviations only, that were not considered to affect the study results. Dosage levels (Range finding phase) were 500, 1000, 1500, and 2000 mg/kg/day for Groups I, II, III, and IV respectively. Dosage levels (Definitive phase) of 1000, 1500, and 2000 mg/kg/day were administered by oral gavage for 3 consecutive days to Groups II, III, and IV respectively. Group I was a concurrent vehicle control group and a positive control group (Group V) received a single oral dose of 60 mg/kg bwcyclophosphamide monohydrate (CPS)

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Sacrifice Time (hours)*
I (vehicle control)	6 M, 6 F	0	24
II (low dose)	6 M, 6 F	1000	24
III (mid dose)	6 M, 6 F	1500	24
IV (high dose)	6 M, 6 F	2000	24
V (positive control, CP)	6 M, 6 F	60	24

CP = cyclophosphamide.

RESULTS	
Doses Producing Toxicity	All animals survived to the scheduled necropsy in both the range finding study and the main study. In the range-finding study, there were no test article-related clinical observations or effects on body weights or food consumption. In the main study, there were no test substance-related clinical observations. All clinical findings in the treated groups were similar to the vehicle control animals. There were no statistically significant differences when the vehicle control and treated groups were compared except for higher mean body weight gains from study day 2 to 3 and 0 to 3 at 2000 mg/kg/day males. Differences in body weight gain were likely due to biological variability and were not considered related to test substance administration. Food consumption was unaffected by test substance administration.
Genotoxic Effects	None
Remarks – Results	The test substance did not produce an increase in the mean number of micronucleated polychromatic erythrocytes (MN-PCEs) in treated animals compared to the vehicle control group. No bone marrow cytotoxicity (decreases in the ratio of polychromatic to total erythrocytes, PCE:TE ratio) was noted in any test substance treated group. The group mean values for both MN-PCEs and PCE:TE ratios for the vehicle and positive control groups were within the respective historical control ranges. As there were no clinical signs of toxicity, or changes in MN-PCE ratios, it was not demonstrated in the study that the chemical reached the bone marrow.
CONCLUSION	The notified chemical was not clastogenic under the conditions of this *in vivo* Assay

TEST FACILITY	Wil Research (2013)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready Biodegradability

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO2 Evolution Test
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Theoretical Carbon Dioxide (ThCO2)
Remarks – Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported.

RESULTS

Test Substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
3	3.2	3	12.6
14	8.6	14	74.2
28	13.6	28	90.0

Remarks – Results	All validity criteria for the test were satisfied. The mean biodegradation of notified chemical was 13.6% during the 28 day window. The test substance is, therefore, considered to be not readily biodegradable
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	GDCM-LEES (2018a)

C.2. Ecotoxicological Investigations

C.2.1. Acute Toxicity to Fish

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-static
Species	*Danio rerio* (Zebra fish)
Exposure Period	96 hrs
Auxiliary Solvent	None
Water Hardness	132 mg $CaCO_3$/L
Analytical Monitoring	HPLC-DAD (diode array detection)
Remarks – Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported. A saturated solution of 100 mg/L was prepared, then filtered (0.45 µm) from the solid phase. The test solutions were renewed every 24 hours during the 96 hour test period.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*			
Nominal	*Actual WAF**		*24 h*	*48 h*	*72 h*	*96 h*
Control	Control	7	0	0	0	0
100	13.1	7	0	0	0	0

* Water accommodated fraction

LC50	> 13.1 mg/L at 96 hours
NOEC (or LOEC)	13.1 mg/L at 96 hours
Remarks – Results	All validity criteria for the test were satisfied. The dissolved O_2 concentration remained between 61 and 105%. The results of the study were based on the WAF geometric mean of measured test concentrations. The limit test for the median lethal concentration causing 100% mortality was LC_{100} is > 13.1 mg/L.
CONCLUSION	The notified chemical is not toxic to fish to the limit of its water solubility.
TEST FACILITY	GDCM-LEES (2018b)

C.2.2. Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	160-180 mg $CaCO_3$/L
Analytical Monitoring	HPLC-DAD
Remarks – Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. A saturated solution of 100 mg/L was prepared, then siphoned from the solid phase. No significant deviations from the test guidelines were reported. A positive control ($K_2Cr_2O_7$) was run approximately 1 month prior to the current study.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual WAF**		*24 h*	*48 h*
Control	Control	20	0	0
1.94	0.0750	20	0	1
4.27	0.212	20	1	2
9.39	0.454	20	1	7
20.7	1.02	20	1	8
45.5	2.19	20	4	9
100	4.34	20	5	12

* Water accommodated fraction

EC50	2.18 mg/L at 48 hours (95% CI), sigmoidal dose response.
Remarks – Results	All validity criteria for the test were satisfied. The dissolved O_2 concentration was ≥ 7.77 mg/L in the 24 hours old media in all concentration levels and control. The 24h EC50 of positive control was 1.64 mg/L (within the accepted range). Test concentrations were measured at the beginning and end of the test. The 48 h EC50 is 2.18 mg/L (95% CI), based on geometric mean of the measured loading concentrations (WAF).
CONCLUSION	The notified chemical is considered to be toxic to aquatic invertebrates.
TEST FACILITY	NL (2018d)

C.2.3. Algal Growth Inhibition Test

TEST SUBSTANCE	Notified Chemical

METHOD	OECD TG 201 Alga, Growth Inhibition Test
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal (WAF): 12.5, 25, 50, 100, 200 mg/L Actual: 0.391, 0.779, 1.55, 3.19, 6.49 mg/L
Auxiliary Solvent	None
Water Hardness	0.24 mg $CaCO_3$/L
Analytical Monitoring	HPLC-DAD
Remarks – Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported. A saturated solution of 200 mg/L was prepared, then separated from the solid phase. 6.49 mg/L was the maximum achievable concentration under the test conditions. A positive control ($K_2Cr_2O_7$) was run approximately 6 months prior to the current study.

RESULTS

Biomass		*Growth*	
EyC50 (mg/L at 72 h)	*NOEC (mg/L)*	*ErC50 (mg/L at 72 h)*	*NOEC (mg/L)*
> 6.49	1.55	> 6.49	1.55

Remarks – Results	All validity criteria of the test guideline were satisfied. The cell growth increased by 102 fold in the control cultures. The ErC50 and EyC50 for growth and yield were 0.498 mg/L and 0.398 mg/L respectively (at 95% CI) for the positive control (within the expected range). The results for the notified chemical showed that the cell density started to decrease at a geometric mean of 3.19 mg/L compared to the control sample. The ErC50 and EyC50 values were calculated, on the basis of the geometric mean measured WAF loading rates.
CONCLUSION	The notified chemical inhibits the growth of freshwater algae, but an ErC50 value could not be established at the limit of water solubility.
TEST FACILITY	NL (2018e)

C.2.4. Inhibition of Microbial Activity

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 10, 32, 100, 1000 mg/L Actual: Not determined
Remarks – Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported.
RESULTS	
EC50	> 1000 mg/L
NOEC	1000 mg/L
Remarks – Results	All validity criteria for the test were satisfied. The treatment mixture dosed with 1000 mg/L of the notified chemical had a respiration rate of 28.1 mg O_2/L/hr showing there was no significant uptake or release of oxygen resulting from reactions of the test substance compared to the control. The EC50 value for the notified chemical was greater than 1000 mg/L, the highest concentration tested.
CONCLUSION	The notified chemical does not inhibit microbial activity.

TEST FACILITY	NL (2018f)

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