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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Acetamide, *N*-[2-(2-hydroxyethoxy)ethyl]- (INCI Name: Acetamidoethoxyethanol)

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
STD/1605	Nouryon Chemicals Australia Pty Ltd	Acetamide, *N*-[2-(2-hydroxyethoxy)ethyl]- (INCI Name: Acetamidoethoxyethanol)	No	≤ 10 tonnes per annum	Cosmetic ingredient

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is not recommended for classification using the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS), as adopted for industrial chemicals in Australia.

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 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 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 safe work practices to minimise occupational exposure during handling of the notified chemical during reformulation:
  - 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:
  - Impervious gloves
  - Protective clothing
  - Respiratory protection if aerosols are formed

  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.

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if
- information on skin sensitisation becomes available on the notified chemical;
- the notified chemical is proposed to be used in spray products

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from cosmetic 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 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
Nouryon Chemicals Australia Pty Ltd (ABN: 64 621 806 273)
Unit 12, 44 Lakeview Drive
SCORESBY VIC 3179

NOTIFICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne 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)
Variation to the schedule of data requirements is claimed as follows: hydrolysis as a function of pH, absorption/desorption, dissociation constant, flammability, explosive and oxidising properties and *in vivo* genotoxicity.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME
ElfaMoist AC

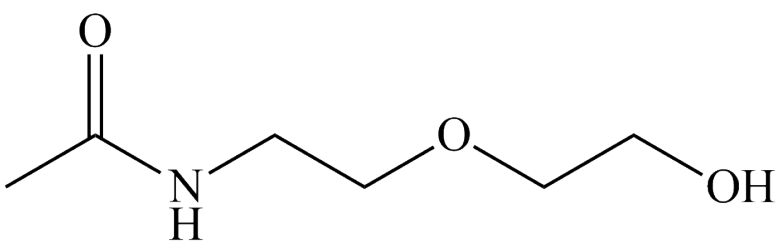
CAS NUMBER
118974-46-2

CHEMICAL NAME
Acetamide, *N*-[2-(2-hydroxyethoxy)ethyl]-

OTHER NAMES
Acetamidoethoxyethanol (INCI name)
DGA acetamide
5-(Acetylamino)-3-oxapentan-1-ol

MOLECULAR FORMULA
$C_6H_{13}NO_3$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
147.18 g/mol

ANALYTICAL DATA
Reference FT-IR spectra was provided.

3. COMPOSITION

DEGREE OF PURITY
70-80 %

IDENTIFIED IMPURITIES

Chemical Name	Ethanol, 2-(2-aminoethoxy)-		
CAS No.	929-06-6	*Weight %*	1.8-2.3
*Hazardous Properties**	H312 (harmful in contact with skin) H314 (Causes severe skin burns and eye damage)		
Chemical Name	Diglycolamine acetate		
CAS No.	Unknown	*Weight %*	1.0-1.1
*Hazardous Properties**	H312 (harmful in contact with skin) H314 (Causes severe skin burns and eye damage)		
Chemical Name	Acetamide, *N*-[2-[2-(acetyloxy)ethoxy]ethyl]-		
CAS No.	1862537-07-2	*Weight %*	1.6-6.7
*Hazardous Properties**	H312 (harmful in contact with skin) H314 (Causes severe skin burns and eye damage)		

*hazard statements provided by the notifier.

Chemical Name	1,2,3-Propanetriol, monoacetate		
CAS No.	26446-35-5	*Weight %*	0.3-2.8
Chemical Name	1,2,3-Propanetriol (other names: glycerine, glycerol)		
CAS No.	56-81-5	*Weight %*	10-20

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: clear viscous liquid with mild odour

Property	Value	Data Source/Justification
Melting Point/Freezing Point	< -70 °C	Measured
Boiling Point	178 °C at 101.3 kPa	Measured
Density	1146 kg/m^3 at 20 °C	Measured*
Vapour Pressure	2.8 x 10^{-5} kPa at 25 ºC	Measured (vapour pressure balance method)*
Water Solubility	> 0.6 g/g	Measured (in-house method; details not provided)
Hydrolysis as a Function of pH	Not determined	The notified chemical is unlikely to hydrolyse in the environmental pH (4-9)
Partition Coefficient (n-octanol/water)	log Pow = -2.7 at 20 °C	Measured
Adsorption/Desorption	log Koc ≤ 1	Estimated by using KOCWIN v2.00, US EPA 2011.
Dissociation Constant	Not determined	The notified chemical is not expected to be ionised under environmental conditions (pH 4-9)
Flash Point	198.5 °C (closed cup)	Measured*
Flammability	Not determined	-

Property	Value	Data Source/Justification
Autoignition Temperature	376 ± 5 °C	Measured*
Explosive Properties	Not determined	Contains no functional groups that would imply explosive properties
Oxidising Properties	Not determined	Contains no functional groups that would imply oxidising properties

* - only study summary provided

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

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

Physical hazard classification
Based on the submitted physico-chemical data depicted in the above table, the notified chemical is not recommended for hazard classification according to the *Globally Harmonised System 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 as a raw material for reformulation into end-use cosmetic products. It may also be imported in end-use cosmetic products at concentrations of up to 20%.

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

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

PORT OF ENTRY
Melbourne, Sydney

TRANSPORTATION AND PACKAGING
The notified chemical will be imported into Australia in neat form (80% purity) or in end-use cosmetics by sea and transported by road to the site of formulation and retail sales. The notified chemical in raw material form will be packaged in 20 kg plastic containers or 208 L drums. The end-use cosmetic products containing the notified chemical will be packaged in containers of various sizes (up to 500 mL) suitable for retail sales.

USE
The notified chemical will be used in leave-on and rinse-off cosmetic products at concentrations ≤ 15% and ≤ 20% respectively. The notified chemical is not proposed to be used in aerosols.

OPERATION DESCRIPTION
The notified chemical will be imported as a raw material (up to 80% purity) for reformulation in Australia or as a component of end-use cosmetic products.

The reformulation procedures for incorporating the notified chemical into end-use products will likely vary depending on the nature of the cosmetic products formulated. This may involve both automated and manual processes including transferring and blending the notified chemical with other formulations. However, a typical blending operation is expected to be highly automated and occur in a fully enclosed/contained environment, followed by automated filling into containers of various sizes suitable for retail sales.

The end-use products containing the notified chemical may be used by consumers and professionals such as hairdressers and workers in beauty salons.

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	4	12
Professional compounder	8	12
Chemist	3	12
Packers (dispensing and capping)	8	12
Store person	4	12
Professional end user	8	365

EXPOSURE DETAILS

Transport and storage
Transport and storage workers may come into contact with the notified chemical in neat form or as a component of the imported cosmetic products, only in the event of accidental rupture of containers. Incidental exposure to the notified chemical may occur via skin or eyes during the clean-up of accidental spills.

Reformulation
Compounders, chemists and maintenance workers may come in contact with the neat form of the notified chemical (or at lower concentrations) during weighing and addition of the notified chemical to blending equipment for reformulation, testing for quality control and equipment cleaning and maintenance, respectively. Packaging workers may come in contact with the notified chemical at up to 20% concentration during packaging of end-use cosmetic products. The principal routes of exposure are expected to be dermal, accidental ocular and inhalation (if aerosols are formed). According to the notifier, worker exposure to the notified chemical will be reduced through the implementation of control measures including closed systems with local exhaust ventilation and use of personal protective equipment (PPE) such as coveralls, impervious gloves, goggles, and full face protection if potential exists for formation of aerosols or splashes or handling of hot substance.

Professional end-users
Beauticians and salon workers may come in contact with the notified chemical at up to 20% concentration during application of the cosmetic products to customers. The workers may experience dermal and ocular exposure. No inhalation exposure is expected due to the notified chemical not being proposed to be used in sprays generating aerosols. No PPE is proposed by the notifier for professional workers but good hygiene practices followed in beauty salons would assist in reduction of exposure to the notified chemical. Under normal circumstances, the exposure experienced by this category of workers is expected to be similar to that experienced by consumers, described under section 6.1.2.

6.1.2. Public Exposure
There will be widespread and repeated exposure of the public to the notified chemical through the use of a wide range of cosmetic products with ≤ 15% concentration in leave-on cosmetics and ≤ 20% concentration in rinse-off cosmetics except aerosol generating spray products. The principal route of exposure will be dermal, while accidental ocular and oral exposure are also possible, particularly from facial use products.

Data on typical use patterns of cosmetic product categories (SCCS, 2012) in which the notified chemical may be used are shown in the following table. For the purposes of the exposure assessment via the dermal route, Australian use patterns for the various product categories are assumed to be similar to those in Europe. A dermal absorption (DA) study was conducted on the notified chemical and a DA value of 4% was used for the systemic exposure assessment, based on the study outcome (maximum of 3.4% on skin and 0.48% in receiver chamber). A lifetime average female body weight (BW) of 64 kg (enHealth, 2012) was used for calculation purposes.

Estimated systemic exposure from use of cosmetic products containing the notified chemical

Product type	Amount (mg/day)	C (%)	RF	Daily systemic exposure (mg/kg bw/day)
Body lotion	7820	15	1	0.73313
Face cream	1540	15	1	0.14438
Hand cream	2160	15	1	0.20250
Deodorant (non-spray)	1500	15	1	0.14063
Fragrances	750	15	1	0.07031
Liquid Foundation	510	15	1	0.04781
Lipstick, lip salve	57	15	1	0.00534
Mascara	25	15	1	0.00234
Eyeliner	5	15	1	0.00047
Eye shadow	20	15	1	0.00188
Makeup remover	5000	20	0.1	0.06250
Hair styling products	4000	15	0.1	0.03750
Shower gel	18670	20	0.01	0.02334
Hand wash soap	20000	20	0.01	0.02500
Shampoo	10460	20	0.01	0.01308
Hair conditioner	3920	20	0.01	0.00490
Facial cleanser	800	20	0.01	0.00100
Total				**1.5161**

C = concentration (%); RF = retention factor (SCCS, 2012).
Daily systemic exposure = (Amount x C x RF x DA)/BW

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above table that contain the notified chemical. This would result in a combined internal dose of 1.5161 mg/kg bw/day.

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 dermal toxicity – rat	LD50 > 2,000 mg/kg bw; low toxicity
In vitro Phototoxicity Test	no phototoxicity
Skin irritation – *in vitro* EpiDerm™ Skin irritation test (10% test substance)	non-irritating
Skin irritation – *in vitro* EpiDerm™ Skin irritation test (100% test substance)	non-irritating
Eye irritation – *in vitro* Bovine Corneal Opacity Test (BCOP) (100% test substance)	non-irritating
Eye irritation – *in vitro* EpiOcular™ Test (10% test substance)	non-irritating
In vitro Dermal Penetration Study	4% dermal penetration (maximum of 3.4% on skin and 0.48% in receiver chamber)
Skin sensitisation – *in chemico* Direct Peptide Reactivity Assay (DPRA)	negative
*S*kin sensitisation – *in vitro* ARE-Nrf2 Luciferase Test	negative
*S*kin sensitisation – *in vitro* human Cell Line Activation Test (h-CLAT)	positive
Combined Repeated Dose Toxicity Test With Reproduction / Developmental Toxicity Screening Test – Oral route	NOAEL > 1,000 mg/kg bw/day for parental toxicity, reproduction toxicity and developmental toxicity
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* Mammalian Cell Micronucleus Test	non clastogenic
Genotoxicity – *in vitro* Mammalian Cell Gene Mutation Test	non mutagenic

Toxicokinetics, metabolism and distribution
No data on toxicokinetics, metabolism and distribution of the notified chemical were provided. An *in vitro* skin penetration study was carried out on skin from human cadaver. After 24 hours, the maximum amount in the stratum corneum/epidermis was reported as 3.4%, and 0.48% in the receiver chamber, totalling ~4% of the chemical available for absorption. However, dermal penetration of the chemical may vary based on the other ingredients in a product formulation. The metabolism potential of the skin tissue was not evaluated. The possibility of the notified chemical breaking down on the skin resulting in dermal penetration of a metabolite cannot be ruled out, as it was not investigated in the dermal penetration study.

Metabolism/transformation prediction conducted by the notifier using QSAR Toolbox (v3.3) does not list acetamide among the likely metabolites for the structure. Hydrolysis simulation indicates possible breakdown to acetic acid and diglycolamine, or even ethanediol and n-2-hydroxyethylacetamide, but not acetamide.

Acute toxicity
The notified chemical had low toxicity in an acute dermal toxicity study with an LD_{50} > 2,000 mg/kg bw. A Cosmetic Ingredient Review (CIR) report on an analogue, Acetamide MEA (acetamide, *N*-(2-hydroxyethyl)– ; CAS No. 142-26-7) reported low acute oral and dermal toxicity (CIR, 1993).

Irritation
Two *in vitro* skin irritation studies and two *in vitro* eye irritation studies were conducted to evaluate the irritation potential of the notified chemical. When tested at 10% concentration in one *in vitro* skin irritation study, decrease in cell viability along with increase in IL-α was observed after 24 hours' exposure, which suggests the notified chemical might have slight irritating effects at this concentration. In the second *in vitro* skin irritation test, conducted using the notified chemical at 100% concentration, a slight reduction in cell viability was noted when compared to the control. The reduction in cell viability was not sufficient for hazard classification according to the OECD test guideline. The notified chemical was non-irritating to the eyes when tested at 10% concentration in EpiOcular™ test and at 100% in BCOP test. No reduction in cell viability was observed in both in vitro eye irritation tests when compared to controls.

Based on the results of the *in vitro* studies, the notified chemical is not classified as a skin or eye irritant.

Sensitisation
A battery of tests consisting of one *in chemico* and two *in vitro* cell based assays were conducted to evaluate the sensitisation potential of the notified chemical. The tests are part of an Integrated Approach to Testing and Assessment (IATA) which address specific events on the Adverse Outcome Pathway (AOP) leading to development of skin sensitisation (OECD, 2015). The tests are thus considered relevant for assessment of the skin sensitisation potential of the notified chemical, together with other supporting information.

The first key event, commonly referred to as the molecular initiating event in the AOP for sensitisation, is the covalent binding of electrophilic chemical to nucleophilic centres in skin proteins. The *in chemico* Direct Peptide Reactivity Assay (DPRA) measures the interaction of a test substance with small synthetic peptides containing Cysteine and Lysine (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 ARE-Nrf2 Luciferase Assay measures change in expression of the 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 sensitisation.

The third key event in the AOP for sensitisation is the activation of dendritic cells resulting in change in cell surface expression of markers such as CD54 and CD86. The *in vitro* h-CLAT assay measures the change in expression of cell surface markers CD54 and CD86 upon activation of human monocyte leukaemia cell line (THP-1) with proper stimuli. The assay addresses the third key event in the AOP for sensitisation.

The notified chemical showed negative responses in two of the three tests (DPRA and ARE-Nrf2 Luciferase Assay). With the h-CLAT assay, test substance mediated increases in the levels of CD54 and CD86 were noted. The increase in CD54 above the threshold of 200% was noted in 2 of the 3 independent tests (252% and 234%). For CD86 an increase above threshold of 150% was noted in one test (168%). The increases in expression of CD54 and CD86 were noted at very high concentrations (≥ 4167 µg/mL) of the chemical. The positive results in the h-

CLAT test results suggest the notified chemical may be a skin sensitiser. In addition, according to the OECD test guidelines (TG 442c, 442d and 442e), the suite of *in vitro* tests based on the AOP may not detect pre-haptens (chemicals that can become sensitisers following auto-oxidation) and pro-haptens (chemicals requiring enzymatic activation to become sensitisers). The notifier commented that the positive result in the h-CLAT test may be due to the use of high concentrations under the test guideline 442e (due to no cytotoxicity at low concentration), and that it is likely that any sensitisation potential is weak.

According to the notifier, QSAR Toolbox versions 3.3 and 4.2, TOPKAT and DEREK have predicted no structural alerts for the chemical for skin sensitisation. However, the QSAR modelling has indicated a possibility of metabolism to a protein-reactive metabolite and if this was the reason for the positive result in the h-CLAT assay, the notifier states that it is a very weak effect and may occur only at extremely high concentrations.

No other studies related to skin sensitisation are available on the notified chemical. The analogue acetamide MEA was negative in a guinea pig maximisation test, using a dermal induction concentration of 100% (CIR, 1993). When acetamide MEA was tested at 7.5% in a human repeat insult test (HRIPT), erythema was seen in two subjects during the induction phase but not during challenge, and the study author concluded that it was not sensitising.

Overall there is some uncertainty regarding the sensitisation potential of the notified chemical. The weight of evidence suggests that even if there is potential for skin sensitisation through metabolism, it is likely to be very weak and elicited only at very high concentration. Therefore, a hazard classification is not warranted.

Repeated dose toxicity
A repeated dose toxicity study with reproduction / developmental toxicity screening test was conducted on the notified chemical. No test substance mediated adverse effects were observed up to the highest test concentration of 1,000 mg/kg bw/day. No treatment related effects on reproduction and development of offspring were noted up to the highest test substance concentration of 1,000 mg/kg bw/day. Based on the results of the study, a no observed adverse effect level (NOAEL) > 1,000 mg/kg bw/day was established for repeated dose toxicity, reproduction and developmental toxicity.

Mutagenicity/Genotoxicity
The notified chemical was not mutagenic in a bacterial reverse mutation assay and in an *in vitro* mammalian cell gene mutation test and not clastogenic in an *in vitro* mammalian cell micronucleus test.

Impurities
The notified chemical is of 70-80% purity. It contains a number of impurities, with glycerine (1,2,3-propanetriol) occurring at the highest level (10-20%). Some other impurities contain free amine groups (see Section 3). Some of the impurities classified by the notifier as causing skin burns and eye damage (GHS classification H314) may cause corrosive effects at concentrations ≥ 3% (according to GHS cut-off concentrations for corrosion). However, the notified chemical tested presumably with these impurities did not show corrosive/irritation effects in the *in vitro* studies to warrant classification.

Health hazard classification
Based on the available information, the notified chemical is not classified as hazardous according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

6.3. Human Health Risk Characterisation
Based on the data provided by the notifier, no risks are identified from the use of the notified chemical at the proposed concentration of 15% in leave-on and 20% in rinse off cosmetic products.

6.3.1. Occupational Health and Safety
Exposure to the notified chemical (at up to 80% concentration as the neat form) of workers involved in product formulation may occur during blending operations, quality testing and equipment cleaning and maintenance. The potential for skin sensitisation when exposed to the neat chemical cannot be ruled out. As inhalation toxicity of the notified chemical has not been determined, adverse respiratory effects from inhalation of aerosolised form of the notified chemical during the formulation cannot be ruled out.

Provided that control measures are in place to minimise worker exposure, including the use of automated processes and personal protective equipment (PPE) such as impervious gloves, coveralls and respiratory

protection if aerosols are generated, the risk to the health of workers during the handling of the notified chemical is not considered to be unreasonable.

Exposure to the notified chemical (at up to 20% concentration in formulated products) of professional end-users such as beauticians and salon workers may occur during application of the cosmetic products to customers. The notifier does not propose that PPE should be used by professional workers, but good hygiene practices followed in beauty salons would assist in reduction of exposure to the notified chemical.

6.3.2. Public Health

Members of the public may experience repeated exposure to the notified chemical through the use of cosmetic products containing the notified chemical at up to 15% concentration in leave-on and 20% concentration in rinse off products. No inhalation exposure is expected due to the chemical not being used in aerosol generating cosmetic products.
An exposure scenario estimate by using a 4% dermal absorption rate indicates an internal dose of 1.5161 mg/kg bw/day when the notified chemical is used at 15% in leave-on cosmetics and at 20% in rinse off cosmetics (see Section 6.1.2). As the dermal absorption rate of the chemical could vary with other ingredients in cosmetic product formulations and the notified chemical is a small molecule (MW = 147.18 g/mol), using a 4% dermal absorption rate to estimate systemic dose may not be the worst case. The chemical is expected to have a humectant effect in some cosmetic products, allowing it to be on the skin for extended periods. As the NOAEL of the repeated dose toxicity study was > 1,000 mg/kg bw/day, the highest dose tested, a margin of exposure (MOE) for the proposed use in cosmetics has not been calculated.

Based on available information, 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 into Australia in neat form, as a component in finished products or in mixtures for reformulation into finished leave-on and rinse-off cosmetic formulations. There is unlikely to be any significant release to the environment from transport and storage, except in the case of accidental spills and leaks. Accidental leaks and spills of the product containing the notified chemical are expected to be collected by inert absorbent material and disposed of to landfill in accordance with local government regulations.

The reformulation processes will involve both automated and manual blending operations that are expected to occur within a fully enclosed system. Therefore, significant release of the notified chemical from this process to the environment is not expected. Wastes containing the notified chemical generated during reformulation include equipment wash water, residues in empty import containers (estimated to be approximately 1% of the annual import volume by the notifier) and spilt materials. These will be collected and released to on-site wastewater treatment facilities, sewers, or disposed of to landfill in accordance with local government regulations. Empty import containers are expected to be recycled or be disposed of to landfill in accordance with local government regulations.

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 cosmetic products.

RELEASE OF CHEMICAL FROM DISPOSAL

It is estimated by the notifier that 3% of the import volume of the notified chemical may remain in end-use containers once the consumer products are used up. Wastes and residues of the notified chemical in empty containers are likely to either share the fate of the container and be disposed of to landfill, or be released to the sewer system when containers are rinsed before recycling through an approved waste management facility.

7.1.2. Environmental Fate

Following its use in cosmetic formulations in Australia, the majority of the notified chemical will enter into the sewer system before potential release to surface waters nationwide. The submitted biodegradation studies indicate

that the mixture containing the notified chemical exhibits relatively high biodegradation within 28 days, 78% and 89% biodegradation in 28 days. Therefore, the notified chemical is expected to significantly degrade in the sewage treatment plant (STP) and only a small portion of the notified chemical may be released to surface waters. For details of the environmental fate study, refer to Appendix C.

A proportion of the notified chemical may be applied to land when effluent is used for irrigation or when sewage sludge is used for soil remediation, or disposed of to landfill. The notified chemical residues in landfill and soils are expected to have high mobility based on its high water solubility, low n-octanol/water partition coefficient (log P_{OW} = -2.7) and low predicted soil adsorption coefficient log Koc ≤ 1 (KOCWIN v2.00, US EPA 2011). The notified chemical is not expected to bioaccumulate based on its low n-octanol/water partition coefficient. In the aquatic and soil compartments, the notified chemical is expected to degrade through biotic and abiotic processes to form water and oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

The calculation for the predicted environmental concentration (PEC) is summarised in the table below. Based on the reported use in cosmetic products, it is assumed that 100% of the total import volume of the notified chemical is released to the sewer. The release is assumed to be nationwide over 365 days per year. The extent to which the notified chemical is removed from the effluent in STP processes based on the properties of the notified chemical has not been considered for the worst-case scenario. The resulting PECs in receiving waters is displayed in the table below.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	10,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	10,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	27.40	kg/day
Water use	200.0	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:	5.62	µg/L
PEC - Ocean:	0.56	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 5.62 µg/L may potentially result in a soil concentration of approximately 37.45 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 187.3 µg/kg and 374.5 µg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Acute toxicity		
Fish Toxicity	96 h EC50 > 100 mg/L	Not harmful to fish
Daphnia Toxicity	48 h EC50 > 100 mg/L	Not harmful to aquatic invertebrates
Algal Toxicity	72 h E_rC50 > 100 mg/L	Not harmful to algae
Chronic Toxicity		
Daphnia Toxicity	21 d NOEC = 100 mg/L	Not harmful to aquatic invertebrates on a chronic basis
Algal Toxicity	72 h NOEC = 100 mg/L	Not harmful to algae on a chronic basis

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

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) for the notified chemical has been calculated from the lowest chronic endpoint for Daphnia and algae. An assessment factor of 50 was used given acute endpoints for three trophic levels and chronic endpoints for two trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
NOEC (Algae and Daphnia)	100.00	mg/L
Assessment Factor	50.00	
Mitigation Factor	1.00	
PNEC:	2,000.00	µg/L

7.3. Environmental Risk Assessment

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

Risk Assessment	***PEC µg/L***	***PNEC µg/L***	***Q***
Q - River:	5.62	2000	0.003
Q - Ocean:	0.56	2000	0.0003

The Risk Quotients (Q = PEC/PNEC) for discharge of treated effluents containing the notified chemical have been calculated to be < 1 for both river and ocean compartments indicating that the notified chemical is unlikely to reach ecotoxicologically significant concentrations in surface waters based on its maximum annual importation quantity. Therefore, based on the low toxicity to aquatic life and assessed use pattern, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point < -70 °C

Method	Method similar to OECD TG 102 Melting Point/Melting Range – thermal analysis method
Remarks	Study summary only provided. No freezing point was observed to a temperature of -70 °C.
Test Facility	In house

Boiling Point 178 °C at 101.3 kPa

Method	Method similar to OECD TG 103 Boiling Point – thermal analysis method
Remarks	Study summary only provided. The boiling point of the test substance was broad with an onset calculated to be 178 °C. Thermogravimetric analysis (TGA) detected weight loss at ~155°C, progressing continuously until 235°C, where the sample completely volatilised.
Test Facility	In house

Water Solubility > 0.6 g/g

Method	In-house method (details not provided)
Remarks	Although the test indicates ready solubility of the notified chemical, notifier did not follow any standard test method.
Test Facility	AkzoNobel (2016a)

Partition Coefficient (n-octanol/water) log Pow = -2.7 at 20 °C

Method	OECD TG 117 Partition Coefficient (n-octanol/water). EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	HPLC Method. Partition Coefficient of the notified chemical was obtained by extrapolation. In the calibration graph the lower limit for log Pow was reported to be 0.9 for aniline.
Test Facility	AkzoNobel (2016b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute Dermal Toxicity – Rat

Test Substance	Notified chemical
Method	OECD TG 402 Acute Dermal Toxicity – Fixed Dose Procedure (2017)
Species/Strain	Rat/Crl:WI(Han)
Vehicle	None
Type of dressing	Semi-occlusive.
Remarks – Method	No significant deviations from the OECD test guideline were evident. A range finding study was performed in order to select the dose causing no mortality or significant toxicity to be used in the main study. One female rat was dosed at 2,000 mg/kg. Based on the results of the range finding study, two additional female rats were dosed at 2,000 mg/kg in main study. The test substance was applied undiluted and the application period was 24 hours.

Results

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
Range finding	1 female	2,000	0/1
Main	2 female	2,000	0/2

LD50	> 2,000 mg/kg bw
Remarks – Results	Chromodacryorrhoea (snout) was noted for two animals on Day 1. No other test substance related local or systematic effects were observed. No abnormalities were found at macroscopic post mortem examination. All the test animals gained weight within the range expected for rats in the sex and group.
Conclusion	The notified chemical is of low acute toxicity via the dermal route.
Test Facility	CRL (2018)

B.2. Irritation – skin (*in vitro*)

TEST SUBSTANCE	Notified chemical at 10% concentration
METHOD	Similar to OECD TG 439 *In Vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method: EpiDerm™ Skin irritation Test Model
Vehicle	Tissue culture grade water
Remarks - Method	No significant deviations from the OECD test guideline were evident, except that additional exposure times were tested, and a test measuring inflammatory processes was added. The volume of test substance was slightly higher than recommended in the test guideline. MatTek EpiDerm™ tissues were used for the study. The test substance (100 µL) at 10% concentration was applied to the tissues in duplicates. Following 1, 4 and 24 hour exposure periods, the tissues were rinsed with phosphate buffered saline (PBS) and then incubated with MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) for 3 hours at 37 °C. The tissues were rinsed again with PBS and treated with extraction solution overnight. Following overnight extraction at room temperature, the optical densities were determined at 570 nm using a reference wavelength of 690 nm to measure the conversion of MTT to formazan. The cell viability was expressed as percentage of negative control values. Quantification of Interleukin-1-alpha (IL-1α), an inflammatory cytokine released by the cells in response to chemical stress, was also conducted in the assay medium post exposure to the test substance and negative control.

1% Triton X-100 was used as positive control and vehicle was used as negative control.

The test substance was considered by the study authors to be an irritant if the ET_{50} value (exposure time required to reduce cell viability by 50%) was < 24 hours.

RESULTS

Test material	*Exposure period (hours)*	*Mean OD_{570} of duplicate tissues*	*Relative mean viability (%)*	*ET_{50} Value (hours)*	*Mean IL-1α concentration (pg/ml)*
Negative control	4	1.658	100	-	13.9
Test substance	1	1.519	91.6		20.5
	4	1.520	91.7	> 24	18.6
	24	1.200	72.3		89.4
Positive control	4	1.551	93.5	6.1	N.D.
	9	0.174	10.5		N.D.

OD = optical density; N.D. = Not determined

Remarks - Results

Tissue viability after exposure to the test substance was not reduced to ≤ 50% in any of the exposure periods. Thus the classification criteria for irritation were not met.

Marked increase in production of the cytokine IL-1α was seen in tissues exposed to the test substance for 24 hours which was the highest incubation time. This supports the MTT viability data; at 24 hours, where a marked decrease in cell viability was seen when compared to negative control.

The negative and positive control performed as expected confirming the validity of the assay.

CONCLUSION

The test substance did not meet the criteria for classification as irritating to the skin under the conditions of the test.

TEST FACILITY

MB Research Labs (2016a)

B.3. Irritation – skin (*in vitro*)

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 439 *In Vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method: EpiDerm™ Skin irritation Test Model

Vehicle

Tissue culture grade water

Remarks - Method

Reconstructed human skin tissue model EpiDerm™ was incubated with 30 µL (47 µL/cm^2) test substance for 60 min in triplicates. The skin irritation potential of the test substance was determined by measuring the dehydrogenase conversion of MTT in cell's mitochondria, into a blue formazan salt that was quantitatively measured after extraction from the tissues. The amount of extracted formazan was determined photometrically by measuring its optical density at 570 nm.

Five percent sodium lauryl sulphate in deionised water was used as positive control and phosphate buffered saline (PBS) was used as negative control.

The test substance was considered to be irritating to the skin in accordance with the UN GHS (Category 2), if the tissue viability after exposure and post-treatment incubation was ≤ 50%. The cell viability was expressed as percentage of the negative control values.

The study was performed in accordance with GLP.

RESULTS

Test material	*Exposure period (hours)*	*Mean OD_{570} of three tissues blank corrected*	*Mean OD_{570} of triplicate tissues*	*Relative standard deviation (%)*	*Relative mean cell viability (%)*
Test item 1	1	1.121			
Test item 2	1	0.984	1.076	7.4	80.6
Test item 3	1	1.124			
Negative control 1	1	1.469			
Negative control 2	1	1.254	1.336	8.7	100.0
Negative control 3	1	1.283			
Positive control 1	1	0.079			
Positive control 2	1	0.076	0.072	14.8	5.4
Positive control 3	1	0.059			

Remarks - Results

Compared to the relative absorbance value of the negative control, the mean relative absorbance value was 80.6% after exposure of the skin tissues to the test item. This value is higher than the threshold for skin irritants (≤ 50%).

The optical pre-experiment (colour interference pre-experiment) to investigate the test item's colour change potential in water did not lead to a change in colour.

Optical evaluation of the MTT-reducing capacity of the test item after 1 hour incubation with MTT-reagent did not show blue colour, indicating that it does not react directly with MTT.

After treatment with the negative control the absorbance values were well within the required acceptability criterion of mean OD ≥ 0.8 and ≤ 2.8 for the 60 min treatment interval, thus showing the quality of the tissues.

The relative mean cell viability of the positive control (5.4%) confirmed the validity of the test system.

CONCLUSION

The test substance did not meet the criteria for classification as irritating to the skin under the conditions of the test.

TEST FACILITY

Envigo (2017)

B.4. Irritation – eye (*in vitro*)

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

0.9% Sodium Chloride solution

Remarks - Method

No significant deviations from the OECD test guideline. The negative control used was 0.9% NaCl in deionised water. The positive control used was 2-ethoxyethanol.

RESULTS

Test material	*Opacity Value = Difference (t_{130}-t_0) of Opacity*	*Mean permeabilities of triplicate tissues*	*Mean IVIS*
Vehicle control	0.00	0.067	1.00
Test substance	0.00*	0.024*	0.37
Positive control	75.67*	1.24*	94.25

IVIS = in vitro irritancy score
*Corrected for background values

Remarks - Results: No change in corneal opacity and permeability was noted in test substance exposed eyes when compared to vehicle control.

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

CONCLUSION: The test substance did not meet the criteria for classification as irritating to the eye under the conditions of the test.

TEST FACILITY: Envigo (2017a)

B.5. Irritation – eye (*in vitro*)

TEST SUBSTANCE: Notified chemical at 10% concentration

METHOD: Determination of Ocular Irritation Potential Using the MatTek EpiOcular™ MTT Viability Assay (similar to OECD Test Guideline 492)

Vehicle: Tissue culture grade water

Remarks - Method: MatTek EpiOcular tissues were used for the study. The test substance (100 µL) at 10% concentration was applied to the tissues in duplicates. Following 16, 64 and 256 min exposure periods, the tissues were rinsed with phosphate buffered saline (PBS) and then incubated with MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) for 3 hours at 37 °C. The tissues were rinsed again with PBS and treated with extraction solution overnight. Following overnight extraction at room temperature, the optical densities were determined at 570 nm using a reference wavelength of 690 nm to measure the conversion of MTT to formazan.

Quantification of the cytokine Interleukin-1-alpha (IL-1α), an inflammatory cytokine released by the cells in response to chemical stress, was also conducted in the assay medium post exposure to the test substance and negative control.

0.3% Triton X-100 was used as the positive control and vehicle was used as the negative control.

The mean percent viability for each time point was used to calculate an ET_{50}, which represent the time at which the EpiOcular tissues viability was reduced to 50% compared to control tissues.

RESULTS

Test material	*Exposure period (mins)*	*Mean OD_{570} of duplicate tissues*	*Relative mean viability (%)*	*ET_{50} Value (mins)*	*Mean IL-1α concentration (pg/ml)*
Negative control	16	1.497	100		2.1
Test substance	16	1.529	102.1		1.4
	64	1.723	115.1	> 256.0	2.1
	256	1.765	117.9		2.8

Positive control	15	1.080	72.1	27.9	N.D.
	45	0.493	32.9		N.D.

OD = optical density; N.D. = Not determined

Remarks - Results

Tissue viability after exposure to the test substance was not altered when compared to the negative control. This was confirmed by no significant change in the production of the cytokine IL-1α.

The negative and positive controls performed as expected confirming the validity of the assay.

CONCLUSION

The test substance did not meet the criteria for classification as irritating to the eye under the conditions of the test.

TEST FACILITY

MB Research Labs (2016b)

B.6. *In Vitro* Dermal Penetration Study

TEST SUBSTANCE

Notified chemical

METHOD

Similar to OECD TG 428 – Skin Absorption: *in vitro* Method

Remarks - Method

No significant deviations from the OECD test guidance. The test was conducted under non-GLP (good laboratory practice) condition. ^{3}H-radiolabeled test substance was used in the study. The two reference used were ^{14}C-Benzoic acid (high dermal penetration rate) and ^{14}C-Mannitol (low dermal penetration rate).

The test substance and controls were measured in quadruplicates. Dermal penetration was measured up to 24 hours. Samples were collected at 1, 2, 4, 6, 12 and 24 h into the exposure. The presence of test substance and controls was measured in the application chamber after 24 hour exposure, the receiver chamber at various time intervals which was later cumulated to measure yield and in the skin by tape-stripping the skin epidermis 10-20 times and measuring the amount in the tape after extraction in acetone. Skin from single human cadaver thigh was used for the study.

The solubility of the test substance in the buffer used in the receiver chamber (Dulbecco's phosphate buffered saline pH 7.4 with 1mM sodium azide and 0.1% Brij 98) was not tested as it was considered necessary by the study authors. This was due to the very high solubility of the test substance in water (500 mg/mL) which was above the test guideline recommendation of 10 times the maximum concentration achievable in the experiment of 9.5 mg/mL indicating that the receiver buffer was appropriate for test material.

RESULTS

% Mean radioactivity remaining in donor chamber: 96.6% ± 7.8%
% Mean radioactivity recovered from skin (stratum corneum/epidermis): 3.28% ± 0.15%
% Mean radioactivity in receiver chamber: 0.38% ± 0.1%*
Mean total absorbable dose: 3.37% ± 0.15%
* the amount of test substance expected to be available for systemic exposure.

Remarks - Results

One of the test substance replicate for recovery was not met as per the OECD test guideline. The total yield was outside the acceptable range of 100 ± 10% (85.6%). However the replicate was not excluded from the calculations by the study authors. It was believed to have occurred due to the variability in the dose volume applied.

The recovery of controls and the dermal penetration rates fell within the historical range confirming the validity of the study and the integrity of the human cadaver skin.

CONCLUSION The dermal absorption of the test substance was considered to be approximately 3.37%

TEST FACILITY Cyprotex (2017)

B.7. *In Chemico* Skin Sensitisation (DPRA Test)

TEST SUBSTANCE Notified chemical

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

Remarks - Method No significant deviations from the OECD test guideline.

The test substance was prepared in water (100mM stock solution). Cinnamic aldehyde (100mM in acetonitrile) was used as positive control. Solvent reference controls were setup and used in parallel to sample preparation in order to verify the validity of the test run. 0.667mM stock solutions of cysteine and lysine peptides were prepared in phosphate (pH 7.4) and ammonium acetate (pH 10.2) buffers respectively. The test substance was incubated in dark with the peptide solutions for 24 ± 2 h at 25 ± 2.5 °C for the reaction to take place. The ratios of test substance: peptides were 1:10 cysteine peptide and 1:50 lysine peptide. After incubation, peptide depletion was monitored by HPLC coupled with a UV detector at wavelength of 220 nm using a reverse-phase HPLC column.

RESULTS

Sample	*Cysteine Peptide Depletion (% ± SD)*	*Lysine Peptide Depletion (% ± SD)*
Vehicle	0.00*	0.00*
Test Substance	0.59 ± 0.64	0.33 ± 0.14
Positive Control	70.06 ± 0.48	57.48 ± 1.25

* – normalised to 100%; SD = Standard Deviation

Remarks - Results Depletion of peptides was less than 6.38% indicating no or minimal reactivity (negative prediction for skin sensitisation).

Phase separation was observed after 24 h incubation of lysine peptide with the positive control cinnamic aldehyde. The precipitates did not interfere with the evaluation.

The positive controls and references fulfilled all quality criteria confirming the validity of the test.

CONCLUSION The test substance was not considered a skin sensitiser in this adverse outcome pathway (AOP) key event (KE) 1 assay.

TEST FACILITY Eurofins (2017a)

B.8. *In Vitro* Skin Sensitisation (ARE-Nrf2 Luciferase Test)

TEST SUBSTANCE Notified chemical

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

Remarks - Method No significant deviations from the OECD test guideline. KeratinoSens™ test method was used.

A 200mM stock solution of test substance was prepared in dimethyl sulphoxide (DMSO). A set of twelve master solutions were prepared in DMSO from the stock solution using 1:2 serial dilutions. These master solutions were used in

assay where they were further diluted to give a final concentration DMSO to be 1% in the test system. DMSO and cinnamic aldehyde were used as negative and positive controls respectively. Three independent experiments were conducted with samples tested in triplicates in each test. The mean ± standard deviations for cell viability and luciferase induction calculated from three independent experiments are depicted below.

RESULTS

Sample	*Concentration (µM)*	*% Cell viability (mean ± SD, n=3)*	*% Luciferase Induction (mean ± SD, n=3)*
Control			
	-	100*	1.00*
Test substance			
	0.98	100.8 ± 23	1.33 ± 0.23
	1.95	89.8 ± 1.5	1.11 ± 0.15
	3.91	99.4 ± 16.2	1.14 ± 0.15
	7.81	100.4 ± 20.7	1.15 ± 0.18
	15.63	102.9 ± 16.9	1.14 ± 0.15
	31.25	97.5 ± 15.8	1.03 ± 0.12
	62.500	101.0 ± 18.8	1.13 ± 0.20
	125.00	101.9 ± 17.6	1.10 ± 0.16
	250.00	102.6 ± 18.6	1.22 ± 0.37
	500.00	100.1 ± 16.0	1.05 ± 0.15
	1000.00	93.6 ± 12.6	1.06 ± 0.19
	2000.00	98.2 ± 12.8	1.01 ± 0.13
Positive Control			
	4.00	106.0 ± 1.7	1.16 ± 0.13
	8.00	106.5 ± 2.8	1.23 ± 0.09
	16.00	115.3 ± 2.8	1.62 ± 0.27†
	32.00	123.6 ± 3.5	1.96 ± 0.35†
	64.00	123.3 ± 5.3	3.88 ± 1.11†

* – normalised to 100%
† – statistically significant increase ($p < 0.05$)

Remarks - Results Luciferase induction > 1.5 at cell viability > 70% is required for identification as a skin sensitiser. This criterion was met for the positive control but not for the test substance.

In experiment 1 (value not shown in the table), the mean luciferase induction level for cells exposed to 250 µM of test substance was 1.61. This was greater than 1.5 and the mean cell viability for the cells was > 70%. The parameters meet the categorisation criteria. However one of the replicates showed luciferase induction level (2.37) significantly higher than the other two replicates (1.21 and 1.25). Again no dose response was observed.

CONCLUSION The test substance was not considered a skin sensitiser in this AOP KE2 assay.

TEST FACILITY Eurofins (2017b)

B.9. *In Vitro* Skin Sensitisation (h-CLAT test)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 442e *In Vitro* Skin Sensitisation: human Cell Line Activation Test (h-CLAT; 2016)

Vehicle 0.9% sodium chloride

Remarks - Method No significant deviations from the OECD test guideline were evident.

Human monocytic leukaemia cell line (THP-1; TIB202^{TM}) from American Type Culture Collection (ATCC) was used for the assay. Two positive controls 4 µg/mL 1-chloro-2, 4-dinitrobenzene (DNCB) and 100 µg/mL nickel sulfate (NiSO4) and a negative control 1000 µg/mL lactic acid (LA) were used. Stimuli mediated increase in expression of the cell surface markers CD86 and CD54 was measured using fluorescence tagged antibodies.

Three dose finding tests (single replicates) were conducted to find the test substance concentration at which cell viability is reduced to 75% (CV75) to decide the test substance concentrations for CD54 and CD86 expression test. The following test substance concentrations were used:
Test 1 & 2: 7.81, 15.63, 31.25, 62.50, 125, 250, 500 and 1000 µg/mL
Test 3: 39.06, 78.13, 156.25, 312.50, 625, 1250, 2500 and 5000 µg/mL

Three main tests were conducted to evaluate the ability of the test substance to induce expression of CD54 and CD86. The following concentrations were used: 1395.41, 1674.49, 2009.39, 2411.27, 2893.52, 3472.22, 4166.67 and 5000 µg/mL

Test acceptance criteria:
Positive Control: Relative Fluorescence Intensity (RFI) > 150 for CD86 and > 200 for CD54 with cell viability ≥ 50%.
Negative Control: RFI < 150 for CD86 and < 200 for CD54 with cell viability ≥ 50%.
Cell Viability: > 50% for Positive control, > 75% for test substance and > 90% for negative and vehicle controls

RESULTS

Reactivity check: the positive controls DNCB and NiSO4 led to upregulation of the cell surface markers CD54 and CD86. The negative control LA did not induce an upregulation of CD54 and CD86. The acceptance criterion was met.

Dose finding study: none of concentrations up to 5000 µg/mL tested produced cell viability less than 96.8%. The test substance concentration at which cell viability was reduced to 75% could not be calculated. Therefore, the test substance was tested at various concentrations up to the maximum recommended dose of 5000 µg/mL in the test guideline.

Main study: upregulation of CD54 marker, RFI greater than 200, was observed in experiment 1 at test substance concentrations of 4166.67 µg/mL and 5000 µg/mL and at 5000 µg/mL in experiment 3.

Upregulation of CD86 marker, RFI greater than 150, was seen in experiment 3 at concentrations above 2411.27 µg/mL. No dose response was observed.

Remarks - Results

Marked increase in expression of CD54 and CD68 were observed. The increase in RFI was sufficiently high to classify the test substance as a skin sensitiser. The negative and positive controls met the acceptance criteria.

CONCLUSION

The test substance was considered a skin sensitiser in the AOP KE3 assay.

TEST FACILITY

Eurofins (2017c)

B.10. Repeat Dose Oral Toxicity – Rats

Test Substance	Notified chemical
Method	OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test
Species/Strain	Rat / Crl: WI(Han)
Route of Administration	Oral – gavage

Exposure Information	Total exposure days: Males – 29 days Females – 50-56 days, except for one from mid dose and one from high dose, which were exposed for 63 days (post-natal day 14-16). Dose regimen: 7 days per week
Vehicle	Purified water
Remarks – Method	A 10-day dose range finding study was conducted to decide the dose levels in main study. Two groups of 3 female rats each were fed with the test substance via oral gavage once daily at the doses of 500 and 1,000 mg/kg bw/day. No clear clinical signs relevant to the test substance administration were observed during the preliminary study. Dose levels for the main study were selected at 100, 300 and 1000 mg/kg bw/day.

Results

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	10 F & 10 M	0	1 (F) /20
Low Dose	10 F & 10 M	100	0 / 20
Mid Dose	10 F & 10 M	300	0 / 20
High Dose	10 F & 10 M	1000	1 (F) / 20

Mortality and Time to Death
No test substance related mortality was observed.

One female from the control group was euthanized in extremis on day 19 post-coitum for humane reasons.

One female rat from the high dose group died after blood sample collection on the day of scheduled necropsy. The death was regarded to be related to the blood sampling procedure under anaesthesia.

Clinical Observations
One male rat from a mid dose group had severe scabs on day 24 and one female rat from mid dose group exhibited severe scabs and neck wound in the final week of exposure. One female rat from the mid dose group exhibited alopecia during week 4 of exposure which persisted till the end of the study.

One female rat from the control group was sacrificed in extremis.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Blood samples from five test animals from each group were analysed except for female rats from high dose group where samples from only four treated animals were tested. Blood sample from a female rat in high dose group that died after blood collection procedure was not tested.

A 4% decrease in mean corpuscular volume (MCV) and 6% decrease in mean corpuscular haemoglobin (MCH) levels were reported in male rats from mid dose group when compared with control group male rats. These changes were considered unrelated to administration of the test substance by the study authors due to the minimal magnitude of the changes and the absence of a dose response.

In females from high dose group, the mean eosinophils level was significantly lower when compared with the control group. However, the values remained within the historical control range of the test facility and thus the finding was considered unrelated to treatment.

Small but statistically significant reduction in mean total protein level was observed for male rats from mid dose group. Similarly, slight but statistically significant reduction in mean albumin levels were observed for male rats from low and high dose groups. These changes were not considered test substance related by the study authors due to a lack of dose response and the values fell within the historical range for control animals in the test facility.

Significant reduction in mean potassium levels were noted for male rats from low dose group. One rat from this group had significantly lower level of potassium, which may have resulted in a reduction in the mean value for the group. However, no change was noted for mid and high dose groups when compared to control.

No urinalysis was conducted.

Effects in Organs
All test animals were subjected to gross necropsy. Organ weights were taken of five male and five female test rats from each group. No treatment related changes in organ weights and organ to body weight ratios were noted.

During macroscopic examination, several foci were noted in the thymus of one male rat from the control group and one male rat from the mid dose group. One male rat from high dose group had several granular foci in the stomach. One female rat from low dose group had isolated scab formation at the back of the neck. One female rat from mid dose group rat had alopecia on both sides of foreleg and another female rat had a yellowish hard nodule in the vagina. The observations were isolated and did not show a dose response relationship.

The female rat from control group, which had to be euthanised on day 19, exhibited pale appearance, dark red discolouration of the gastro-intestinal tract, enlarged liver, gelatinous pancreas, enlarged iliac and renal lymph nodes, and a red discolouration of the mesenteric and renal lymph nodes. The uterus contained in total 12 dead foetuses. Microscopic examination revealed marked necrosis of the liver and marked tubular degeneration of the kidneys. These alterations in liver and kidney were regarded the main cause of moribundity.

Small but statistically significant reduction in mean thymus weight was observed in female rats from low dose group. The changes were not considered to be test item related by the study authors due to the lack of dose response. Small reduction in mean thymus weight was also observed in male rats from high dose group. However, this change did not reach statistical significance when compared to the control mean.

The mean relative spleen weight of females in the high dose group was deceased by 17% when compared with concurrent control. The value remained within the historical control range and hence this change was considered by the study authors not to be toxicologically relevant.

No macroscopic and microscopic findings were noted for the female rat from high dose group that died after the blood collection procedure on the day of scheduled necropsy.

Histopathology
Detailed histopathological studies were conducted on five female and five male rats from control and high dose groups only. According to the study authors, no test substance related effects were observed. All of the macroscopic and microscopic findings were isolated and within the range of background gross observations encountered in rats of this age and strain. There was no test item-related alteration in the prevalence, severity, or histologic character of those incidental tissue alterations.

Grade 1 cysts were observed in the pituitary gland of two male rats from high dose groups.

Functional observations
Five female and five male rats from each group were selected for functional observations. Parameters such as hearing ability, pupillary reflex and static righting reflex were not affected by treatment.

In male groups, mean grip strength of the fore legs was decreased in high dose group by 11% when compared to the control. As all values remained well within the historical control data range of the test facility and the concurrent control mean was relatively high, this change was reported to be attributed to biological variation.

Motor activity was similar between treated and control groups. All groups showed a similar motor activity habituation profile with a decreasing trend in activity over the duration of the test period.

Effects on Reproduction and Off-springs
No test substance related effects were observed. No changes in reproductive organs of test animals were observed. No treatment related changes in serum thyroxine (Thyroid hormone) levels were noted.

Length and regularity of the oestrous cycle were considered not to have been affected by treatment. Most females had regular cycles of 4 days with the exception of one female in low dose group for which the regularity could not be determined and one female in mid dose group which had an irregular cycle. Given the incidental nature, absence of a dose-related incidence and absence of an apparent correlation to pregnancy status, these findings did not indicate a relation with treatment.

There was no indication for abnormal spermatogenesis in the treated males.

Precoital time and number of implantation sites were not affected by the treatment. The mating and fertility indices were 100% for control and all test groups. No changes in gestational index and duration, parturition, post implantation survival index, litter size, live birth index, viability index and lactation index were observed.

No test substance mediated changes in the offspring were observed. No clinical signs were noted. No change in body weight, sex ratio, anogenital distance, nipple retention, thyroid hormone levels and macroscopic findings were observed in the offspring.

Remarks – Results
Body weight and weight gains were not affected in animals treated with the test substance when compared to the control.

Some isolated observations in haematology, clinical chemistry, macroscopic and histopathology observations were noted. These findings were considered to be not treatment related by the study authors due to either lack of dose response or falling within the historical control range data of the test facility.

Conclusion
The No Observed Adverse Effect Levels (NOAELs) for parental toxicity, reproduction toxicity and developmental toxicity were established as > 1,000 mg/kg bw/day by the study authors in this study, based on no adverse effects observed at any dose tested.

Test Facility CRL (2019)

B.11. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

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

Species/Strain	*Salmonella typhimurium*: TA1535, TA97a, TA98, TA100 *Echerichia 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 or without metabolic activation: 0.05-5 µL/plate
Vehicle	Tissue culture grade water
Remarks - Method	A preliminary cytotoxicity test (0.001-5 µL/plate) was performed to determine the toxicity of the test material in the presence and absence of metabolic activation in the TA100 bacterial strain only. In the main test, based on the preliminary cytotoxicity results, five concentrations of the test material (0.05-5 µL/plate) were used in triplicates against each strain. Test 1 (main test): All strains with and without S9: 0.005, 0.1, 0.5, 1 and 5 µL/plate. Test 2 (confirmatory test): TA97a with and without S9: 0.005, 0.1, 0.5, 1 and 5 µL/plate. The criterion for a positive response was a 2-fold or higher increase in revertant colonies.

RESULTS

Metabolic Activation	*Test Substance Concentration (µL/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				

Test 1	> 5 µL/plate	> 5 µL/plate	None observed	Negative
Test 2*		> 5 µL/plate	None observed	Negative
Present				
Test 1	> 5 µL/plate	> 5 µL/plate	None observed	Negative
Test 2*		> 5 µL/plate	None observed	Negative

* Independent repeat test conducted on bacterial strain *S. typhimurium* TA97a only

Remarks - Results

There was no 2-fold increase or dose-dependent increase of the number of revertant colonies in any tester strain treated with the test substance in the presence and absence of S9.

In the TA97a strain, there appeared to be a borderline positive response with S9, with responses at all test concentrations increased by 1.6 to 1.8-fold compared to the vehicle control, close to the 2-fold criteria for a positive response. However a dose-response effect was not observed, and the positive control, 2-aminoanthracene, showed a much higher increase (11.2-fold). An independent repeat assay (confirmatory test) was conducted in this strain (with or without S9), using test substance concentrations of 0.05 – 5 µL/plate. Once again, equivocal increases of 1.5-1.7-fold were observed in the presence of S9, with no dose response effect being evident.

The positive and negative controls gave satisfactory results confirming the sensitivity of the test system.

CONCLUSION

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

TEST FACILITY

MB Research Labs (2016c)

B.12. Genotoxicity – *in vitro*

TEST SUBSTANCE

Notified Chemical

METHOD

OECD TG 487 *In Vitro* Mammalian Cell Micronucleus Test (2016).

Species/Strain: Human
Cell Type/Cell Line: Peripheral blood lymphocytes
Metabolic Activation System: S9-mix from Phenobarbital/β-Naphtha flavone induced rat liver
Vehicle: none

Remarks - Method

No significant deviations from the OECD test guideline. Three independent experiments were performed. Cytokinesis block proliferation Index (CBPI) was calculated for the toxicity assessment of the test substance to cultured human lymphocytes. At least 500 cells were used per culture. The test item was prepared minimal essential medium at 14.72 mg/ml. The positive controls used were: Mitomycin C (MMC) without S9 mix and cyclophosphamide monohydrate, (CPA) with S9 mix.

A preliminary test was conducted to measure test substance toxicity and precipitation.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 46, 92, 184*, 368*, 736*, 1472*	4 h	28 h
Test 2	0*, 46, 92, 184*, 368*, 736*, 1472*	24 h	48 h
Present			
Test 1	0*, 46, 92, 184*, 368*, 736*, 1472*	4 h	28 h

*Dilutions selected for scoring of micronuclei

RESULTS

Metabolic Activation	*Cytotoxicity in Preliminary Test*	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 1472 µg/ml	> 1472 µg/ml	Nil	Negative
Test 2	> 1472 µg/ml	> 1472 µg/ml	Nil	Negative
Present				
Test 1	> 1472 µg/ml	> 1472 µg/ml	Nil	Negative

Remarks - Results

All vehicle controls had frequencies of cells with micronuclei within the range expected for normal human lymphocytes.

The positive controls induced statistically significant increases in the frequency of cells with micronuclei. Thus the sensitivity of the assay and the efficacy of the S9-mix were validated.

The test substance was non-toxic and did not induce any statistically significant increases in the frequency of cells with micronuclei up to the maximum dose tested.

CONCLUSION

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

TEST FACILITY

Envigo (2017b)

B.13. Genotoxicity – *in vitro*

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 490 *In Vitro* Mammalian Cell Gene Mutation Test Using the Thymidine Kinase Gene (2016).

Species/Strain: Mouse

Cell Line: L5178Y TK $^{+/-}$ -3.7.2c lymphoma cells (heterozygous at the thymidine kinase locus)

Metabolic Activation System: S9-mix from Phenobarbital/β-Naphtha flavone induced rat liver

Vehicle: none

Remarks - Method: No significant deviations from the OECD test guideline. The positive controls used were: Ethyl methane sulphonate (EMS) without S9 mix and cyclophosphamide (CP) with S9 mix.

A preliminary test was conducted to measure test substance toxicity and precipitation.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0, 46, 92, 184, 368, 736, 1472	4 h	10–14 d
Test 2	0, 46, 92, 184, 368, 736, 1472	24 h	10–14 d
Present			
Test 1	0, 46, 92, 184, 368, 736, 1472	4 h	10–14 d

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*

Absent				
Test 1	> 1472	> 1472	> 1472	Negative
Test 2	> 1472	> 1472	> 1472	Negative
Present				
Test 1	> 1472	> 1472	> 1472	Negative

Remarks - Results

The maximum test substance concentration (10mM) used in the study was the maximum recommended dose according to the test guideline.

The positive controls induced marked increases in the mutant frequency validating the sensitivity of the assay and the efficacy of the S9-mix.
The test substance was non-toxic and did not induce any toxicologically significant increases in the mutation frequency at any of the test concentrations either with or without metabolic activation.

CONCLUSION

The notified chemical was not mutagenic to L5178Y TK $^{+/-}$ -3.7.2c lymphoma cells treated in vitro under the conditions of the test.

TEST FACILITY

Envigo (2017c)

B.14. Phototoxicity (*in vitro*)

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 432 *In vitro* – 3T3 NRU Phototoxicity Test

Vehicle

1% v/v EBSS.

Remarks - Method

The study was performed in accordance with the GLP principles.
The only alteration from the OECD test guidelines was the cell number seeded into the cavities of the 96-well plate (2×10^4 cells instead of the recommended 1×10^4 cells).

Irradiation was performed with a solar simulator with UVB filter. The produced wavelength of the simulator was > 320 nm. 96-well plates were irradiated through the lid at 1.65 mW/cm^2 UVA, resulting in a dose of ~5 J/cm^2, previously shown to be non-cytotoxic to 3T3 cells but sufficiently potent to excite chemicals to elicit phototoxic effect.

Negative and positive (chlorpromazine) controls, with or without irradiation with artificial sunlight, were used in parallel with the test substance.

A preliminary, range finding experiment (RFE), was conducted with the notified chemical at 7.81 – 1,000 µg/mL with or without irradiation.

The main experiment (ME) (6 trials) was conducted at the same concentrations range as the RFE: 7.81 – 1,000 µg/mL with or without irradiation.

In both experiments, one test group of cells treated with the test item was irradiated with artificial sunlight for 50 min. Another test group of test item treated cells were kept in the dark for 50 min.

Cytotoxicity was expressed as a concentration dependent reduction of the uptake of the Neutral Red dye 24 h after treatment with the test substance and irradiation. The absorbance was determined at 550 nm.

RESULTS

Test material	*IC_{50} Value (+ UV) [µg/mL]**	*IC_{50} Value (- UV) [µg/mL]**	*PIF**	*MPE**

RFE	*Positive control*	0.60	11.88	20.4	0.540
	Test substance	-	-	-	-0.044
ME	*Positive control*	0.77	10.72	13.89	0.548
	Test substance	-	-	-	-0.029

IC_{50} = half maximal Inhibitory Concentration; the concentration of the test chemical by which the cell viability is reduced by 50%
PIF = Photo-Irritation Factor
MPE = Mean Photo Effect
* Calculated on the basis of the results in 6 trials.

Remarks - Results	In both experiments no cytotoxic effects were observed after treatment of cells with the test substance, neither in the presence nor in the absence of irradiation with artificial sunlight. Therefore, IC_{50}-values or PIFs could not be calculated. The resulting MPE was -0.044 or -0.029, respectively, and the test item was classified as not phototoxic. The positive control induced phototoxic responses within the expected range, confirming the validity of the test system.
CONCLUSION	The notified chemical did not have any phototoxic effects on BALB/c 3T3 fibroblast cells, under the test conditions.
TEST FACILITY	Envigo (2017d)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	3:1 molar mixture of notified chemical and glycerol
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test.
Inoculum	Activated sludge
Exposure Period	28 Days
Auxiliary Solvent	None
Analytical Monitoring	Dissolved oxygen concentrations (oxygen electrode and meter)
Remarks - Method	The Closed Bottle tests are performed according to slightly modified OECD Test Guidelines. The notified chemical was added at a concentration of 3 mg/L into biological oxygen demand (BOD) bottles containing the inoculum and nutrient medium, with ammonium chloride omitted to prevent oxygen consumption due to nitrification. Biodegradation was measured by following the course of the oxygen decrease. No toxicity control experiment was conducted in parallel to the activated sludge biodegradation test. A river water biodegradation test was run in parallel. The actual concentration of the test solution was not determined.

RESULTS

Notified Chemical in activated sludge		*Notified chemical in river water*	
Day	*% Degradation*	*Day*	*% Degradation*
7	11	7	9
14	17	14	14
21	54	21	31
28	78	28	48
42	79	42	71

Remarks - Results	The validity of the test was demonstrated by oxygen concentrations > 0.5 mg/L in all bottles during the test period. Inhibition of the endogenous respiration of the inoculum was not detected with the notified chemical. Biodegradation of ≥ 60% (78%) was found within 28 days with activated sludge. However, test of ready biodegradability and 10-day window pass level is not applicable to mixtures containing different types of chemical.
CONCLUSION	The notified chemical exhibits high biodegradation within 28 days
TEST FACILITY	AkzoNobel (2016c)

C.1.2. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test.
Inoculum	Activated sludge
Exposure Period	28 Days
Auxiliary Solvent	None
Analytical Monitoring	Dissolved oxygen concentrations (oxygen electrode and meter)
Remarks - Method	The notified chemical was added at a concentration of 2 mg/L into biological oxygen demand (BOD) bottles containing the inoculum and nutrient medium, with ammonium chloride omitted to prevent oxygen consumption due to nitrification.. The actual concentration of the test substance was not determined.

RESULTS

Test substance		*Sodium acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
0	0	0	0
7	18	7	76
14	32	14	80
21	68	21	
28	89	28	

Remarks - Results: The validity of the test was demonstrated by oxygen concentrations > 0.5 mg/L in all bottles during the test period. Inhibition of microorganisms capable of degrading the reference compound did not occur, however, the inhibition of endogenous respiration of the inoculum by the test substance was detected at day 7 of the test. Biodegradation of ≥ 60% (89%) was found within 28 days with activated sludge. Test of ready biodegradability and 10-day window pass level is not applicable to mixtures containing different types of chemical.

CONCLUSION: The notified chemical exhibits high biodegradation within 28 days

TEST FACILITY: AkzoNobel (2016d)

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: *Oncorhynchus mykiss*

Exposure Period: 96 hours

Auxiliary Solvent: None

Water Hardness: 140 mg $CaCO_3$/L

Analytical Monitoring: HPLC-MS/MS

Remarks – Method: The method used followed the OECD 201 guideline recommendations except the organisms were added to the test system within approximately 45 minutes and this was considered not to have affected the outcome or integrity of the study. Based on the results of the range-finding test, the test was conducted at a concentration of 100 mg/L. Test preparations were renewed daily.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*						
Nominal	*Actual*		*1 h*	*3h*	*6h*	*24h*	*48h*	*72h*	*96h*
Control	Control	7	0	0	0	0	0	0	0
100	98.6-102*	7	0	0	0	0	0	0	0

*Measured concentrations at 24, 72 and 96 hr.

LC50: >100 mg/L at 96 hours.

NOEC (or LOEC): 100 mg/L.

Remarks – Results: The actual concentrations of the test substance were within 99-102% of nominal concentrations of notified chemical in test preparations. However, analysis of the 100 mg/L test preparations at 0 hours showed concentrations of less than the Limit of Quantification of the analytical method due to unknown reasons. The system was assumed to be dosed correctly based on measured concentrations in the old test media at 24 hours. LC50 was greater than 100 mg/L based on the nominal test concentrations.

CONCLUSION	The notified chemical is not considered harmful to fish
TEST FACILITY	Envigo (2017e)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test - Static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	Not given
Analytical Monitoring	None
Remarks - Method	The tests were carried out in accordance with the OECD 202 Guideline for testing of chemicals with the following exceptions: No chemical analysis was conducted GLP was not claimed for the test.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	ND	20	0	0
0.1	ND	20	0	0
1.0	ND	20	0	0
10	ND	20	0	0
100	ND	20	0	0

EC50	> 100 mg/L at 48 hours (CI not determined)
NOEC	100 mg/L at 48 hours
Remarks - Results	The test solution was not renewed during the 48 h test period. The actual concentrations of the test substance were not measured. The 48 h EC_{50} and NOEC for Daphnia was determined to be > 100 mg/L and 100 mg/L, respectively, based on the nominal concentration.
CONCLUSION	The notified chemical is not considered to be harmful to aquatic invertebrates.
TEST FACILITY	AkzoNobel (2016e)

C.2.3. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 211 *Daphnia magna* Reproduction test.
Species	*Daphnia magna*
Exposure Period	21 days
Auxiliary Solvent	None
Water Hardness	246-270 mg $CaCO_3$/L
Analytical Monitoring	HPLC-MS/MS
Remarks - Method	No significant deviations to the test protocol were reported. Based on the results of the range-finding test, *Daphnia magna* were exposed (10 replicates of a single daphnid per group) to a solution of the test item at a nominal concentration of 100 mg/L for a period of 21 days. The test solutions were renewed 3 times per week on Days 3, 5, 7, 10, 12, 14, 17 and 19.

	Test Concentration (mg/L)	
	Control	*100*
Total No. of Offspring Released by Survived *Daphnia*	1481	1325
Average No. of Offspring Released by Survived *Daphnia* by Day 21	148±7.3*	147±14*
Body Lengths of Surviving Adults (mm)	4.2±0.1*	4.2±0.1*
Survival (%)	100	90

*Standard Deviation

NOEC	100 mg/L at 21 days
Remarks - Results	All validity criteria for the test were satisfied except the temperature exceeded maximum deviation of ±1 °C, which was considered no to have affected the validity and integrity of the study. The actual concentrations of the test substance were within 87-100% of nominal concentrations during the 21 d test period, therefore, the results were based on nominal concentrations. There was not significant effect based on the size and color of the daphnids. The numbers of live young produced per adult by the control group were not significantly different from the 100 mg/L test group. The 21 d EC50 and NOEC were determined to be > 100 mg/L and 100 mg/L respectively based on immobilization and reproduction.

CONCLUSION The notified chemical is not considered to be harmful to aquatic invertebrates on a chronic basis

TEST FACILITY Envigo (2017f)

C.2.4. 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: 1, 10 , 100 and 1000 mg/L
Auxiliary Solvent	None
Water Hardness	Not determined
Analytical Monitoring	None
Remarks - Method	The method used followed the OECD 201 guideline recommendations with the following exceptions: Use of fewer replicates, controls and absorbance/physical chemical measurements than specified. Chemical analysis was not conducted. GLP was not claimed for the test.

RESULTS

Biomass		*Growth*	
E_bC50	*NOEC*	*E_rC50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
> 100	ND	> 100	ND

Remarks - Results	Validity criteria for the test were satisfied. The test solution was not renewed during the 72 h test period. The actual concentrations of the test substance were not measured. The 72 h E_bC50 and E_rC50 for the alga were both determined to be > 100 mg/L. As there was almost no observable effect on algae growth up to a concentration of 1000 mg/L of the notified chemical, actual test concentration was not determined. Due to the limited number of test concentrations used in a screening study results should be considered as a toxicity estimate only.

CONCLUSION	The notified chemical is not considered to be harmful to algae.
TEST FACILITY	AkzoNobel (2016f)

C.2.5. 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: 100 mg/L
Auxiliary Solvent	None
Water Hardness	Not determined
Analytical Monitoring	HPLC-MS/MS
Remarks - Method	The method used followed the OECD 201 guideline recommendations. Based on the results of the range-finding test, the test was conducted at a concentration of 100 mg/L.

RESULTS

Biomass		*Growth*	
E_yC50 mg/L at 72 h	*NOEC mg/L*	*E_rC50 mg/L at 72 h*	*NOEC mg/L*
> 100	100	> 100	100

Remarks - Results	Validity criteria for the test were satisfied. The results from the positive control were within the normal ranges for potassium dichromate. The actual concentrations of the test substance were within 91-92% of nominal concentrations, therefore, the results were based on nominal concentrations. As there was almost no observable effect on algae growth up to a concentration of 100 mg/L of the notified chemical, the 72 h E_bC50 and E_rC50 for the alga were both determined to be > 100 mg/L.
CONCLUSION	The notified chemical is not considered to be harmful to algae
TEST FACILITY	Envigo (2017g)

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