File No.: STD/1684

June 2020

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

1-Propanamine, 3,3'-[oxybis(2,1-ethanediyloxy)]bis-, (2*Z*)-2-butenedioate (1:2)
Bis-aminopropyl diglycol dimaleate (INCI Name)

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 Agriculture, Water and the Environment.

This Public Report is available for viewing and downloading from the NICNAS website or available on request, free of charge, by contacting NICNAS. For requests and enquiries please contact the NICNAS Administration Coordinator at:

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Director
NICNAS

TABLE OF CONTENTS

SUMMARY

The following details will be published on our website:

ASSESSMENT REFERENCE	APPLICANTS	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1684	Privity Pty Ltd trading as Healthcare Australia Sephora Australia Pty Ltd	1-Propanamine, 3,3'-[oxybis(2,1-ethanediyloxy)]bis-, (2*Z*)-2-butenedioate (1:2) (INCI name: bis-aminopropyl diglycol dimaleate)	Yes	≤ 50 tonnes per annum	Component of haircare products

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

*potency data not available to confirm Category 1A or 1B.

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 Category 3	H412 - Harmful 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 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

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Skin sensitisation (Category 1): H317 – May cause an allergic skin reaction

The above should be used for products/mixtures containing the notified chemical, if applicable, based on the concentration of the notified chemical present. As potency data were not available and the available hazard data indicated the chemical to have high reactivity, the notified chemical could be a skin sensitiser at or above 0.1% concentration.

Health Surveillance

- As the notified chemical is a skin sensitiser, employers should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of skin sensitisation.

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical:
  - Avoid contact with skin

- 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.

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.

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 final use concentration of the notified chemical is ≥ 0.1% in haircare products;
  - the notified chemical is to be used as a component of cosmetic products, other than for hair care;
  - additional toxicological information becomes available on the notified chemical, in particular, studies on genotoxicity or skin sensitisation;
  - the notified chemical has begun to be reformulated in Australia to be used in haircare products;

or

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

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

Safety Data Sheet

The SDS of the notified chemical, and products containing the notified chemical, provided by the notifier were reviewed by NICNAS. The accuracy of the information on the SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANTS
Privity Pty Ltd trading as Haircare Australia (ABN: 23 007 887 729)
17-21 Commercial Street
MARLESTON SA 5033

Sephora Australia Pty Ltd (ABN: 37 169 030 737)
Suite 3, Level 7, 133 Castlereagh Street
SYDNEY NSW 2000

NOTIFICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne per year)

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details exempt from publication include: specific other names, analytical data, impurities, additives/adjuvants, study references, import volume, and identity of manufacturer.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Schedule data requirements are varied for water solubility, hydrolysis as a function of pH, dissociation constant, flammability, oxidising properties, explosive properties, acute oral and dermal toxicity, and repeated dose toxicity.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANTS
None

NOTIFICATION IN OTHER COUNTRIES
EU (2018)

2. IDENTITY OF CHEMICAL

MARKETING NAME
Bis-aminopropyl diglycol dimaleate (INCI Name)

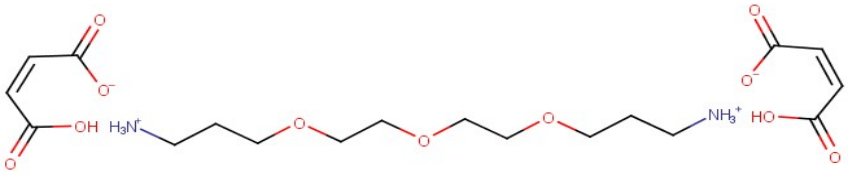
CAS NUMBER
1629579-82-3

CHEMICAL NAME
1-Propanamine, 3,3'-[oxybis(2,1-ethanediyloxy)]bis-, (2*Z*)-2-butenedioate (1:2)

OTHER NAME(S)
3-{2-[2-(3-azaniumylpropoxy)ethoxy]ethoxy}propan-1-aminium di[(2*Z*)-3-carboxyprop-2-enoate]

MOLECULAR FORMULA
$C_{10}H_{24}N_2O_3.2C_4H_4O_4$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
452.46 g/mol

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

3. COMPOSITION

DEGREE OF PURITY
> 99%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Clear transparent yellow liquid

Property	*Value*	*Data Source/Justification*
Melting Point	-25.4 °C	Measured
Boiling Point	Decomposes without boiling at > 175 °C	Measured
Density	1,180 kg/m^3 at 20 °C	Measured
Vapour Pressure	2.4×10^{-7} kPa at 25 °C	Measured
Water Solubility	Not determined	Expected to be readily soluble based on the chemical structure
Hydrolysis as a Function of pH	pH at 8 half-life: 11.1 hours pH at 7 half-life: 4.63 days	QSAR calculation
Partition Coefficient (n-octanol/water)	log Pow = -2.8 to -2.4 at 20 °C	Measured
Surface tension	72.8 mN/m at 20 °C	Measured
Adsorption/Desorption	log K_{oc} < 1.26 at 35 °C	Measured
Dissociation Constant	Not determined	Notified chemical is present in the ionised salt form
Flash Point	> 175 °C	Measured
Flammability	Not determined	Not expected to be highly flammable based on measured flash point
Autoignition Temperature	455 °C	Measured
Explosive Properties	Not determined	Contains no functional groups that imply explosive properties
Oxidising Properties	Not determined	Contains no functional groups that imply oxidative properties

DISCUSSION OF PROPERTIES
For 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 into Australia as a component of finished haircare products at < 0.1% concentration.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤ 50	≤ 50	≤ 50	≤ 50	≤ 50

PORT OF ENTRY
Adelaide, Brisbane, Sydney and Melbourne

TRANSPORTATION AND PACKAGING
Finished haircare products containing the notified chemical at < 0.1% concentration will be imported in containers suitable for retail sale (i.e. ≤ 500 mL plastic bottles or tubes).

USE
The notified chemical will be used as a component of haircare products at < 0.1% concentration, including shampoos, conditioners and hair styling products. The products will not be applied by spray application.

OPERATION DESCRIPTION
The notified chemical will be imported as a component of finished haircare products.

End-use
Finished haircare products containing the notified chemical at < 0.1% concentration will be used by consumers and professionals such as beauticians and hairdressers.

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 storage	4	12
Compounder	8	12
Chemist	3	12
Packers (dispensing and capping)	8	12
Store persons	8	12
Professional end users	8	365

EXPOSURE DETAILS

Transport and storage
Transport, storage and warehouse workers may come into contact with the notified chemical at < 0.1% concentration in finished haircare products, only in the unlikely event of an accidental rupture of containers.

End-use
Workers involved in professions which involve application of haircare products containing the notified chemical to clients (e.g. hairdressers and beauty salon workers) may be exposed to the notified chemical at < 0.1% concentration. The principal route of exposure will be dermal, while accidental ocular exposure is also possible. Such professionals may use some PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using the products containing the notified chemical.

6.1.2. Public Exposure
There will be widespread and repeated exposure of the public to the notified chemical at < 0.1% concentration through the use of haircare products containing it. The main route of exposure will be dermal, while accidental ocular exposure is also possible.

6.2. Human Health Effects Assessment
The results from toxicological investigations conducted on the notified chemical and an analogue chemical (Analogue 1) are summarised in the following table. For details of the studies, refer to Appendix B.

Endpoint	***Result and Assessment Conclusion***
Acute oral toxicity – rat*	LD50 > 2,000 and < 3,160 mg/kg bw; low toxicity
Acute dermal toxicity – rat*	LD50 > 2,150 mg/kg bw; low toxicity
Skin irritation – *in vitro* reconstructed human epidermis method	non-irritating at 26% concentration

Endpoint	*Result and Assessment Conclusion*
Eye irritation – *in vitro* bovine corneal opacity and permeability test	no prediction can be made at 26% concentration
Eye irritation – *in vitro* EpiOcular™ test	non-irritating at 26% concentration
Skin sensitisation – *in chemico* DPRA test	positive (in the highest reactivity class)
Skin sensitisation – *in vitro* ARE-Nrf2 luciferase test	positive
Skin sensitisation – *in vitro* U937 cell line activation test (U-SENS™)	positive
Repeat dose oral toxicity – rat, 28 days*	NOAEL (local) < 100 mg/kg bw/day in males and 100 mg/kg bw/day in females NOAEL (maternal/reproductive/development) = 600 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* micronucleus test in human lymphocytes	clastogenic

*studies on Analogue 1

Toxicokinetics
Given the relatively low molecular weight (452.46 g/mol) of the notified chemical, absorption across biological membranes may occur. However, dermal absorption may be limited based on low partition coefficient (log Pow = -2.8 to -2.4 at 20 °C).

Acute Toxicity
No acute toxicity data were submitted for the notified chemical. The acute toxicity of the notified chemical is estimated from information available on its two components, Analogue 1 and 2.

Analogue 1 is of low acute oral and dermal toxicity based on studies conducted in rats.

Analogue 2, however, was found to be harmful via the oral route (LD50 ~1,000 mg/kg bw in rats) and harmful to low toxicity via the dermal route (acute dermal toxicity LD50=1,600-2,600 mg/kg bw in rabbits) (OECD, 2004).

No acute inhalation toxicity data were provided on the notified chemical. The notified chemical's vapour pressure is low therefore inhalation exposure is not expected to be significant. Moreover, inhalation exposure to aerosols of the notified chemical is not expected under the proposed use.

Irritation and Sensitisation
In an *in vitro* skin irritation test using the EpiSkin™ reconstructed human epidermis model, the notified chemical at 26% concentration in water was determined not to be a skin irritant requiring hazard classification according to the test guideline.

In an *in vitro* bovine corneal opacity and permeability (BCOP) test, the *in vitro* irritancy score (IVIS) for the notified chemical at 26% concentration in water was 4.8, within in the range where no prediction can be made (i.e. > 3 and ≤ 55). However, in an *in vitro* eye irritation study using the EpiOcular™ cornea-like epithelial model, the notified chemical at 26% concentration in water was considered to be non-irritating to the eyes.

One *in chemico* and two *in vitro* cell based assays were conducted to evaluate the skin sensitisation potential of the notified chemical. The tests are part of Integrated Approach to Testing and Assessment (IATA) which address specific events of the Adverse Outcome Pathway (AOP) leading to development of skin sensitisation (OECD, 2016). The tests are thus considered relevant for assessment of the skin sensitisation potential of the notified chemical.

The *in chemico* direct peptide reactivity assay (DPRA) aims to address the first key event (KE) (molecular initiation) of the AOP by measuring the interaction of the notified chemical with cysteine and lysine, small synthetic peptides representing the nucleophilic centres in skin proteins. The ARE-Nrf2 luciferase assay aims to address the second key event (keratinocyte activation) of the AOP by measuring the expression of a reporter luciferase gene under the control of a promoter from the antioxidant response element (ARE), a responding gene known to be upregulated by contact sensitisers. The *in vitro* U937 cell line activation test (U-SENS™) aims to address the third key event (dendritic cell activation) of the AOP by measuring the expression of the CD86 cell surface marker in the U937 cell line upon stimulation with the notified chemical.

The notified chemical showed positive responses in all three tests of the AOP for skin sensitisation (DPRA assay, ARE-Nrf2 luciferase assay and U-SENS™ assay), suggesting potential for skin sensitisation. In the first KE assay (DPRA), the notified chemical showed that it belongs to the highest reactivity class for protein binding (with cysteine depletion of 92.3% at the concentration tested) according to the test guideline.

Two negative human repeat insult patch tests (HRIPTs) conducted in 2014 (n=50) and 2015 (n=50) were provided with the notification. The introducer indicated that the test formulations contained 3% and 20% of the notified chemical, respectively; however, there is uncertainty as to the exact test substance identity used in these studies. Given this uncertainty and the low number of subjects tested (< 100), the two HRIPTs were not considered as reliable to consider in the hazard assessment, to contradict the positive results obtained through the skin sensitisation AOP results available for the notified chemical.

Furthermore, Analogue 2 was reported to be a skin sensitiser with an EC3 value of 4.1% in a mouse Local Lymph Node Assay (LLNA) (Confidential, 2016).

Overall, based on the results of the AOP studies, the notified chemical is considered a skin sensitiser, requiring hazard classification. Although there is no potency data, the DPRA results indicated the notified chemical to have high potency.

Repeated Dose Toxicity
No repeated dose toxicity data were submitted for the notified chemical. The repeated dose toxicity of the notified chemical is estimated from information available on its two components, Analogue 1 and 2.

In a combined repeated dose oral toxicity study with the reproduction/developmental screening test (OECD TG 422), rats received Analogue 1 daily by oral gavage at doses of 100, 300 and 1,000 mg/kg bw/day (up to day 6 only) and 600 mg/kg bw/day (from day 7 onwards) for 59 days for males and 62 days for females.

The No Observed Adverse Effect Level (NOAEL) was established as < 100 mg/kg bw/day in males and 100 mg/kg bw/day in females based on local irritation effects, degeneration of squamous cells in the forestomach and inflammatory cell infiltrates in the glandular stomach. The NOAEL for maternal, reproductive and developmental toxicity was established as 600 mg/kg bw/day (no adverse effects at the highest dose tested). Statistically significant increased mean absolute kidney weight in high dose females and increased relative mean liver weight in mid dose females were reported. However, no associated histopathological changes were observed at necropsy.

For Analogue 2, the NOAEL for reproductive effects was determined to be 55 mg/kg bw/day (highest dose tested due to parental death at 150 mg/kg bw/day) (OECD, 2004). However, adverse effects in kidneys and bladder of parental animals (first generation only) were observed at all doses, and a low observed adverse effect level (LOAEL) of 20 mg/kg bw/day for parental effects was established. No developmental toxicity was observed when pregnant rats were dosed with maleic anhydride via gavage at 0, 30, 90 and 140 mg/kg bw/day. The dams in all dose groups either lost weight or failed to gain weight between days 6 and 9 of gestation, however, this effect was not statistically significant at any interval and was reversible. As a result, the NOAEL for maternal toxicity was determined to be 140 mg/kg bw/day (OECD, 2004).

Overall, based on the results for Analogue 1 and 2, the notified chemical may have potential for systemic toxicity at high concentrations with potential effects on kidney and bladder.

Mutagenicity/Genotoxicity
The notified chemical tested negative in a bacterial reverse mutation assay, however, in an *in vitro* micronucleus assay in human lymphocytes, the notified chemical showed equivocal results.

When human peripheral lymphocytes were exposed to the notified chemical, with or without metabolic activation, the following results were observed:

- in test 1, no statistically significant increase in mono- or binucleated cells with micronuclei, both with or without metabolic activation, at up to 900 µg/mL; however, the results of mid and high concentrations were 95% above the control limits of the distribution of historical negative control values.
- in test 1a, statistically significant increases in mono- or binucleated cells with micronuclei at dose levels of 900 µg/mL with metabolic activation and at ≥ 600 µg/mL without metabolic activation.
- in tests 1b and 1c, a statistically significant increase in the number of binucleated cells with micronuclei at ≥ 100 µg/mL, both with or without metabolic activation.

- in test 2 without metabolic activation, no statistically significant or biologically relevant increase in mono- or binucleated cells with micronuclei were observed at a concentration range of 10-150 µg/mL.

Based on the results of the two *in vitro* genotoxicity assays, clastogenicity potential of the notified chemical cannot be ruled out. As the notified chemical induced statistically significantly higher frequency of micronuclei in binucleated cells in test 1a and 1b, with or without metabolic activation, it may be a clastogenic compound. Therefore, further data, preferably on clastogenicity, are required to confirm the genotoxic potential of the notified chemical.

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

*potency data were not available to confirm the Category as 1A or 1B; however, the DPRA results indicated the notified chemical to have high potency (Category 1A).

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The notified chemical will only be imported in finished haircare products at up to 0.1% concentration. There will be no reformulation or repackaging of the notified chemical in Australia.

Hairdressers and beauty care professionals will handle the notified chemical at < 0.1% concentration, similar to public use. Such professionals may use PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. Therefore, the risk to workers who use products containing the notified chemical is expected to be of a similar or lesser extent than consumers who use such products on a regular basis. For details of the public health risk assessment see section 6.3.2 below.

6.3.2. Public Health
Members of the public may experience repeated exposure to the notified chemical through the use of haircare products containing the notified chemical at < 0.1% concentration.

Sensitisation
The notified chemical is considered be a strong sensitiser, however significant sensitisation effects are not expected from the use of products containing the notified chemical at the proposed low use concentration (< 0.1%) in haircare products.

Repeated dose toxicity
The repeated dose toxicity effects of the notified chemical have not been determined, however based on analogue chemicals the notified chemical may have potential for systemic toxicity at high concentrations. Given the low end use concentration (< 0.1%) and limited potential for dermal absorption based on physico-chemical properties, systemic toxicity effects are not expected under the proposed use.

Therefore, based on the information available, the risk to the public associated with use of the notified chemical at < 0.1% concentration in haircare products is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will not be manufactured or reformulated in Australia, therefore no release is expected from these activities. Any releases into the environment will be during accidental spills during transport of the products containing the notified chemical. Any accidental spills are to be collected and disposed of in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE
The majority of the notified chemical is expected to be washed into sewers as a part of its use in various haircare products, where it will be treated in sewage treatment plants nationwide before being released into surface waters.

RELEASE OF CHEMICAL FROM DISPOSAL
The notifier estimates that up to 3% of the notified chemical may remain as residues in empty product containers. These containers are expected to be either recycled or disposed of to domestic landfill. Collected wastes of the notified chemical will be disposed of by licensed waste contractors to eventually be disposed of to landfill.

7.1.2. Environmental Fate
Following its use in haircare products, the notified chemical is expected to be primarily released into sewers and treated at sewage treatment plants before release to surface waters nationwide.

A ready biodegradability study was conducted which determined that the notified chemical is not readily biodegradable, however it is expected to be inherently biodegradable (51% degradation after 28 days). For further details on the biodegradation study refer to Appendix C. The notified chemical is not expected to bioaccumulate due to its low log Pow (log Pow -2.8 to -2.4). Approximately 3% of the notified chemical may remain in the end use containers, which are either recycled or disposed of to landfill. In surface waters and landfill, the notified chemical is expected to eventually degrade into water and oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)
The use pattern will result in most of the notified chemical being washed into the sewer. The predicted environmental concentration (PEC) has been calculated assuming the realistic worst-case scenario with 100% release of the notified chemical into sewer systems nationwide over 365 days per annum. 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 this scenario, and therefore no removal of the notified chemical during sewage treatment processes, is assumed. The PEC in sewage effluent on a nationwide basis is estimated as follows:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import Volume	50,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	50,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	136.99	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	24.386	million
Removal within STP	0%	
Daily effluent production:	4,877	ML
Dilution Factor - River	1	
Dilution Factor - Ocean	10	
PEC - River:	28.1	µg/L
PEC - Ocean:	2.81	µg/L

7.2. Environmental Effects Assessment
The results from ecotoxicological investigations conducted on the notified chemical and its analogues (Analogues 3 and 4) are summarised in the table below. Details of these studies can be found in Appendix C. The fish toxicity endpoint for Analogue 3 is derived from modelled data (ECOSAR) and the fish toxicity endpoint for Analogue 4 is derived from several studies conducted under neutralised conditions (Confidential, 2004).

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	LC50 > 100 mg/L	Analogue 3 is not expected to be harmful to fish
Fish Toxicity	LC50 > 100 mg/L	Analogue 4 is not harmful to fish
Acute Daphnia Toxicity	EC50 > 80 mg/L	The notified chemical is harmful to daphnia
Algal Toxicity	EC50 = 96 mg/L	The notified chemical is harmful to algal growth
Inhibition of Bacterial Respiration	EC50 = 509 mg/L NOEC > 180 mg/L	The notified chemical is harmful to bacterial respiration

Based on the above ecotoxicological endpoints for the notified chemical and its analogue chemicals, the notified chemical is expected to be harmful to daphnia and algae. Therefore, the notified chemical is classified as Acute Category 3 (H402): Harmful to aquatic life according to the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009). The notified chemical is not readily biodegradable and is not expected to bioaccumulate. Therefore, the notified chemical is formally classified under the GHS for its long-term hazard as Chronic Category 3 (H412): Harmful to aquatic life with long lasting effects.

7.2.1. Predicted No-Effect Concentration

A Predicted No-Effect Concentration (PNEC) was calculated based on the most sensitive acute endpoint for daphnia (EC50 > 80 mg/L) using an assessment factor of 100 as three acute trophic endpoints are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Invertebrates).	80.00	mg/L
Assessment Factor	100.00	
Mitigation Factor	1.00	
PNEC:	800.00	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	***PEC µg/L***	***PNEC µg/L***	***Q***
Q - River:	28.1	800	0.035
Q - Ocean:	2.81	800	< 0.01

The risk quotient (Q=PEC/PNEC) has been calculated based on the worst-case assumption of complete release into the waterways with no removal in STPs. As the Q value is less than 1 the notified chemical is unlikely to reach ecotoxicologically significant concentrations. Therefore, on the basis of the PEC/PNEC ratio, the notified chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point -25.4 °C

Method	OECD TG 102 Melting Point/Melting Range
Remarks	Determined using differential scanning calorimetry (DSC)
Test Facility	Confidential (2018a)

Boiling Point Decomposes without boiling at > 175 °C

Method	OECD TG 103 Boiling Point
Remarks	Determined using DSC
Test Facility	Confidential (2018a)

Density 1,180 kg/m^3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids
Remarks	Pycnometer method
Test Facility	Confidential (2018a)

Vapour Pressure 2.4×10^{-7} kPa at 25 °C

Method	OECD TG 104 Vapour Pressure
Remarks	Effusion method
Test Facility	Confidential (2018a)

Partition Coefficient (n-octanol/water) log Pow = -2.8 at 20 °C (cation)
log Pow = -2.4 at 20 °C (anion)

Method	Equivalent to OECD TG 107 Partition Coefficient (n-octanol/water).
Remarks	Shake Flask Method. Test was conducted at pH 4.3.
Test Facility	Confidential (2018b)

Surface Tension 72.8 mN/m at 20 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions
Remarks	Concentration: 1 g/L
Test Facility	Confidential (2018a)

Adsorption/Desorption – screening test log K_{oc} = < 1.26 at 35 °C

Method	OECD TG 106 Adsorption – Desorption Using a Batch Equilibrium Method
Remarks	HPLC method. Test was conducted at pH 4
Test Facility	Confidential (2018b)

Flash Point > 175 °C

Method	EC Council Regulation No 440/2008 A.9 Flash Point
Remarks	Closed cup method. No flash point was observed below the temperature at which decomposition occurred.
Test Facility	Confidential (2018a)

Autoignition Temperature 455 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases)
Test Facility	Confidential (2018a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute Oral Toxicity – Rat

TEST SUBSTANCE	Analogue chemical 1
METHOD	Not specified
Species/Strain	Rat/Wistar
Vehicle	Distilled water
Remarks – Method	Test substance was administrated orally via gavage. Observations were made up to day 14.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	5 M/5 F	1,470	0/10
2	5 M/5 F	2,150	0/10
3	5 M/5 F	3,160	6/10

LD50	> 2,150 and < 3,160 mg/kg bw
Signs of Toxicity	Six (four males and two females) high dose animals were found dead on day 1 observation. Dyspnoea, apathy and staggering were observed in high dose males and females (number of animals with these symptoms not specified) at 1 hour to day 6 observations. In addition to these symptoms, high dose females (number of animals affected not specified) also showed wheezing at the 4 hour to day 2 observations.
Effects in Organs	Bleeding gastritis in glandular stomach and atonic, reddened diarrhoea content was observed in bowel during autopsy in the high dose dead animals.
Remarks – Results	Normal average body weight gain was observed in low (both sexes), mid (both sexes) and high dose males. Average body weight loss was observed in surviving high dose females in days 2 and 7.
CONCLUSION	Analogue 1 is of low acute toxicity via the oral route.
TEST FACILITY	Confidential (1984a)

B.2. Acute Dermal Toxicity – Rat

TEST SUBSTANCE	Analogue chemical 1
METHOD	Not specified
Species/Strain	Rat/Wistar
Vehicle	Not stated
Type of dressing	Semi-occlusive
Remarks – Method	

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	5 M/5 F	2,150	1/10

LD50	> 2,150 mg/kg bw
Signs of Toxicity	One male rat was found dead at the day 2 observation. Deep necrosis and oedema were observed in male and female animals on days 1-14. Dyspnoea, apathy, aggressiveness and staggering were observed in both sexes from days 2-9.

	Moderate erosion (in one male), and surface erosion and crust formation (in one male), were observed.
Effects in Organs	Severe oedema was observed in the lungs of the dead animal at necropsy.
Remarks – Results	Normal average bodyweight gain was observed in males, but bodyweight gain was reduced in females on day 6.
CONCLUSION	Analogue 1 is of low acute toxicity via the dermal route.
TEST FACILITY	Confidential (1984b)

B.3. Skin Irritation – *In Vitro* Reconstructed Human Epidermis Method

TEST SUBSTANCE	Notified chemical at 26% concentration in water
METHOD	OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method EPISKIN-SM™ model
Vehicle	Nil
Remarks – Method	No correction was made for the concentration of the test substance. The MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, thiazolyl blue] assay was used to determine cell viability Negative control: phosphate buffered saline Positive control: sodium dodecyl sulphate (5%)

RESULTS

Test Material	*Mean OD_{570} of Triplicate Tissues*	*Relative Mean Viability (%)*	*SD of Relative Mean Viability*
Negative control	0.880	100	1
Test substance	0.863	98	4.8
Positive control	0.137	16	8

OD = optical density; SD = standard deviation

Remarks – Results	The test substance was shown not to directly reduce MTT. The relative mean viability of the test substance treated tissues was 98% after a 15-minute exposure period. Since the mean relative tissue viability for the test substance was > 50%, the test substance is considered to be a non-irritant. The positive and negative controls gave satisfactory results, confirming the validity of the test system.
CONCLUSION	The notified chemical at 26% concentration was considered non-irritating to the skin under the conditions of the test.
TEST FACILITY	Confidential (2018c)

B.4. Eye Irritation – *In Vitro* Bovine Corneal Opacity and Permeability Test

TEST SUBSTANCE	Notified chemical at 26% concentration in water
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 (2017)

Vehicle	Nil
Remarks - Method	No correction was made for the concentration of the test substance. Negative control: physiological saline Positive control: ethanol

RESULTS

Test Material	*Mean Opacities of Triplicate Tissues*	*Mean Permeabilities of Triplicate Tissues*	*IVIS*
Negative control	1.4	-0.018	1.2
*Test substance**	2.8	0.136	4.8
*Positive control**	28	1.536	51

IVIS = *in vitro* irritancy score
*Corrected for background values

Remarks – Results	The IVIS for the test substance was 4.8 and was within > 3 and ≤ 55 (in the range where no prediction can be made). The positive control (ethanol) gave IVIS of 51 but this IVIS was within the mean values of the positive historical control data with two standard deviation. The negative control did not cause irritation to the corneas as expected.
CONCLUSION	No prediction on eye irritation could be made for the test substance
TEST FACILITY	Confidential (2018d)

B.5. Eye Irritation – *In Vitro* EpiOcular™ Cornea-like Epithelial Method

TEST SUBSTANCE	Notified chemical at 26% concentration in water
METHOD	OECD TG 492: Reconstructed Human Cornea-like Epithelium (RhCE) Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage (2015)
Vehicle	Nil
Remarks – Method	No correction was made for the concentration of the test substance. Positive and negative controls were run in parallel with the test substance: - negative control: Milli-Q water - positive control: methyl acetate The MTT assay was used to determine cell viability.

RESULTS

Test Material	*Mean OD_{570} of Duplicate Tissues*	*Relative Mean Viability (%)*
Negative Control	1.613 +/-0.086 SD	100
Test Substance	1.308 +/- 0.033 SD	81
Positive Control	0.078 +/- 0.078 SD	33

OD = optical density; SD = standard deviation

Remarks – Results	The test substance was shown not to directly reduce MTT. The relative mean tissue viability for the test substance as compared to the negative control was 81%. Given that the relative mean tissue viability for the test substance was > 50%, it is considered as a non-irritant.

	The positive and negative controls gave satisfactory results, confirming the validity of the test.
CONCLUSION	The notified chemical at 26% concentration was considered non-irritating to the eye under the conditions of the test.
TEST FACILITY	Confidential (2018e)

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

TEST SUBSTANCE	Notified chemical at 26% concentration in water
METHOD	OECD TG 442c *In Chemico* Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA) (2015)
Vehicle	Acetonitrile
Remarks – Method	The test substance was prepared in acetonitrile (100 mM stock solution). Cinnamic aldehyde (100 mM in acetonitrile) was used as positive control. The concentration of the stock solution prepared for testing was 72 mM, instead of 100 mM as required according to the test guideline, as an incorrect correction factor of 2.778 was used instead of 3.84. However, as the lower concentration indicated classification in the highest reactivity class according to the test guideline, this error had no impact on the test result.

RESULTS

Sample	*Cysteine Peptide Depletion (% ± SD)*	*Lysine Peptide Depletion (% ± SD)*
Vehicle Control	0.00*	0.00*
Test Substance	92.3 (±1.4)	0.0 (±0.0)
Positive Control	72.8 (±1.0)	60.8 (±0.9)

*normalised to 100%. SD = Standard Deviation

Remarks – Results	The mean depletion of peptides was 46.2% for the test substance, which is > 42.47%, indicating high reactivity (positive prediction for skin sensitisation). The positive and vehicle controls gave satisfactory results, confirming the validity of the test.
CONCLUSION	The notified chemical 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	Confidential (2017a)

B.7. Skin Sensitisation – *In Vitro* ARE-Nrf2 Luciferase Test

TEST SUBSTANCE	Notified chemical at 26% concentration in water
METHOD	OECD TG 442d *In Vitro* Skin Sensitisation Assays Addressing the AOP Key Event on Keratinocyte Activation (2015) - The ARE-Nrf2 luciferase KeratinoSens™ test method
Vehicle	Dimethyl sulfoxide (DMSO)
Remarks – Method	Positive control: ethylene dimethacrylate glycol Negative control: DMSO

Two independent tests were conducted with samples tested in triplicates in each test. The first test (conducted using a correction factor of 1.49 [inadvertently calculated the water content as 33% instead of 74%]) gave a positive response. The test was repeated at the correct correction factor of 3.85 (74% water content) and the test gave a negative result. As a result of inconsistent results, a third test was conducted at another test facility.

In test 3 the test substance was tested at 12 concentrations ranging from 0.38 to776 µM.

No significant deviations from the OECD test guideline.

RESULTS

Test 3 (main study)

Sample	*Concentration (µM)*	*Mean Cell viability (%)*	*Mean Luciferase Induction (%)*
Vehicle Control	-	100	1
Test substance			
	0.38	98.9	0.99
	0.76	101.8	1.06
	1.5	90.4	1.02
	3.0	92.5	1.14
	6.0	88,1	1.14
	12.0	87.1	1.1
	24.0	92.9	1.11
	49.0	93.6	1.13
	97.0	114.8	1.18
	194.0	112.9	1.34
	388.0	138.8	1.75*
	776.0	171.3	2.36*
Positive Control			
	7.8	96.2	0.82
	16.0	100.5	0.93
	31.0	97.6	1.08
	63.0	108.4	1.25
	125.0	112.5	1.72*
	250.0	116.3	3.17*

*Statistically significant increase ($p < 0.05$)

Remarks – Results

The viability of cells in the three tests were above 70% (no toxicity) and therefore IC30 and IC50 values were not calculated.

A biologically relevant, dose related induction of luciferase activity (EC1.5 = 269 µM) with a maximum induction of 2.36 fold was determined in test 3.

Test 1 was positive (EC1.5 = 757 µM) and test 2 was negative (EC1.5 = 1427 µM, no induction at < 1000 µM).

As positive responses (> 1.5 fold induction) were observed in 2 out of 3 tests at test concentrations of ≤ 1,000 µM with a cell viability of > 70%, the test substance was considered to be positive in this key event assay.

The positive and vehicle controls gave satisfactory results, confirming the validity of the test.

CONCLUSION	The notified chemical was positive in the second key event (keratinocytes response) of the adverse outcome pathway (AOP) for skin sensitisation as defined in the test guideline.
TEST FACILITY (TESTS 1 AND 2)	Confidential (2017b)
TEST FACILITY (TEST 3)	Confidential (2018f)

B.8. Skin Sensitisation – *In Vitro* Human Cell line Activation Test (U-SENS™)

TEST SUBSTANCE	Notified chemical at 26% concentration in water
METHOD	OECD TG 442e *In Vitro* Skin Sensitisation Assay Addressing the Key Event on Activation of Dendritic Cells on the Adverse Outcome Pathway for Skin Sensitisation *In Vitro* Skin Sensitisation (2015) - U937 Cell Line Activation Test (U-SENS™)
Vehicle	Dimethyl sulfoxide (DMSO)
Remarks – Method	A correction factor of 3.85 was used for the water content (74%) of the notified chemical. No dose range finding test was conducted. Two independent main tests were conducted to evaluate the ability of the test substance to induce expression of CD86 in the U937 cell line. The concentrations used were 1, 10, 20, 50, 100 and 200 µg/mL in the first test and 1, 5, 10, 50, 100 and 200 µg/mL in the second test. All assays were performed using two replicates. At the end of the incubation period, cells were stained with fluorescein isothiocyanate (FITC) labelled anti CD86 and mouse IgG1 antibodies (for measurement of nonspecific background signal). Vehicle control: Roswell Park Memorial Institute (RPMI) medium Negative control: lactic acid Positive control: 2,4,6-trinitrobenzenesulfonic acid The negative and positive controls were prepared in RPMI medium. No significant deviations from the OECD test guideline

RESULTS

Test 1

Sample	*Concentration (µg/mL)*	*CD86-IgG1 Stimulation Index*	*Colour Interference Stimulation Index*	*Relative Viability (%)*
Vehicle Control 1	-	80	103	99
Vehicle Control 2	-	101	102	99
Negative Control 1	200	104	105	99
Negative Control 2	200	95	112	99
Test substance				
Dose Level 1	1.0	125	105	99
Dose Level 2	10	254	111	98
Dose Level 3	20	266	114	98
Dose Level 4	50	573	134	96
Dose Level 5	100	706	160	92
Dose Level 6	200	790	190	79
Positive Control 1	50	600	153	97
Positive Control 2	50	691	155	97

Test 2

Sample	*Concentration (µg/mL)*	*CD86-IgG1 Stimulation Index*	*Colour Interference Stimulation Index*	*Relative viability (%)*
Vehicle Control 1	-	109	96	98
Vehicle Control 2	-	86	106	98

Sample	*Concentration (µg/mL)*	*CD86-IgG1 Stimulation Index*	*Colour Interference Stimulation Index*	*Relative viability (%)*
Negative Control 1	200	77	99	98
Negative Control 2	200	98	103	98
Test substance				
Dose Level 1	1.0	122	97	98
Dose Level 2	5	107	130	98
Dose Level 3	10	154	116	97
Dose Level 4	50	316	129	95
Dose Level 5	100	644	155	86
Dose Level 6	200	563	191	68
Positive Control 1	50	305	145	96
Positive Control 2	50	407	171	96

Remarks – Results

No precipitation was observed in both tests at the end of the incubation period.

In test 1, the test substance showed no toxicity. The cell viability (CV) was > 70% at all concentrations tested and therefore the CV70 values were not calculated (> 200 µg/mL).

In test 2, the calculated CV70 value was 182 µg/mL and therefore the test substance is considered to be toxic.

In both tests, the test item showed a biologically relevant increase in the expression of CD86 (EC150 was 2.8 µg/mL and 9.5 µg/mL in test 1 and test 2, respectively).

Colour interference by the test substance was observed in both tests at 100 and 200 µg/mL.

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

CONCLUSION

The notified chemical was positive in the third key event (dendritic cell activation) of the adverse outcome pathway (AOP) for skin sensitisation as defined in the test guideline.

TEST FACILITY

Confidential (2018g)

B.9. Repeat Dose Oral Toxicity – Rats

TEST SUBSTANCE

Analogue chemical 1

METHOD

OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test

Species/Strain

Rat/Wistar

Route of Administration

Oral – gavage

Exposure Information

Males: 59 days (2 weeks premating, during mating and ~ 4 weeks post-mating)
Females: 62 days (2 weeks premating, during mating, entire gestation period, 4 days of lactation and until 1 day before sacrifice).
Total exposure days: 59 days for males and 62 days for females
Dose regimen: 7 days per week
Post-exposure observation period: females and pups – up to postnatal day (PND) 4.

Vehicle

Water

Remarks – Method

Due to substantial bodyweight loss in several high dose (1,000 mg/kg bw/day) animals during first 6 days of treatment, these animals were

subsequently treated at a concentration of 600 mg/kg bw/day from day 7 onwards.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	10 M/10 F	0	0/20
Low Dose	10 M/10 F	100	0/20
Mid Dose	10 M/10 F	300	0/20
High Dose	10 M/10 F	1,000 (up to day 6) and 600 (from day 7 onwards)	0/20

A male treated at 600 mg/kg bw/day showed piloerection during week 2 and another male showed a blood discharge from nose on mating day 7. No other significant clinical findings were reported in treated animals.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There were some statistically significant clinical chemistry, haematology and urinalysis parameters (group mean values) observed in the treated males but the study authors considered these to be incidental and not treatment related. These included increase in mean blood urea levels in low, mid and high dose males; increase in phosphate levels in mid and high dose males and reduction in mean glucose levels and albumin levels in low and mid dose males.

Effects in Organs

Statistically significant increase mean absolute kidney weight in high dose female group and increase relative mean liver weight in mid dose female group were observed. However, no associated histopathological changes were observed at necropsy.

One low dose male showed enlarged kidneys and one low dose female showed dilated uterus.

Discoloured liver lobe was observed in two mid dose pups (in one male and female pup) and the study authors stated this effect was spontaneous in nature and not biologically relevant. Due to cannibalism, 2 pups from this dose group were not assessed.

Fundic area of the glandular stomach of some treated rats had hyperemia (excess of blood in vessel) in the mucosa (in 2 high, 1 mid and 1 low dose males) and submucosal oedema (2 of each sex at high dose, 3 males and 1 female at mid dose, and 2 males at low dose). These findings correlated with discolouration (in 3 high dose males) and thickening of walls (in one mid dose male and in 3 high dose males) of glandular stomach observed macroscopically. Three high dose males also showed thickening of duodenum walls. Increase thickness of the lamina muscularis of the duodenum was also observed in 5 high and 1 mid dose males.

The study authors considered these adverse findings were due to local irritating effects of the test substance.

Reproductive and developmental findings

Two sperm positive low dose females and one sperm positive mid dose female did not have implantation sites and did not deliver any pups. The male and female mating index was 100% for all dose groups. The male and female fertility indices were 100%, 80%, 90% and 100% for control, low, mid and high dose groups, respectively.

The gestation index was 100% in all groups. A control group female delivered one stillborn pup and the live birth indices were 99% for control group and 100% for all treatment groups.

One (on PND 0) and two (during PND 1-4) out of 113 pups were found dead in mid dose group and one pup found dead (PND 0-4) in control group. The viability index was 97% for mid and 99% for control groups. A 100% viability index was achieved for low and high dose groups.

CONCLUSION

The NOAEL for local effects was established as < 100 mg/kg bw/day for males and 100 mg/kg bw/day for females, based on the irritation effects observed in the digestive system of males and females.

The NOAEL for parental, reproductive and developmental toxicity was established as 600 mg/kg bw/day.

TEST FACILITY	Confidential (2013)

B.10. Genotoxicity – Bacteria

TEST SUBSTANCE	Notified chemical at 26% concentration in water
METHOD	OECD TG 471 Bacterial Reverse Mutation Test Plate incorporation (tests 1 and 2) and pre incubation (test 3) procedures
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98 and TA100 *Escherichia coli*: WP2uvrA
Metabolic Activation System	S9 mix from Aroclor 1254 induced rat liver
Concentration Range in Main Test	Test 1 a) With metabolic activation: 41, 129, 403, 1,259, 3,935 and 5,000 µg/plate b) Without metabolic activation: 41, 129, 403, 1,259, 3,935 and 5,000 µg/plate Test 2 a) With metabolic activation: 52, 164, 512, 1,600 µg/plate b) Without metabolic activation: 52, 164, 512, 1,600 µg/plate
Vehicle	Water (Milli-Q)
Remarks – Method	A correction factor of 3.85 was used for the water content (74%) of the test substance. A preliminary toxicity test was conducted using 0.44, 1.4, 4.4, 14, 43, 133, 416, 1,299, 3,935 and 5,000 µg/plate concentrations in strains TA100 and WP2uvrA. The two highest concentrations (3,935 and 5,000 µg/plate) tested in the preliminary toxicity test were reported (test 1) as part of the main study. Negative control: Milli-Q water Positive control: with S9-mix: 2-aminoanthracene (TA98, TA100, TA1535, TA1537 and WP2uvrA) without S9-mix: sodium azide (TA1535), 2-nitrofluorene (TA1537 and TA98), methylmethanesulfonate (TA100). 4-nitroquinoline N-oxide (WP2uvrA) and 2-methoxy-6-chloro-9-(3-(2-chloroethyl)aminopropylamino)acridine (ICR-191) (TA1537).

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 1,299	> 5,000	> 5,000	Negative
Test 2		> 1,600	> 5,000	Negative
Present				
Test 1	>1,299	> 5,000	> 5,000	Negative
Test 2		> 1,600	> 5,000	Negative

Remarks – Results	No relevant increase in the number of revertant colonies of any of the tested strains were observed following treatment with the test substance at any dose level, with or without metabolic activation, in the three mutation tests. The positive controls induced a distinct increase of revertant colonies during the study indicating the validity of the test system.

CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Confidential (2018h)

B.11. Genotoxicity – *In Vitro* Mammalian Cell Micronucleus Test

TEST SUBSTANCE	Notified chemical at 26% concentration in water
METHOD	OECD TG 487 Mammalian Cell Micronucleus Test (July 2016)
Species/Strain	Human
Cell Type/Cell Line	Peripheral lymphocytes
Metabolic Activation System	Rat liver
Vehicle	Water
Remarks – Method	A correction factor of 3.85 was used for the water content (74%) in the test substance. Negative control: water Positive control: without metabolic activation: mitomycin C and colchicine with metabolic activation: cyclophosphamide Concentration, stability, and homogeneity of test substance formulations were not determined in the study. Preparations were visually inspected for homogeneity prior to use and all preparations were used within 4 hours after preparation of the formulation. In a preliminary dose range finding test, human peripheral lymphocytes were treated with the test substance at 0 (solvent, pH = 7.5), 125, 250, 500, 1000 and 2,000 µg/mL (pH = 5.9) for 3 and 24 hours without metabolic activation, and for 3 hours with metabolic activation. In the first test (test 1), the test substance did not induce a statistically significant increase in the number of mono- and binucleated cells with micronuclei. The results of intermediate and high concentrations tested, however, are outside the 95% upper control limits of the historical control negative data range. Therefore an additional test (test 1b) was conducted. The pH of the test substance in test 1a was lower (6.5 at 1,000 µg/mL) than the solvent control (7.5). The pH of the test substance and the vehicle control was therefore adjusted to 7.7-7.8 by adding sodium hydroxide in test 1b.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0, 25*, 250, 500*, 600, 700, 800* and 900	3 h	27 h
Test 1a	0, 250, 500, 600*, 700*, 800*, 900 and 1,000	3 h	27 h
Test 1b	0, 100*, 300, 600*, 700, 800*, 900 and 1,000	3 h	27 h
Test 2	0, 1, 10*, 50, 75, 100*, 125* and 150*	24 h	24 h
Present			
Test 1	0, 25*, 250, 500, 600*, 700, 800 and 900*	3 h	27 h
Test 1a	0, 250, 500, 600, 700*, 800*, 900* and 1,000	3 h	27 h
Test 1b**	0, 100, 300, 600, 700, 800, 900 and 1,000	3 h	27 h
Test 1c	0, 100*, 500, 1000*, 1250, 1500*, 1750 and 2000	3 h	27 h

*cultures selected for metaphase analysis.
**cultures not suitable for metaphase analysis and therefore test 1c was conducted. The pH values were between 7.2 and 7.3 for test concentrations, and 7.3 for the solvent control.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation**	*Genotoxic Effect*
Absent				
Test 1	≥ 500	≥ 800	Not stated	Equivocal
Test 1a	≥ 125	≥ 800	Not stated	Positive
Test 1b		≥ 800	Not stated	Positive
Test 2		≥ 800	Not stated	Negative
Present				
Test 1	≥ 1,000	≥ 900	Not stated	Equivocal
Test 1a		≥ 900	Not stated	Positive
Test 1b		> 1,000	Not stated	Positive
Test 1c		≥ 1,500	Not stated	Positive

*No precipitation was observed at 2,000 µg/mL in the dose range finding study.

Remarks – Results

No statistically significant increase in mono- or binucleated cells with micronuclei, both with or without metabolic activation, was observed in test 1. However, the results obtained in mid and high doses, both with or without metabolic activation were 95% above the control limits of the distribution of historical negative control values.

In test 1a, without metabolic activation a statistically significant increase in mono- and binucleated cells was observed at concentrations ≥ 600 µg/mL (dose related increase was reported) and with metabolic activation at 900 µg/mL. The study authors stated that the mean number of mononucleated cells with micronuclei were within the 95% control limits of the distribution of the historical negative control and therefore not biologically relevant. However, the number of binucleated cells with micronuclei were above the 95% control limits of the distribution of the historical negative control. Therefore this increase is considered to be biologically relevant. The study authors further stated the pH of the test substance was lower than the negative control and the low pH, rather the test substance, may have increased the chromosome damage.

In Tests 1b and 1c (following pH correction), a statistically significant increase in the number of binucleated cells with mincronuclei was observed at ≥ 100 µg/mL, both with or without metabolic activation. For the number of binucleated cells with micronuclei, the test substance induced a statistically significant and dose dependent increase, which was considered to be biologically relevant since the results were outside the 95% control limits of the distribution of the historical negative control database. Statistically significant increase in the number of mononucleated cells with micronuclei was observed at ≥ 1,500 µg/mL without metabolic activation, but these increases were within the 95% control limits of the distribution of the historical negative control and therefore these increases were not considered to be biologically relevant. No statistically significant or biologically relevant increase in mono- or binucleated cells with micronuclei was observed in test 2, without metabolic activation, up to150 µg/mL.

The test substance induced the formation of micronuclei in binucleated cells in human lymphocytes.

Positive and negative controls performed as expected.

CONCLUSION

The notified chemical was clastogenic to human lymphocytes treated *in vitro* under the conditions of the test.

TEST FACILITY	Confidential (2019)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready Biodegradability

TEST SUBSTANCE	Notified chemical at 26% concentration in water
METHOD	OECD TG 301 B Ready Biodegradability: CO2 Evolution Test
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	LC-MS
Remarks – Method	Sodium acetate was used as a reference substance. A toxicity control was also conducted.

RESULTS

Test Substance		*Sodium acetate*		*Toxicity control*	
Day	*% Degradation*	*Day*	*% Degradation*	*Day*	*% Degradation*
2	0	2	2	2	1
8	25	5	33	5	20
15	37	8	51	8	36
23	44	12	64	12	49
29	51	15	73	15	58

Remarks – Results	All validity criteria were met. The reference compound (sodium acetate) reached the pass level by day 12, and the difference in extremes between replicates was < 3%. The inorganic carbon in test suspension was < 5% of total carbon and the total CO_2 evolution in the control sample was 19.3 mg/L. The toxicity test indicated that the test substance was not considered inhibitory as the control sample reached 58% degradation after 15 days.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	Confidential (2018i)

C.2. Ecotoxicological Investigations

C.2.1. Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE	Notified chemical at 26% concentration in water
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	180 mg $CaCO_3$/L
Analytical Monitoring	LC-MS
Remarks – Method	Based on a range finding test, concentrations were prepared from a stock solution. The stock solution was created using a correction factor of 3.85. All concentrations are expressed as the notified chemical. A reference test was run less than one month prior to the definitive study using potassium dichromate.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	-	20	0	0
2.2	2.19	20	0	0
4.6	4.44	20	0	0
10	9.82	20	0	0
22	22.9	20	0	0
46	46.3	20	0	0
100	99.5	20	7	10

EC50	> 80 mg/L at 48 hours
NOEC	46 mg/L at 48 hours
Remarks – Results	All validity criteria were met. Dissolved oxygen was maintained at > 8 mg/L, pH remained within 1.5 units in each test and temperature was maintained between 19 – 20 °C. The reference test showed an EC50 of 0.59 mg/L for potassium dichromate, which is within the expected range.

CONCLUSION The notified chemical is harmful to aquatic invertebrates

TEST FACILITY Confidential (2018j)

C.2.2. Algal Growth Inhibition Test

TEST SUBSTANCE Notified chemical at 26% concentration in water

METHOD	OECD TG 201 Alga, Growth Inhibition Test
Species	*Raphidocelis subcapitata* (formerly known as *Pseudokirchneriella subcapitata*)
Exposure Period	72 hours
Concentration Range	Nominal: 0.32 - 100 mg/L Actual: 0.13 - 115 mg/L
Auxiliary Solvent	None
Analytical Monitoring	LC-MS
Remarks – Method	Based on a range finding test, concentrations were prepared from a stock solution. The stock solution was created using a correction factor of 3.85. All concentrations are expressed as the notified chemical. A reference test was run less than one month prior to the definitive study using potassium dichromate.

RESULTS

Growth rate		*Yield*	
NOEC (mg/L)	*ErC50 (mg/L)*	*NOEC (mg/L)*	*EyC50 (mg/L)*
29	96	4.3	39

Remarks – Results	All validity criteria were met. The control cell density increased by a factor of 96, the mean coefficient of variation for section-by-section specific growth was 16% and the coefficient of variation for the average specific growth rates was 1.7%. The reference test showed an EC50 of 0.90 mg/L for potassium dichromate, which is within the expected range.

CONCLUSION The notified chemical is harmful to algal growth.

TEST FACILITY	Confidential (2018k)

C.2.3. Inhibition of Microbial Activity

TEST SUBSTANCE	Notified chemical at 26% concentration in water
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test
Inoculum	
Exposure Period	3 hours
Concentration Range	Nominal: 100 - 1000 mg/L
Remarks – Method	A stock solution was prepared using a correction factor of 3.85 and it was diluted to prepare the test concentrations. All concentrations are expressed as the active ingredient. A reference test was conducted using 3, 5-Dichlorophenol.
RESULTS	
EC50	509 mg/L
NOEC	180 mg/L
Remarks – Results	All validity criteria were met. the oxygen uptake in the blank control was 29 mg/g of activated sludge, the coefficient of variation of oxygen in control replicates was 8.69% and the reference test showed an EC50 of 5.9 mg/L for 3, 5-Dichlorophenol, which is within the expected range.
CONCLUSION	The notified chemical is harmful to bacterial respiration
TEST FACILITY	Confidential (2018l)

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