File No: LTD/1704

January 2014

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Disubstituted Alaninamide

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment.

For the purposes of subsection 78(1) of the Act, this Public Report may be inspected at our NICNAS office by appointment only at Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/1704	Novozymes Australia Pty Ltd	Disubstituted Alaninamide	Yes	≤0.4 tonne/s per annum	Enzyme stabiliser in liquid laundry and dishwashing detergents

*ND = not determined

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard classification	*Hazard statement*
Skin Sensitisation (Category 1)	H317: May cause an allergic skin reaction

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

R43: May cause skin sensitisation by skin contact

Human health risk assessment
Under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

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 and the reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

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

- The following should be used for products/mixtures containing the notified chemical:
  - Con. ≥ 1%: H317

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

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 isolation and engineering controls to minimise occupational exposure to the notified chemical during reformulation process:
  - Enclosed automated processes, where possible

- 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 process:
  - Avoid contact with skin and eyes
  - Clean up any spills promptly

- 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 process:
  - Impervious gloves
  - Goggles
  - Protective clothing

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

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

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

Public Health

- The following measures should be taken to minimise public exposure to the notified chemical:
  - Adequately label the products containing the notified chemical to avoid contact with skin.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

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

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the

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

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

(1) Under Section 64(1) of the Act; if
- the importation volume exceeds one tonne per annum notified chemical;
- the concentration of the notified chemical in automatic dishwashing detergents or laundry detergents is intended to exceed 0.01%;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from an enzyme stabiliser in liquid laundry detergents and automatic dishwashing detergents, 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;
- the method of manufacture of the chemical in Australia has changed, or is likely to change, in a way that may result in an increased risk of an adverse effect of the chemical on occupational health and safety, public health, or the environment;
- additional information has become available to the person as to an adverse effect of the chemical/polymer on occupational health and safety, public health, or the environment.

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

(Material) Safety Data Sheet

The (M)SDS of the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Novozymes Australia Pty Ltd (ABN: 48 001 420677)
3/22 Loyalty Road
North Rocks NSW 2151

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: chemical name, CAS number, molecular and structural formulae, analytical data and purity/impurities.

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, adsorption/desorption and dissociation constant.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
US EPA (2012)
ECHA (2013)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Disubstituted alaninamide

MOLECULAR WEIGHT
427.5 Da

ANALYTICAL DATA
Reference spectra and reports for 1H and 13C NMR, HPLC, MS, UV-VIS, ICP-OES and Anion analysis were provided.

3. COMPOSITION

DEGREE OF PURITY
>70%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Solid, off white

Property	Value	Data Source/Justification
Melting Point/Freezing Point	133-143 °C (with decomposition)	Measured
Boiling Point	Not determined	Substance decomposes at melting
Density	1,170 kg/m^3 at 20 °C (Relative density 1.17)	Measured
Vapour Pressure	2 x 10^{-9} kPa at 25 °C	Measured
Water Solubility	1.4 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	The notified chemical contains hydrolysable functionality. However, no significant hydrolysis is expected to occur in the environmental pH range of 4 – 9.

Partition Coefficient (n-octanol/water)	log Pow = 1.8.at 20 °C	Measured
Surface tension	61 mN/m at 20 °C	Measured
Adsorption/Desorption	Not determined	Expected to be mobile in soil based on its high water solubility and low potential to partition to the organic phase (log Pow = 1.8).
Dissociation Constant	Not determined	The notified chemical contains phenolic functionalities with typical pKa of ~9. It is expected to be ionised in the environmental pH range (4–9).
Particle Size	Inhalable fraction (< 100 μm): <16.4 % Respirable fraction (< 10 μm): 0 %	Measured
Flash Point	Not determined	The notified chemical is a solid.
Solid Flammability	Not highly flammable	Measured
Autoignition Temperature	>400 °C	Measured
Explosive Properties		Not expected to be explosive based on structure.
Oxidising Properties		Not expected to be oxidising based on structure.

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

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as a component of enzyme products at a concentration of ≤0.5%.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	0.4	0.4	0.4	0.4	0.4

PORT OF ENTRY
Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
Novozymes Australia Pty Ltd

TRANSPORTATION AND PACKAGING
The product containing the notified chemical (at up to 0.5% concentration) will be imported in 1000 kg immediate bulk containers and 25 kg jerry cans and distributed within Australia by road.

USE
The notified chemical will be used at up to 0.01% concentration in heavy duty liquid laundry detergents and automatic dishwashing detergents, as an enzyme stabiliser.

OPERATION DESCRIPTION

Repackaging

The liquid enzyme product containing the notified chemical (at up to 0.5% concentration) may be repackaged by plant operators under local exhaust ventilation.

Reformulation
The liquid enzyme product containing the notified chemical (at up to 0.5% concentration) is weighed and blended by plant operators under local exhaust ventilation, during the formulation of heavy duty liquid laundry detergents and automatic dishwashing detergents. Blending may also be automated. The detergents will be packaged either in bottles or in plastic pouches of manageable size for end-users.

End-use
The finished liquid detergent containing the notified chemical at up to 0.01% will be used by consumers and professional workers such as commercial laundry workers. The end-use products will be dispensed into laundry and dish washing machines. Users will either pour the detergent or will add a plastic pouch containing the detergent into the washing machine or dishwashing machine. It is possible that consumers may also use the liquid laundry detergent to manually hand-wash clothing.

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	4	30
Distribution	4	30
Warehouse	4	30
Plant operation	6-8	240

EXPOSURE DETAILS
Transport and storage
Transport and storage workers may come in contact with the notified chemical as a component of liquid enzyme products at up to 0.5% concentration only in the event of accidental rupture of containers.

Repackaging and reformulation
During reformulation of the notified chemical to heavy duty liquid detergents and automatic dish washing detergents, dermal, ocular and inhalation exposure of workers to the notified chemical in liquid enzyme products may occur during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. Exposure is expected to be minimised through the use of mechanical ventilation and/or enclosed systems and through the use of personal protective equipment (PPE) such as coveralls, safety glasses and impervious gloves. Controls are expected to be in place to minimise inhalation exposure to the enzyme, which has a workplace exposure standard of 60 ng/m^3.

End-use
Commercial laundry workers may be exposed to a maximum of 0.01% of notified chemical when handling the laundry liquid containers and adding the detergent to the washing machine. The main route of exposure will be dermal and ocular. Inhalation exposure is not expected, as the notified chemical will be part of a liquid mixture, and generation of aerosols is not expected. Precautions to reduce worker exposure in commercial laundries are likely to be based on the other ingredients of the laundry detergents.

6.1.2. Public Exposure
Incidental dermal and ocular exposure of the public to liquid laundry and dish washing detergents containing up to 0.01% of notified chemical may occur through spills and splashes. It is expected that any spilt material would be washed from the skin. Public exposure is expected to be minimal if the detergents are pre-packaged in pouches, unless this packaging is breached.

In addition, household consumers carrying out laundry hand washing have potential for dermal and accidental ocular exposure to the diluted detergent containing very low levels of the notified chemical (< 0.0001%). The

public may also come into incidental contact with wash water containing the laundry or dish washing detergents at low dilutions.

Significant exposure to the notified chemical from washed clothing/linen and dishes/cutlery is not expected to occur as the chemical is of very low concentration (up to 0.01% in detergent), further diluted in the wash, and is expected to be rinsed from the washed articles prior to drying.

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
EPISKIN reconstructed human epidermis – in vitro skin irritation	not classifiable as a skin irritant
Bovine cornea – in vitro serious eye damage /eye irritation	not classifiable for serious eye damage/eye irritation
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
Mutagenicity – bacterial reverse mutation	non mutagenic

Toxicokinetics, metabolism and distribution.
The potential for dermal and systemic (via GI tract or lungs) absorption of the notified chemical exists, due to its partition coefficient (log P_{ow} = 1.8 at 20°C) and molecular weight (<500).

Acute toxicity.
The notified chemical was of low toxicity via the oral route in a study in rats.

Irritation and sensitisation.
No *in vivo* animal study reports on skin and eye irritation were provided. *In vitro* studies for irritation were conducted using the neat chemical. An EPISKIN test using reconstructed tissue indicated that the chemical is not classifiable for skin irritation. Similarly, the results of a bovine corneal opacity and permeability (BCOP) assay suggested that the notified chemical would not be classified for eye irritation or serious eye damage. BCOP focusses on corneal injury and does not consider conjunctival and iridial injuries. The potential for skin and/or eye irritation cannot be ruled out.

The notified chemical was a skin sensitiser in a local lymph node assay (LLNA) in mice, with reported stimulation indices of 3.9, 7.3 and 4.1 at 10, 25 and 50% concentration, respectively (EC_3 = 3.6%).

Mutagenicity/Genotoxicity.
The notified chemical was not mutagenic in a bacterial reverse mutation assay.

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

Hazard classification	***Hazard statement***
Skin Sensitisation (Category 1)	H317: May cause an allergic skin reaction

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase(s):
R43: May cause sensitisation by skin contact.

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The notified chemical was a skin sensitiser in a mouse local lymph node assay (LLNA). It may also have slight irritation potential, but not sufficient for classification. Workers at greatest risk of skin sensitisation are those exposed to the chemical at up to 0.5% in the enzyme solution, during formulation of detergent products.

During reformulation and repackaging, several control measures will be in place to reduce exposure to the enzyme, including compliance with an Occupational Exposure Level (OEL) of 60 ng/m^3. These would also reduce exposure to the notified chemical. Provided that control measures are in place to minimise worker exposure, such as automated processes and PPE, the risk to the health of workers from the use of the notified chemical is not considered to be unreasonable.

Commercial laundry workers will handle the notified chemical at a much lower concentration (≤ 0.01%), similar to public use. Therefore, the risk to these workers is expected to be similar to that experienced by members of the public who use such products on a regular basis. For details of the public health risk assessment see section 6.3.2.

6.3.2. Public Health

The notified chemical has skin sensitisation potential, based on a LLNA study in mice. It may also have slight irritation potential. During use of liquid laundry detergents and automatic dishwashing detergents, consumers may have incidental exposure to the chemical at low concentrations (≤ 0.01%). They may also have exposure to very low concentrations (≤ 0.0001%) during hand-washing of laundry. Exposure from washed and dried clothing/linen is not anticipated, as any chemical residues are expected to be removed during rinsing. The risk to consumers is considered low because of the low concentration of use, and the likelihood that spilt material would be washed from the skin.

Based on available information, the risk to the public associated with the notified chemical in the use of heavy duty liquid laundry detergents and automatic dishwashing detergents at ≤0.01% 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 is imported into Australia at a concentration of less than 1% in enzyme products. The imported products will be reformulated into liquid detergents at a concentration of less than 0.1%.

RELEASE OF CHEMICAL FROM USE

Given its use as an enzyme stabilizer in laundry and dishwashing detergents, the notified chemical is expected to be discharged to sewer via domestic waste water.

RELEASE OF CHEMICAL FROM DISPOSAL

Residue of the notified chemical in empty containers will share the fate of the container and will either be disposed of to landfill, or washed to sewer when containers are rinsed before recycling. Waste and expired material is expected to be disposed of to landfill.

7.1.2. Environmental Fate

The notified chemical will be blended into laundry and dishwashing products in Australia. Residues are likely to be blended with similar products, or otherwise may be discarded in landfill where it may be expected to have moderate solubility and slowly degrade. The notified chemical contained in detergent products will be released in a diffuse manner into the sewer when spent wash-water is released. The notified chemical is very slightly volatile (2×10^{-9} kPa at 25°C) and loss to the atmosphere is unlikely to be significant from sewers and the aquatic environment. The majority of the notified chemical is expected to remain in effluent and be released into surface water. It is classified as readily biodegradable and the notified chemical is not expected to bioaccumulate due to the low n-octanol/water partition coefficient (log Pow 1.8), despite its low molecular weight of < 1000 Da. In sewage treatment plants, the notified chemical may be partially associated with the sludge that will eventually be sent to landfill or used for bioremediation. In landfill or surface water, the notified chemical is expected to degrade slowly via biotic and abiotic pathways, forming water and oxides of carbon and nitrogen. For the details of the environmental fate studies please refer to Appendix C.

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 laundry and dishwashing detergents, it is assumed that 100% of the total import volume of the chemical is released to sewer on a nationwide basis over 365 days per year. For the worst case scenario it is assumed that none of the notified chemical will be removed during STP processes.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	400	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	400	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	1.10	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	0%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.24	µg/L
PEC - Ocean:	0.02	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.242 µg/L may potentially result in a soil concentration of approximately 1.61 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 8.077 µg/kg and 16.15 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*
Daphnia Toxicity	EC50 > 88.4 mg/L (actual); EC50 > 100 mg/L (nominal)	Not Harmful
Algal Toxicity	EC50 > 101 mg/L	Not Harmful

The notified chemical is not considered harmful to aquatic invertebrates or alga. 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 or chronic hazards.

7.2.1. Predicted No-Effect Concentration

The endpoint for the most sensitive species (*Daphnia*) from the reported results is used to calculate the Predicted no-Effect Concentration (PNEC). An assessment factor of 1000 was used as end points were provided for only two trophic levels. The lower limit of the acute toxicity endpoint for *Daphnia* was used because it provides the most conservative PNEC value.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Invertebrates).	> 88.4	mg/L
Assessment Factor	1000	
PNEC:	> 88.4	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.24	88.4	< 0.0027

Q - Ocean	0.024	88.4	< 0.00027

The Risk Quotients (Q = PEC/PNEC) for the worst case scenario have been calculated to be < 1 for the river and ocean compartments. Although the notified chemical may be released into waterways, it is unlikely to pose a risk to the aquatic environment given that it is not expected to bioaccumulate nor is it expected to be released at ecotoxicologically relevant concentrations. Therefore, on the basis of the PEC/PNEC ratio and the assessed use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 133-143 °C (with decomposition)

Method	OECD TG 102 Melting Point/Melting Range. EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks	Determined using the metal block method. The test substance decomposed at melting.
Test Facility	Huntingdon Life Sciences (2009g)

Density 1170 kg/m^3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids. EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks	Pycnometer method, using heavy distillate petroleum as displacement liquid. Relative density was calculated to be 1.17.
Test Facility	Huntingdon Life Sciences (2009g)

Vapour Pressure 2 x 10^{-9} kPa at 25 °C

Method	OECD TG 104 Vapour Pressure. EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks	Determined using a vapour pressure balance.
Test Facility	Huntingdon Life Sciences (2009g)

Water Solubility 1.4 g/L at 20 °C

Method	OECD TG 105 Water Solubility. EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks	Flask Method
Test Facility	Huntingdon Life Sciences (2009g)

Partition Coefficient (n-octanol/water) log Pow = 1.8.at 20 °C

Method	OECD TG 107 Partition Coefficient (n-octanol/water). EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	Flask Method. Test was conducted at pH 4 in order to examine the notified chemical in its non-ionised form (the notified chemical contains an ionisable phenolic functional group).
Test Facility	Huntingdon Life Sciences (2009g)

Surface Tension 61 mN/m at 20 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions. EC Council Regulation No 440/2008 A.5 Surface Tension.
Remarks	OECD harmonised ring method. Concentration tested: 1 g/L. As the result was > 60 mN/m, the test substance was considered not to be surface active.
Test Facility	Huntingdon Life Sciences (2009g)

Particle Size

Method OECD TG 110 Particle Size Distribution/Fibre Length and Diameter Distributions.

Range (μm)	*Mass (%)*
>400	54.5
400-12529.3	29.3
125-75	11.8
75-30	4.2
30-10	0.4

<10	0.0

Remarks Examined using sieve analysis.
Test Facility Huntingdon Life Sciences (2009g)

Solid Flammability Not highly flammable

Method EC Council Regulation No 440/2008 A.10 Flammability (Solids)
Remarks Determined using a test mould and an ignition source.
Test Facility Huntingdon Life Sciences (2009g)

Autoignition Temperature >400°C

Method EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids.
Remarks There was no significant exothermic reaction of the test substance. The small exotherm at approximately 130°C was consistent with the melting temperature with decomposition.
Test Facility Huntingdon Life Sciences (2009g)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method. EC Council Regulation No 440/2008 B.1 tris Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat / CD (Crl:CD 'SD')
Vehicle	Methylcellulose (1% m/v in water)
Remarks - Method	No control animals were included in the study.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
300a	3F	300	0/3
300b	3F	300	0/3
2000a	3F	2000	0/3
2000b	3F	2000	0/3

LD50	>2000 mg/kg bw
Signs of Toxicity	One animal treated at 300 mg/kg bw/day displayed salivation after dosing which disappeared after 30 minutes. Piloerection was observed in the first group (three animals) of animals treated at 2000 mg/kg bw/day four hours after dosing; this was resolved approximately six hours after dosing.
Effects in Organs	One animal treated at 300 mg/kg bw/day displayed an atrophic stomach at necropsy but no other abnormalities were observed in other animals.
Remarks - Results	All animals were considered to have achieved satisfactory bodyweight gains throughout the study.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Huntingdon Life Sciences (2009b)

B.2. Irritation – skin (in vitro) human reconstructed EPISKIN

TEST SUBSTANCE	Notified chemical
METHOD	The EPISKIN Skin Irritation Test $^{-42\ hours}$, performed according to draft OECD Guideline "*In vitro* skin irritation" 2008. This method is similar to OECD 439 adopted in July 2013
Vehicle	Distilled water
Remarks - Method	The test involves the application of test substance for 15 minutes to the EPISKIN three-dimensional human skin model. The chemical was ground and applied in powder form. The tissues were wetted with distilled water before application of the test substance.Cell viability was measured by mitochondrial dehydrogenase activity, assessed by the reduction of 3 -(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to a soluble, coloured formazan product (blue). Triplicate tissues (0.38 cm^2) dosed with the test substance and positive/negative controls were incubated in a maintenance medium for 42 hours before incubation with MTT for 3 hours. The formazan is extracted and the optical density of the extractant read at 540 nm. Irritant and non-irritant substances are identified by comparing the viability values with the negative control. A mean tissue viability of ≤50% classifies the test substance as an irritant (R38) and >50% classifies it as a non irritant, defined as not classified as

a skin irritant.

RESULTS

Test material	*Mean OD_{540} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	0.91	100.0	7.5
Test substance	0.88	97.0	4.4
Positive control	0.15	16.4	5.6

OD = optical density; SD = standard deviation

Remarks - Results: There was no change in the colour of the test substance/MTT solution or the water control/MTT solution after 3-hr incubation, precluding the possibility that the MTT was reduced by the test substance, instead of mitochondrial dehydrogenase activity.
The positive and negative controls gave satisfactory results confirming the validity of the test system.

CONCLUSION: The notified chemical was non-irritating to the skin (not classified as a skin irritant) under the conditions of the test.

TEST FACILITY: Huntingdon Life Sciences (2009f)

B.3. Irritation – eye (in vitro)

TEST SUBSTANCE: Notified chemical

METHOD: Similar to OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants (2009)

Vehicle: Sodium chloride (0.9% w/w in water)

Remarks - Method: The time between the excision of eyes from slaughtered animals to incubation of the mounted corneas was 4 hours and 16 minutes. The test substance and the positive control (imidazole) were tested at 20% (w/w) in 0.9% sodium chloride solution. The negative control used was 0.9% sodium chloride solution.

RESULTS

Test material	*Mean opacities of triplicate tissues (SD)*	*Mean permeabilities of triplicate tissues (SD)*	*IVIS (SD)*
Vehicle control	0.00 (1.00)	0.02 (0.01)	N/A
Test substance	0.33 (2.08)	0.03 (0.06)	0.70 (2.40)
Positive control	90.33 (5.51)	2.82 (0.51)	132.7 (2.6)

SD = Standard deviation; IVIS = in vitro irritancy score
N/A = not applicable

Remarks - Results: Throughout the assay the corneas were examined for irregularities and one cornea treated with the test substance was slightly opaque around the edge. For comparison, corneas treated with the positive control were very opaque and corneas treated with the negative control were clear.
The positive and negative controls gave satisfactory results confirming the validities of the test systems.
The study authors concluded that the test substance was not corrosive and not a severe irritant, based on the IVIS score of <55.1. An updated version of the OECD test guideline 437 (2013) states that IVIS values of ≤ 3 can be used as indications that the test substance would not be classified under the

UN GHS category 1.

CONCLUSION The notified chemical was non-corrosive and non-irritating (not classifiable) under the conditions of the test.

TEST FACILITY Huntingdon Life Sciences (2009i)

B.4. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)

Species/Strain Mouse / CBA/CA

Vehicle Dimethylformamide (DMF)

Remarks - Method The lymph nodes from each ear were pooled for each treatment group (pooled treatment group approach).
The highest concentration of the test substance (50% w/v in DMF) was selected based on the practicality of preparing suspensions for testing. A concurrent positive control study was run using hexyl cinnamic aldehyde (HCA) in DMF (25% v/v).

RESULTS

Concentration (%)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	254.0	
10 (w/v)	991.5	3.9
25 (w/v)	1847.4	7.3
50 (w/v)	1047.4	4.1
Positive Control (HCA)		
25 (v/v)	1076.6	4.2

Remarks - Results There were no deaths and no signs of systemic toxicity observed.
As the sensitisation index of 3 or more was recorded for all three concentrations tested, the notified chemical was considered to have the potential to cause skin sensitisation. Based on the results for the two lowest concentrations, the EC_3 value was calculated to be 3.6%.
The result for the concurrent positive control confirmed the validity of the test system.

Conclusion There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY Huntingdon Life Sciences (2009e)

B.5. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.
Plate incorporation procedure/Pre incubation procedure

Species/Strain *S. typhimurium*: TA1535, TA1537, TA98, TA100
E. coli: WP2uvrA (pKM101)

Metabolic Activation System Phenobarbital/5,6-benzoflavone induced rat liver S9

Concentration Range in Main Test	a) With metabolic activation:	50 - 5000 µg/plate
	b) Without metabolic activation:	50 - 5000 µg/plate
Vehicle	Dimethyl sulphoxide (DMSO)	

Remarks - Method A standard plate incorporation assay, with and without metabolic activation, was reported as Test 1. A pre-incubation assay (Test 2) was conducted as a variation of Test 1, using only five concentrations starting from 50 µg/plate.

RESULTS

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

Remarks - Results Toxicity was not seen up to a maximum dose of 5000 µg/plate.

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

TEST FACILITY Huntingdon Life Sciences (2009h)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Closed system oxygen consumption measuring apparatus to determine Biochemical Oxygen Demand (BOD). Automated respirometer was used to monitor the cumulative amount of oxygen consumed by the mixture.
Remarks - Method	Conducted according to the guidelines above with no significant deviations to the protocol and in compliance with GLP protocol.

Test substance		*Sodium benzoate*	
Day	*% Degradation (BOD)*	*Day*	*% Degradation (BOD)*
7	55	7	73
14	82	14	80
21	90	21	83
28	92	28	79

Remarks - Results	All validity criteria for the test were satisfied. The test substance was readily biodegradable in this test as it passed the criteria given in the guideline (i.e. > 60% degradation after 28 days reached within a 10 day period). Based on its carbon content, the results demonstrated that the test substance was degraded to 92% after 28 days.
CONCLUSION	The notified chemical is readily biodegradable.
TEST FACILITY	Huntingdon Life Sciences (2009d)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test - Static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 and 50 mg $CaCO_3$/L
Analytical Monitoring	pH, temperature, and dissolved oxygen
Remarks - Method	The test was conducted according to the guidelines above. No significant deviations from the test guidelines were reported.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*
Nominal	*Actual*	*20*	*48 h*
100	88.4	20	0

EC50	>88.4 mg/L at 48 hours
NOEC	>88.4 mg/L at 48 hours
Remarks - Results	All validity criteria for the test were satisfied. No immobilization or adverse effects on *Daphnia* were noted at a measured level of 88.4 mg/L (nominal concentration 100 mg/L).

According to test validity criteria, the results should be based on measured concentrations if the deviation from the nominal concentration is greater than 20%. However, the intended exposure concentration was adequately achieved (between 81 and 95% of its nominal value and maintained during the test (at 99% of the starting value). Therefore, the notified chemical is considered as not harmful to aquatic invertebrates at the nominal concentration of 100 mg/L.

CONCLUSION The notified chemical is not harmful to aquatic invertebrates.
TEST FACILITY Huntingdon Life Sciences (2009c)

C.2.2. 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: 4.27, 9.39, 20.7, 45.5 and 100 mg/L
Measured: 4.21, 7.69, 17.2, 36.8 and 84.9 mg/L
Auxiliary Solvent None
Water Hardness None reported
Analytical Monitoring Algal cell densities were measured at 24, 48 and 72 h using Coulter Z Series Particle Count and size analyzer.
The concentration of the notified chemical were measured using Liquid Chromatographic Mass Spectrometric method of analysis.
Remarks - Method The tests were conducted according to the guidelines above with no significant deviations from the protocol.

RESULTS

Biomass		*Growth*	
E_bC50 *mg/L at 72 h*	*NOEC mg/L*	E_rC50 *mg/L at 72 h*	*NOEC mg/L*
> 101	43.9	> 101	43.9
E_bC50	*NOEC*	E_rC50	*NOEC*

Remarks - Results All validity criteria for the test were satisfied.
CONCLUSION The notified chemical is not harmful to algae.
TEST FACILITY Huntingdon Life Sciences (2009a)

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