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September 2013

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Imidazolium compounds, 2-(C9-19 and C9-19-unsatd. alkyl)-1-[(C10-20 and C10-20-unsatd. amido)ethyl]-4,5-dihydro-1-Me, Me sulfates (INCI name: Quaternium-87)

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 Ageing, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of Sustainability, Environment, Water, Population and Communities.

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
STD/1451	L'Oreal Australia Pty Ltd	Imidazolium compounds, 2-(C9-19 and C9-19-unsatd. alkyl)-1-[(C10-20 and C10-20-unsatd. amido)ethyl]-4,5-dihydro-1-Me, Me sulfates (INCI name: Quaternium-87)	Yes	≤ 5 tonne/s per annum	Component of hair care products

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System 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 irritation (category 2)	H315 – Causes skin irritation.

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):
R38: Irritating to skin

The environmental hazard classification according to the *Globally Harmonised System for the 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*
Acute Category 2	H401, Toxic to aquatic life

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 assessed 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 Irritation (Category 2): H315 – Causes skin irritation

CONTROL MEASURES
Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical during reformulation processes:
  - Enclosed, automated processes, where possible

- 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 processes:
  - Avoid contact with skin and eyes

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical during reformulation processes:
  - Coveralls, goggles, impervious gloves

  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 should be considered by formulators to minimise public exposure to the notified chemical:
  - Take account of the stability of the notified chemical at different pHs
  - Take account of the skin irritation potential of the notified chemical
  - Take account of the surfactant nature of the notified chemical, that may enhance the absorption of other substances in the formulation

Disposal

- The notified chemical should be disposed of to landfill.

Storage

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

Emergency procedures

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

Regulatory Obligations

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

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

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

(1) Under Section 64(1) of the Act; if
- the concentration in hair care products exceeds 7.5%;
- the chemical is proposed to be used in products other than hair care cosmetics.

(2) Under Section 64(2) of the Act; if
- 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.

(Material) Safety Data Sheet
The (M)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 (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
L'Oreal Australia Pty Ltd (ABN: 40 004 191 673)
564 St Kilda Road
Melbourne VIC 3004

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: Some analogue trade names, molecular weight, analytical data, degree of purity, residual monomers, impurities, additives/adjuvants, specific use details, import volume, site of manufacture/reformulation and identity of manufacturer/recipients.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: All physical and chemical properties except water solubility, partition coefficient, adsorption desorption and dissociation constant and a variation to the schedule of data requirements was also claimed for all human health endpoints except eye irritation and bacterial reverse mutation. Finally a variation to the schedule of data requirements was also claimed for bioaccumulation.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

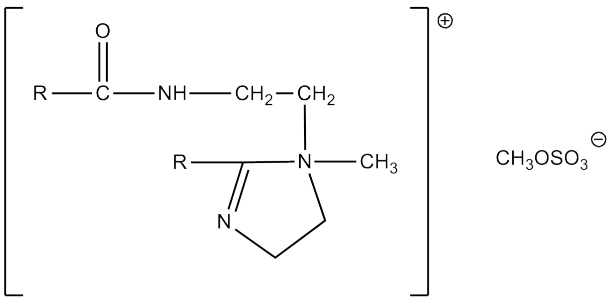
MARKETING NAME(S)
Varisoft 575 PG
Rewoquat W 575 PG (product containing 75% notified chemical)
Varisoft 575 PG N (product containing 75% notified chemical)

CAS NUMBER
92201-88-2

CHEMICAL NAME
Imidazolium compounds, 2-(C9-19 and C9-19-unsatd. alkyl)-1-[(C10-20 and C10-20-unsatd. amido)ethyl]-4,5-dihydro-1-Me, Me sulfates

OTHER NAME(S)
Quaternium-87 (INCI name)

STRUCTURAL FORMULA



R = palm oil derivatives.
An isomeric mixture exists in equilibrium where the double bond and the methyl group alternate between ring nitrogen atoms.

MOLECULAR WEIGHT
> 500 Da

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

3. IDENTITY OF ANALOGUES

Four analogues were provided for the notified chemical.

Analogue 1

This chemical has the same INCI name as the notified chemical and also is marketed under the same names. The structure of analogue 1 as described by its CAS name would essentially be a large subset of the chemicals possible under the name of the notified chemical as palm oil while predominantly C16 and C18 fatty acids also include fatty acids with other carbon chain lengths in lower ratios. In this assessment there are a number of studies where the test substance was not described using the CAS name or number and therefore it was not possible to determine whether it was analogue 1 or the notified chemical that was used in the study.

CAS NUMBER
98219-51-3

CHEMICAL NAME
Palm oil, reaction products with diethylenetriamine, di-Me sulfate-quaternized

Analogue 2

CAS NUMBER
86088-85-9

CHEMICAL NAME
Imidazolium compounds, 4,5-dihydro-1-methyl-2-nortallow alkyl-3-(2-tallow amidoethyl), Me sulfates.

AND

CAS NUMBER
68122-86-1

CHEMICAL NAME
Imidazolium compounds, 4,5-dihydro-1-methyl-2-nortallow alkyl-1-(2-tallow amidoethyl), methyl sulfates

OTHER NAME(S)
Quaternium-27 (INCI name)
Varisoft W75, Rewoquat W75 and Rewoquat W7500. (75% Quaternium-27, 25% Isopropanol)
Varisoft W90, Rewoquat W90 (90% Quaternium -27, 10% Isopropanol)
Varisoft W75 PG Rewoquat W75 PG. (75% Quaternium -27, 25% 1,2-propanediol)
Varisoft W90 PG, Rewoquat W90 PG. (90% Quaternium -27, 25% 1,2-propanediol)
BO493-01
Onium compounds, 4,5-dihydro-1-methyl-2-nortallow alkyl-1-(2-tallow amidoethyl) imidazolium, Me sulfates

Analogue 3

CAS NUMBER
91723-55-6.

CHEMICAL NAME
Imidazolium compounds, 4,5-dihydro-2-(hydrogenated nortallow alkyl)-1-[2-(hydrogenated tallow amido)ethyl]-1-methyl, methyl sulfates

OTHER NAME(S)
Quaternium-83 (INCI name)
Rewoquat W7500H (75% Quaternium -83, 25% Isopropanol). Uses Hydrogenated tallow in place of tallow as used in Rewoquat W7500 (75% Quaternium -83, 25% Isopropanol)
Onium compounds, 4,5-dihydro-2-(hydrogenated nortallow alkyl)-1-[2-(hydrogenated tallow amido)ethyl]-1-methylimidazolium Me sulfates

Analogue 4

CAS NUMBER
72749-55-4.

CHEMICAL NAME
Imidazolium compounds, 2-(C17 and C17 unsatd. Alkyl)-1-{2-(C18 and C18 unsatd. Amido)ethyl]-4,5-dihydro-1-methyl, Me sulfates

OTHER NAME(S)
Quaternium-72. (INCI name)
Rewoquat V 2815 (98% Purity)

4. COMPOSITION

DEGREE OF PURITY > 95%

5. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Colourless to yellow / beige paste (analogue 1)

Property	Value	Data Source/Justification
Melting Point/Freezing Point	76°C at 98.5-99.0 kPa	Measured (analogue 1)
Boiling Point	> 310°C at 98.5 to 99.0 kPa	Measured (analogue 1). Decomposed prior to boiling
Density	1037 kg/m^3 at 20.1°C	Measured (analogue 1)
Vapour Pressure	1.1×10^{-5} kPa at 25 °C	Measured (analogue 1)
Water Solubility	2.6×10^{-3} g/L at 20 °C	Measured.
Hydrolysis as a Function of pH	$t_{1/2}$ > 1 year at 25 °C, pH 4 $t_{1/2}$ = 2.5 days at 25 °C, pH 7 $t_{1/2}$ = 1.2 days, at 20 °C,pH 9	Measured (analogue 3)
Partition Coefficient (n-octanol/water)	log Pow > 6.5	Measured. The notified chemical is surface active and thus is expected to partition to phase boundaries.
Adsorption/Desorption	log K_{oc} > 5.63	Measured. The notified chemical is expected to sorb to soil sediment and sludge based on its surface activity.
Dissociation Constant	Not determined…	The notified chemical is a salt and will be ionised under the environmental conditions
Particle Size	Not determined	Chemical is a paste and imported in liquid mixtures
Flash point	Not determined	Decomposes before boiling and hence cannot vapourise to form a flammable mixture in the air.
Flammability	Not highly flammable	Measured (analogue 1)
Autoignition Temperature	Not auto-flammable	Measured (analogue 1)
Explosive Properties	Not expected to be explosive	Estimated (analogue 1)
Oxidising Properties	No oxidising properties	Measured (analogue 1)

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, however hydrolysis may occur at other than low pH.

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.

6. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported into Australia neat or as a component of formulated hair care products containing ≤ 7.5% notified chemical.

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

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

PORT OF ENTRY
Melbourne and Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
L'Oreal Australia Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemical or products containing the notified chemical (at ≤ 7.5% concentration) are generally shipped to Australia by sea in containers. The containers are transported from the wharf by road to central distribution centres or reformulators warehouses (if chemical is imported neat) and finally retailer's sites. The end use containers are predominantly HDPE plastic bottles or tubes of up to 500 mL in size.

USE
The notified chemical will be used in leave on and rinse off hair care products at ≤ 7.5% concentration.

OPERATION DESCRIPTION
The notified chemical will not be manufactured within Australia. The notified chemical may be imported neat for reformulation or in hair care products containing the notified chemical at a concentration of ≤ 7.5%.

Reformulation
If imported neat, the notified chemical will be weighed and added to the mixing tank where it will be blended with additional additives to form the finished personal hair care products. The mixing facilities are expected to be mostly automated, well ventilated (local exhaust ventilation) and use closed systems. After being reformulated, the finished products containing the notified chemical will be transferred into the retail packaging.

End use
The finished hair care products containing the notified chemical will be used by the public and may also be used occupationally by hairdressers and beauticians.

7. 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
Professional compounder	8	12
Chemist	3	12
Packers (dispensing and capping)	8	12
Store persons	4	12
Hairdressers	Unspecified	Unspecified

EXPOSURE DETAILS
Transport workers and store staff may come into contact with the neat notified chemical only in the event of an accidental rupture of containers.

During reformulation, exposure to the neat notified chemical may occur during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. The principal route of exposure would be dermal, while ocular and inhalation exposure are also possible. 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.

Hairdressers may be exposed to the notified chemical at ≤ 7.5% concentration when applying products containing it to clients. The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible, particularly if products are applied by spray or used in powder form. Such professionals may use some PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, dermal exposure of such workers is expected to be of either a similar or lesser level than that experienced by consumers using products containing the notified chemical.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemical (at ≤ 7.5% concentration) through the use of hair care products. The principal route of exposure will be dermal, while oral, ocular and inhalation exposure is also possible.

Data on typical use patterns of cosmetic product categories in which the notified chemical may be used are shown in the following table (SCCS, 2012). For the purposes of the exposure assessment, Australian use patterns for the various product categories are assumed to be similar to those in Europe. An adult bodyweight of 60 kg was used for calculation purposes. Based on the physical and chemical properties of the notified chemical and supported by the percutaneous absorption data on analogue 4, a dermal absorption of 10% was assumed for the notified chemical. The basis for this assumption is described in the toxicokinetics section below.

Product type	**Amount** (mg/day)	**C** (%)	**RF**	**Daily systemic exposure** (mg/kg bw/day)
Hair styling products	4000	7.5	0.1	0.05
Shampoo	10460	7.5	0.01	0.01
Hair conditioner	3920	7.5	0.01	0.00
Total				**0.07**

C - concentration; RF - retention factor.
Daily systemic exposure = Amount × C × RF × dermal absorption/body weight

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above table that contain the notified chemical. This would result in a combined internal dose of 0.07 mg/kg bw/day.

The notified chemical will also be used in hair dyes. Typical exposure to hair dyes includes application of 20 mg/cm^2 product to a scalp surface area of 580 cm^2 (SCCS, 2012). Given the proposed concentration of up to 7.5% in hair dye products, an amount of 870 mg notified chemical is estimated to be used per application. Using a retention factor of 0.1, body weight of 60 kg, dermal absorption of 10%, and frequency of use of one application per 28 days, the equivalent daily systemic exposure is 0.005 mg/kg bw/day.

The potential systemic exposure to members of the public from the combined use of rinse-off cosmetics and hair dyes is 0.08 mg/kg bw/day.

6.2. Human Health Effects Assessment

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

Endpoint	*Test substance*	*Result and Assessment Conclusion*
Rat, acute oral toxicity	Analogue 1 (100%)	LD50 > 2,000 mg/kg bw; low toxicity
Rat, acute oral toxicity	Analogue 2 (75%)	LD50 > 13,650 mg/kg bw; low toxicity
Rat, acute dermal toxicity	Analogue 1 (100%)	LD50 > 2,000 mg/kg bw; low toxicity
Skin irritation (in vitro- EpiDerm)	Analogue 1 (100%)	Non-corrosive
Rabbit, skin irritation	Analogue 1 (100%)	irritating
Rabbit, skin irritation	Analogue 3 (75%)	slightly irritating
Eye irritation (in vitro- HET-CAM)	Analogue 1 (100%)	non-irritating
Rabbit, eye irritation	Notified chemical (75%)	slightly irritating
Rabbit, eye irritation	Analogue 2 (75%)	slightly irritating
Guinea pig, skin sensitisation – adjuvant test	Analogue 1 (100%)	no evidence of sensitisation
Guinea pig, skin sensitisation – adjuvant test	Analogue 2 (75%)	no evidence of sensitisation
Guinea pig, skin sensitisation – adjuvant test	Analogue 2 (75%)	inadequate evidence of sensitisation
Mouse, skin sensitisation – Local lymph node assay	Analogue 1 (100%)	inadequate evidence of sensitisation
Rat, repeat dose oral toxicity – 90 days.	Analogue 2 (100%)	NOAEL = 94.1 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	Notified chemical (76.9%)	non mutagenic
Genotoxicity – in vitro mammalian cell gene mutation test	Analogue 4 (98%)	non genotoxic
Genotoxicity – in vitro mammalian chromosome aberration test	Analogue 2 (75%)	non genotoxic
Genotoxicity – in vitro mammalian chromosome aberration test	Analogue 1 (100%)	non genotoxic
Rat, developmental toxicity	Analogue 2 (75%)	NOAEL > 1,000 mg/kg bw/day

Toxicokinetics, metabolism and distribution.
Based on the moderately high molecular weight (> 500 Da) and the lipophilicity of the notified chemical (water solubility 2.6 × 10^{-3} g/L at 20 °C; log Pow > 6.5) dermal absorption is expected to be slow. However, it is noted that the notified chemical is surface active, which may affect dermal absorption of other compounds.

There is no toxicokinetic data available on the notified chemical, however there is a percutaneous absorption test conducted on a ^{14}C labelled version of analogue 4. In this study two groups of 5 or 8 female wistar rats had 200 mg solutions of 0.1% (group 1) or 0.5% (group 2) concentrations applied to 10 cm^2 of skin for 48 hours. At the end of the application < 0.51% of the radiolabelled analogue 4 had been absorbed through the skin in group 1 while 2-3% had been absorbed in group 2.

A conservative dermal absorption of 10 % was used in the exposure calculations in section 6.1.2, based on the physical and chemical properties of the notified chemical and supported by the percutaneous absorption data on analogue 4.

Acute toxicity.
There is no acute toxicity data available on the notified chemical. Analogues 1 and 2 were both of low toxicity via the oral route with analogue 1 also being of low toxicity via the dermal route.

Irritation and sensitisation.
The notified chemical has structural alerts for corrosion and sensitisation. There is no skin irritation or sensitisation data available on the notified chemical.

An *in vitro* skin irritation test with analogue 1 similar to OECD TG 431 showed no evidence of corrosion under the conditions of the test; however, analogue 1 was found to be irritating to the skin of rabbits. Also, in the acute dermal toxicity test with analogue 1 at a dose of 2,000 mg/kg, moderate to marked erythema, scaling, crusts and fissures were noted. In a skin irritation test on rabbits that was not conducted to OECD guidelines analogue 3 was estimated to be slightly irritating to the skin. Based on the skin irritation for analogue 1 in rabbits, and the structural alerts of the notified chemical, the notified chemical is considered to be irritating to the skin.

The notified chemical was slightly irritating to the eyes of rabbits in a study carried out to OECD TG 405. Analogue 2 was slightly irritating to the eyes of rabbits. An *in vitro* eye irritation test (non-validated) with analogue 1, using the Hen's Egg Test utilizing the Chorioallantoic Membrane (HET-CAM) method, showed no effects due to the test substance.

Analogues 1 and 2 were not skin sensitisers in guinea pig maximisation tests. In a second guinea pig maximisation test on analogue 2 and in a LLNA with analogue 1 no conclusion on the sensitising ability could be obtained due to inadequate test conditions. The available evidence indicates that the notified chemical is not a skin sensitiser.

Repeated Dose Toxicity.
There is no available repeated dose toxicity data on the notified chemical. In a 90 day oral toxicity study in rats with analogue 2 male animals treated with a nominal dose of 1,000 mg/kg bw/day and an actual dose of 939 mg/kg bw/day had significantly elevated serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase levels as well as significantly lower mean and absolute liver weights. These effects were considered to be adverse and hence the lower nominal concentration of 100 mg/kg bw/day (94.1 mg/kg bw/day actual) was selected as the NOAEL.

Mutagenicity/Genotoxicity.
The notified chemical was found to not be mutagenic using a bacterial reverse mutation test. Analogue 4 was not clastogenic to mouse lymphoma L5871Y cells *in vitro*, analogue 2 was not clastogenic to Chinese hamster ovary cells *in vitro* and analogue 1 was not clastogenic to human lymphocytes *in vitro*. The results do not indicate a concern for mutagenicity/genotoxicity for the notified chemical.

Toxicity for reproduction.
In a developmental toxicity study where analogue 2 was administered by gavage to rats during gestation days 6-15 there were no adverse treatment related effects noted and hence the NOAEL was considered to be the maximum dose of 1,000 mg/kg bw/day that was administered in this study.

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 irritation (category 2)	H315 – Causes skin irritation.

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):
R38: Irritating to skin

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Hairdressers will handle the notified chemical at ≤ 7.5% concentration in hair care cosmetic products, similar to public use. Therefore, the risk for beauty care professionals who regularly use products containing the notified chemical is expected to be of a similar or lesser extent than 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.

Compounders and laboratory staff involved in the formulation of hair care cosmetic products may come in contact with the neat notified chemical. Exposure is expected to be limited during product formulation by the engineering controls and PPE used, and the enclosed and automated processes. Under the proposed occupational settings and provided that formulation control measures are being adhered to, the notified chemical is not considered to pose an unreasonable risk to workers.

Based on the information available, the risk to workers associated with use of the notified chemical at ≤ 7.5% concentration in cosmetic products is not considered to be unreasonable.

6.3.2. Public Health

The general public will be repeatedly exposed to the notified chemical during the use of hair care products containing the notified chemical at up to 7.5% concentration.

Local effects

Based on the information available, the notified chemical is considered to be a skin irritant. However, as the notified chemical will be present in cosmetic products at concentrations ≤ 7.5%, skin and eye irritation effects are expected to be reduced. Adverse effects may be increased if the chemical is used at high concentrations, or used in leave-on products for application to the skin.

Systemic effects

The potential systemic exposure to the public from the use of the notified chemical in hair care cosmetic products was estimated to be 0.08 mg/kg bw/day. Using a NOAEL of 94.1 mg/kg bw/day, which was derived from a repeated dose toxicity study on analogue 2, the margin of exposure (MOE) was estimated to be 1,286. A MOE value greater than or equal to 100 is considered acceptable to account for intra- and inter-species differences; therefore, the MOE is considered to be acceptable.

As the notified chemical may also increase the dermal absorption of other components of cosmetic products, due to its surfactant nature, care should be taken when reformulating the notified chemical into the end-use products.

Therefore, based on the information available, the risk to the public associated with the use of the notified chemical at ≤ 7.5% in hair care products is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported into Australia as a component of finished cosmetic products or as a raw material to produce the final products. No significant release of the notified chemical to the environment is expected from reformulation or repackaging. Accidental spills or releases are expected to be collected and subsequently disposed of, most likely to landfill.

RELEASE OF CHEMICAL FROM USE

The notified chemical is a component of hair care cosmetic products, which are expected to be rinsed off and enter the sewer after use. Therefore, the total import volume of the notified chemical is expected to be discharged to sewer.

RELEASE OF CHEMICAL FROM DISPOSAL

Residues of the notified chemical in end-use containers for cosmetics (3%) are expected to share the fate of the

container and be disposed of to landfill, or to be washed to sewer when containers are rinsed before recycling.

7.1.2. Environmental Fate

The notified chemical is not readily biodegradable based on the performed biodegradation test. However, the reliability of the test is uncertain and the notified chemical has functionalities with the potential to biodegrade. A hydrolysis study conducted on a close analogue chemical indicates that the notified chemical has potential to hydrolyse under environmental conditions. Therefore, the notified chemical is not expected to persist in the environment. For the details of the environmental fate studies please refer to Appendix A and C.

The majority of the notified chemical is expected to be released to the sewage system after use. In waste water treatment processes at sewage treatment plants (STPs), the majority of the notified chemical is expected to be removed from influent by hydrolysis, or by partitioning to sludge based on its surface activity and cationic properties. Sludge from STPs containing the notified chemical and its hydrolysis products is expected to be disposed of to landfill or applied to agricultural soils. Notified chemical released into surface waters is expected to degrade or disperse in the aqueous environment and is not expected to bioaccumulate based on its surface activity. In sludge, soil and water, the notified chemical is expected to be degraded by abiotic and biotic processes to form water and oxides of carbon, nitrogen and sulphur.

7.1.3. Predicted Environmental Concentration (PEC)

The notified chemical will be released to sewers following its use in hair care cosmetic products. Therefore, under a worst case scenario, it is assumed that 100% of the total import volume of the notified chemical will be discharged into sewers nationwide over 365 days per year. Assuming no removal of the notified chemical in the sewage treatment processes for the worst case scenario, the resultant Predicted Environmental Concentration (PEC) in sewage effluent on a nationwide basis is estimated as follows:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	5,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	5,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	13.7	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	0%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1	
Dilution Factor - Ocean	10	
PEC - River:	3.03	□µg□L
PEC - Ocean:	0.30	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 3.03 µg/L may potentially result in a soil concentration of approximately 20.2 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 0.10 mg/kg and 0.20 mg/kg, respectively. However, the actual concentration is expected to be lower as the notified chemical is likely to hydrolyse under environmental conditions and has potential to adsorb strongly in sludge at STPs.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations are summarised in the table below. The daphnia and algae toxicity studies were conducted on water accommodated fractions (WAF) of the notified chemical. The fish toxicity study was conducted on analogue 1. The slight difference in alkyl chain length and branching between analogue 1 and the notified chemical is not expected to significantly alter their ecotoxcity profiles. Therefore, it is acceptable to predict the ecotoxcity for the notified chemical using the analogue results. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish	LC50 (96 h) = 9.84 -10.24 mg/L	At worst, toxic to fish
Daphnia	EL50 (48 h) = 3.7 mg/L WAF	Toxic to aquatic invertebrates
Algal	E_rL50 (72 h) = 4.8 mg/L WAF	Toxic to algae

The concentrations of the test item in the test solutions were measured throughout the duration of the daphnia and algae toxicity tests. However, the reliability of the measured data is questionable. The method used in the tests is not believed to be suitable to accurately determine the concentration of the test substance in solution because: 1) the test substance is cationic surfactant and it is expected to adsorb to surfaces, particularly those in the test vessel and potentially the membrane used to filter the solutions; 2) the test substance is a surface active substance capable of forming a dispersion in solution, filtration of the water accommodated fraction (WAF) is not considered the most appropriate approach to measuring the effects of the test substance; 3) potential hydrolysis products were not characterised and their concentrations are unknown. Therefore, it is unclear whether the test substance or its hydrolysis products are causing the observed toxicity.

Therefore, based on the uncertainty in the reliability of the measured data, the nominal loading rate was used as the best representative of the toxicity for the whole product under the conditions of the experiment.

The toxicity results reported in the above table are also consistent with the expected toxicity of other cationic surfactants. Therefore, based on weight of evidence approach, the nominal concentrations will be used for the purpose of classification under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009).

The available data indicates that the notified chemical is expected to be toxic to fish, toxic to aquatic invertebrates and toxic to algae on an acute basis and, therefore is formally classified as “Acute Category 2: Toxic to aquatic life” under GHS. Based on the expected lack of persistence in the environment, the notified chemical is not formally classified for long-term hazard.

7.2.1. Predicted No-Effect Concentration

The Predicted No-Effect Concentration (PNEC) has been calculated using the most sensitive species, daphnia (EL50 (48) = 3.7 mg/L). An assessment factor of 1000 was used as the fish toxicity was based on analogue data and the daphnia and algae toxicity were based on nominal concentrations determined in tests that may not be reliable.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Invertebrates).	3.70	mg/L
Assessment Factor	1,000	
PNEC:	3.70	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	***PEC µg/L***	***PNEC µg/L***	***Q***
Q - River:	3.03	3.7	0.82
Q - Ocean:	0.30	3.7	0.082

The Risk Quotients (Q = PEC/PNEC) for a worst case discharge scenario have been calculated to be 0.82 for the river. However, the actual risk is expected to be lessened as the notified chemical is expected to be efficiently removed during STP processes based on the likelihood that the notified chemical will sorb strongly to sludge and potentially hydrolyse.

The notified chemical is not expected to be readily biodegradable according to the test conducted but it has potential to hydrolyse in the environment. Based on its low water solubility and surface activity, the notified chemical is not expected to bioaccumulate. Therefore, on the basis of 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 76.0°C

Method OECD TG 102 Melting Point/Melting Range.
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks A thermal analysis test was conducted using a calorimeter and visual tests
Test Facility Harlan (2010a)

Boiling Point > 310°C at 98.5 kPa and 99.0 kPa

Method OECD TG 103 Boiling Point.
EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks The capillary method was used to determine the boiling point.
The test substance started to decompose prior to boiling.
Test Facility Harlan (2010a)

Density 1.037×10^3 kg/m^3 at 25 °C

Method OECD TG 109 Density of Liquids and Solids.
EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks A gas comparison pycnometer was used to determine the relative density.
Test Facility Harlan (2010b)

Vapour Pressure 1.1×10^{-5} kPa at 25 °C

Method OECD TG 104 Vapour Pressure.
EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks The vapour pressure was determined using a vapour pressure balance.
Test Facility Harlan (2010c)

Water Solubility 2.65×10^{-3} g/L at 22 °C

Method OECD TG 105 Water Solubility, column elution method and a slow stirring method.
Remarks Due to the cationicity of the test substance, the test substance adsorbed on the glass beads of the column. The column elution method was therefore not applicable for the determination of water solubility for the test substance. Therefore, a slow stirring method was performed according to the "Guidance on information requirements and chemical safety assessment, chapter R.7a: Endpoint specific guidance, 2008".

The test substance (notified chemical) forms an emulsion when mixed with water due to its surface activity. The pH for the test solutions was not reported in the test.
Test Facility Harlan (2010d)

Hydrolysis as a Function of pH

Method OECD TG 111 Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$*
4	25	> 1 year
7	25	2.5 days
9	20	1.2 days

Remarks A high performance liquid chromatographic method with tandem mass spectrometric detection (HPLC-MS/MS) was used for the quantitative analysis of the test substance, Analogue 3.

At pH 4.0, less than 10% hydrolysis was observed for the test substance at 50 °C. Therefore, the half-life of the test substance at 25°C was concluded to be > 1 year.

At pH 7.0, the half-life of the test substance was determined according to the model for pseudo-first order reactions.

At pH 9.0, the test substance decreased rapidly first and then became more stable but degradation was still observed at 50 °C and 60 °C. The half-life of the test substance at 20 °C was determined according to the model for pseudo-first order reaction.

Test Facility NOTOX (2010)

Partition Coefficient (n-octanol/water) log Pow > 6.5

Method OECD TG 117 Partition Coefficient (n-octanol/water).

Remarks HPLC Method. In the preliminary test, the log Pow of the test substance was estimated to be > 3.1 based on the individual solubilities in water and n-octanol. Therefore, the HPLC method was selected.

The retention time of the test substance is higher than the retention time of the reference item which has a log Pow = 6.5. Therefore, the log Pow of the notified chemical was reported to be > 6.5. However, due to the surface activity of the test substance and its cationic effects on the stationary phase, the HPLC method used here may not be applicable. Being a surfactant, the notified chemical is expected to partition to phase boundaries.

Test Facility Harlan (2010d)

Adsorption/Desorption log K_{oc} > 5.63

Method OECD TG 121 Adsorption - Desorption HPLC Method.

Remarks It was observed that the test substance (notified chemical) did not elute from the column under the test conditions. The retention time of the test substance is higher than the retention time of the reference item which has a log K_{oc} = 5.63. Therefore the determined log K_{oc} was reported to be > 5.63. However, due to the surface activity of the test substance and its cationic effects on the stationary phase, the HPLC method used here may not be applicable. The notified chemical is a cationic surfactant and expected to sorb strongly to sludge, sediment and surface.

Test Facility Harlan (2010e)

Flammability Not highly flammable

Method EC Council Regulation No 440/2008 A.10 Flammability (Solids)

Remarks The sample was ignited with the aim to determine the burning time over a distance of 200 mm if applicable. The sample melted slightly but could not be ignited with a flame during the preliminary test (contact time of about 2 minutes). After the experiment a yellow brown melt remained. Therefore, no main test was performed.

Test Facility Harlan (2010f)

Flammability Not flammable

Method EC Council Regulation No 440/2008 A.12 Flammability (Contact with Water).

Remarks Studies were performed in aqueous test systems. The test item did not show any spontaneous ignition or formation of flammable gases when in contact with water or humid air and shows no risk with respect to pyrophoric properties.

Test Facility Harlan (2010f)

Autoignition Temperature Not auto-flammable

Method EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids.

Remarks The test item was filled in a cube and heated in an oven. The temperature of the oven and the sample were continuously recorded while the temperature of the oven was increased to approximately 400 ºC.

Test Facility Harlan (2010g)

Explosive Properties Not explosive

Method EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks The exothermic decomposition energy was determined using Differential Scanning Calorimetry in a closed, gold plated high pressure vessel (DSC). The total decomposition energy was 67 J/g which is below the 500 J/g limit.
Test Facility Harlan (2010h)

Oxidizing Properties No oxidising properties

Method EC Council Regulation No 440/2008 A.17 Oxidizing Properties (Solids)
Remarks The test item and cellulose powder were mixed in various ratios and a pile of 250 mm length was ignited at one end. The maximum burning rate was compared with the highest burning rate of the reference mixtures, containing barium nitrate and cellulose in an optimal ratio (60% barium nitrate).
Test Facility Harlan (2010i)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Analogue 1 (100%)
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/HanRcc: WIST(SPF)
Vehicle	Corn oil
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	3 female	2,000	0/3
II	3 female	2,000	0/3

LD50	> 2,000 mg/kg bw
Signs of Toxicity	There were no unscheduled deaths during the study. There were no observed adverse clinical signs.
Effects in Organs	No adverse macroscopic findings were recorded at necroscopy.
Remarks - Results	The body weights were within the range commonly recorded for this strain and age of rats.

CONCLUSION	Analogue 1 is of low toxicity via the oral route.
TEST FACILITY	Harlan (2010j)

B.2. Acute toxicity – oral

TEST SUBSTANCE	Analogue 2 (75%)
METHOD	No test guideline was specified in the study report although protocols were similar to OECD TG 401 Acute Oral Toxicity.
Species/Strain	Rat/BOR: WISW (SPF TNO)
Vehicle	Test substance administered as supplied.
Remarks - Method	Animals were observed for 14 days after administration of the test substance.

RESULTS

Group	*Number and Sex of Animals*	*Dose mL/kg bw*	*Mortality*
I	10 per sex	15	0/20

LD50	> 15 mL/kg bw (13,650 mg/kg bw, based on a density of ~ 910 mg/mL)
Signs of Toxicity	There were no deaths or adverse clinical signs in any of the animals during the duration of the study.
Effects in Organs	No adverse macroscopic findings were recorded at necroscopy.
Remarks - Results	The body weights were within the range commonly recorded for this strain and age of rats.

CONCLUSION	Analogue 2 is of low toxicity via the oral route.
TEST FACILITY	IBR (1982a)

B.3. Acute toxicity – dermal

TEST SUBSTANCE	Analogue 1 (100%)
METHOD	OECD TG 402 Acute Dermal Toxicity. EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/HanRcc: WIST(SPF)
Vehicle	Corn oil
Type of dressing	Semi-occlusive.
Remarks - Method	The experiment was conducted at 2 different dose levels as at the original dose all the animals had to be euthanized on day 10 due to the severe irritation present. No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5 per sex	2,000	0/10*
II	1 per sex	200	0/2
III	4 per sex	200	0/8

LD50	> 2,000 mg/kg bw.
Signs of Toxicity - Local	Animals in the 2,000 mg/kg bw dose group had marked erythema present at the application site upon removal of the dressing, which gradually declined to moderate or slight erythema by the end of the study. Moderate to marked scaling and crusts was also noted on most animals from day 3, 4 or 5 up until they were euthanized. Fissures were noted in all male animals and 3 female animals for a number of days but were not present at day 10. Oedema was present in all animals but had cleared by day 6. Necrosis was noted in all the female animals and 2 males. Other signs of local toxicity included screaming at the removal of the dressing (all animals), haemorrhagic spotted sores (2 males) and water discharge from the wound (3 males and 1 female). Animals dosed with 200 mg/kg bw of the test substance showed moderate erythema upon removal of the dressing, and in 1 animal per sex this declined to slight erythema, with no signs of erythema by the end of the study. In 6 remaining animals slight erythema was still present at the end of the study and 2 female animals still had moderate erythema present at the end of the study. Slight to moderate crusts and slight to moderate scaling was observed in all animals and declined to be slight or to have resolved by the end of the study. Fissures were present in 2 animals per sex and in 2 of these animals it was still present at the end of the study. Oedema was present in 1 male on days 3 and 4 and 1 female on days 2 and 3.
Signs of Toxicity - Systemic	There were no signs of systemic toxicity observed in any of the test animals. Female animals in the 2,000 mg/kg bw dose group showed negligible weight gain over the first week of the study as did 1 female animal dosed with 200 mg/kg bw of the test substance.
Effects in Organs	No macroscopic findings were noted at necropsy at either dose.
Remarks - Results	Although all the animals in the 2,000 mg/kg bw dose group were euthanized on day 10 for ethical reasons due to severe irritant effects, there is no evidence that these animals would have died from effects caused by the test substance if they were allowed to survive to the end of the 14 day test period. This is supported by the lack of systemic effects noted at necroscopy in these animals.

CONCLUSION	Analogue 1 is of low toxicity via the dermal route.

TEST FACILITY Harlan (2010k)

B.4. Irritation – skin (in vitro)

TEST SUBSTANCE Analogue 1 (100 %)

METHOD Similar to OECD TG 431 In vitro Skin Corrosion - Human Skin Model Test –EpiDerm.

Vehicle Water

Remarks - Method The test substance (25 µL) wetted with 25 µL of water was applied to the tissues in duplicate. Following exposure periods of 3 minutes or 1 hour, the tissues were rinsed, treated with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolim bromide; 1.0 mg/mL] and then incubated at 37.5 °C for 3 hours.

Positive and negative controls were run in parallel with the test substance:
- Negative control (NC): water
- Positive control (PC): potassium hydroxide (8M)

RESULTS

Test material	*Test 1 (3 minute exposure period)*		*Test 2 (1 hour exposure period)*	
	Mean OD_{570} of duplicate tissues	*Relative mean viability (%)*	*Mean OD_{570} of duplicate tissues*	*Relative mean viability (%)*
Negative control	1.538	100	1.476	100
Test substance	1.705	110.9	1.501	101.7
Positive control	0.276	18.0	0.006	0.4

OD = optical density

Remarks - Results The positive and negative controls gave satisfactory results, confirming the validities of the test systems.

CONCLUSION Analogue 1 was non-corrosive to the skin under the conditions of the test.

TEST FACILITY RCC (2007)

B.5. Irritation – skin

TEST SUBSTANCE Analogue 1 (100%)

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Directive 2004/73/EC B.4 Acute Toxicity (Skin Irritation).

Species/Strain Rabbit/New Zealand White

Number of Animals 3 (2 female, 1 male)

Vehicle Water

Observation Period 14 Days

Type of Dressing Semi-occlusive.

Remarks - Method No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	3	3	3	3	> 14 days	1
Oedema	2.3	2.3	2	3	< 14 days	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results Well-defined erythema was seen in all animals 1 hour after removal of the dressing. This increased to moderate/severe erythema in all animals at the

24 – 72 hour observations before subsiding to very slight erythema at the 7 and 10 day observations. At the 14 day observation erythema was absent from 2 of the animals and only very slight in the remaining animal. At the 1 hour observation slight oedema was noted in 2 animals with moderate oedema in the other animal, this increased to moderate oedema in 2 animals at the 24 hour observation before subsiding to slight oedema in all animals at the 48 and 72 hour observations. At the 7 and 10 day observations very slight oedema was present in 2 of the animals with the remaining animal being free of oedema and at the 14 day observation all animals were free of oedema. Scaling was noted in all the animals from the day 7 observation and persisted until the end of the study.

There were no deaths or test substance-related clinical signs or remarkable body weight changes during the study period.

CONCLUSION Analogue 1 is irritating to the skin.

TEST FACILITY Harlan (2010l)

B.6. Irritation – skin

TEST SUBSTANCE Analogue 3 (75%)

METHOD Variation on OECD TG 404 Acute Dermal Irritation/Corrosion.

Species/Strain	Rabbit/New Zealand White
Number of Animals	6 male
Vehicle	Test substance administered as supplied.
Observation Period	72 hours
Type of Dressing	Occlusive
Remarks - Method	The test substance was applied and held in place by the dressing for 24 hours. The test substance was applied to both intact and scarified skin. Observations were only performed at 1 and 48 hours after the removal of the dressing. Bodyweight was not measured at the end of the test and clinical observations were not made.

RESULTS

Lesion	*Mean Score**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Erythema/Eschar	2	2	> 72 hours	2
Oedema	0.5	1	< 72 hours	0

*Calculated on the basis of the scores at 1 and 48 hours for ALL animals.

Remarks - Results The observed effects were identical in both the scarified and intact skin. Very slight oedema was observed 1 hour after the removal of the dressing but had cleared by 48 hours. Very visible redness was observed at both observations. There were no unscheduled deaths during the study.

CONCLUSION The number of observations and the duration of the observations in the test are not sufficient to allow for the classification of the test substance under the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. However, the effects that were observed during the limited study period were not sufficient for classification. Analogue 3 is slightly irritating to the skin.

TEST FACILITY Hazleton (1984)

B.7. Irritation – eye (in vitro)

TEST SUBSTANCE: Analogue 1 (100%)

METHOD: The Hen's Egg Test – Utilizing the Chorioallantoic Membrane (HET-CAM) Test. Modification of that described by Kemper and Luepke (1986).

Species/Strain: Chicken/Lohmann Selected Leghorn

Remarks - Method: In this HET-CAM test the test substance, negative and positive controls were applied to the chorioallantoic membrane (CAM) of eggs and observations for 300 seconds were taken. Amounts between 126 and 175 mg of the material being tested was applied to the CAM, with at least 25% of the CAM being covered. The three endpoints observed were time until haemorrhage, lysis and coagulation. The faster the effect is seen the stronger the irritating effect. Each reaction type can be recorded only once for each CAM, therefore the maximum score per CAM is 21. The mean score was determined for all CAMs similarly tested using the following equation:

$$R = \frac{(301-h)\times 5}{300} + \frac{(301-l)\times 7}{300} + \frac{(301-c)\times 9}{300}$$

h=time in seconds at which haemorrhages appear
l=time in seconds at which lysis first occurs
c=time in seconds in which coagulation firs

Positive controls used were aqueous solutions of 1% sodium dodecyl sulfate (SDS) and a 0.1 mol/L NaOH. The negative control was a 0.9% (v/v) solution of NaCl in water. Eggs were fertilised and incubated for 9 days at 37.5 ± 0.5°C prior to use. The test substance was tested using 6 eggs and positive and negative controls used 3 eggs each.

The HET-CAM assay has not yet been fully validated as a replacement test for the *in vivo* Draize test.

Results

Test material	Haemorrhage (s)	Lysis (s)	Coagulation (s)	Mean irritancy index
Negative control	> 300	> 300	> 300	0
Test substance	> 300	> 300	> 300	0
Positive control SDS	16.3	126.3	> 300	8.8
Positive control NaOH	11.3	50	16.7	19.2

Remarks - Results: There were no signs of haemorrhage, lysis and coagulation in any of the CAM treated with the test substance during the 300 second observation period. Effects were seen with the positive control substances.

CONCLUSION: Under the conditions of the test analogue 1 showed no effects on the hen chorioallantoic membrane.

TEST FACILITY: RCC (2008)

B.8. Irritation – eye

TEST SUBSTANCE: Notified chemical (75%)

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion.
EC Council Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation).

Species/Strain: Rabbit/New Zealand White

Number of Animals: 3 (2 female, 1 male)

Observation Period	7 Days
Remarks - Method	No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1.3	1.3	1.3	2	< 7 days	0
Conjunctiva: chemosis	1.7	2	1	2	< 7 days	0
Conjunctiva: discharge	0.3	0.3	0.3	1	< 48 hours	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results	A single application of 0.1 mL of the test material to the non-irrigated eye of three rabbits produced moderate conjunctival irritation in all animals. All treated eyes appeared normal at the 7 day observation. There were no effects from the test substance on the cornea or iris. There were no signs of systemic toxicity and bodyweight gains were with the normal range.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	Evonik (2010)

B.9. Irritation – eye

TEST SUBSTANCE	Analogue 2 (75%)
METHOD	Similar to OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	6
Observation Period	7 Days
Remarks - Method	Observations were recorded after 1, 2 and 8 hours and after 1, 2, 3, 4, 5, 6 and 7 days. There was no indication in the test report that clinical signs or bodyweight changes were measured.

RESULTS

Lesion	*Mean Score**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Conjunctiva: redness	0	1	< 24 hours	0
Conjunctiva: chemosis	0	0	-	0
Conjunctiva: discharge	0	0	-	0
Corneal opacity	0	0	-	0
Iridial inflammation	0	0	-	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for ALL animals.

Remarks - Results	A single application of 0.1 mL of the test material to the non-irrigated eye of 6 rabbits produced slight conjunctival redness in all animals at the 1, 2 and 8 hour observations. All treated eyes appeared normal at the 24 hour observation. There were no effects from the test substance on the cornea or iris.
CONCLUSION	Analogue 2 is slightly irritating to the eye.
TEST FACILITY	IBR (1981a)

B.10. Skin sensitisation (1)

TEST SUBSTANCE	Analogue 1 (100%)
METHOD	OECD TG 406 Skin Sensitisation – Maximisation Test. EC Directive 96/54/EC B.6 Skin Sensitisation - Maximisation Test.
Species/Strain	Guinea pig/albino Dunkin Hartley, CRL:(HA)BR, SPF, Charles River
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: < 0.1% topical: 1%
MAIN STUDY	
Number of Animals	Test Group: 10 Control Group: 5
INDUCTION PHASE	Induction Concentration: intradermal: 0.5% topical: 10%
Signs of Irritation	Discrete/patchy to moderate/confluent erythema was seen in all test animals at the 24 h and 48 h observations after topical induction, with scaling in 70% of the animals at 48 h. The expected irritation effects of FCA were seen in both control and test groups after intradermal induction.
CHALLENGE PHASE	
1st challenge	topical: 1%
Remarks - Method	The test substance was diluted in PEG300. Several pre-tests were carried out to determine a non-irritating concentration. At the lowest levels tested for the intra-dermal pre-test, mild irritation was still seen at the lowest concentrations tested (0.1% and 0.5%). A second epidermal challenge was not performed.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: 1st challenge*	
		24 h	*48 h*
Test Group	1% in PEG300	1/10	0/10
	PEG300	0/10	0/10
Control Group	1% in PEG300	0/5	0/5
	PEG300	0/5	0/5

Remarks - Results	No skin reactions were observed in the control animals treated with PEG300 alone or with the test item at 1%. Discrete patchy erythema was observed at the 24 h observation in 1/10 test animals when challenged with the test substance at 1%, but no skin reactions were seen in the test animals at 48 h. No reaction was seen in the test animals when treated with PEG300 only. A separate study on the positive control α-hexylcinnamaldehyde gave sensitising results, confirming the validity of the test system.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Harlan (2009a)

B.11. Skin sensitisation (2)

TEST SUBSTANCE	Analogue 2 (assumed 75%)
METHOD	Method similar to OECD TG 406 Skin Sensitisation – Maximisation Test.
Species/Strain	Guinea pig/Pirbright, HOE:DHPK(SPE-LAC) /Boe.

PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: - topical: 10%	
MAIN STUDY		
Number of Animals	Test Group: 15	Control Group: 15
INDUCTION PHASE	Induction Concentration: intradermal: 5% topical: 5%	
Signs of Irritation	None	
CHALLENGE PHASE		
1st challenge	topical: 2.5%, 5%, 10%	
Remarks - Method	The skin was pre-treated with 10% sodium lauryl sulphate in white vaseline prior to the epidermal induction. The solvent used for dilution and controls was distilled water. Methyl methacrylate was used as a positive control. A second epidermal challenge was not performed.	

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: 1st challenge*	
		24 h	*48 h*
Test Group	2.5%, 5% or 10%	0/15	0/15
Control Group	Distilled water	0/15	0/15

Remarks - Results	There were no skin reactions on epidermal challenge, either after treatment with 2.5%, 5% or 10% test substance, or in the distilled water negative control group. The positive control produced sensitising results in 15/15 animals after challenge with 2.5%, 5% or 10% methyl methacrylate.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	IBR (1981b)

B.12. Skin sensitisation (3)

TEST SUBSTANCE	Analogue 2 (assumed 75%)	
METHOD	Method similar to OECD TG 406 Skin Sensitisation – Maximisation Test.	
Species/Strain	Guinea pig/ Pirbright White (albino), male	
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: : <0.1% topical: < 10% (inconsistent information was provided regarding the irritation, or lack thereof, observed at 10% concentration)	
MAIN STUDY		
Number of Animals	Test Group:	Control Group:
INDUCTION PHASE	Induction Concentration: intradermal: 0.1% in liquid paraffin topical: 25% in liquid paraffin	
Signs of Irritation	Moderate redness of the skin of test and control animals was seen after epidermal induction. Slight to moderate redness of the skin of test and control animals was seen after intradermal induction.	
CHALLENGE PHASE		
1st challenge	topical: 10%	

Remarks - Method	The pre-test for irritation was carried out in liquid paraffin at 50%, 25% and 10% v/v for epidermal application, and 5%, 1%, 0.5% and 0.1% v/v for intradermal application. A second epidermal challenge was not performed. The challenge results were evaluated at 48 h and 72 h (rather than 24 h and 48 h in the OECD protocol)

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: 1st challenge*	
		48 h	*72 h*
Test Group		9/10	8/10
Control Group		5/5	4/5

Remarks - Results	The average irritation scores after challenge tended to reduce between 48 h and 72 h. The challenge results may be due to irritation rather than sensitisation, as similar effects occurred in test and control animals. The study authors state that the results are not indicative of sensitisation, but do not provide an explanation of the irritation scores.
CONCLUSION	On the basis of inadequate evidence, no conclusion can be made regarding the skin sensitising ability of the analogue chemical.
TEST FACILITY	Hazelton (1983a)

B.13. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Analogue 1 (100%)
METHOD	Variation on OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain	Mouse/CBA/CaOlaHsd
Vehicle	2-Butanone
Remarks - Method	No concurrent positive control group was added to the study. The challenge phase was delayed to day 21 due to swelling of the ears in animals treated with the test substance. The aim of the study was to show if the lymphocyte proliferation seen in a previous study was due to sensitisation or irritation. The animals were divided into 5 groups with 5 animals per group. The test substance at a 1.5% concentration was applied to 2 of the groups on 3 consecutive days with the remaining 3 groups (controls) receiving the vehicle only. On day 6, 1 group that had been treated with the test item and 1 control group were injected with ^{3}H-methyl thymidine and the euthanized 5 hours later and the proliferative responses measured. On day 21 one of the control groups and the group treated with the test item at 1.5% with the remaining control receiving the vehicle only. On day 23 the remaining 3 groups were injected with ^{3}H-methyl thymidine and the euthanized 5 hours later and the proliferative responses measured.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)*	564.0	1

1.5%*	6403.6	11.35
0 (vehicle control)	321.5	1
0†	2026.5	6.30
1.5%	2622.1	8.16

* The proliferative response of these 2 groups was measured on day 6 of the experiment.
† This group was only treated with the control during the induction phase of the experiment, but was challenged with the test substance at a concentration of 1.5%.

Remarks - Results

There was no mortality and no clinical signs of toxicity were observed in the test subjects. Measurements of ear thickness showed a strong increase (~30%) in animals that had been treated with the test substance and swelling did not start to decrease until day 16. The OECD TG 429 states that if excessive local skin irritation is indicated by an increase in ear thickness of ≥ 25% on any day of measurement during the pre-screen test the highest dose selected for the main LLNA study should be the next lower dose in the pre-screen concentration series that does not induce systemic toxicity and/or excessive local skin irritation. Therefore under current OECD guidelines the test should have been conducted at a lower concentration.

A stimulation index (SI) of 11.5 was determined for the treatment group sacrificed on day 6 after three applications of the test substance on consecutive days 1-3. After 1 application of the test substance on day 21 of the test substance a SI of 6.30 was measured on day 23, whilst the treatment group which was also challenged on day 21 had a SI of 8.16. The results show that a single application of the test substance at 1.5% is sufficient to induce lymphoproliferation in the draining lymph nodes.

The difference in the SI responses between the control and treatment groups (6.30 and 8.16 respectively) that both received an application of the test substances on day 21 was not large, suggesting that the lymphoproliferation observed was due to the irritating nature of the test substance rather than induction of a lymphocyte proliferative response indicative of skin sensitisation. However, due to the test substance being tested at a concentration greater than recommended in OECD TG 429 as indicated by the ear thickness, the irritancy of the test substance affected the results, making an adequate determination of the lymphocyte proliferative response of the test substance in this study not possible.

CONCLUSION

Analogue 1 may have skin sensitising ability but the test conditions employed are inadequate. Therefore, on the basis of inadequate evidence, no conclusion is made.

TEST FACILITY

Harlan (2008)

B.14. Repeat dose toxicity

TEST SUBSTANCE

Analogue 2 (100%)

METHOD

Similar to OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents.

Species/Strain: Rat/CD-Crl:CD(SD)BR
Route of Administration: Oral – diet
Exposure Information: Total exposure days: 91 days
Dose regimen: 7 days per week
Post-exposure observation period: none
Remarks - Method: No functional parameters were measured. The only organ weights recorded were kidneys and liver.

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration mg/kg bw/day*			*Mortality*
		Nominal	*Actual (standard deviation)*		
			Male	*Female*	
control	20 per sex	0	0	0	0/40
low dose	20 per sex	10	9.5 (1.5)	9.3 (1.0)	0/40
mid dose	20 per sex	100	94.1 (12.1)	90.3 (6.4)	0/40
high dose	20 per sex	1000	939 (134)	864 (56)	0/40

Mortality and Time to Death

There were no unscheduled deaths during the study.

Clinical Observations

The following clinical signs were noted in both male and female animals: rhinorrhea (discharge from the nasal mucous membrane), malocclusion, alopecia, sores, aural irritation, chromodacryorrhea and thinning hair coat. 1 male animal showed signs of sensitivity to touch and 1 female animal expistaxis (bleeding from the nose) with another being thin. The effects were seen in both control and test animals and no dose response relationship was evident in the incidence of the clinical signs and hence these changes were considered of no toxicological importance.

There were no significant differences in the bodyweight gain between the control and treated groups. There were no significant differences in the food consumption between the control and treated groups for male animals. For female animals treated with a nominal dose of 10 mg/kg bw/day, food consumption values were significantly higher than controls during weeks 1-6 and 12. For female animals treated with a nominal dose of 100 mg/kg bw/day food consumption values were significantly higher than controls during weeks 1 – 8 and 11 – 12. Significantly lower food efficiency values were seen in male animals in the 100 mg/kg bw/day and 1,000 mg/kg bw/day dose groups during week 1 and weeks 1 – 2 respectively.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There were no significant differences in any of the measured parameters for all female animals in any of the groups and male animals in the 100 mg/kg bw/day dose group. Total protein was significantly higher in male animals in the 10 mg/kg bw/day treatment group but significantly lower for male animals in the 1,000 mg/kg bw/day treatment group. Serum glutamic-oxaloacetic transaminase were significantly elevated in the male the 10 and 1,000 mg/kg bw/day treatment groups, while serum glutamic-pyruvic transaminase was significantly increased in the male 1,000 mg/kg bw/day treatment group. No statistically significant differences were detected across a range of bone marrow parameters.

Effects in Organs

Significantly lower mean and absolute liver weights were present in male animals in the 1,000 mg/kg bw/day treatment group, but were not associated with histopatholgical changes. No adverse effects were noted in the organs of any of the other treatment groups.

Remarks – Results

Female animals showed no test substance related adverse effects at any dose level. Male animals treated with a nominal dose of 1,000 mg/kg bw/day and an actual dose of 939 mg/kg bw/day had significantly elevated serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase levels as well as significantly lower mean and absolute liver weights. These effects were considered to be adverse and hence the lower nominal concentration of 100 mg/kg bw/day (94.1 mg/kg bw/day actual) was the dose where no adverse treatment related effects were observed.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 94.1 mg/kg bw/day in this study, based on adverse effects seen in male animals in the higher dose group.

TEST FACILITY Hazleton (1983b)

B.15. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical (76.9%)

METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA (pKM101),
Metabolic Activation System	S9 from phenobarbital/naphthaflavone induced rat liver.
Concentration Range in Main Test	a) With metabolic activation: up to 2000 or 2500 µg/plate b) Without metabolic activation: up to2000 or 2500 µg/plate
Vehicle	Distilled water
Remarks - Method	Concentrations were chosen on the basis of a preliminary test with TA100 (assumed to be without metabolic activation)

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 2500	>2000	not stated	no
Test 2		≥2500		no
Present				
Test 1	≥ 2500	>2000	not stated	no
Test 2		≥2500		no

Remarks - Results	Precipitation occurred at ≥ 1250 µg/plate in the preliminary test, but was not reported in the main tests. The positive controls performed as expected. No significant increase in revertants was seen in any strain, in the presence and absence of metabolic activation.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	LSAG (1998)

B.16. Genotoxicity – in vitro (1)

TEST SUBSTANCE	Analogue 4 (98%)
METHOD	OECD TG 476 In vitro Mammalian Cell Gene Mutation Test. EC Directive 2000/32/EC B.17 Mutagenicity - In vitro Mammalian Cell Gene Mutation Test.
Species/Strain	Mouse
Cell Type/Cell Line	Lymphoma L5871Y cells
Metabolic Activation System	S9 rat liver microsome fraction induced with phenobarbital / β-naphthoflavone
Vehicle	DMSO
Remarks - Method	Selection of doses was done on the basis of a pre-experiment.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	1, 3, 5, 10, 12, 16, 18, 20, 25, 30	4 h	48 h	11-14 d
Test 2	0.5, 1, 2, 4, 6, 8, 10, 12, 16	24 h	48 h	11-14 d
Present				
Test 1	2.5, 5, 7.5, 10, 12.5, 15, 20, 40, 60	4 h	48 h	11-14 d
Test 2	10, 15, 20, 25, 30, 35, 40, 45, 50, 55	4 h	48 h	11-14 d

Positive controls were methyl methanesulfonate (without metabolic activation) and benzo[a]pyrene (with metabolic activation)

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 25	≥16	> 30	no
Test 2		≥ 12	> 16	no
Present				
Test 1	-	≥ 40	≥ 60	no
Test 2		≥ 40	≥ 50	no

Remarks - Results: The positive controls showed increased mutation frequency, confirming the validity of the test system. The mutation frequencies in the test groups did not show a biologically relevant increase, compared to the controls. A higher mutation factor of 1.73 seen in Test 1 without metabolic activation, was considered not biologically relevant as the dose (60 µg/mL) was associated with high toxicity. A higher mutation factor of 2 at 0.5 µg/mL was seen in Test 2 without metabolic activation, however was within historical controls. No indication of clastogenicity was found in the analysis of colony sizing, carried out on the highest few concentrations in each dose group.

CONCLUSION: The notified chemical was not clastogenic to the mouse lymphoma thymidine kinase locus using the cell line L5178Y treated in vitro under the conditions of the test.

TEST FACILITY: BSL Bioservice (2003)

B.17. Genotoxicity – in vitro (2)

TEST SUBSTANCE: Analogue 2 (75%)

METHOD: Method similar to OECD TG 473 In vitro Mammalian Chromosome Aberration Test.

Species/Strain: Chinese hamster
Cell Type/Cell Line: Ovary cell line (CHO/WBL)
Metabolic Activation System: S9 from Aroclor 1254 induced rat liver
Vehicle: Deionized water
Remarks - Method: Concentrations were chosen on the basis of a range-finding study for toxicity and cell cycle delay. Only one test was performed.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	24.9*, 37.4*, 49.9*, 74.8*	7.25 h	10 h
Test 2	-		
Present			
Test 1	49.9*, 99.7*, 150*, 199*	7.25 h	10 h
Test 2	-		

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 49.6	≥ 74.8	≥ 49.9	no

Test 2				
Present				
Test 1	≥ 165	≥ 199	≥ 49.9	no
Test 2				

Remarks - Results

CONCLUSION The notified chemical was not clastogenic to Chinese hamster ovary cells treated in vitro under the conditions of the test.

TEST FACILITY Hazelton (1989)

B.18. Genotoxicity – in vitro (3)

TEST SUBSTANCE Analogue 1 (100%)

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.
EC Directive 2000/32/EC B.17 Mutagenicity - In vitro Mammalian Cell Gene Mutation Test.

Cell Type/Cell Line	Human lymphocytes (blood from donors)
Metabolic Activation System	S9 from Phenobarbital / β-naphthoflavone induced rat liver
Vehicle	Ethanol
Remarks - Method	No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	20.8*, 36.4*, 63.7, 111.4, 195.0, 341.2*, 597.1*, 1044.9, 1828.6, 3200.0	4 h	22 h
Test 2	8.1, 14.2, 25.9, 43.5*, 76.2*, 133.3*, 233.2*, 408.2, 714.3, 1250.0	22 h	22 h
Present			
Test 1	11.9*, 20.8*, 36.4, 63.7, 111.4, 195.0, 341.2*, 597.1*, 1044.9, 1828.6, 3200	4 h	22 h
Test 2	10.0, 20.0*, 50.0*, 125.0*, 250.0, 500.0, 750.0, 1000.0, 1250.0, 1500.0	22 h	22 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*		
	Cytotoxicity in Main Test	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	> 597.1	≥ 36.4	no
Test 2	≥ 233.2	≥ 76.2	no
Present			
Test 1	> 597.1	≥ 20.8	no
Test 2	> 125	≥ 50.0	no

Remarks - Results The positive controls performed as expected, confirming the validity of the test system. The aberration rates of the cells after treatment were slightly above the solvent controls. A single statistically significant increase in aberrant cells was noted at one dose level in Experiment 2, with metabolic activation. This result was not considered biologically relevant as it was not dose related and it was within the range of the historical control data. No increases in polyploidy cells were seen in the test groups.

CONCLUSION	The notified chemical was not clastogenic to human lymphocytes treated in vitro under the conditions of the test.
TEST FACILITY	Harlan (2009b)

B.19. Developmental toxicity

TEST SUBSTANCE	Analogue 2 (75%)
METHOD	OPP Guideline 83-3 (Similar to US EPA OPPTS 870.3700 Prenatal Developmental Toxicity Study and OECD TG 414 Prenatal Developmental Toxicity Study)
Species/Strain	Rat/CD Sprague-Dawley
Route of Administration	Oral – gavage
Exposure Information	Exposure days: gestation days 6-15
Vehicle	None
Remarks - Method	No significant protocol deviations. Dosage was adjusted to account for the purity of the test material.

RESULTS

Group	*Number of Animals*	*Dose* mg/kg bw/day*	*Mortality*
control	25	0	0/25
low	25	100	0/25
mid	25	300	1/25
high	25	1000	0/25

*Dose of notified/analogue chemical

Mortality and Time to Death

One female treated at 300 mg/kg bw/day was killed *in extremis* on gestation day 10 but was not considered to be treated related. Swelling of the face, urogenital wetness, gasping, and perinasal and perioral encrustation was observed prior to sacrifice.

Effects on Dams

Low incidence of audible respiration was observed in dams treated at 300 and 1000 mg/kg bw/day. There were no statistically significant differences in absolute body weights, body weight gains or food consumption between treated and control groups.

There were no treatment related macroscopic findings at necropsy. Gravid uterine weight, corrected body weights and body weight gains, or absolute or relative liver weights were unaffected by treatment.

The pregnancy rate was similar in treated and control groups. There were no treatment related changes between treated and control groups in the number of corpora lutea, the number of viable or nonviable implantations per litter, or on sex ratio. The proportion of preimplantation loss and live foetuses were similar in treated and control groups.

Effects on Foetus

There were statistically significant decreases in male and female foetal body weights in the 100 mg/kg bw/day group. This was not considered to be treatment related due to the lack of a dose response.

There were no treatment related external, visceral or skeletal foetal malformations. There was a statistically significant increase in the number of litters with an unossified anterior arch of the atlas in the 1000 mg/kg bw/day group (96% compared to 71% in controls). The study authors did not consider this finding to be treatment related, based on the inherent variability of the finding.

Remarks – Results

Under the conditions of the study, there was no evidence developmental toxicity.

CONCLUSION
The maternal and foetal NOAEL was established as 1000 mg/kg bw/day in this study, based on the lack of adverse effects.

TEST FACILITY BRRC (1992)

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 reported
Analytical Monitoring	CO_2 evolution
Remarks - Method	The test was conducted according to the guidelines above using good laboratory practice (GLP). No significant deviations from the test guidelines were reported.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
3	0	3	67
7	-2.0	7	76
10	-2.5	10	82
14	-2.0	14	83
28	-1	28	86

Remarks - Results	The reference compound reached the 60% pass level by day 3 indicating the suitability of the inoculum. The toxicity control exceeded 25% biodegradation within 14 days showing that toxicity was not a factor inhibiting the biodegradability of the test substance. However, based on structural considerations and the potential for the test substance to hydrolyse to form biodegradable hydrolysis products, it would be expected that at least some degradation of the test substance would be observed. The test substance is a cationic surfactant that is likely to sorb strongly to the sludge which may inhibit its ability to biodegrade. Therefore, the reliability of the test is unknown.
CONCLUSION	The notified chemical is not readily biodegradable based on the test results
TEST FACILITY	Harlan (2010m)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Analogue 1
METHOD	OECD TG 203 Fish, Acute Toxicity Test .
Species	Rainbow trout (*onchorhynchus mykiss*)
Exposure Period	96 hour
Auxiliary Solvent	Not reported
Water Hardness	114 mg $CaCO_3$/L
Analytical Monitoring	UV-absorption
Remarks – Method	The test was conducted according to the guidelines above using good laboratory practice (GLP). It is reliable with restrictions. No control was performed in the test. The minor deviation is not considered to invalidate

the study.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality (%)*		
Nominal	*Actual*		*24 h*	*48 h*	*96 h*
10	Not reported	10	0	0	0
13.3	Not reported	10	10	30	50
17.6	Not reported	10	60	80	100
23.3	Not reported	10	70	90	100
30.8	Not reported	10	90	100	100

LC50	9.84-10.24 mg/L at 96 hours (confidence limit: 2.7-11.73 mg/L)
NOEC	Not reported
Remarks – Results	The test material contains isopropanol. The toxicity of isopropanol against fish is about 100 to 1000 times lower than the toxicity of the test material. Therefore, it can be concluded in the first approach, neglecting additive effects, that the observed effect can be attributed to the active ingredient itself. Sublethal effects were not observed during the exposure time period.

CONCLUSION Analogue 1 and, by inference, the notified chemical are, at worst, toxic to fish

TEST FACILITY IBR (1982b)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 *Daphnia* sp. Acute Immobilisation Test - Semi-static

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	LC-MS/MS (liquid Chromatography/Mass Spectrometry)
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. Water Accommodated Fractions (WAFs) at the two highest loading rates of 3.2 and 10 mg/L dispersions of the test item were individually prepared by a three -hour stirring period. The dispersions were filtered through membrane filters (0.45 µm) and the undiluted filtrates were used as 10 mg/L and 3.2 mg/L WAFs preparations. The WAFs with the lower loading rates of 0.01, 0.032, 0.1, 0.32 and 1.0 mg/L were prepared as dilutions of the WAFs with the loading rate of 3.2 mg/L. All the test media were clear solutions throughout the entire test period.

RESULTS

Concentration		*Number of D. magna*	*% Immobilised*
Nominal (mg/L)	*Mean measured concentrations(mg/L)*		*(48 h)*
Control	n.a.	20	0
0.010	--	20	0
0.032	--	20	0
0.1	--	20	0
0.32	0.015	20	0

1.0	0.048	20	15
3.2	0.152	20	30
10	1.924	20	100

n.a.: Not applicable
--: Not analysed, since below NOEC

EL50	3.7 mg/L WAF at 48 hours (95% confidence limit: 2.6-4.9 mg/L WAF)
NOEL	0.32 mg/L WAF at 48 hours
Remarks - Results	The concentrations of the test item in the solutions were measured throughout the duration of the test. However the reliability of the measured data is questionable. The method used is not believed to be suitable to accurately determine the concentration of the test substance in solution. Based on the cationicity of the test substance, it is expected to adsorb to surfaces, particularly those in the test vessel and potentially the membrane used to filter the solution. The adsorption, along with the potential for the test substance to hydrolyse, explains the depletion of the measured concentration of the test substance over time and the low concentration of the test substance observed in test solutions. Being a surface active substance capable of forming a dispersion in solution, filtration of the water accommodated fraction (WAF) is not considered the most appropriate approach to measuring the effects of the test substance. A dispersion should be formed by mixing the test substance with the aqueous phase and allowing the dispersion to stabilise before introducing the test organisms. Only non-dissolved material with the potential to cause physical effects should be removed. Potential hydrolysis products were also not characterised and their concentrations are unknown. Therefore, it is unclear whether the test substance or its hydrolysis products are causing the observed toxicity. Therefore, based on the uncertainty in the reliability of the measured data, the nominal loading rate of WAF instead of the measured concentrations for the notified chemical was used as the best representative of the toxicity for the whole product under the conditions of the experiment. The results are also consistent with the expected toxicity of other cationic surfactants. Therefore, the test substance is considered toxic to daphnia.
CONCLUSION	The notified chemical is toxic to aquatic invertebrates
TEST FACILITY	Harlan (2010n)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test - Static.
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 0.01, 0.032, 0.1, 0.32, 1.0, 3.2 and10 mg/L Measured: 0.31, 0.52, 2.8, 7.6, 21, 42 and 95 µg/L
Auxiliary Solvent	None
Water Hardness	24 mg $CaCO_3$/L
Analytical Monitoring	LC-MS/MS
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. Water Accommodated Fractions (WAFs) at the two highest loading rates of 3.2 and 10 mg/L dispersions of the test item were individually prepared by a three -hour stirring period. The dispersions were filtered

through membrane filters (0.45 µm) and the undiluted filtrates were used as 10 mg/L and 3.2 mg/L WAFs preparations. The WAFs with the lower loading rates of 0.01, 0.032, 0.1, 0.32 and 1.0 mg/L were prepared as dilutions of the WAFs with the loading rate of 3.2 mg/L. All the test media were clear solutions throughout the entire test period.

RESULTS

Biomass (72 h)		*Growth (72 h)*	
E_yC_{50} *(mg/L)*	NOE_yC *(mg/L)*	E_rC_{50} *(mg/L)*	NOE_rC *(mg/L)*
1.5 (1.2 – 2.0)	0.032	4.8 (4.3-5.5)	0.32

Remarks - Results

The concentrations of the test item in the solutions were measured throughout the duration of the test. However the reliability of the measured data is questionable. The method used is not believed to be suitable to accurately determine the concentration of the test substance in solution. Based on the cationicity of the test substance, it is expected to adsorb to surfaces of test vessels, potentially the membrane used to filter the solution, as well as algae itself. The adsorption, along with the potential for the test substance to hydrolyse, explains the significant depletion of the measured concentration of the test substance over time and the low concentration of the test substance observed in the test solutions. .

Being a surface active substance capable of forming a dispersion in solution, filtration of the water accommodated fraction (WAF) is not considered the most appropriate approach to measuring the effects of the test substance. A dispersion should be formed by mixing the test substance with the aqueous phase and allowing the dispersion to stabilise before introducing the test organisms. Only non-dissolved material with the potential to cause physical effects should be removed.

Potential hydrolysis products were also not characterised and their concentrations are unknown. Therefore, it is unclear whether the test substance or its hydrolysis products are causing the observed toxicity.

Therefore, based on the uncertainty in the reliability of the measured data, the nominal loading rate of WAF instead of the measured concentrations for the notified chemical was used as the best representative of the toxicity for the whole product under the conditions of the experiment. The results are also consistent with the expected toxicity of other cationic surfactants. Therefore, the test substance is considered toxic to algae.

CONCLUSION The notified chemical is toxic to algae

TEST FACILITY Harlan (2010o)

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