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December 2007

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

AE 425/03 (Dioleoylethyl hydroxyethylammonium methosulfate)

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 the Environment and Water Resources.

For the purposes of subsection 78(1) of the Act, this Full Public Report may be inspected at our NICNAS office by appointment only at 334-336 Illawarra Road, Marrickville NSW 2204.

This Full 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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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANTS
Salkat Australia Pty Ltd (ABN 30 318 540 786), 262 Highet Road, Highett VIC 3190

Procter & Gamble Australia Pty Ltd (ABN 91 008 396 245), Level 4, 1 Innovation Road, Macquarie Park NSW 2113

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: Marketing Name, Analytical Data, Purity, Hazardous Impurities/Residual Monomers, Non-Hazardous Impurities/Residual Monomers, Additives/Adjuvants, Introduction Volume

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
USA, Canada, Korea, China

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
AE425/03

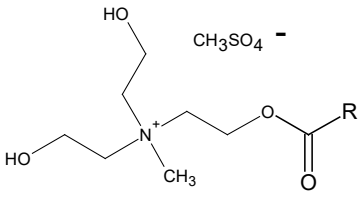
CAS NUMBER
157905-74-3

CHEMICAL NAME
Ethanaminium, 2-hydroxy-N,N-bis(2-hydroxyethyl)-N-methyl-, esters with C16-18 and C18-unsatd. fatty acids, Me sulfates (salts)

OTHER NAME(S)
2-Hydroxy-N,N-bis(2-hydroxyethyl)-N-methylethanaminium esters with (C16-18) and (C18)-unsatd. Fatty acids, Me sulfates (salts)
Fatty acids, C_{16-18} and C_{18} unsat'd. reaction products with triethanol amine, dimethyl sulfate-quaternized
TEA Esterquat
Triethanolamin Esterquat
Dioleoylethyl hydroxyethylammonium methosulfate

STRUCTURAL FORMULA

Monoester:



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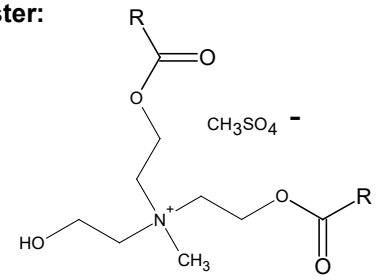

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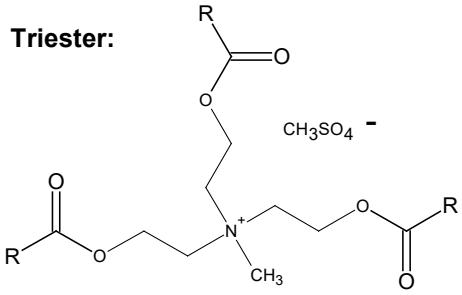

R= C16 -C18, C18 unsaturated

MOLECULAR FORMULA
Not known

MOLECULAR WEIGHT
733.5 (weighted average)

ANALYTICAL DATA
Reference NMR, IR, HPLC, UV-Vis spectra were provided.

3. COMPOSITION

DEGREE OF PURITY >85%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Slightly yellowish solid

Property	Value	Data Source/Justification
Melting Point	> 85°C	Measured
Boiling Point	≥ 260°C (decomposition)	Measured
Density	1059 kg/m^3 at 20°C	Measured
Vapour Pressure	6.7 x 10^{-7} kPa at 25°C	Calculated from measured values
Water Solubility	2.244 g/L at unbuffered pH 3.86 and 20°C 3.39 mg/L at buffered pH 7.08 and 20°C	Measured
Hydrolysis as a Function of pH	$t_{1/2}$ >1 year (pH 4), 17.0 days (pH 7) and 11.3 days (pH 9) at 25°C	Measured
Partition Coefficient (n-octanol/water)	log Pow = >6.5 at 20°C	Estimated
Adsorption/Desorption	log K_{oc} = >5 at 20°C	Calculated
Dissociation Constant	pKa = 1.14 (methylsulfuric acid) pKa = 12.42 and 13.68 (monoester with C18 esterchain) pKa = 12.52 (diester with C18 esterchain)	Calculated

Surface tension	41.8 mN/m at 20ºC	Measured
Particle Size	Not determined	The notified chemical is a waxy solid (compact) substance.
Flash Point	Not determined	The notified chemical is not a liquid.
Flammability	Not highly flammable	Measured
Autoignition Temperature	> 402°C	Measured
Explosive Properties	Not explosive	Based on the structural formula, the notified chemical is not explosive.
Oxidising Properties	Not oxidising	The notified chemical has no oxidising properties based on its structural groups, thermodynamic calculations and negative oxygen balance.

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

Reactivity
No information on reactivity was provided. However, based on the structural formula, the notified chemical is stable under normal environmental conditions.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. It will be imported as a neat material for further formulation and as a component of a finished cosmetic product.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	<5	<5	5-10	5-10	<100

PORT OF ENTRY
Sydney, Melbourne

IDENTITY OF MANUFACTURER/RECIPIENTS
Salkat Australia Pty Ltd and Procter and Gamble Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a neat material in 180kg drums and transported from dockside to storage facilities in NSW before distribution to formulators by road. The formulated product (fabric softeners) are in 1-2.5L plastic bottles and 350g sachets and shipped to retail outlets directly. The notified chemical will also be imported as a component of a facial cleanser in 125mL packs which will be transported to the Procter and Gamble bunded warehouse before distribution to retail outlets.

USE
Eighty percent of the imported notified chemical will be used in the formulation of fabric softeners (at a concentration of 5%) and 20% as a facial cleanser component (at a concentration of 1.7%).

OPERATION DESCRIPTION

Formulation of fabric softeners

At the formulation site, drums containing the neat notified chemical are stored on racks after quality control analysis by laboratory personnel. The notified chemical will be heated in water baths to reduce the viscosity prior to being transferred by forklifts to the formulation site. It will then be manually added to an open mixing tank and mechanically mixed with other ingredients. The mixing tank will be sealed after a sample is taken for quality control analysis and the concentration of the notified chemical in the final product is 5%. With quality approval, the fabric softener will then be pumped to stainless steel production transfer lines and filled into 1-2.5L plastic bottles and 350g sachets. The finished product will be packed into boxes, transported to and sold to retail outlets.

End use of fabric softeners and facial cleansers

The formulated fabric softeners containing 5% of the notified chemical and the imported facial cleansers containing 1.7% of the notified chemical are sold to retail outlets for public uses.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration (hours / day)*	*Exposure Frequency (days)*
Transport workers	4-6	4	4
Warehouse workers	2-4	2	160
Storage workers at reformulation site	1	0.5	12
Laboratory technicians	2	0.5	24
Compounders	3	2	24
Line setters	3	2	24
Other production staff	6	6	24
Retail workers	8,000	1	240

Transport and storage workers will not be exposed to the notified chemical and the product containing the notified chemical except in the event of an accidental spill.

Laboratory technicians and floor workers may be exposed to the notified chemical via the dermal and ocular routes during formulation, especially when the neat notified chemical is handled during sampling and manual addition to the mixing tank. Workers' exposure after the mixing process will be lower due to the lower concentration of the notified chemical (5%). In addition, exposures to the notified chemical are expected to be reduced by the use of appropriate personal protective equipment (PPE) including overalls/protective clothing, safety glasses, and gloves. Worker's exposure via inhalation is unlikely due to the low vapour pressure of the notified chemical and the process is conducted in a well-ventilated area.

Dermal and ocular exposure to the formulated fabric softeners and imported facial cleansers containing the notified chemical are not expected for workers involved in the retail industry except in the event of accidental breaching of the packaging of the end products.

6.1.2. Public exposure

There will be widespread and repeated exposure of the public to the notified chemical.

In the case of the fabric softener, members of the public are likely to make dermal contact and/or accidentally ocular exposure via spills during use given that the general public do not usually wear gloves during use of fabric softener. However, the potential for exposure is expected to be low due to relatively infrequent uses and low concentrations of the notified chemical (up to 5%). It is also possible for the general public to have dermal contact via wearing the clothes treated with the fabric softener, but the potential for exposure is low due to very low concentrations of the notified chemical after dilution during washing.

In the case of the facial cleanser, members of the public will dispense the product from bottles into the fingers and then massage through the face and neck. Since this is a wash-off product, the majority of the notified chemical should be removed afterward. Accidental ocular exposure to the notified chemical may also occur, especially during rinse-off. However, the notified chemical is present at a relatively low concentration (up to 1.7%).

Again, public exposure via inhalation is unlikely due to the low vapour pressure of the notified chemical. Since laundry and cosmetic products are stored and used in a domestic environment, there is the possibility of accidental ingestion by a child.

6.2. Human health effects assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity[a]	LD50 > 2000 mg/kg bw, low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw, low toxicity
Rabbit, skin irritation[a]	slightly irritating
Rabbit, eye irritation[a] (undiluted test substance)	severely irritating
Rabbit, eye irritation[a] (undiluted test substance, low volume procedure)	slightly irritating
Rabbit, eye irritation[a] (5% diluted test substance)	not irritating
Guinea pig, skin sensitisation – non-adjuvant test[a]	Inconclusive
Rat, repeat dose oral toxicity – 28 days.	NOAEL = 1000 mg/kg bw
Genotoxicity – bacterial reverse mutation[b]	non mutagenic
Genotoxicity – in vitro Mammalian Chromosome Aberration Test	non genotoxic

[a] Toxicological investigations were conducted using analogue 1 – Rewoquat WE18. Details are in Appendix B.
[b] Toxicological investigation was conducted using analogue 2 – Rewoquat WE20. Details are in Appendix B.

Toxicokinetics, metabolism and distribution

No pharmacokinetic data were provided. However, based on the physical and chemical properties, the notified chemical is expected to be absorbed via following routes:

- low dermal absorption due to low water solubility (at pH of 7), high partition coefficient (>6), and high surface tension;
- moderate oral absorption due to high water solubility (at pH of 4);
- low inhalation potential due to very low vapour pressure.

Analogue chemicals used in toxicity studies
The two analogues used in the majority of the toxicity studies are considered acceptable based on its close similarity in chemical structure to the notified chemical. However, both analogues contain 10% 2-Propanol (CAS no. 67-63-0) which is classified as an eye and respiratory irritant (OASCC, 2007). Therefore, it should be noted that the toxicity of the analogue could be biased by the toxicity of this impurity.

A previous NICNAS assessment on Analogue 1 evaluated a number of toxicity studies that were not provided by the notifier. These studies are summarised below and are used for hazard classification where applicable.

It should be also noted that Analogue 1 is readily degraded by the cleavage of the ester linkages to give fatty acids and the main degradation intermediate, methyl-triethanol-ammonium, ion (MTEA). While no data is available on the toxicokinetics of this analogue, the toxicokinetics of this degradation intermediate have been studied. MTEA was found to be almost completely excreted within 3 days of administration by both the oral and intravenous route in rats. In pharmacological investigations of MTEA in a variety of laboratory animals, effects on blood pressure were observed only at high doses (8 mg/kg). Long term oral toxicity studies for the degradation product found MTEA to be of low toxicity with a NOEL of 0.096% when administered *via* drinking water (literature sources).

Acute toxicity, Irritation and Sensitisation
Analogue 1 is of low acute toxicity (LD50 of >2000 mg/kg bw/day) via oral and dermal routes. The previous NICNAS assessment on Analogue 1 indicated a LD50 of >5000 mg/kg bw/day via oral route. Acute effects by inhalation are unlikely due to its low inhalation potential.

A dermal irritation study provided by the notifier indicated that the undiluted Analogue 1 is slightly irritating to the skin (very slight erythema and very slight to well-defined oedema in some of test animals). Analogue 1 was also tested in two other skin irritation studies. One study using undiluted Analogue 1 showed cutaneous reactions which were slight in one animal and marked in 2 animals (erythema scores from 1 to 3 and oedema scores from 1 to 4). Another study using 20% Analogue 1 in water showed slight irritation. Based on the scores, Analogue 1 was classified as a skin irritant in the previous NICNAS assessment.

Three eye irritation studies were submitted by the notifier. In one study using undiluted Analogue 1, irreversible severe eye effects were observed in conjunctiva, cornea and iris in 5 out of 6 test animals. This observation cannot be fully attributed to the possible effect of the impurity, 2-Propanol (an eye irritant). The second eye irritation study using undiluted Analogue 1 showed only slight eye irritation. However, the test volume used was 10 folds lower than the first study. The third study indicated that diluted Analogue 1 (5%) is not irritating to the eyes. Analogue 1 was also evaluated in two other eye irritation studies. One study using undiluted Analogue 1 showed moderate to marked conjunctival reactions, but all the ocular lesions resolved between day 4 and day 8. Another study using 20% Analogue 1 in water showed slight redness and slight chemosis of the conjunctiva. On the basis of the severity of effects, Analogue 1 meets the criteria for hazard classification ie. R41 – Risk of serious eye damage.

The guinea pig maximisation test submitted by the notifier did not provide an evidence for skin sensitisation. No definite conclusion can be made due to the limitations of this study (test conditions inadequately or insufficiently documented). The previous NICNAS assessment on Analogue 1 indicated that the chemical is not sensitising in a Beuhler test and a human repeated insult patch test, although evidence of sensitisation was inconclusive in a guinea pig maximisation test.

Repeated dose toxicity
In a 28-day repeated oral rat study using the notified chemical, no significant effects were observed up to the highest dose tested. The few treatment-related changes (increased forelimb and hindlimb grip strength in males, decreased thromboplastin time in females) were considered to be not biologically relevant due to lack of corresponding pathological changes. The No Observed Adverse Effect Level (NOAEL) was established at 1000 mg/kg bw/day based on this study.

Mutagenicity
The Analogue 2 was not mutagenic to bacteria and in vitro under the conditions of the test. The previous NICNAS assessment on Analogue 1 indicated that this chemical was not genotoxic/mutagenic both in bacteria and in vivo.

Hazard Classification
Based on the similarity of the hazardous profile to Analogue 1, the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).
R41 – Risk of serious eye damage
R38 - Irritating to skin

6.3. Human health risk characterisation

The critical health effect of the notified chemical is skin irritation and severe eye irritation.

6.3.1. Occupational health and safety
The risk to the health of **transport and storage workers** and **retailer workers** is not considered to be significant, due to the lack of probable exposure.

Risk of local effects
Formulation workers will likely experience the greatest risk of skin irritation and severe eye irritation posed by the notified chemical, especially when the neat notified chemical is handled and when spills during handling are likely, for example, sampling of the neat notified chemical and manual addition into mixing tanks. Although use of PPE will reduce the potential risk, recommendations on engineering controls and safe work practices will be made to minimise workers' potential exposure.

Risk of systemic effects
Workers' risk of systemic effects from repeated exposure is considered to be low due to 1) NOAEL of 1000 mg/kg bw/day; 2) the notified chimerical is poorly absorbed via dermal based on its properties; 3) workers' repeated exposures should be largely reduced by the control measures proposed by the notifier and recommended by NICNAS.

6.3.2. Public health
Risk of local effects
In the case of fabric softeners, the risk of skin irritation and severe eye irritation from accidental spillages onto the skin and eyes is considered to be low due to the low concentrations of the notified chemical in the finished product (up to 5%), short use duration and relatively infrequent use of the product. The risk of skin irritation and severe eye irritation to the general public when using the washed materials treated with the fabric softener is considered low on the basis that negligible level of residual product is expected on the washed materials.

In the case of facial cleansers, the concentration of the notified chemical is up to 1.7%. Although dermal exposure is inevitable during use of the facial cleanser product and eye exposure is also likely, especially during rinse-off of the product, the risk of skin irritation and severe eye irritation is considered to be low based on the negative skin irritation study using 20% Analogue 1 and negative eye irritation animal study using 5% Analogue 1.

However, due to the toxicity of the accepted analogue causing severe eye irritation when used undiluted, recommendations will be made to review the notified chemical if importation of consumer products containing higher concentrations of the notified chemical is intended.

Risk of systemic effects
The public risk of systemic effects from use of both fabric softener and facial cleansers is considered to be low due to the poor dermal absorption of the notified chemical at pH of 7 and its low concentration in these products. The risk of systemic effects from accidental ingestion of the fabric softeners and facial cleansers by young children cannot be ruled out, but is expected to be low as the incidence of such events occurring is low. Nevertheless, appropriate warning statement (such as 'keep out of reach of children') should be made available on product labels.

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 in as a neat material in 180 kg drums for use (80%) in the formulation of the product as fabric softeners in 1-2.5 L plastic bottles and 350 g sachets. The notified chemical will also be imported as a component of a facial cleanser for use as a skin care product (20%) in 125 mL packs. Thus there will be no environmental implications associated with the manufacture of the notified chemical.

In the formulation of fabric softeners, the notified chemical from the imported drums are decanted directly into mixing vessels with other raw materials. Spillage at blending facilities are cleaned up with absorbent and disposed of in accordance with local state and federal regulations. Following decanting the drums are rinsed into the blending process and up to 3% could remain in the drums which are removed to an approved drum wash facility where injected steam would remove the residues. Sludge from this operation will be removed for appropriate waste treatment. It is expected that 0.05% will be left in the mixers which is rinsed into onsite treatment processes which are not released to sewer. Sludge from the onsite waste water treatment will be disposed of by approved waste operators.

RELEASE OF CHEMICAL FROM USE

The notified chemical will be used in household, laundry, and skin care products. In these applications, it is anticipated that the entire product is eventually washed into the sewer system. The majority of the imported notified chemical (>99%) is therefore expected to be disposed of to sewers. The notifier indicated that approximately 0.7% (1 mL in every 150 mL) will remain after use as fabric softener. Based on the maximum import volume of 100 tonnes, this will corresponds to ~560 kg (700 kg X 0.8) of the notified chemical per annum to be disposed of to landfill via domestic garbage collection. Similarly, it is expected that approximately 0.8% (1 mL in every 125 mL) will remain for use as skin care product corresponding to ~160 kg (800 kg X 0.2) of the notified chemical to be disposed of by landfill.

RELEASE OF CHEMICAL FROM DISPOSAL

It is anticipated that <1% of the import volume will be lost as residues in consumer containers, which are primarily sent to landfill.

7.1.2 Environmental fate

The notified chemical is considered a surface active substance. It is relatively insoluble in water buffered at pH 7 but highly soluble at unbuffered pH 3.73-3.86, and is hydrolysable in the environmental pH range of 4-9. Its relatively high log Kow of >6.5 indicates that it is likely to partition to the soil or sediment. The notified chemical is considered to be readily biodegradable. In landfill, the residue is expected to degrade by biotic and abiotic processes to oxides of carbon and nitrogen, and water. For the details of the environmental fate studies please refer to Appendix C.

7.1.3 Predicted Environmental Concentration (PEC)

It is anticipated that essentially all of the notified chemical will be released into the sewer system from the wash-off of products containing the chemical in domestic applications. As the notified chemical is to be used domestically, it is anticipated that release will occur on 365 days per year across Australia. The mitigated PEC arising from this domestic release pattern was modelled using the SIMPLETREAT approach (EC, 2003). Removal within STP is based on the water solubility of 3.39 mg/L, log H of -0.839 $Pa/m^3/mol$ (based on the water solubility and vapour pressure of 6.7 x 10^{-7} kPa), log Kow of 6 and a molecular weight of 733.5 for the notified chemical. The details of the PEC calculation with mitigation from STP removal are presented below:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	100,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	100,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	273.97	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	20.496	million
Removal within STP		
(a) Volatilisation	0%	
(b) Degradation	11%	
(c) Partition to sludge	79%	
(d) Remain in effluent	10%	
Daily effluent production:	4,099	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	6.68	µg/L
PEC - Ocean:	0.668	µg/L

Partitioning to biosolids in STPs Australia-wide may result in an average biosolids concentration of 528 mg/kg (dry wt). Biosolids are applied to agricultural soils, with an assumed average rate of 10 t/ha/year. Assuming a soil bulk density of 1300 kg/m3 and a soil-mixing zone of 10 cm, the concentration of the notified chemical may approximate 4.062 mg/kg in applied soil. This assumes that degradation of the notified chemical occurs in the soil within 1 year from application. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated biosolids application, the concentration of notified chemical in the applied soil in 5 and 10 years may approximate 20.31 mg/kg and 40.62 mg/kg, respectively.

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 (soil bulk density 1300 kg/m3). Using these assumptions, irrigation with a concentration of 6.684 mg/L may potentially result in a soil concentration of approximately 6.684 X 10^{-2} mg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 3.342 X 10^{-1} mg/kg and 6.684 X 10^{-1} mg/kg, respectively.

7.2. Environmental effects assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 = 1.91 mg/L	Toxic
Daphnia Toxicity	48 h EC50 = 6.05 mg/L	Toxic
Algal Toxicity	72 h ErC50 = 22.3 mg/L	Harmful
Inhibition of Bacterial Respiration	3 h EC50 >243 mg/L	Non-toxic

The results indicate that the notified chemical is toxic to aquatic organisms.

7.2.1 Predicted No-Effect Concentration

The Predicted No-Effect Concentration has been calculated from the most sensitive fish toxicity (96 h LC50 = 1.91 mg/L) of the notified chemical. As the results are available for three trophic levels, the assessment factor of 100 has been used.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
96 h LC50 for rainbow trout	1.91	mg/L
Assessment Factor	100	
Mitigation Factor	1.00	
PNEC:	19.1	µg/L

7.3. Environmental risk assessment

Insert the Risk Quotient Table (PEC/PNEC)

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River:	6.68	19.1	**0.35**
Q - Ocean:	0.668	19.1	**0.035**

The mitigated Risk Quotients are <1 for both the river and ocean disposal scenarios. Therefore, the notified chemical is not expected to pose an unacceptable risk to the aquatic environment based on the current use pattern and the maximum import volume of 100 tonnes/year.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

Based on the available data relating to the analogue, the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)]. The classification are:

- R41 Risk of serious eye damage
- R38 Irritating to skin

and

As a comparison only, the classification of the notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations, 2003) is presented below. This system is not mandated in Australia and carries no legal status but is presented for information purposes.

	Hazard category	***Hazard statement***
Health		
- Serious eye damage	Category 1	Causes severe eye damage
- Skin irritation	Category 3	Causes mild skin irritation
Environment		
	Acute II	Toxic to aquatic life
	Chronic II	Toxic to aquatic life with long lasting effect

Human health risk assessment

Based on the available data, the notified chemical is not expected to pose an unreasonable risk to workers under the condition of current and recommended control measures.

Based on the available data, the notified chemical is not expected to pose an unreasonable risk to the public when used in the proposed manner with appropriate product labelling.

Environmental risk assessment

On the basis of the PEC/PNEC ratio, the notified chemical is not considered to pose a risk to the environment based on its reported use pattern.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- The Office of the ASCC, Department of Employment and Workplace Relations (DEWR), should consider the following health hazard classification and safety phrases for the notified chemical:
  - R41 Risk of serious eye damage
  - R38 Irritating to skin

  Safety phrases
  - S25 Avoid contact with eyes
  - S26 In case of contact with eyes, rinse immediately with plenty of water and contact a doctor or Poison Information Centre
  - S28 After contact with skin, wash immediately with plenty of soap suds
  - S 37 Wear suitable gloves
  - S39 Wear eye protection

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - 5%≤conc<10% R36
  - ≥10% R41
  - ≥20% R41, R38

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical as introduced:
  - *Prevent leaks and spills.*
  - *Wherever possible, direct handling of the notified chemical should be avoided; rather, some remote handling apparatus should be used.*
  - *Minimise manual processes.*

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced:
  - *Avoid contact with skin, eyes and contaminated clothing.*
  - *A shower and eyewash station should be available.*
  - *Avoid spills and splashing during use.*
  - *After exposure, any contaminated PPE should be thoroughly cleaned before re-use.*

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced:
  - *Protective clothing*
  - *Chemical resistant gloves*
  - *Face-shield*

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

- A copy of the MSDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Public Health

- The following measures should be taken by the manufacturer of consumer products containing the notified chemical to minimise public exposure to the notified chemical:
  - Advice on the label of products containing the notified chemical should include information on the possibility of skin and eye irritation and recommend washing of the skin and eyes immediately following exposure to the product.
  - A warning statement of 'Keep out of reach of children' should be on product labels.

Environment

- The notified chemical should be disposed of by landfill.

- Do not allow entering drains or waterways. Do not discharge into the subsoil/soil.

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 of the notified chemical >5% in consumer products;
  - The notified chemical is intended for use in leave-on products.

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from formulation of fabric softener (up to 5%) and facial cleansers (up to 1.7%), or is likely to change significantly;
  - the amount of chemical being introduced has increased from 100 tonnes or is likely to increase, significantly;
  - if 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.

No additional secondary notification conditions are stipulated.

Material Safety Data Sheet
The MSDS 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 MSDS remains the responsibility of the applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point > 85°C

Method	OECD TG 102 Melting Point/Melting Range. EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks	Test was conducted concurrent with the determination of the boiling point. According to the observations made with three different test methods i.e. Differential Scanning Calorimetry (DSC), Capillary Method and Penetrometer Test, the following conclusion was given as the final result: the test item is a solid at ambient temperature and has the character of a waxy, viscous solidified liquid. The test item has no specific melting point. With increasing temperature, the viscosity of the test item decreases. According to the Penetrometer Test, the test item is a solid up to a temperature of 85°C.
Test Facility	SIEMENS (2004a)

Boiling Point ≥ 260°C (decomposition)

Method	OECD TG 103 Boiling Point. EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks	Test was conducted concurrent with the determination of the melting point. Two test methods were used: DSC and Capillary Method. The boiling point was not observed at atmospheric pressure. Decomposition of the test item begins at temperatures at and above 260°C.
Test Facility	SIEMENS (2004a)

Density 1059 kg/m^3 at 20°C

Method	OECD TG 109 Density of Liquids and Solids. EC Directive 92/69/EEC A.3 Relative Density.
Remarks	The air comparison pycnometer was used. There were no significant deviations from the protocol.
Test Facility	GAB (2004a)

Vapour Pressure 6.7 x 10^{-7} kPa at 25°C

Method	OECD TG 104 Vapour Pressure. EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks	Test conducted concurrent with the screening test for thermal stability and stability in air. The vapour pressure balance (Effusion method) was used. The vapour pressure was measured in the temperature range of 16°C to 138°C. No signal was observed up to a temperature of 39°C. Above 43°C, a vapour pressure could be measured. The vapour pressure at 25ºC was extrapolated from vapour pressure measurements at above 43°C.
Test Facility	SIEMENS (2004b)

Water Solubility 2.244 g/L at unbuffered pH 3.86 and 20ºC (saturated with atmospheric CO_2) but 3.39 mg/L at buffered pH 7.08 and 20ºC

Method	OECD TG 105 Water Solubility. EC Directive 92/69/EEC A.6 Water Solubility.
Remarks	On the basis of a preliminary test performed with water in equilibrium with CO_2, the test item was dissolved at 10, 20 or 30ºC in distilled water being in equilibrium with atmospheric CO_2 (no pH adjustment, pH = 3.86). The pH dependency in the pH range of 4-9 was also determined at 20ºC using buffered water. Six replicates at each test temperature and each test medium were prepared and incubated under agitation over a period of 120 h at the specified test temperature. Once saturation was achieved, the mixture was maintained at the test temperatures and the actual concentrations of the test item were determined by HPLC/MS-MS analysis. The water solubility was found not to be dependent on temperature as no clear trend in

solubility was observed with change in temperature. The data also suggests that the water solubility may be slightly dependent on pH values, increasing at alkaline pH. However, no reasons were advanced as to the much greater solubility in unbuffered versus buffered water.

Test Facility GAB (2005a)

Hydrolysis as a Function of pH

Method OECD TG 111 Hydrolysis as a Function of pH.
EC Directive 92/69/EEC C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$*
4	50	27.3 days
4	25	> 1 year
7	50	72.3 hour
7	25	17.0 days
9	50	47.8 hours
9	25	11.3 days

Remarks On the basis of a preliminary test performed at 50ºC at pH of 4, 7 and 9, samples were taken at the beginning of the test and after 24, 48, 72, 120, 144 and 168 h at 50ºC. The concentrations in buffer solutions were determined by HPLC/MS-MS. The abiotic degradation of the notified chemical in aqueous solution was measured as a function of pH at 50°C. The results were subsequently extrapolated to 25°C.

At pH 4 and 50ºC, the degradation of the main components of the notified chemical was <10% over a period of 120 h whereas at pH 7 and 9 and 50ºC >10% were hydrolysed within 120 h, though the main test was not performed.

Test Facility GAB (2005b)

Partition Coefficient (n-octanol/water) log Pow = >6.5 at 20ºC

Method OECD TG 117 Partition Coefficient (n-octanol/water).
EC Directive 92/69/EEC A.8 Partition Coefficient.

Remarks A preliminary assessment of the partition coefficient was based on the ratio of the solubility of the test item in pure octanol and water. The test item forms micelles above 0.5 mg/L in water but is characterised by an apparent solubility of 2531 mg/L at 20ºC in pure water. An attempt was made to determine partition coefficient based on elution behaviour with 7 other reference substance by HPLC method. The sample was injected three times. No elution of the test item from the column could be observed. The log P_{ow} of the test item was determined to be > 6.5 at pH 5.9 and 20°C based on the assumption it eluted after the higher reference substance. However, in view of the surface active properties of the test item, the estimate may be considered of only indicative value.

Test Facility GAB (2005c)

Adsorption/Desorption log K_{oc} = >5 at 20°C

Method OECD 121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using HPLC
EC Directive 2001/59/EC C.19 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using HPLC

Remarks The determination of the adsorption coefficient of the test item on soil was determined by HPLC method using 8 reference substances. The analysis was conducted four times. No elution of the test item could be observed. The log K_{oc} value of the test item was calculated based on the results of the HPLC determination to be > 5 at pH 5.9 and 20.0°C, again assuming it eluted beyond the higher reference substance.

Test Facility GAB (2005d)

Dissociation Constant pKa = 1.14 (methylsulfuric acid)
pKa = 12.42 and 13.68 (monoester with C18 esterchain)
pKa = 12.52 (diester with C18 esterchain)

Method OECD TG 112 Dissociation Constants in Water.
Remarks The test substance is a surface active substance which dissociates in water to a quaternary ammonium ion and methyl sulphate. The pKa value of methylsulfuric acid was calculated to be 1.14. Therefore, the salt can be regarded as dissociated over almost the complete pH-range. However, the dissociation constants for the OH-groups of the mono- (12.42, 13.68) and diester (12.52) could be calculated with a QSAR using the SPARC On-line Calculator v3.1.

Test Facility Goldschmidt GmbH (2006)

Flammability Not highly flammable

Method EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks Test was conducted concurrent with the determination of the auto ignition temperature. The test substance could not be ignited with a flame before the test substance was melted, thus, the main test was not necessary.
Test Facility SIEMENS (2004c)

Autoignition Temperature > 402°C

Method EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks Test was conducted concurrent with the determination of the flammability. There was no exothermal reaction of the test substance observed up to a maximum test temperature of 402°C.
Test Facility SIEMENS (2004c)

Surface Tension 41.8 mN/m at 20°C

Method OECD TG 115 Surface Tension of Aqueous Solutions.
EC Directive 92/69/EEC A.5 Surface Tension.
Remarks The test substance was prepared at a concentration of 1 g/L. The surface tension was measured using the ring method. It was determined after 20 min equilibration time. Further measurements were conducted in intervals of 5 min. The test item is considered to be surface active.
Test Facility GAB (2004b)

Stability Testing

Method OECD TG 113 Screening Test for Thermal Stability and Stability in Air.
Remarks Test was conducted concurrent with the determination of the vapour pressure. DSC measurement in a closed glass crucible showed an exothermal decomposition in the temperature range 275-310°C.
Test Facility SIEMENS (2004b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

Some of the tests below were conducted using following accepted analogues:

Analogue 1 - Fatty acids, C10-20 and C16-18-unsatd., reaction products with triethanolamine, di-Me sulfate-quaternized (CAS No. 91995-81-2, Rewoquat WE18). This is a triethanolamine-based esterquat with the main constituents of C16, C18 and C18 unsaturated fatty acids; minor amounts of C10-14 and C20 fatty acids (depending on the source of the raw material); and contains about 10% isopropanol (CAS No. 67-63-0).

Analogue 2 - Rewoquat WE20, a preparation consisting of about 84% Rewoquat 18 and 6% of 1-Propanaminium, 3-1mino-N,N,N-trimethyl-, N-C12-18 acyl derivatives, and Me sulfates (CAS No. 68514-93-2); and contains 10% isopropanol.

B.1. Acute toxicity – oral

TEST SUBSTANCE	Analogue 1
METHOD	OECD TG 401 Acute Oral Toxicity. EC Directive 84/449/EEC.
Species/Strain	Rat / Wistar
Vehicle	None
Remarks - Method	There were no significant deviations from the protocol.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5 males	2000	0
II	5 females	2000	0

LD50	> 2000 mg/kg bw
Signs of Toxicity	There were no abnormal clinical signs observed.
Effects in Organs	Necropsy revealed no test article-dependent findings.
Remarks - Results	Body weight gains were normal in all test animals.

CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	International BioResearch (1992a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal) – Limit Test.
Species/Strain	Rat / Crl:CD
Vehicle	Purified water
Type of dressing	Semi-occlusive
Remarks - Method	There were no significant deviations from the protocol.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5 males	2000	0
II	5 females	2000	0

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	None

Signs of Toxicity - Systemic	None
Effects in Organs	No macroscopical changes were noted at necropsy.
Remarks - Results	The animals gained the expected body weights throughout the observation period except for one animal in Group II where the body weight gain is slightly reduced.

CONCLUSION The notified chemical is of low toxicity via the dermal route.

TEST FACILITY LPT (2004).

B.3. Irritation – skin

TEST SUBSTANCE Analogue 1

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Directive 84/449/EEC.

Species/Strain	Rabbit / New Zealand White
Number of Animals	6
Vehicle	None
Observation Period	9 days
Type of Dressing	Semi-occlusive
Remarks - Method	There were no significant deviations from the protocol.

RESULTS

Lesion	*Mean Score**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Erythema/Eschar	1.0	1	<9 days	0
Oedema	0.56	2	<6 days	0

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

Remarks - Results Very slight erythema was observed in all test animals for five consecutive days after patch removal. By the sixth to the eighth day, no erythema to very slight erythema was manifested in some of the animals. Very slight to well-defined oedema was observed in some of the animals up to day 5 after patch removal. The observed findings were reversible within 9 days after patch removal. No other toxic effects were observed.

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY International BioResearch (1992b)

B.4.1 Irritation – eye

TEST SUBSTANCE Analogue 1 (undiluted, pasty with pH value of 4.5)

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 84/449/EEC.

Species/Strain	Rabbit / New Zealand White
Number of Animals	6
Observation Period	5 days
Remarks - Method	There were no significant deviations from the protocol.

RESULTS Conjunctival redness and chemosis as well as corneal opacity and iris damage were observed during the observation period. In three of the test animals, the treated eye was completely closed. In five test animals, purulent ocular secretion occurred. Since the clinical symptoms were so severe, the test was terminated 24-48 hours after treatment. In these five

test animals, results showed no signs of reversibility during the 24-hour and 48-hour observation times. Therefore, the ocular reactions observed may be indicative of irreversible ocular corrosion.

CONCLUSION — The notified chemical is severely irritating to the eye.

TEST FACILITY — International BioResearch (1992c)

B.4.2 Irritation – eye

TEST SUBSTANCE — Analogue 1 (undiluted, off-white waxy solid, pH value not reported)

METHOD — OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 84/449/EEC.

Species/Strain	Rabbit / New Zealand White
Number of Animals	3
Observation Period	22 days
Remarks - Method	This test followed low volume procedure – 10mg of the test substance was used on Day 1, which is 10 folds lower than the dose used in study B.4.1 and B.4.3. There were no other significant deviations from the protocol.

RESULTS

Lesion	*Mean Score**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Conjunctiva: redness	0.2	1	<72 hours	0
Conjunctiva: chemosis	0	0	NA	NA
Conjunctiva: discharge	0	0	NA	NA
Corneal opacity	0	0	NA	NA
Iridial inflammation	0	0	NA	NA

*Calculated on the basis of the scores at 24, 48, and 72 hours for ALL animals. NA, not applicable.

Remarks - Results — Slight redness of conjunctival tissue was observed which resolved within 24 hours in two test animals and within 72 hours in one. A small amount of discharge was noted in two animals on day 1 only. No other signs of irritation were observed.

No mortality and signs of systemic toxicity were noted during the test period.

CONCLUSION — The notified chemical is slightly irritating to the eyes under the low volume test condition.

TEST FACILITY — Notox (1994)

B.4.3 Irritation – eye

TEST SUBSTANCE — Analogue 1 (5%, turbid white liquid with pH value of 4.5)

METHOD — OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 84/449/EEC.

Species/Strain	Rabbit / New Zealand White
Number of Animals	6
Observation Period	72 hours
Remarks - Method	There were no significant deviations from the protocol.

RESULTS — All test animals showed slight redness and chemosis of the conjunctivae one hour after treatment, but resolved within 24 hours. No other signs of

irritation were observed.

No mortality and signs of systemic toxicity were noted during the test period.

CONCLUSION — The notified chemical is not irritating to the eye.

TEST FACILITY — International BioResearch (1992d)

B.5. Skin sensitisation

TEST SUBSTANCE — Analogue 1

METHOD — OECD TG 406 Skin Sensitisation – Magnusson & Kligman Maximisation Test.

Species/Strain — Guinea pig / Pirbright White

PRELIMINARY STUDY — Maximum Non-irritating Concentration:
intradermal: 5% (w/w) dilution with purified water (vehicle)
topical: 10% (w/w) test substance in vehicle

MAIN STUDY

Number of Animals — Test Group: 20 Control Group: 20

INDUCTION PHASE — Induction Concentration:
intradermal: 5% (w/w) test substance in vehicle
topical: 25% (w/w) test substance in vehicle

Signs of Irritation — Not reported

CHALLENGE PHASE

1st challenge — topical: 10% (w/w) test substance in vehicle

Remarks - Method — The positive controls used in the study were 2,4-dinitrochlorobenzene and benzocaine. No skin irritation data was presented concerning the positive controls. The laboratory stated that the reactions to the positive controls were tested periodically and that there was an acceptable level of response.

No skin reactions were observed or reported in the induction phase of the study.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st challenge		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	10%	0	0	NA	NA
Control Group	0%	0	0	NA	NA

Remarks - Results — At 10% concentration, no skin reactions were observed in all of the test animals. All animals gained bodyweight after the observation period.

CONCLUSION — There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test. However, no definite conclusion can be made because the test conditions were inadequately or insufficiently documented.

TEST FACILITY — International BioResearch (1992e)

B.6. Repeat dose toxicity

TEST SUBSTANCE — Notified chemical

METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/ Crl:CD
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	0.8% aqueous hydroxypropylmethylcellulose gel
Remarks – Method	No significant deviations from the test protocol.

RESULTS

Dose *mg/kg bw/day*	*Number and Sex* *of Animals*	*Mortality*
0	5 /sex	0
100	5 /sex	0
300	5 /sex	0
1000	5 /sex	0
0 (recovery)	5 /sex	0
1000 (recovery)	5 /sex	0

Mortality and Time to Death
No test item related mortality was noted.

Clinical Observations
A significant dose-related increase in forelimb and hindlimb grip strength was found in treated male groups. No other treatment-related changes of behaviour or external appearance were observed during the study.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Significant decreases in mean corpuscular volume (MCV) (at dose of 100 and 1000 mg/kg bw/day) and mean corpuscular haemoglobin (MCH) (at 1000 mg/kg bw/day) were found in females, but these changes were not dose-related. A dose-related decrease of thromboplastin time (TPT) in females was noted with a statistically significant decrease at 1000 mg/kg bw/day only. No other treatment-related laboratory findings were noted.

Effects in Organs
Macroscopical changes during both treatment and recovery period included red discoloured periphery cervical lymph nodes, colon and uterus filled with liquid, and reduced testicle size. However, they were either isolated changes, comparable with the control group, or not dose-related. There was a dose-related increased lobular pattern in the liver in male rats, but it was reversible during the recovery period. Therefore, these changes are not considered biologically relevant.

No treatment-related microscopical changes in organs were noted.

Remarks – Results
In general, no significant effects were observed up to the highest dose tested. The few treatment-related changes (increased forelimb and hindlimb grip strength in males, decreased TPT in females) were considered to be not biological relevant due to lack of corresponding pathological changes.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established at 1000 mg/kg bw/day based on this study.

TEST FACILITY LPT (2005)

B.7 Genotoxicity – bacteria

TEST SUBSTANCE Analogue 2

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

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100
Metabolic Activation System	Aroclor 1254-induced rat S9 liver homogenate
Concentration Range in Main Test	a) With metabolic activation: 8-5000 µg/plate b) Without metabolic activation: 8-5000 µg/plate
Vehicle	Purified water
Remarks - Method	A preliminary test was conducted using the TA100 strain of *S. typhimurium*. The doses chosen for range finding were the following: 10, 32, 100, 320, 1000, 3200 and 10000 µg/plate. In order to improve the solubility, the test substance was subjected o ultrasonic treatment and heated to 40 °C for 15 minutes. It was not possible to dissolve the test substance completely due to adhesion of the substance on the walls of the vessel. Therefore, the reported concentrations were based on calculated values. Two concentrations of the metabolic activation system (S9) were used: 4% for Test 1 and 10% for Test 2.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	>32			
Test 1 (4% S9)		>200	5,000	Negative
Test 2 (10% S9)		>200	5,000	Negative
Present	>32			
Test 1 (4% S9)		>200	5,000	Negative
Test 2 (10% S9)		>1000	5,000	Negative

CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	International BioResearch (1993)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosomal Aberration Test. EC Directive 2000/32/EC B.10 Mutagenicity - *In vitro* Mammalian Chromosome Aberration Test
Species/Strain	Chinese Hamster
Cell Type/Cell Line	V79 Cell line
Metabolic Activation System	S9, Phenobarbital/beta-Naphthoflavone induced rat liver
Vehicle	Deionised water
Remarks – Method	

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Present			
Test 1	4.7, 9.4, 18.8, 37.5*, 75*, 150, 300*, 600*	4h	18h
Test 2	18.8*, 37.5*, 75, 150*, 300*, 600	4h	28h
Test 3	100, 200, 300*, 400*, 500*, 600	4h	28h
Absent			
Test 1	12.5, 25*, 50*, 100*, 150, 200	4h	18h
Test 2 a	3.1, 6.3*, 12.5*, 25*, 50, 100	18h	18h
Test 2 b	18.8*, 37.5, 75, 150	28h	28h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in PreliminaryTest	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Present	>312.5			
Test 1		>300	≥75	None
Test 2		>300	≥37.5	Borderline
Test 3		>400	≥100	None
Absent	>78.1			
Test 1		>100	≥100	None
Test 2 a		≥100	100	None
Test 2 b		>75	≥75	None

Remarks – Results

In Test 1 in the presence of S9 mix, a single significant increase in the number of cells carrying structural chromosomal aberrations (4 %) was observed at 37.5 µg/mL only. The aberration rates at higher doses were the same or lower than the negative and solvent controls. Therefore, this isolated change is not considered biologically relevant.

In Test 2 in the presence of S9 mix, a significant increase in aberration rates (4.8 %) was observed at the highest dose tested (300 µg/mL). Although it slightly exceeded the historical control range (0 - 4 %), this finding was accompanied with a dose-related increase in the aberration rate (0.5%, 2.5% and 4.8 % at dose levels of 37.5, 150, and 300 µg/mL). A confirmatory experiment (Test 3, in the presence of S9 mix) was performed to proof these observations. Although a dose-related pattern was found again in Test 3, all increases in the aberration rate were within the historical control range. Therefore, the borderline change in Test 2 is considered biologically irrelevant.

No significant increase in the number of cells carrying structural chromosomal aberrations was observed in other tests after treatment with the test item.

CONCLUSION

The notified chemical was not clastogenic to Chinese Hamster V79 Cells treated in vitro under the conditions of the test.

TEST FACILITY

RCC (2004)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test. EC Directive 92/69/EEC C.4-E Biodegradation: Determination of the " Ready" Biodegradability: Closed Bottle Test
Inoculum	Municipal activated sludge from a plant in Pforzheim /Germany
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	COD
Remarks - Method	No significant protocol deviations.

RESULTS

Test substance		*Na-benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	22.5	7	69.9
14	45.4	14	76.6
21	63.2	21	84.2
28	66.2	28	94.4

Remarks - Results

The notifier indicates that the level of 60% degradation was reached within a 14-day window (day 7-21), which according to OECD guideline 301 (adopted 17.07.1992), subchapter 10 (pass levels) is considered to be equivalent to the 10-d window in the case that sampling is only performed in 7-day intervals. In the case of surfactants, the 10 days window criterion is not a requirement for the desired stringency of OECD 301 type screening tests (CESIO, 2003). Therefore, the test substance can be considered as readily biodegradable.

No inhibitory effects of the test item were observed (more than 25% degradation occurred within 14 days). The degradation of the reference substance had reached 77% within 14 days and thus validating the test.

CONCLUSION — The notified chemical is considered to be ready biodegradable.

TEST FACILITY — GAB (2005e)

C.1.2. Bioaccumulation

A calculation was performed using the calculation program BCFWIN v2.15, which takes the ionic structure and the length of the side chains into account. This has been established particularly for quaternary ammonium compounds, according to Meylan et al (1999). The following BCF values, using the C18-ester chain as the chemical lead compound, were estimated:

Methyl sulphate: BCF = 3.162
Monoester of triethanolmethylammonium: BCF = 70.79
Diester of triethanolmethylammonium: BCF = 70.79
Triester of triethanolmethylammonium: BCF = 70.79

QSAR predictions adapted to quaternary ammonium compounds (and the underlying experimental data base) suggest that none of the components of the notified chemical will have BCF values greater than 100, and thus there is little potential for bioaccumulation. Furthermore, BCF of surface active substance cannot be measured or calculated and bioconcentration is not expected to pose an unacceptable risk based on the present knowledge (CESIO, 2003).

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test - semi-static, renewal after 48 h. EC Directive 92/69/EEC C.1 Acute Toxicity for Fish - semi-static, renewal after 48 h.
Species	Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period	96 h
Auxiliary Solvent	None
Water Hardness	214 mg $CaCO_3$/L
Analytical Monitoring	HPLC-MS-MS
Remarks – Method	No significant protocol deviations.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality (%)*					
Nominal	*Mean measured*		*3 h*	*6 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	<LOQ	10	0	0	0	0	0	0
1	n.d.	10	0	0	0	0	0	0
1.6	n.d.	10	0	0	0	0	0	0
2.56	1.34	10	0	0	0	0	0	0
4.1	2.68	10	0	10	90	100	100	100
6.55	n.d.	10	0	40	100	100	100	100
10.5	n.d.	10	40	100	100	100	100	100

n.d. = not determined
LOQ = 0.1 mg/L

LC50	1.91 (mean measured) mg/L at 96 hours.
NOEC	1.51 (mean measured) at 96 hours.
Remarks – Results	Foaming of the test solution was observed at the nominal concentrations of 2.56, 4.10, 6.55 and 10.5 mg/L. Sub-lethal effects of No mortality was observed at the nominal concentration of 2.56 mg/L and below after 96 h. 100% mortality of fish was observed at the nominal concentration of 4.1 mg/L and above after 96 h. Sub-lethal effects of reduced activity and/or orientation to bottom or surface of the test vessels and difficulties with maintenance of balance were observed at nominal concentrations of 4.1 and 10.5 mg/L at 24 and 3 h, respectively. There was a decline in test substance detected in the water after 24 and 48 hours of exposure with

analysis detecting between 10 and 76% of the nominal concentration. Since the measured concentrations of the test substance in the water samples fell gradually below 80%, the toxicological endpoints were additionally evaluated using the mean measured concentration of 59%. The decrease of test substance concentrations can be explained by the significant hydrolysis anticipated to occur under the conditions of this test. The water quality (pH, temperature and dissolved oxygen) was within acceptable limits.

CONCLUSION — The notified chemical is considered toxic to rainbow trout (*Oncorhynchus mykiss*).

TEST FACILITY — GAB (2005f)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE — Notified chemical

METHOD — OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - static, 48 h.
EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - static, 48 h.

Species	*Daphnia magna Straus Clone V*
Exposure Period	48 hours
Auxiliary Solvent	none
Water Hardness	178 mg $CaCO_3$/L
Analytical Monitoring	HPLC-MS-MS
Remarks – Method	No significant protocol deviations.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Mean measured*		*24 h (acute)*	*48 h (acute)*
0	<LOQ	20	0	0
0.20	n.d.	20	0	0
0.44	n.d.	20	0	0
0.97	n.d.	20	0	0
2.13	0.74	20	0	0
4.69	n.d.	20	0	1
10.3	4.86	20	2	4
22.7	11.7	20	17	18

n.d. = not determined
LOQ = 0.1 mg/L

LC50	6.05 mg/L (CI: 4.98-7.34 mg/L) [mean measured] at 48 hours
NOEC	0.948 mg/L (mean measured) at 48 hours
Remarks – Results	It was observed in the stock solutions turbid dispersion containing small agglomerates of the test item. After 48 h, 90% of the daphnids were immobilised at the nominal concentration of 22.7 mg/L. No immobilisation was observed at the nominal concentration of 2.13 mg/L and below after 48 h. Test concentrations at test initiation were between 53% and 65% of the nominal values declining to test concentrations between 16% and 39% of the nominal values by 48 h. The mean measured concentrations during the test were between 34.7 and 51.7% of the nominal values, corresponding to an average of 44.5% of the nominal values. Since measured concentrations of the test substance in the water samples were below 80 %, the toxicological endpoints were evaluated using the mean measured concentrations during the test period. The EC50 of the reference substance was within the acceptable range and the water quality (pH, temperature and dissolved oxygen) was within

acceptable limits.

CONCLUSION	The notified chemical is considered to be toxic to *Daphnia magna*.
TEST FACILITY	GAB (2005g)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test. EC Directive 92/69/EEC C.3 Algal Inhibition Test.
Species	Green alga (*Desmodesmus subspicatus*)
Exposure Period	72 hours
Concentration Range Nominal	1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg/L
Concentration Range Actual	Nominal concentrations were analytically verified at test start (95.2 – 99.7%). Measured concentration reached 5% of the nominal value at test end (72 h).
Auxiliary Solvent	None
Water Hardness	Not stated
Analytical Monitoring	HPLC-MS-MS
Remarks – Method	No significant protocol deviations.

RESULTS

Biomass		*Growth*	
EbC50 mg/L at 72 h	*NOEC mg/L*	*ErC50 mg/L at 72 h*	*NOEC mg/L*
n.d.	n.d.	22.3 (CI: 20.2-24.9) [mean measured concentration]	9.25 (mean measured concentration)

n.d. = not determined

Remarks – Results	Significant effects were observed at the nominal concentrations of 50-100 mg/L. No effects were observed at the nominal concentration of 25 mg/L and below after 72 h. The nominal concentrations of the test substance in the water samples were verified by initial measured concentrations. The content of the test substance rapidly decreased during the test, reaching ~5% of nominal after 72 h. The decrease of test item concentrations can be explained by the significant hydrolysis expected to occur under the conditions of this test. The mean measured concentration during the test was about 37% of the nominal concentrations. The pH and temperatures were within acceptable ranges.
CONCLUSION	The notified chemical is moderately toxic to *Desmodesmus subspicatus*.
TEST FACILITY	GAB (2005h)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test.
Inoculum	Activated sludge from the municipal wastewater treatment plant of Pforzheim, Germany, was used as microbial inoculum for the test. This plant predominantly is treating domestic sewage. 2 L of sludge with an initial content of MLSS (mixed liquid suspended

	solids) of 8 g/L was collected at the day of the test. It was washed with tap water by centrifugation, resuspended in 4 L of tap water and aerated with an air pump. The MLSS were adjusted to a final concentration of 1.6 g/L in the test medium.
Exposure Period	3 hours
Concentration Range Nominal	1 – 243 mg/L
Remarks – Method	No significant protocol deviations.
RESULTS	
IC50	> 243 mg/L
NOEC	243 mg/L
Remarks – Results	There was no inhibitory effect of the test substance at any test concentrations. The EC50 for DCP was between 5 and 30 mg/L after 3 h which fulfilled the criterion of validity.
CONCLUSION	The notified chemical is considered to be non-toxic to micro-organisms.
TEST FACILITY	GAB (2004c)

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