Existing Chemical Secondary Notification Assessment Report STD/1258S

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Australian Government
Department of Health
National Industrial Chemicals Notification and Assessment Scheme

Ethanaminium, 2-hydroxy-N,N-bis(2-hydroxyethyl)-N-methyl-, esters with C_{16-18} and C_{18}-unsatd. fatty acids, Me sulfates (salts)

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

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Preface

This assessment was carried out under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS). This scheme was established by the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act), to aid in the protection of the Australian people and the environment by assessing the risks of industrial chemicals, providing information and making recommendations to promote their safe use. NICNAS assessments are carried out by staff employed by the Australian Government Department of Health in conjunction with the Australian Government Department of the Environment and Energy.

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

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Background

Ethanaminium, 2-hydroxy-N,N-bis(2-hydroxyethyl)-N-methyl-, esters with C_{16-18} and C_{18}-unsatd. fatty acids, Me sulfates (salts), Chemical Abstracts Service Registry Number (CAS RN) 157905-74-3, is a chemical of unknown or variable composition (UVCB). It was assessed by NICNAS under the standard notification category in 2007 (STD/1258) for use in fabric softeners and facial cleansers. It is now listed on the Australian Inventory of Chemical Substances (AICS).

In 2017, NICNAS was notified that the introduction volume and concentration of the notified chemical in fabric softeners available to the public significantly exceed those previously assessed. The change in introduction may result in increased risk of adverse effects to the environment and to users of those end-use products. New toxicity data are also available, which warrant a review of the hazard classification of the chemical.

This secondary notification assessment reassesses the risks posed to the public, workers and the environment from the chemical based on this new information.

Exempt Information (Section 75 of the Act)

No applications for exempt information were made for the secondary notification assessment.

Importation/manufacturing volume and uses

The notified chemical is not manufactured in Australia and is imported in finished products.

The chemical was originally notified as being imported as a neat material for further formulation. However, no applicants for the secondary notification assessment indicated importation of the chemical as neat material.

The maximum reported import volume of the chemical is up to 504 tonnes per annum, compared to an annual introduction of up to 100 tonnes in the original new chemical assessment. The chemical is used as a component of fabric softeners at a concentration of up 21.1%, compared to the originally assessed concentration of up to 5%.

Human health effects

The new human health toxicity data on the notified chemical submitted for the secondary notification consist of one study each for dermal and eye irritation in rabbits and an in vitro bovine corneal opacity permeability (BCOP) test. Analogue data were provided for these endpoints for the new chemical assessment. Summaries of studies on the chemical and other analogues from the Human and Environmental Risk Assessment (HERA) report on esterquats (HERA, 2009) for all endpoints were also available for the secondary notification assessment.

Data on chemical analogues available from the HERA report for this secondary notification assessment indicate that the chemical is of low acute oral and dermal toxicity. These confirm the findings from the new chemical assessment. No data for the secondary notification assessment or new chemical assessment were available to evaluate the acute inhalation hazard.

The submitted study on the notified chemical and data for chemical analogues from the HERA report for skin irritation confirm the findings from the new chemical report that the chemical is a skin irritant. However, studies submitted for the secondary notification assessment on the notified chemical for eye irritation only reported mild irritant effects, in contrast to analogue studies submitted for the new chemical assessment that reported irritation ranging from slight to severe.

From the HERA report, human and animal studies for skin sensitisation using analogues reported only weak or equivocal evidence for skin sensitisation in experimental animals and no reactions indicative of skin sensitisation in humans. None of the analogue studies available for the new chemical assessment indicated a potential for skin sensitisation.

Results from a 90 day repeat dose study of an analogue chemical from the HERA report available for the secondary notification assessment could not be interpreted due to bacterial infections in treatment groups. In the new chemical assessment, no significant effects were observed in a 28-day oral repeated dose rat study on the notified chemical up to the highest dose tested (1000 mg/kg bw/day).

The HERA report contained analogue studies indicating no genotoxicity/mutagenicity in vitro or in vivo, which confirm the findings from the new chemical assessment that the chemical is not genotoxic.

Occupational exposure and health risks

The notified chemical is imported as a component of finished fabric softeners at a concentration of up to 21.1%. The finished fabric softeners containing the chemical will be transported by road to warehouse facilities for temporary storage before distribution to retail outlets for sale to the general public. For workers involved in transport, storage and retail, dermal and ocular exposures to the finished fabric softeners containing the chemical are not expected except in the event of accidental breaching of the packaging of the finished products. The risk to the health of transport and storage workers and retailer workers is therefore expected to be low.

Public exposure and health risks

Exposure of the public to the notified chemical at up to 21.1% concentration may occur when using the fabric softeners during laundry activities. The principal route of exposure is dermal, while incidental ocular exposure is also possible. The risk of skin and eye irritation following such exposures is potentially of concern. However, considering the relatively infrequent use of the product and short use duration during which exposures may occur, the overall risk to public health from use of the chemical in fabric softeners is considered to be low.

The risk of skin and eye irritation when using washed materials treated with the fabric softener is also considered low on the basis that a negligible level of residual product is expected on the washed materials.

In addition, restrictions are in place for consumer products containing the chemical in Australia. The chemical is covered by the entry for ‘Quaternary ammonium compounds’ listed in the Poisons Standard (SUSMP, 2018). Quaternary ammonium compounds are listed in Schedules 5 and 6 requiring substances to be labelled with the signal word “Caution” for ≤ 20% concentration or “Poison” for concentration greater than 20%, respectively. Preparations containing ≤ 5% concentration of quaternary ammonium compounds do not require labelling.

Environmental effects

Ecotoxicity data were available in the original new chemical assessment. No new ecotoxicological studies were provided for this secondary notification. Therefore, the ecotoxicity results in the new chemical assessment are reproduced in this secondary notification assessment. The chemical is toxic to fish and aquatic invertebrates and harmful to algae. It is non-inhibitory to microbial respiration. The notified chemical is toxic to aquatic life for the purpose of regulatory risk assessment.

Environmental exposure and risks

The notified chemical will be used in fabric softeners and after use will be released in wastewater to sewers. Based on the maximum import volume, assessed use pattern, and a conservatively

determined PEC/PNEC ratio, the release of the chemical may approach ecotoxicologically significant concentrations in the aquatic environment. However, the calculated risk quotient is an upper limit given the chemical will likely degrade further in the environment than is assumed in the conservative environmental modelling. Therefore, based on the current annual import volume, the overall risk to the environment from release of the chemical is expected to be low. However, any further increase in import volume will lead to a commensurate increase in risk.

Recommendations

This section outlines the recommendations arising from the secondary notification assessment of the notified chemical, and incorporates the applicable recommendations from the new chemical assessment report (NICNAS, 2007). The hazard classification presented below is according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009).

Recommendations are directed principally to:

- regulatory bodies
- importers
- reformulators of the notified chemical.

Implicit in these recommendations is that best practice is implemented to minimise occupational and environmental exposures.

Recommendations to regulatory bodies

Based on the assessment findings, an amended hazard classification of the chemical, according to the GHS, is recommended to Safe Work Australia as below:

- Skin irritation (Category 2): H315 – Causes skin irritation
- Acute aquatic toxicity (Category 2): H401 – Toxic to aquatic life
- Chronic aquatic toxicity (Category 2): H411 – Toxic to aquatic life with long lasting effects

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

- Concentration ≥ 10%: Causes skin irritation
- 1% ≤ Concentration < 10%: Causes mild skin irritation

Recommendations to importers and state and territory governments

Hazard communication

Labels

Importers of the notified chemical should ensure their labels reflect the hazards identified by this assessment and comply with the *Labelling of workplace hazardous chemicals – Code of practice* (Safe Work Australia, 2015).

Safety Data Sheets (SDSs)

Under the *Model Work Health and Safety Regulations* (Safe Work Australia, 2016a) and the Commonwealth, state and territory regulations introduced in accordance with these model regulations, employees must have easy access to SDSs for hazardous substances at their workplace. SDSs provide information to those who use the hazardous substance.

Importers of the notified chemical should:

- ensure their SDSs reflect the hazards identified by this assessment and comply with the

Preparation of safety data sheets for hazardous chemicals - Code of practice (Safe Work Australia, 2016b)

- ensure that employees exposed to the chemical have easy access to a copy of the SDS.

Control measures

Occupational controls

The following recommendations for reformulation processes stipulated in the new chemical assessment (NICNAS, 2007) are applicable and included in this assessment.

A person conducting a business or undertaking (PCBU) at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical during reformulation processes:

- prevent leaks and spills
- enclosed, automated processes, where possible.

A PCBU at a workplace should implement the following safe work practices to minimise occupational exposure during reformulation processes:

- 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 (personal protective equipment) PPE should be thoroughly cleaned before re-use.

A PCBU at a workplace should ensure that the following PPE is used by workers to minimise occupational exposure to the notified chemical during reformulation processes:

- protective clothing
- chemical resistant gloves
- face-shield.

Guidance in the selection of PPE can be obtained from Australian, Australian/New Zealand or other approved standards.

If products and mixtures containing the notified chemical are also classified as hazardous to health in accordance with the GHS (United Nations, 2009) 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

Products containing the notified chemical should be labelled in accordance with state and territory legislation. The chemical is covered by the entry for 'Quaternary ammonium compounds' listed in the Poisons Standard. Quaternary ammonium compounds are listed in Schedules 5 and 6 requiring substances to be labelled with the signal word "Caution" for ≤ 20% concentration or "Poison" for concentration greater than 20%, respectively (SUSMP, 2018).

The following additional measures should be taken by the supplier of consumer products containing the 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 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

Any direct release of the notified chemical to surface waters or soils should be avoided.

Disposal

Where reuse or recycling are not appropriate, disposal of the notified chemical should occur in accordance with relevant Commonwealth, state, territory and local government legislation.

Storage

Containers should be securely closed and stored according to container label instructions.

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 the secondary 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 Act) an introducer (importer/manufacturer) of the notified chemical, has post-assessment regulatory obligations to notify NICNAS when any of these circumstances change.

Therefore, the Director of NICNAS must be notified in writing within 28 days by the notifiers, other importers or manufacturers:

(1) Under Section 64(1) of the Act; if

- importation by either of the current applicants increases, or a person commences introduction of the notified chemical

The following secondary notification condition stipulated in the new chemical assessment (NICNAS, 2007) is applicable and reproduced in this assessment:

- the notified chemical is intended for use in leave on products.

or

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

- the function or use of the chemical has changed or is likely to change significantly from being a component of fabric softeners,
- if the chemical has begun to be manufactured in Australia
- additional information becomes available to the person on the adverse effects of the chemical on human health or the environment.

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

Abbreviations and acronyms

Act, the	Commonwealth Industrial Chemicals (Notification and Assessment) Act 1989
AICS	Australian Inventory of Chemical Substances
BCOP	bovine corneal opacity and permeability
bw	body weight
CAS RN	Chemical Abstracts Service Registry Number
Da	Daltons (units of molecular weight)
DOC	dissolved organic carbon
EC50	median effective concentration or half maximal effective concentration
EL50	effective loading rate resulting in 50% effect
g	gram
g/cm³	grams per cubic centimetre
GHS	Globally Harmonized System of Classification and Labelling of Chemicals (United Nations)
GPMT	guinea pig maximisation test
ha	hectare
hazard	inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub)population is exposed to that agent; intrinsic property of a substance to cause harm
HCIS	Hazardous Chemical Information System
HERA	Human and Environmental Risk Assessment
HET-CAM	hen's egg test chorioallantoic membrane
HPLC	high-performance liquid chromatography
HRIPT	human repeated insult patch test
IR	infrared
IVIS	in vitro irritancy score
kg	kilogram
L	litre
LC50	median lethal concentration
LD50	median lethal dose
m^2	square metre
m^3	cubic metre
µg	microgram
mg	milligram
mg/kg bw	milligram per kilogram bodyweight
mL	millilitre
ML	megalitre

MSDS	(Material) Safety Data Sheet, also see SDS
MTEA	methyl-triethanol-ammonium
MW	molecular weight
NAMW	number-average molecular weight
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NMR	nuclear magnetic resonance
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
Pa	pascal
PCBU	person conducting a business or undertaking
PEC	predicted environmental concentration
PNEC	predicted no effect concentration
PPE	personal protective equipment
ppm	parts per million
QA	quality assurance
risk	probability or likelihood of harm and the likely extent of the harm; the probability of an adverse effect in an organism, system or (sub)population caused under specified circumstances by exposure to an agent
RF	retention factor
RQ	risk quotient
SDS	Safety Data Sheet (also see MSDS)
STP	sewage treatment plant
SUSMP	Standard for the Uniform Scheduling of Medicines and Poisons
TG	test guideline
UVCB	chemical of unknown or variable composition, complex reaction products and biological materials
UV-Vis	ultraviolet-visible spectroscopy
WAF	water accommodated fraction
WAMW	weight-average molecular weight
w/v	weight to volume
w/w	weight to weight

1. Introduction

1.1 Background

Ethanaminium, 2-hydroxy-N,N-bis(2-hydroxyethyl)-N-methyl-, esters with C_{16-18} and C_{18}-unsatd. fatty acids, Me sulfates (salts), CAS RN 157905-74-3, is a chemical of unknown or variable composition (UVCB), consisting of C_{16-18} and C_{18} unsaturated mono, di and tri esters of triethanolamine, quaternised with dimethyl sulfate. It was assessed by NICNAS as a new chemical under Section 32 of the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act) in the standard notification category. The new chemical assessment report STD/1258 (NICNAS, 2007) was published in December 2007, and the notified chemical was listed on the Australian Inventory of Chemical Substance (AICS) in December 2012.

In 2017, NICNAS was notified that the introduction volumes and the concentration of the notified chemical in fabric softeners significantly exceed those previously assessed. There may therefore be increased risk of adverse effects to the environment and to users of those end-use products. Furthermore, new toxicity data are available, which warrant a review of the hazard classification of the chemical. Therefore, a reassessment of the human health and environmental risks for the chemical is required. This secondary notification assessment focuses on the new data provided.

Data submitted for the original assessment on use, exposure and toxicity are summarised in this report in the relevant sections. Details of the studies provided for assessment as a new chemical are reproduced in the Appendix. New data submitted for this assessment are discussed in detail and identified by the abbreviation **ND.**

1.2 Declaration

A notice was published in the Chemical Gazette of November 2017, requiring a secondary notification of ethanaminium, 2-hydroxy-N,N-bis(2-hydroxyethyl)-N-methyl-, esters with C_{16-18} and C_{18}-unsatd. fatty acids, Me sulfates (salts), in accordance with Section 65(2) of the Act. The secondary notification applied to all importers of the chemical for use in fabric softeners, and importers of fabric softeners containing the chemical. The declaration required the provision of any information relevant to the assessment of the notified chemical that was not covered in the new chemical assessment and included the following:

1) Identity, properties and uses:
    a. Trade name(s) under which the chemical is marketed by the introducers
    b. Annual import volumes of the chemical
    c. The concentration of the chemical as imported and in fabric softener products
    d. Description of transportation and storage of the imported chemical and the end-use product
    e. If importers of the chemical are formulating fabric softeners, the description of the formulation/packing process and disposal of wastes resulting from the process
    f. The percentage of total imported volume of the chemical that is expected to be released as:
        i. residues in empty containers (both from import and in end-use)
        ii. accidental leaks and spills
        iii. washings from equipment used to formulate fabric softeners
    g. The expected fate for each of the above releases of the chemical (e.g. landfill, sewer

etc.)

2) Human health and environmental data:
    a. Any additional toxicology data for the chemical, or a suitable analogue
    b. Any additional ecotoxicology data for the chemical, or a suitable analogue
    c. Any additional environmental fate studies for the chemical, or a suitable analogue
    d. Composition of the substance tested in each study including:
        i. Concentration of the chemical/suitable analogue in the test substance
        ii. Any hazardous impurities or additives.

1.3 Objectives

The objectives of this assessment are to review the new data made available since the publication of the new chemical assessment report and reassess the:

- human health hazards
- environmental hazards
- risks of adverse effects resulting from exposure to workers, the public and the environment from the use of the notified chemical.

Based on the above, appropriate recommendations will be made to control exposures and reduce risks for workers, the public and the environment, as required.

1.4 Peer review

During all stages of preparation, this report has been subject to internal peer review by NICNAS.

1.5 Applicants

Following the secondary notification declaration of ethanaminium, 2-hydroxy-N,N-bis(2-hydroxyethyl)-N-methyl-, esters with C16-18 and C18-unsatd. fatty acids, Me sulfates (salts), two companies applied for assessment of the chemical. The original notifier, Salkat Australia Pty Ltd, is no longer importing the chemical.

In accordance with the Act, NICNAS provided the applicants with a draft copy of the report for comment during the corrections/variations phase of the assessment.

The applicant details are as follows:

Amway of Australia
7-9 Irvine Place
Bella Vista, NSW 2153

Unilever Asia Private Ltd
219 North Rocks Road
North Rocks, NSW 2151

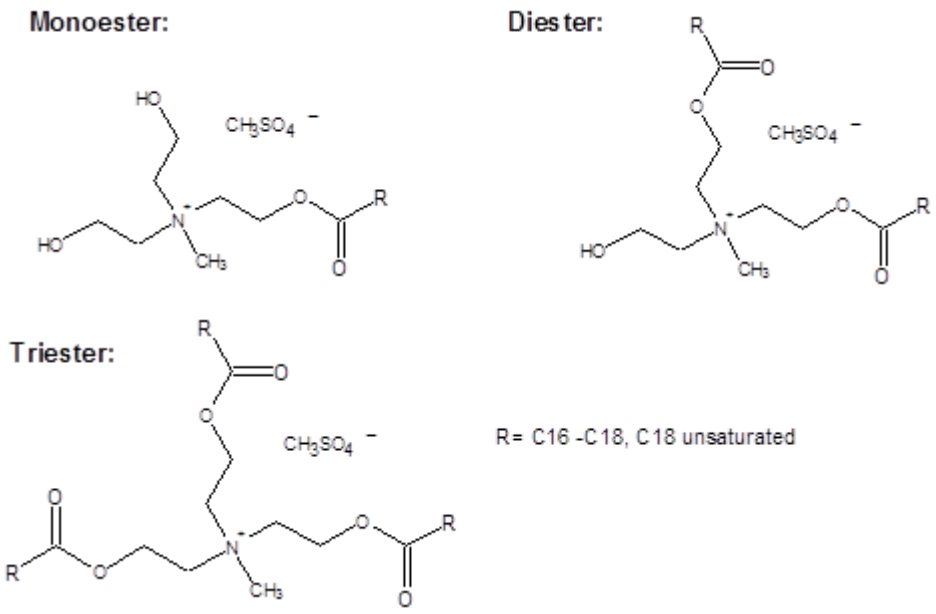
1.6 Exempt information

No application for exempt information was made for the secondary notification assessment.

2. Chemical identity, physical and chemical properties

The chemical identity, physical and chemical data assessed by NICNAS in the new chemical assessment report (NICNAS, 2007) are reproduced in this report. New data submitted for this secondary notification assessment are indicated as **ND**.

2.1 Chemical identity

Chemical name:	Ethanaminium, 2-hydroxy-N,N-bis(2-hydroxyethyl)-N-methyl-, esters with C_{16-18} and C_{18}-unsatd. fatty acids, Me sulfates (salts)
CAS number:	157905-74-3
Marketing names:	STEPANTEX SP-88-2 (**ND**) STEPANTEX VT-90 (**ND**) STEPANTEX VM-90 (**ND**)
Other names:	2-Hydroxy-N,N-bis(2-hydroxyethyl)-N-methylethanaminium esters with (C_{16-18}) and (C_{18})-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 Triethanolamine Esterquat Dioleoylethyl hydroxyethylammonium methosulfate
Molecular formula:	Unspecified
Structural formula:	
Molecular weight:	733.5 (weighted average)

Method of detection and determination: Reference NMR, IR, HPLC, UV-Vis spectra were provided for the new chemical assessment.

2.2 Composition

Degree of purity: >80%

Additives:

Formulation (ND)	Chemical name	CAS number	Weight percentage (ND)
STEPANTEX SP-88-2	Ethanol	64-17-5	10-20%
STEPANTEX VT-90	2-Propanol	67-63-0	9-11%
STEPANTEX VM-90	2-Propanol	67-63-0	5-15%

2.3 Physical and chemical properties

The physical and chemical properties of the notified chemical are shown in the table below. The robust summaries of tests on physical and chemical properties are provided in Appendix C of this report.

Summary of physical and chemical properties

Property	Value	Data Source
Appearance at 20°C and 101.3 kPa:	Slightly yellowish solid	Measured
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 P_{ow} = >6.5 at 20°C	Estimated
Adsorption/Desorption	log K_{oc} = >5 at 20°C	Calculated
Surface tension:	41.8 mN/m at 20°C	Measured
Dissociation constant:	pKa = 1.14 (methylsulfuric acid) pKa = 12.42 and 13.68 (monoester with C18 ester chain) pKa = 12.52 (diester with C18	Calculated

	ester chain)	
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 chemical is not explosive
Oxidising properties:	Not oxidising	The chemical has no oxidising properties based on its structural groups, thermodynamic calculations and negative oxygen balance.
Reactivity:	Chemical has a half-life of 17 days at pH 7 at 25ºC	Based on the hydrolysis study.

3. Importation and use

Importation

The notified chemical is not manufactured in Australia and is imported in finished products. The chemical was originally notified in 2007 as being imported as a neat material for further formulation. The original notifier, Salkat Australia Pty Ltd, is no longer importing the neat material.

No importation of the neat chemical was reported for this assessment. The maximum import volume of the chemical is up to 504 tonnes per annum, compared to an initial annual introduction volume of up to 100 tonnes.

Use

The new chemical assessment (NICNAS, 2007) assessed the use of this chemical in fabric softeners and cosmetic facial cleansers. This secondary notification assessment is reviewing the significant increase in the concentration of the chemical in fabric softeners from the initial concentration of 5%. The applicants reported importing fabric softeners containing the chemical at a concentration of up to 21.1%.

Use of the chemical in cosmetic facial cleansers is not discussed in this assessment.

4. Exposure

New information on the use of the chemical reported for the secondary notification assessment has significantly altered the public, occupational and environmental exposures from that originally assessed. Therefore, the public, occupational and environmental exposure sections have been updated.

4.1 Occupational exposure

4.1.1 Operational description

The finished fabric softeners containing the notified chemical at concentrations of up to 21.1% will be transported by road to warehouse facilities for temporary storage before distribution to retail outlets for sale to the general public.

4.1.2 Estimates of occupational exposure

Transportation and storage

Transport and warehouse workers may come into contact with the notified chemical in the finished products in the event of accidental rupture of containers. However, the likelihood of such an event is expected to be low.

End-use

For workers involved in the retail industry, dermal and ocular exposures to the finished fabric softeners containing the chemical are not expected except in the event of accidental breaching of the packaging of the finished products.

4.2 Public exposure

Exposure of the public to the notified chemical at up to 21.1% concentration may occur when using the fabric softeners during laundry activities. The principal route of exposure is dermal, while incidental ocular exposure is also possible. The public may also be exposed to the residual product on washed clothes via dermal contact. However, the level of residual product on washed materials is expected to be negligible.

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

4.3 Environmental exposure

4.3.1 Releases

Release of chemical at site

For this secondary notification assessment, the notified chemical will be imported as a component in fabric softeners. The chemical is not manufactured in Australia and no reformulation was reported for this assessment. Therefore, the only potential for release to the environment at occupational sites is in the case of accidental spills and leaks of product during transport and storage. In the event of accidental releases, the product containing the chemical is expected to be collected with adsorbents, and disposed of to landfill in accordance with local government regulations.

Release of chemical from use

The notified chemical will be used as a component in fabric softeners and more than 99% of the annual volume of the chemical will be eventually released into the sewer system.

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.

4.3.2 Fate

No new environmental fate studies were submitted for the secondary notification assessment. The following discussion on fate is reproduced from the new chemical assessment (NICNAS, 2007).

Following use, the majority of the notified chemical is expected to enter sewer systems before potential release to surface waters on a nationwide basis. The chemical is readily biodegradable and hydrolysable at slightly alkaline conditions. It may also partition to suspended matter or sludge in the sewage treatment plant (STP) due to its surface active properties. The chemical remaining in treated sewage effluents is likely to be released to surface waters, or applied to land when used for irrigation. In the aquatic and soil compartments, the chemical was expected to further degrade by biotic and abiotic processes to form oxides of carbon, nitrogen, sulphur, and water. Based on its potential surface activity and expected biodegradability, the chemical is not expected to bioaccumulate.

4.3.3 Predicted environmental concentration (PEC)

The original new chemical assessment (NICNAS, 2007) assumed 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. 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. The maximum percentage of the chemical remaining in the effluent was estimated to be 7% by using the SimpleTreat Model (SimpleTreat model, Struijs, 1996). Removal within STP was based on log H of -0.839 Pa/m^3/mol (based on the water solubility of 3.39 mg/L, vapour pressure of 6.7 x 10^{-7} kPa, and a molecular weight of 733.5 g/mol for the notified chemical), a log K_{ow} of 6 and ready biodegradability.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import Volume	**504,000**	**kg/year**
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	504,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	1380.82	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	24.386	million
Removal within STP	93%	Mitigation
Daily effluent production:	4,877	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	

PEC - River:	19.82	µg/L
PEC - Ocean:	1.98	µg/L

Partitioning to biosolids in STPs Australia-wide may result in an average biosolids concentration of 2038 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 1500 kg/m^3 and a soil-mixing zone of 10 cm, the concentration of the notified chemical may approximate 13.6 mg/kg in applied soil. This assumes that degradation of the notified chemical occurs in the soil within 1 year from application.

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 19.8 µg/L may potentially result in a soil concentration of approximately 0.13 mg/kg.

5. Hazard assessment

5.1 Physicochemical and human health hazard assessment

This section contains a summary of all the data relevant to the physicochemical and human health hazard assessment of the notified chemical, with a focus on new data. The robust summaries of the toxicological data available for the assessment of the notified chemical as a new chemical are reproduced from the new chemical assessment report (NICNAS, 2007) in Appendix A.2 of this report.

The dermal and eye irritation studies on the notified chemical submitted for the secondary notification are summarised in this section and designated as **ND.** The robust summaries of the new studies are provided in Appendix A.1 of this report.

This section also includes summaries of eye irritation studies on the chemical and selected analogues for toxicokinetics, acute toxicity, skin and eye irritation, skin sensitisation, repeated dose toxicity and genotoxicity from the Human and Environmental Risk Assessment (HERA) report on esterquats (HERA, 2009). These studies are all denoted as **ND**.

The analogues are considered acceptable based on the close structural similarity to the notified chemical. The identities of the analogues included in the HERA report are outlined below. Analogue 1 and Analogue 2 are the same analogues as submitted for the new chemical assessment.

- Analogue 1 - Fatty acids, C_{10-20} and C_{16-18}-unsatd., reaction products with triethanolamine, di-Me sulfate-quaternized (CAS RN 91995-81-2)
- Analogue 2 - A preparation consisting of about 84% CAS RN 91995-81-2 and 6% of 1-propanaminium, 3-amino-N,N,N-trimethyl-, N-C_{12-18} acyl derivatives, and Me sulfates (CAS RN 68514-93-2)
- Analogue 3 - 9-Octadecenoic acid (9Z)-, reaction products with triethanolamine, di-Me sulfate-quaternized (CAS RN 94095-35-9)
- Analogue 4 - Fatty acids, tallow, reaction products with triethanolamine, di-Me sulfate-quaternized (CAS RN 93334-15-7)
- Analogue 5 - Fatty acids, C_{12-20}, reaction products with triethanolamine, di-Me sulfate-quaternized (CAS RN 91032-11-0)
- Analogue 6 - Ethanaminium, 2-hydroxy-N,N-bis(2-hydroxyethyl)-N-methyl-, diesters with C_{12-18} fatty acids, Me sulfates (salts) (CAS RN 68921-27-7).

5.1.1 Physicochemical effects assessment

No new physicochemical data were submitted for the secondary notification assessment.

5.1.2 Human health effects assessment

The studies on dermal and eye irritation provided for the secondary notification assessment, as well as the studies on the chemical and analogues for all endpoints from the HERA report (HERA, 2009) are briefly summarised in the following tables and text. The robust study summaries of the newly submitted studies are provided in Appendix A.1.

Studies submitted for the secondary notification

Endpoint	*Test substance*	*Result (ND)*
Rabbit, skin irritation	Notified chemical	Irritating
Rabbit, eye irritation (undiluted test substance)	Notified chemical	Slightly irritating
Eye irritation – in vitro bovine corneal opacity and permeability (BCOP) test	Notified chemical	Slightly irritating

Study summaries available from the HERA report

Endpoint	*Test substance*	*Result (ND)*
Rat, acute oral	Analogue 1	LD50 > 1540 mg/kg bw
	Analogue 3	LD50 > 2000 mg/kg bw
Rat, acute dermal	Analogue 1	LD50 > 2000 mg/kg bw
Rabbit, skin irritation	Analogue 1	Irritating
Human, skin irritation (24 h occluded patch test)	Analogue 1	Slightly irritating
	Analogue 5	Slightly irritating
Human, skin irritation (4 h semi-occluded and 30 min open application patch test)	Analogue 1	Not irritating
Rabbit, eye irritation (undiluted test substance)	Notified chemical	Slightly irritating
	Analogue 1	Irritating
	Analogue 3	Irritating
	Analogue 4	Slightly irritating
Rabbit, eye irritation (undiluted test substance, low volume procedure)	Analogue 2	Slightly irritating
Eye irritation – in vitro hen's egg test chorioallantoic membrane (HET-CAM) test	Notified chemical	Not irritating
Guinea pig, skin sensitisation – guinea pig maximisation test (GPMT)	Analogue 3	Not sensitising
	Analogue 4	Not sensitising
Guinea pig, skin sensitisation – Buehler test	Analogue 1	Inconclusive
	Analogue 4	Inconclusive
	Analogue 5	Inconclusive
	Analogue 6	Inconclusive
Human, skin sensitisation – human repeated insult patch test (HRIPT)	Analogue 1	Not sensitising
	Analogue 5	Not sensitising

Human, skin sensitisation – human maximisation test (HMT) and diagnostic patch test	Analogue 1	Not sensitising
Rat, repeat dose oral toxicity – 90 days	Analogue 4	NOAEL = 300 mg/kg bw
Genotoxicity -bacterial reverse mutation	Analogue 5	Non-mutagenic
Genotoxicity – in vivo mouse micronucleus test	Analogue 1	Not genotoxic

The results from the data submitted for the new chemical assessment (NICNAS, 2007) are summarised in the following table and text. The robust study summaries of these data are provided in Appendix A.2.

Studies available for the new chemical assessment

Endpoint	*Test substance*	*Result and assessment conclusion*
Rat, acute oral	Analogue 1	LD50 > 2000 mg/kg bw, low toxicity
Rat, acute dermal	Notified chemical	LD50 > 2000 mg/kg bw, low toxicity
Rabbit, skin irritation	Analogue 1	Slightly irritating
Rabbit, eye irritation (undiluted test substance)	Analogue 1	Severely irritating
Rabbit, eye irritation (undiluted test substance, low volume procedure)	Analogue 1	Slightly irritating
Rabbit, eye irritation (5% diluted test substance)	Analogue 1	Not irritating
Guinea pig, skin sensitisation – guinea pig maximisation test (GPMT)	Analogue 1	Inconclusive
Rat, repeat dose oral toxicity – 28 days	Notified chemical	NOAEL = 1000 mg/kg bw
Genotoxicity -bacterial reverse mutation	Analogue 2	Non-mutagenic
Genotoxicity – in vitro mammalian chromosome aberration test	Notified chemical	Non-genotoxic

The new chemical assessment (NICNAS, 2007) and the HERA report (2009) noted that the test substances may contain the additive, 2-propanol (CAS no. 67-63-0), which is classified as an eye and respiratory irritant (Safe Work Australia, 2018). Therefore, the toxicity of the analogue could be biased by the toxicity of this impurity.

Toxicokinetics, metabolism and distribution

No toxicokinetic data were provided for the secondary notification. The original new chemical assessment stated that based on the physical and chemical properties, the absorption of the chemical via the various routes is expected to be as follows:

- 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 absorption potential due to very low vapour pressure.

Toxicokinetics studies on the metabolite of Analogue 1, methyl-triethanol-ammonium, ion (MTEA), reported that MTEA was found to be almost completely excreted within 3 days of administration by both the oral and intravenous route in rats (HERA, 2009; **ND**).

Acute toxicity

No acute oral, dermal or inhalation toxicity studies on the notified chemical were submitted for the secondary notification.

The HERA report (2009; **ND**) available for the secondary notification assessment reported acute oral LD50s > 1540 mg/kg bw/day for Analogue 1 and > 2000 mg/kg bw/day for Analogue 3.

An acute oral study for Analogue 1 submitted for the new chemical assessment (NICNAS, 2007) indicated an LD50 > 2000 mg/kg bw/day. Another oral study described in the new chemical assessment indicated an LD50 of >5000 mg/kg bw/day.

The secondary notification assessment confirms this conclusion of the new chemical assessment that the chemical is of low acute oral toxicity.

The HERA report (2009; **ND**) reported a dermal toxicity study on Analogue 1 with an LD50 > 2000 mg/kg bw/day. A dermal toxicity study submitted on the chemical for the new chemical assessment (NICNAS, 2007) indicated an LD50 > 2000 mg/kg bw/day.

The secondary notification assessment confirms this conclusion of the new chemical assessment that the chemical is of low acute dermal toxicity.

No data were available to assess the acute inhalation hazard of the chemical. However, as noted in the new chemical assessment, acute effects by inhalation are unlikely due to the very low vapour pressure of the chemical.

Skin irritation

A dermal irritation study on the notified chemical submitted for the secondary notification (RCC, 2005a; **ND**), indicated that the chemical is irritating to the skin (erythema score of 3 for all 3 tested animals and oedema scores of 2.3-2.7 for 2 of 3 tested animals).

The HERA report (2009; **ND**) reported that Analogue 1 caused a moderate level of irritation to skin of rabbits when applied at concentrations greater than 30% under occluded or semi-occluded conditions. The report also assessed triethanolamine-based esterquats in a range of human patch test studies. The 24 hour patch tests with Analogue 1 and 5 at concentrations up to 10% resulted in only mild and transient irritation. Slight erythema was observed with Analogue 5 in a 30 minute open application patch test (up to 10% concentration) but disappeared 30 minutes post application. No irritation was observed for Analogue 1 in 4-hour semi-occluded patch tests (up to 86% concentration) and 30 minutes open application patch tests (up to 50% concentration).

Data on Analogue 1 provided for the new chemical assessment (NICNAS, 2007) indicated that undiluted Analogue 1 is slightly irritating to the skin (very slight erythema and very slight to well-defined oedema in some test animals). Dermal studies from another NICNAS assessment also using undiluted Analogue 1 were also noted in the new chemical assessment. One study reported cutaneous reactions that 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 with 20% Analogue 1 in water showed slight irritation.

Based on additional data available for the secondary notification, the chemical meets the GHS criteria for classification as a skin irritant (Category 2).

Eye irritation

For the secondary notification assessment, an eye irritation study conducted on rabbits (RCC, 2005b; **ND**) and an in vitro bovine corneal opacity and permeability (BCOP) test (RCC, 2005c; **ND**) on the notified chemical were submitted. These studies tested the chemical in the absence of the additive, 2-propanol, which is classified as an eye irritant.

In the in vivo study, no corneal opacity or iritis were observed during the test period, while slight to moderate reddening and slight chemosis of the conjunctiva were observed in all animals. The effects were reversible within 7 days. The scores were not high enough to classify the test substance as an irritant.

The BCOP test concluded that the chemical was slightly irritating. In vitro irritancy scores (IVIS) were below those required by OECD TG 437 for classification as corrosive or severely irritating, but above those indicating no classification is required. Scores were within the range for which no additional predictions regarding irritancy could be made. The test also compared the IVIS of the notified chemical with and without the additive, 2-propanol. Removal of 2-propanol from the notified chemical led to a reduction in irritancy by about 49%.

The HERA report (2009; **ND**) summarised two more recent eye irritation studies on the notified chemical in the absence of 2-propanol. In an eye irritation study in rabbits, the chemical was only slightly irritating to eyes. In a hen's egg test chorioallantoic membrane (HET-CAM) test, the chemical was considered not irritating.

A majority of the eye irritation studies also summarised in the HERA report (2009; **ND**) for Analogues 1, 3 and 4 at concentrations greater than 80% and in the presence of 2-propanol, reported irritant responses as slight to moderate. The studies also reported that the eyes of the treated animals returned to normal a few days after exposure.

The studies submitted for this assessment and data from the HERA report on the notified chemical without 2-propanol indicate that the chemical induces only slight irritation. Based on the new data available on the notified chemical for the secondary notification assessment, the chemical is assessed as not meeting the GHS criteria for classification as an eye irritant.

The conclusion of the secondary notification assessment differs from the new chemical assessment. The assessment as a new chemical reported that the chemical causes serious eye damage based on analogue data, where the test substance contained 2-propanol. In one study using undiluted Analogue 1, irreversible severe eye effects were observed in the conjunctiva, cornea and iris in 5 out of 6 test animals. The study noted that irritation could not be fully attributed to the possible effects of the impurity, 2-propanol. Other studies reported no or slight irritation only.

Sensitisation

No skin sensitisation studies on the chemical were submitted for the secondary notification. The HERA report (2009; **ND**) evaluated human and animal skin sensitisation data on analogues, showing weak or equivocal evidence for skin sensitisation in experimental animals and no reactions indicative of skin sensitisation in human studies.

None of the analogue studies available for the new chemical assessment (NICNAS, 2007) indicated a potential for skin sensitisation. No definite conclusions could be drawn from a guinea pig maximisation test (GPMT) on Analogue 1 due to the limitations of this study (test conditions inadequately or insufficiently documented). Studies from another NICNAS assessment on Analogue 1 indicated that the chemical is not sensitising in a Buehler test and a human repeated insult patch test

(HRIPT). Another GPMT was inconclusive.

Repeated dose toxicity

No repeated dose toxicity studies on the chemical were submitted for the secondary notification.

In the HERA report (2009; **ND**), the subchronic toxicity of Analogue 4 was evaluated in a 90-day oral gavage study. At 1000 mg/kg bw/day, the animals displayed potentially treatment-related changes (increased levels of blood liver enzyme, signs of gastric irritation and regressive epithelial changes in the urinary bladder). Thus, a No Observed Adverse Effect Level (NOAEL) of 300 mg/kg bw/day was established. However, bacterial infections were reported in all dose groups, which hinder the interpretation of the study.

No significant effects were observed up to the highest dose tested in a 28-day oral repeated dose rat study on the notified chemical provided for the new chemical assessment (NICNAS, 2007). The few treatment-related changes (increased forelimb and hind limb grip strength in males, decreased thromboplastin time in females) were considered not biologically relevant due to lack of corresponding pathological changes. The NOAEL established in this study was 1000 mg/kg bw/day.

Genotoxicity

No new genotoxicity studies on the notified chemical were submitted for the secondary notification.

The HERA report (2009; **ND**) reported studies on Analogue 1 and 5 indicating no genotoxicity/mutagenicity in vitro or in vivo.

In the new chemical assessment (NICNAS, 2007), an in vitro mammalian chromosomal aberration test on the notified chemical and a bacterial reverse mutation test on Analogue 2 concluded that the substances were not mutagenic under the conditions of the test.

5.1.3 Hazard classification

Based on the available information, the hazard classification of the notified chemical according to the GHS (United Nations, 2009) is as presented below.

Hazard classification	Hazard statement
Skin irritation (Category 2)	H315 - Causes skin irritation

5.2 Environmental hazard assessment

This section contains a summary of the data relevant to the environmental hazard assessment of the notified chemical. No new ecotoxicological data were submitted for the secondary notification. Therefore, the environmental effects assessment, predicted no-effect concentration and hazard classification sections have been reproduced from the new chemical assessment report (NICNAS, 2007) without significant modification. The robust summaries of the ecotoxicological data available for the new chemical assessment (NICNAS, 2007) are reproduced in Appendix B.

5.2.1 Environmental effects assessment

Summary of ecotoxicity data

Endpoint	Result	Assessment Conclusion
Fish toxicity	96 h LC50 = 1.91* mg/L	Toxic to fish

Daphnia toxicity	48 h EC50 = 6.05 mg/L	Toxic to aquatic invertebrates
Algal toxicity	72 h ErC50 = 22.3* mg/L	Harmful to algae
Inhibition of bacterial respiration	3 h EC50 > 243 mg/L	Non-inhibitory to microbial respiration

**Results listed are based on mean measured concentrations since the notified chemical concentrations decreased significantly in the test medium over time.*

Based on the acute ecotoxicological studies, the notified chemical is toxic to aquatic life. Based on acute toxicity data, biodegradability, and log P_{ow} value, the notified chemical is also considered toxic to aquatic life with long lasting effects.

5.2.2 Predicted No-Effect Concentration

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

PNEC for the Aquatic Compartment	Result
96 h LC50 for rainbow trout	1.91 mg/L
Assessment Factor	100
Mitigation Factor	1.00
PNEC:	19.1 µg/L

5.2.3 Hazard classification

The environmental hazard classification according to the GHS (United Nations, 2003) is presented in the following table. At the time of this assessment, 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 aquatic toxicity (Category 2)	H401 – Toxic to aquatic life
Chronic aquatic toxicity (Category 2)	H411 – Toxic to aquatic life with long lasting effects

6. Risk characterisation

6.1 Occupational health risk characterisation

The critical health effect of the notified chemical is skin and eye irritation. The risk to the health of transport and storage workers and retailer workers is expected to be low, due to the lack of probable exposure.

No applicants for this secondary notification assessment reported importing the neat material for reformulation. The new chemical assessment indicated that formulation workers will likely be at greatest risk of skin and eye irritation posed by the chemical, especially during handling of the neat chemical and in the event of spills for example, during sampling of the neat chemical and manual addition into mixing tanks. Control measures such as the use of engineering controls and PPE will minimise worker exposure to the chemical.

6.2 Public health risk characterisation

Dermal and/or inadvertent ocular exposures may occur from use of the chemical in fabric softeners. The risk of skin and eye irritation during use of fabric softeners by the public at a concentration of up to 21.1% is of concern. However, considering the short use duration and relatively infrequent use of the product, the risk to the public is considered to be low. The risk of skin and eye irritation to the 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.

The risk of systemic effects from use of fabric softeners is considered to be low due to the poor dermal absorption of the notified chemical at pH 7. The risk of systemic effects from accidental ingestion of the fabric softeners by young children cannot be ruled out, but is expected to be low as the chemical is considered to be of low acute and systemic toxicity.

In Australia, restrictions are in place for consumer products containing the chemical. The chemical is covered by the entry for 'Quaternary ammonium compounds' listed in the Poisons Standard (SUSMP, 2018). Quaternary ammonium compounds are listed in Schedules 5 and 6 requiring substances to be labelled with the signal word "Caution" for ≤ 20% concentration or "Poison" for concentration greater than 20%, respectively. Preparations containing ≤ 5% concentration of quaternary ammonium compounds do not require labelling.

6.3 Environmental risk characterisation

The Risk Quotient (Q = PEC/PNEC) has been calculated based on conservative PEC and PNEC values. The conservatively calculated risk quotient for discharge of treated effluents containing the notified chemical to the aquatic environment indicates that the chemical may approach ecotoxicologically significant concentrations in surface waters based on its maximum annual importation quantity. The calculated risk quotient is an upper limit given the notified chemical will likely further degrade in the environment than is assumed in the conservative environmental modelling. Therefore, based on the current annual import volume, the overall risk to the environment from release of the chemical is expected to be low. However, any further increase in import volume will lead to a commensurate increase in risk.

Risk Assessment	PEC µg/L	PNEC µg/L	Q
River:	19.8	19.1	**1.04**
Ocean:	1.98	19.1	0.10

Appendix A: Toxicological investigations

A.1 Secondary notification assessment

The robust summaries of the toxicological studies submitted for the secondary notification assessment of the notified chemical are presented here.

The tests below were conducted using the following formulations:

STEPANTEX VM 90 - contains about 90% notified chemical and up to 10% 2-propanol (CAS RN 67-63-0).

STEPANTEX VM 100 – similar to STEPANTEX VM 90; however, the 2-propanol is removed by lyophilisation and evaporation.

A1.1 Irritation – skin (ND)

TEST SUBSTANCE	STEPANTEX VM 90
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion
	EC Directive 2004/73/EC B.4 Acute Toxicity: Dermal Irritation/Corrosion
Species/Strain	Rabbit/New Zealand White, SPF
Number of animals	3
Vehicle	None
Observation period	14 days
Type of dressing	Semi-occlusive
Remarks - Method	There were no significant deviations from the protocol.

RESULTS

Lesion	*Mean score* Animal No.*			*Maximum value*	*Maximum duration of any effect*	*Maximum value at end of observation period*
Erythema/Eschar	3	3	3	3	10 days	0
Oedema	1.7	2.3	2.7	3	96 hours	0

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

Remarks - Results	Mild to severe, early-onset and transient signs of irritation such as erythema, oedema and scaling were observed throughout the whole study. The test item caused no staining of the treated skin. No corrosive effects were noted on the treated skin of any animal at any of the measuring intervals and no other clinical signs of substance related effects were observed.
CONCLUSION	The notified chemical is irritating to the skin.
TEST FACILITY	RCC (2005a)

A1.2 Irritation – eye (ND)

TEST SUBSTANCE	STEPANTEX VM 100
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion
	EC Directive 2004/73/EC B.5 Acute Toxicity: Eye Irritation/Corrosion
Species/Strain	Rabbit/New Zealand White, SPF
Number of animals	3
Observation period	17 days
Remarks - Method	There were no significant deviations from the protocol.

RESULTS

Lesion	*Mean score* Animal No.*			*Maximum value*	*Maximum duration of any effect*	*Maximum value at end of observation period*
Conjunctiva: redness	1.3	1	1.7	2	72 hours	0
Conjunctiva: chemosis	0.3	0	0	2	24 hours	0
Corneal opacity	0	0	0	0	NA	NA
Iridial inflammation	0	0	0	0	NA	NA

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

Remarks - Results

No abnormal findings were observed in the cornea or iris of any animal at any of the measurement intervals. Moderate reddening of the conjunctiva was noted in two animals from 1-hour reading to the 24- or 48-hour observation and slight reddening persisted in these two animals up to the 72-hour examination. Slight reddening of the conjunctiva was seen in one animal from the 1-hour to the 72-hour reading.

Chemosis of the conjunctiva with partial eversion of lids was observed in one animal at the 1-hour reading and slight swelling persisted up to the 24-hour observation. Slight swelling was observed in the remaining two animals at the 1-hour examination.

Slight to moderate reddening of the sclera was present in all animals at the 1-hour reading and slight reddening persisted up to the 24- or 72-hour reading in two animals. Slight ocular discharge was observed in all animals at the 1-hour examination.

No abnormal findings were observed in the treated eyes of any animal 7 days after treatment.

CONCLUSION	The notified chemical is slightly irritating to the eyes.
TEST FACILITY	RCC (2005b)

A1.3 Irritation – eye (in vitro; ND)

TEST SUBSTANCE	STEPANTEX VM 90 and STEPANTEX VM 100 (20% w/v dilution)
METHOD	Similar to OECD TG 437 Bovine Corneal Opacity and Permeability Test Method
	INVITOX Protocol no. 98 Bovine Corneal Opacity and Permeability Assay
Controls	Negative/Vehicle: 0.9% physiological sodium chloride
	Positive: 20% w/v Imidazole
Remarks - Method	No significant protocol deviations
	Closed-chamber method
	Negative and positive control items were tested concurrently. Opacity was determined by an opacitometer.

RESULTS

Test material	*Mean opacities of triplicate tissues*	*Mean permeabilities of triplicate tissues*	*IVIS*
Vehicle control	0.3	0.0016	0.6
*STEPANTEX VM 90**	8.7	0.078	9.8
*STEPANTEX VM 100**	4.7	0.025	5.0
*Positive control**	64.7	1.357	85.0

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

Remarks - Results	Before starting the permeability test, the dye solution, sodium fluorescein was checked for quality. The dye solution is valid for use if a dilution of the stock solution containing 10 µg/mL shows an optical density of 1.610 to 1.910. The value found by spectroscopy was 1.699.
	In addition, the test is acceptable if the positive control has an IVIS greater than 55. According to the results obtained in the experiment, this requirement was met.
	Comparing the IVIS of STEPANTEX VM 90 with STEPANTEX VM 100, the study authors stated that the evaporation of the 2-propanol from STEPANTEX VM 90 leads to a reduction in the irritancy by about 49%.
CONCLUSION	The notified chemical is not corrosive or a severe eye irritant but is considered a mild eye irritant under the conditions of the test.
TEST FACILITY	RCC (2005c)

A.2 New chemical assessment

The robust summaries of the toxicological studies submitted for the new chemical assessment of the notified chemical (NICNAS, 2007) are presented here.

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

Analogue 1 - Fatty acids, C_{10-20} and C_{16-18}-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 C_{16}, C_{18} and C_{18} unsaturated fatty acids; minor amounts of C_{10-14} and C_{20} fatty acids (depending on the source of the raw material); and contains about 10% 2-propanol (CAS No. 67-63-0).

Analogue 2 - Rewoquat WE20, a preparation consisting of about 84% Rewoquat WE18 and 6% of 1-Propanaminium, 3-amino-N,N,N-trimethyl-, N-C_{12-18} acyl derivatives, and Me sulfates (CAS No. 68514-93-2); and contains 10% 2-propanol.

A2.1 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*
1	5 males	2000	0
2	5 females	2000	0

LD50	>2000 mg/kg bw
Remarks – Signs of toxicity	No abnormal clinical signs were observed. Necropsy revealed no test substance-dependent findings. Body weight gains were normal in all test animals.
CONCLUSION	The chemical is of low toxicity via the oral route.
TEST FACILITY	International BioResearch (1992a)

A2.2 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*
1	5 males	2000	0
2	5 females	2000	0

LD50	>2000 mg/kg/bw
Remarks – Signs of toxicity	No abnormal clinical signs were observed. No macroscopical changes were noted at necropsy. The animals gained the expected body weights through the observation period except for one animal in Group 2 where the body weight gain was slightly reduced.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	LPT (2004)

A2.3 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 seen 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 chemical is slightly irritating to the skin
TEST FACILITY	International BioResearch (1992b)

A2.4.1 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	
Remarks - Results	Conjunctival redness and chemosis as well as corneal opacity and iris damage were observed. 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, no signs of reversibility during the 24-hour and 48-hour observation times were observed. Therefore, the ocular reactions observed may be indicative of irreversible ocular corrosion.
CONCLUSION	The chemical is severely irritating to the eye.
TEST FACILITY	International BioResearch (1992c)

A2.4.2 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 the low volume procedure – 10 mg of the test substance was used on Day 1, which is 10 folds lower than the dose used in study A.2.4.1. 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 chemical is slightly irritating to the eyes under the low volume test condition.
TEST FACILITY	Notox (1994)

A2.4.3 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	
Remarks	All test animals showed slight redness and chemosis of the conjunctiva one hour after treatment that 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 chemical is not irritating to the eye.
TEST FACILITY	International BioResearch (1992d)

A2.5 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 were presented for 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 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)

A2.6 Repeated 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 peripheral 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 reversed 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 not to be biologically 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)

A2.7 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 to 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		>200	5,000	Negative
Test 2		>200	5,000	Negative
Present	>32			
Test 1		>200	5,000	Negative
Test 2		>1000	5,000	Negative

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

A2.8 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 2a	3.1, 6.3*, 12.5*, 25*, 50, 100	18h	18h
Test 2b	18.8*, 37.5, 75, 150	28h	28h

*Cultures selected for metaphase analysis

RESULTS

Metabolic activation	*Test substance concentration (µg/plate) resulting in:*			
	Cytotoxicity in preliminary test	*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 2a		≥100	100	None

Test 2b	>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 B: Environmental fate and ecotoxicological investigations

The robust summaries of the environmental fate and ecotoxicological studies submitted for the new chemical assessment of the notified chemical (NICNAS, 2007) are presented here.

B.1 Environmental fate

The robust summaries of the ecotoxicological studies analysed for the new chemical assessment of the notified chemical are presented here.

B1.1 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		*Sodium 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 (2005a)

B1.2 Bioaccumulation

Remarks

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 database) 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).

B.2 Ecotoxicological investigations

B2.1 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. 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 (2005b)

B2.2 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
0.2	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 (2005c)

B2.3 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**	
E_bC50 mg/L at 72 h	*NOEC mg/L*	*E_rC50 mg/L at 72 h*	*NOEC mg/L*
n.d.	n.d.	22.3 (CI: 20.2-24.9)	9.25

*Based on geometric mean measured test concentrations; 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 (2005d)

B2.4 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 (2004a)

Appendix C: Investigations of physical and chemical properties

The robust summaries of the physical and chemical properties studies submitted for the new chemical assessment of the notified chemical (NICNAS, 2007) are presented here.

Melting point/freezing 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 (2004b)

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 (2005e)

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

RESULTS

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 (2005f)

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 substances 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 (2005g)

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 (2004c)

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

METHOD OECD TG 121 Estimation of the Adsorption Coefficient (K_{oc}) on Soil and on Sewage Sludge using HPLC

EC Directive 2001/59/EC C.19 Estimation of the Adsorption Coefficient (K_{oc}) 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 (2005h)

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)

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)

References

GAB (2004a) Acute toxicity testing of AE 425/03 on activated sludge with the respiration inhibition test, GAB GmbH, Niefern-Öschelbronn, Germany, report-no.: 20041075/01-AAHT, (unpublished report).

GAB (2004b) Relative density of AE 425/03, GAB GmbH, Niefern-Öschelbronn, Germany, report-no.: 20041075/01-PCRD, (unpublished report).

GAB (2004c) Surface tension of AE 425/03, GAB GmbH, Niefern-Öschelbronn, Germany, report-no.: 20041075/01-PCST, (unpublished report).

GAB (2005a) Assessment of the ready biodegradability of AE 425/03 with the closed bottle test, GAB GmbH, Niefern-Öschelbronn, Germany, report-no.: 20041075/01-AACB, (unpublished report).

GAB (2005b) Acute toxicity testing of AE 425/03 in rainbow trout (Oncorhynchus mykiss) (teleostei, salmonidae), GAB GmbH, Niefern-Öschelbronn, Germany, report-no.: 20041075/01-AAOm, (unpublished report).

GAB (2005c) Assessment of toxic effects of AE 425/03 on Daphnia magna using the 48 h acute immobilisation test, GAB GmbH, Niefern-Öschelbronn, Germany, report-no.: 20041075/01-AADm, (unpublished report).

GAB (2005d) Testing of toxic effects of AE 425/03 to the single cell green alga, Desmodesmus subspicatus (formerly scenedesmus subspicatus), GAB GmbH, Niefern-Öschelbronn, Germany, report-no.: 20041075/01-AADs, (unpublished report).

GAB (2005e) Water solubility of AE 425/03, GAB GmbH, Niefern-Öschelbronn, Germany, report-no.: 20041075/01-PCSB, (unpublished report).

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