File No: STD/1336

January 2010

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Stearoxypropyltrimonium chloride

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

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

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

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Director
NICNAS

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Kao Brands Australia Pty Ltd (ABN 72 111 285 146)
Level 1, 19 Prospect Street
Box Hill VIC 3128

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: Spectral data, Composition, Purity, Use details, Introduction volume.

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Lustrous Touch Conditioner (product containing < 6% notified chemical)

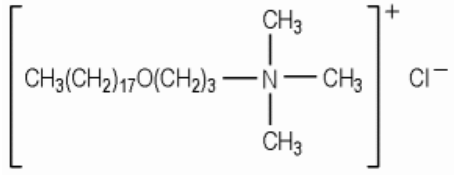
CAS NUMBER
23328-71-4

CHEMICAL NAME
1-Propanaminium, N,N,N-trimethyl-3-(octadecyloxy)-, chloride (1:1)

OTHER NAME(S)
Octadecyloxypropyl trimethyl ammonium chloride
Ammonium, trimethyl[3-octadecyloxy)propyl]-, chloride
3-Octadecyloxypropyl-N,N,N-trimethylammonium chloride

MOLECULAR FORMULA
$C_{24}H_{52}NO.Cl$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
406.14 Da

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

3. COMPOSITION

DEGREE OF PURITY ≥ 98%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (> 1% by weight)
None

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: White solid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	229°C	Measured
Density	1010 kg/m^3 at 20°C	Measured
Vapour Pressure	< 1.4 x 10^{-6} kPa at 25°C	Measured
Water Solubility	4.333 × 10^{-2} mg/L at 25°C	Estimated by Atom/Fragment estimation. Visually estimated to be 6.72 – 6.80 g/L at 20°C. However, based on its structure, the notified chemical is expected to have low water solubility and be surface active.
Hydrolysis as a Function of pH	$t_{½}$ > 1 year	Measured
Partition Coefficient (n-octanol/water)	Not determined.	Test not possible due to the surface active nature of the notified chemical.
Adsorption/Desorption	Not determined.	Test not possible due to the surfactant property of the notified chemical. The notified chemical is expected likely to absorb onto soil sediment from water given the presence of the quaternary ammonium group
Dissociation Constant	Not determined.	The notified chemical is expected to be ionised in the environment due to the presence of ionic moieties.
Particle Size	Inhalable fraction (<100 μm): 18.6% Respirable fraction (<10 μm): 0.4%	Measured
Surface Tension	42.1 mN/m at 22 ± 0.5°C (1.07 g/L solution)	Measured. The notified chemical is surface active.
Flash Point	Not determined	Low vapour pressure solid
Flammability (solid)	Not highly flammable	Measured
Autoignition Temperature	No self-ignition temperature below its melting temperature (229°C)	Measured
Explosive Properties	Not explosive	Predicted
Oxidizing Properties	Not oxidizing	Predicted

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

Reactivity
Stable under normal environmental and usage conditions.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported in finished and packaged hair conditioner.

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

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

PORT OF ENTRY
Sydney

IDENTITY OF RECIPIENTS
Kao Brands Australia Pty Ltd

TRANSPORTATION AND PACKAGING
The product containing the notified chemical will be imported in 250ml plastic bottles or tubes for retail sale. The bottles or tubes will be packed into boxes containing 6 bottles/tubes per box and subsequently transported by road from the wharf to the notifier's site and onto the retailers' warehouses and outlets.

USE
Ingredient in hair conditioner at a maximum concentrations of up to 6% intended for use by the public and perhaps also by hairdressers.

OPERATION DESCRIPTION
The notified chemical will be imported into Australia as a component (< 6%) of finished hair conditioner product and no reformulation or repackaging will take place. The imported product will be sold to the public and perhaps hairdressers, from a range of retailers. Hairdressers will apply the product directly to the wet hair of customers, leave for a number of minutes, and then rinse off the conditioner.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	10	4	Variable
Recycling	5	2	Variable
Hairdressers and hair salon workers	> 1000	1-2	Variable

EXPOSURE DETAILS
Transport and storage workers are not likely to come into contact with the notified chemical as it will be imported as packaged products inside boxes.

Empty plastic bottles may be sent to a recycling plant where workers could experience dermal exposure to the notified chemical when handling the bottles. However the amount of residual hair conditioner in the bottles is considered negligible and hence the level of exposure will be low.

Intermittent repeated dermal exposure to hairdressers is likely to occur on the hands when applying the conditioner (containing up to 6% notified chemical) to the hair of customers in salons, as gloves are not likely to be used for this process. However, hairdressers are expected to rinse their hands following each application and thus the exposure period is expected to be relatively short. Based on similar products, the product quantity used per application will be approximately 14 grams (SCCP 2006). Ocular, oral and inhalation exposure is not expected.

6.1.2. Public exposure

There will be widespread and frequent dermal exposure to the hair conditioner containing up to 6% notified chemical through deliberate application of the products to the hair. Consumers will apply the product directly to wet hair, leave for a number of minutes and then rinse off the conditioner with water. The predominant areas of exposure are the scalp and hands but ocular exposure is also possible through accidental eye contact during use of the conditioner. Oral exposure is not considered to be significant from normal use.

The systemic exposure to the notified chemical is calculated below using EU SCCP default values for rinse-off conditioner products:

Product	*Quantity (g/application)**	*Application Frequency **	*Retention Factor**	*% Notified Chemical*	*Systemic Exposure Dosage (mg/kg bw/day)***
Conditioner	14.0	0.28/day	0.01	6	**0.04**

*data from EU SCCP (2006)

** assuming 60kg body weight and 100% dermal absorption (in the absence of absorption data).

6.2. Human health effects assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	300 < LD50 < 2000 mg/kg bw, harmful toxicity
Rat, acute dermal toxicity	LD50 > 2000mg/kg bw, low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	severely irritating
Guinea pig, skin sensitisation – adjuvant test	no evidence of sensitisation
Mouse, skin sensitisation – local lymph node assay (LLNA)	evidence of sensitisation at > 5%
Rat, repeat dose oral toxicity with reproduction/development toxicity screening test– 45 days.	NOAEL = 5 mg/kg/day NOEL = 2 mg/kg/day NOEL (reproductive toxicity) = 25 mg/kg/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro*, chromosome aberration	non genotoxic
Genotoxicity – *in vivo*, micronucleus test	non genotoxic

Toxicokinetics, metabolism and distribution

No toxicokinetic studies were available. The notified chemical is a quaternary ammonium compound and is likely to be poorly absorbed via the oral route and largely eliminated in the faeces after ingestion (Craig & Stitzel 1994). Dermal absorption of the notified chemical cannot be ruled out, particularly in light of its surface activity.

The notified chemical has ~19% of particles of inhalable size, but only a small proportion of respirable size. Particles may reach the nasopharyngeal region from which they are likely to be coughed or sneezed out or swallowed (due to its expected low water solubility).

Acute toxicity

In an oral toxicity test, a single rat administered 2000 mg/kg showed clinical signs of severe systemic toxicity on day 1 and 2 after dosing, and was killed *in extremis* on day 2. Five rats administered 300 mg/kg bw by oral gavage survived to the end of the study with no sign of toxicity and no abnormalities at necropsy. The notified chemical is considered harmful via the oral route (300 < LD50 < 2000 mg/kg bw).

A group of ten rats were given a single dermal application of 2000 mg/kg bw to assess dermal toxicity. All animals exhibited signs of irritation including well-defined erythema, crust formation, scabs and superficial cracking of the epidermis after 24-hour exposure. There were no signs of systemic toxicity and no deaths, therefore the notified chemical is not considered to be toxic via the dermal route (LD50 > 2000 mg/kg bw).

Irritation

A skin irritation test found that the notified chemical produced very slight erythema in treated skin sites. The treated skin site of one animal appeared normal at the 48-hour observation and the remaining two treated skin sites appeared normal at the 7-day observation. The notified chemical is considered slightly irritating to rabbit skin.

Acute eye exposure to the notified chemical in an eye irritation test caused conjunctival redness, chemosis, discharge and slight corneal opacity and iridial inflammation in one tested rabbit, which persisted to the end of the study period (21 days). Ingrowth of blood vessels, eyelid eversion and haemorrhage of the nictitating membrane were also noted. The notified chemical caused severe irreversible ocular lesions in a rabbit.

Sensitisation

The notified chemical was not sensitising in a Guinea Pig Maximisation Test (GPMT) using intradermal and topical induction concentrations of 0.01% and 10% respectively, with a maximum topical challenge concentration of 0.5%. However, an LLNA showed a greater than three-fold increase of the baseline proliferation. The stimulation index (SI) was determined to be 2.8, 8.0 and 9.1 at 5%, 10% and 25% notified chemical, respectively. The notified chemical should be regarded as a skin sensitiser for the following reasons: (i) there is clear evidence of a dose-related increase in proliferative response indicative of skin sensitisation in the LLNA study; (ii) quaternary ammonium compounds constitute a structural alert for sensitisation and have been known to cause sensitisation following repeated occupational exposure from handling powders or solutions (Oritiz-Frutos et al., 1996; Krogsrud & Larsen, 1997); (iii) the notified chemical was not a skin irritant in the rabbit skin irritation test provided and there was no evidence of significant irritation during the LLNA study, thus irritation effects are unlikely to have caused a false positive LLNA result.

Repeated Dose Toxicity (sub chronic)

There were no treatment-related changes indicative of systemic toxicity in a 45-day oral toxicity test. The effects observed throughout the study were largely attributed to local irritation of the notified chemical on the respiratory and gastrointestinal tracts.

One female control died during normal blood sampling procedures but one high dose (25 mg/kg bw/day) male rat was terminated for humane reasons after two weeks of treatment. Prior to death, the high-dose rat showed respiratory distress, diarrhoea and distended abdomen. Gaseous distension of the gastrointestinal tract was observed at necropsy.

At the highest dose, abnormal organ effects were observed on the oesophagus, stomach, trachea and thymus in some male and female rats. One female rat treated with 5 mg/kg bw/day exhibited lymphoid atrophy and tracheal epithelium changes. The NOAEL was established as 5 mg/kg bw/day. The lymphoid atrophy was not considered adverse in the 5 mg/kg bw/day dose group due to its low incidence. In addition, the tracheal epithelium changes may have been due to dosing errors.

Mutagenicity

The notified chemical was not mutagenic to *Salmonella* and *E.coli* bacteria in a reverse mutation test.

Genotoxicity

The notified chemical was not clastogenic in an *in vivo* mammalian erythrocyte micronucleus test.

A chromosome aberration test using cultured human lymphocytes found no significant increase in the frequency of cells with chromosome aberrations, either in the absence or presence of metabolic activation. However, there was a statistically significant increase in the numbers of polyploid cells at higher dose levels in two separate experiments (one with and one without metabolic activation). As a comparison, the study investigators pointed to a study by Mitchell et al (1995) that suggest some substances (e.g. pharmaceuticals), are known to induce polyploidy in chromosome aberration tests without any correlation to aberrations or mutations indicative of true mutagenicity. A change in the number of chromosomes can occur as a result of many types of biological errors (Tucker & Preston 1996) including errors induced by indirect DNA damage through cytotoxic mechanisms affecting cell division, particularly when testing close to the toxic dose range (Hilliard et al 2007). Given that polyploidy occurred without an increase in aberrations and there was evidence of dose-related cytotoxicity, it was concluded that this result was of no genotoxicological significance. This is further supported by the negative in vivo mammalian erythrocyte micronucleus test performed on the notified chemical, including evidence that the bone marrow of the mice had been exposed to the notified chemical.

Toxicity for reproduction

Rats were given 1-25 mg/kg bw of notified chemical by oral gavage for 45 consecutive days and allowed to mate and produce offspring. There were no adverse effects on reproduction. The offspring were observed for up to 5 days after birth and the investigators found no toxicological effects on growth or development during this period. The NOEL for reproductive toxicity was considered to be 25 mg/kg/day based on the absence of systemic toxicity.

Health hazard classification
Based on the acute oral toxicity, eye irritation and LLNA sensitisation tests, the notified chemical is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrases:
Xn; R22 Harmful if swallowed
Xi; R41 Risk of serious damage to eyes
Xi; R43 May cause sensitisation by skin contact

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
The notified chemical has the potential to cause skin sensitisation (with an EC3 value of 8.2% determined in the LLNA study), is severely irritating to the eyes, and is harmful if swallowed.

Professionals in hair salons may experience frequent dermal exposure to the notified chemical at concentrations up to 6%. Whilst the EC3 value indicates that the notified chemical is not a strong skin sensitiser, the risk of skin sensitisation in hairdressers cannot be ruled out at the proposed use concentrations and due to the repeated exposure experienced by these workers. Hand washing that may occur following application of the product is expected to reduce the risk of skin sensitisation by minimising the skin contact time. In addition, appropriate labelling of the product to warn against the possibility of allergic reactions is expected to further lower the risk of skin sensitisation in hairdressers.

Systemic effects resulting from repeated dermal exposure to the notified chemical are not expected as a 45 day repeat dose oral toxicity study in the rat resulted in no treatment-related changes indicative of systemic toxicity or effects that are likely to occur following dermal exposure.

Ingestion or ocular exposure of hairdressers to products containing the notified chemical at concentrations up to 6% is not expected during normal use, however, accidental exposure may occur. Eye irritation may occur at such concentrations during accidental exposure.

In summary, the risk to hair salon workers associated with exposure to the notified chemical (up to 6%) in hair conditioner products is not considered to be unacceptable if appropriate labelling of the products is in place to warn against the possibility of allergic reactions and eye irritation.

6.3.2. Public health
The public will encounter dermal exposure and occasional ocular exposure to the notified chemical at concentrations up to 6% during use of hair conditioner products. The primary concern associated with use of the notified chemical (a quaternary ammonium compound) in hair conditioners (up to 6%) is skin sensitisation and severe irritation to the eyes. As severe eye irritancy was observed with the undiluted chemical, the potential for adverse effects on the eye at up to 6% cannot be ruled out during accidental exposure. However, the rinse-off nature of the products is expected to reduce the contact time with the eyes and thus the potential for eye irritation.

The potential for skin sensitisation cannot be ruled out, though may be reduced by the rinse-off nature of the hair conditioner product.

The risk of eye irritation and sensitisation may be minimised by the inclusion of appropriate labelling and directions for use to warn against eye contact and the possibility of allergic reactions. When used in the proposed manner (rinse-off hair product), with appropriate safety information on the packaging, the risk to the public associated with eye and skin contact with the notified chemical is not considered to be unacceptable.

Systemic effects resulting from repeated dermal exposure to the notified chemical are not expected as a 45 day repeat dose oral toxicity study in the rat resulted in no treatment-related changes indicative of systemic toxicity or effects that are likely to occur following dermal exposure. This is further supported by calculations using the NOAEL value that was established in a 45-day oral study in the rat (as a dermal NOAEL was not determined). Using a systemic exposure dosage (SED) of 0.04 mg/day and oral NOAEL of 5 mg/kg bw/day, the margin of safety is calculated as 125. Margin of Safety greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. The MOE is based on conservative assumptions (e.g. using 100% dermal absorption) and is therefore likely to overestimate the risk.

In summary, the risk to the public associated with exposure to the notified chemical (up to 6%) in hair conditioner products is not considered to be unacceptable if appropriate labelling of the products is in place to ensure their safe use.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported into Australia as a component of finished hair conditioner product for direct end-use in Australia. No local reformulation or repackaging will take place and therefore no significant release is expected to occur in Australia as a result of these processes.

RELEASE OF CHEMICAL FROM USE

The notified chemical is a component of a hair conditioner, which will be directly applied to the consumer's hair. The hair conditioner will then be rinsed off and go down the sink/drain, and enter the drainage/sewerage system where it will be taken to various waste water treatment facilities.

RELEASE OF CHEMICAL FROM DISPOSAL

The hair conditioner bottles, containing the notified chemical, should be sent for recycling wherever possible once the bottle is no longer to be used. However, as end users are the general public a proportion of containers are anticipated to be sent to landfill. It is expected that there may be minimal amounts of residual notified chemical within the bottles.

7.1.2 Environmental fate

The notified chemical is not considered readily biodegradable. It is not expected to have potential for bioaccumulation based on the molecular structural information. For the details of the environmental fate studies please refer to Appendix C.

It is anticipated that almost all of the imported product containing the notified chemical will go to the sewage system via rinsing after hair application. A study (Games et. al, 1982) indicates that more than 99% of quaternary ammonium surfactant is expected to be removed in a sewage treatment plant via both absorption to solids and biodegradation. Based on this, it is anticipated that no significant amount of the imported notified chemical will be end up in the water environment.

7.1.3 Predicted Environmental Concentration (PEC)

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	5,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	5,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	13.7	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	21.161	Million
Removal within STP	99%	
Daily effluent production:	4,232	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.03	µg/L
PEC - Ocean:	< 0.01	µg/L

The PEC has been calculated assuming that 100% of the notified chemical is disposed of to sewage and 99% of removal from the water column in waste water treatment processes, which is the worst case scenario for the exposure of the notified chemical to the aquatic environment.

7.2. Environmental effects assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	LC50 0.051 mg/L	Acutely very toxic to fish
Daphnia Toxicity	EC50 0.0096 mg/L	Acutely very toxic to daphnia
Algal Toxicity	EC50 0.078 mg/L	Acutely very toxic to algae
Inhibition of Bacterial Respiration	EC50 440 mg/L	Not harmful to sewage sludge bacteria
21-Day Daphnia Toxicity	EC50 0.00063 mg/L (reproduction) EC50 0.001 mg/L (immobilization)	Chronically very toxic to daphnia

The notified chemical is very toxic to the aquatic life on both acute and chronic bases.

7.2.1 Predicted No-Effect Concentration

The PNEC has been calculated based on the most sensitive endpoint of 0.0096 mg/L for the EC50 value for daphnia, and using an assessment factor of 100 since toxicity studies for three species are available for the notified chemical.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Invertebrates).	0.0096	mg/L
Assessment Factor	100.00	
PNEC:	0.096	µg/L

7.3. Environmental risk assessment

Risk Assessment	***PEC µg/L***	***PNEC µg/L***	***Q***
Q - River:	0.03	0.096	0.32
Q - Ocean:	< 0.01	0.096	< 0.1

Based on the above calculation for Risk Quotient, the notified chemical is not expected to pose an unacceptable risk to the environment from the proposed use of the hair conditioner product containing the notified chemical.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available data the notified chemical is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)]. The following risk phrases apply to the notified chemical:

Xn; R22 Harmful if swallowed
Xi; R41 Risk of serious damage to eyes
Xi; R43 May cause sensitisation by skin contact

Human health risk assessment
Based on the occupational settings described and appropriate labelling of products, the notified chemical is not considered to pose an unacceptable risk to the health of workers.

When used in the proposed manner with appropriate product labelling, the notified chemical is not considered to pose an unacceptable risk to public health.

Environmental risk assessment

On the basis of the PEC/PNEC ratio and the notified use pattern, the notified chemical is not expected to pose a risk to the environment.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- The Safe Work Australia, should consider the following health hazard classification for the notified chemical:
  - Xn; R22 Harmful if swallowed
  - Xi; R41 Risk of serious damage to eyes
  - Xi; R43 May cause sensitisation by skin contact

- Use the following risk phrases for products/mixtures containing the notified chemical:
  − Concentration ≥ 25%: R22; R41; R43
  − 10% ≤ concentration < 25%: R41; R43
  − 5% ≤ concentration < 10%: R36; R43
  − Concentration ≥ 1%: R43

- Based on its hazardous properties and intended use in consumer products, the notified chemical should be submitted to the National Drugs and Poisons Schedule Committee (NDPSC) for listing in the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP). However, the notified chemical is a quaternary ammonium compound which is already included in the SUSDP under Schedule 5 or 6 based on its concentration/preparation. All preparations containing quaternary ammonium compounds at 20% or less are included in Schedule 5 of the SUSDP with some exceptions e.g. in preparations containing 5% or less. To promote uniform labelling and packaging requirements throughout Australia, the existing scheduling requirements in the SUSDP for quaternary ammonium compounds are applicable to the notified chemical.

- Products containing ≥ 5% notified chemical and available to the public must carry the following safety directions on the label:
  - Avoid contact with eyes
  - May cause allergy
  - In case of contact with eyes, rinse immediately with plenty of water

Material Safety Data Sheet

- The MSDS for the product provided by the notifier should be amended to reflect the hazardous nature of the chemical:
  - Amend hazard identification to ‘Hazardous substance’.
  - Include the risk phrase R36 Irritating to eyes for products containing ≥ 5% of the notified chemical
  - Include the risk phrase R43 May cause sensitisation by skin contact for products containing ≥ 1% of the notified chemical.
  - Include appropriate safety phrases.
  - Include the full chemical name of the notified chemical in the MSDS.

CONTROL MEASURES
Occupational Health and Safety

- Employers in hair salons should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced:
  - Avoid eye contact

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

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

Public Health

The hazard classification and labelling recommendations provided above will ensure adequate public health control measures.
Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

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

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

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

(1) Under Section 64(1) of the Act; if
  - the concentration of the notified chemical in hair conditioner products exceeds 6%.

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from a component of hair conditioner, or is likely to change significantly;
  - the amount of chemical being introduced has increased from 5 tonnes, or is likely to increase, significantly;
  - the chemical has begun to be manufactured in Australia;
  - additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

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

Material Safety Data Sheet
The MSDS of the product containing the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 229°C

Method OECD TG 102 Melting Point/Melting Range.
EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks Melted with decomposition at 502 ± 0.5 K. As the notified chemical decomposed on melting, no determination of boiling temperature was required.
Test Facility SafePharm Laboratories (2007a)

Density 1010 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 Determined using a gas comparison pycnometer.
Test Facility SafePharm Laboratories (2007a)

Vapour Pressure < 1.4 x 10^{-6} kPa at 25°C

Method OECD TG 104 Vapour Pressure.
EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks Determined using a vapour pressure balance. A sequence of runs was conducted and temperature and pressure readings were taken between 29 and 39°C. No statistical analyses were performed because the balance readings were too low and variable for a line of best fit to have any meaning.

The vapour pressure of the notified chemical was determined to be less than at 1.4 x 10^{-6} kPa at 25°C using linear regression analysis.
Test Facility SafePharm Laboratories (2008a)

Water Solubility 6.72 – 6.80 x 10^3 mg/L at 20°C by Flask method
4.333 x 10^{-2} mg/L at 25°C by Atom/Fragment estimation

Method OECD TG 105 Water Solubility.
EC Directive 92/69/EEC A.6 Water Solubility.
WSKOW version 1.41, ©2000, US EPA.
Remarks Visually estimated based on flask method to be 6.72 – 6.80 x 10^3 mg/L at 20°C, which is not considered to be consistent with the mainly hydrophobic structure of the notified chemical. Considering the surface active nature, the determined result is considered to be consistent with the notified chemical being dispersed as micelles, rather than dissolved in water, due to its behaviour as a surfactant.

Computer soft ware calculation based on Atom/Fragment indicates a water solubility of 4.333 x 10^{-2} mg/L at 25°C which is considered consistent with the mainly hydrophobic molecular structure of the notified chemical.
Test Facility SafePharm Laboratories (2007a)

Hydrolysis as a Function of pH

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

pH	*T (°C)*	*t½*
4	25	> 1 year
7	25	> 1 year
9	25	> 1 year

Remarks Sample solutions of 2 g/L at pH 4, 7 and 9 were maintained at 50 ± 0.5°C for a period of 5 days, and the concentrations were determined using HPLC. Less than 10% hydrolysis after 5 days was detected for all the pH levels, which is equivalent to a half-life greater

than 1 year at 25ºC for all the pH values tested.
Test Facility SafePharm Laboratories (2007a)

Partition Coefficient (n-octanol/water) Not determined

Method OECD 107 Partition Coefficient (n-octanol/water): Shake Flask Method
OECD TG 117 Partition Coefficient (n-octanol/water), HPLC Method.
EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks Testing was not carried out for the following reasons:

The test material exhibited the properties of a surfactant in the n-octanol-water system. Surface active materials are not suitable for estimation by the HPLC method.

The use of computer based estimation programs for materials of this nature are also considered invalid since most of the estimations of logPow are prone to error due to the effects of ionic charge. In addition, prediction methods are likely to be subject to serious error since they do not take into account the potential formation of colloidal aggregates.

Therefore it was considered that in this case the partition coefficient is essentially meaningless and cannot be assessed.

Test Facility SafePharm Laboratories (2007a)

Adsorption/Desorption – screening test Not determined

Method OECD 121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography
Remarks Testing was not carried out using the above methods for the following reasons:

The test material has surface-active properties. The HPLC method is not applicable to substances such as this.

The use of computer-based estimation programs and/or QSARs for materials of this nature are also considered invalid.

The notified chemical is considered likely to absorb onto soil sediment from water given the presence of quaternary ammonium groups.

Test Facility SafePharm Laboratories (2007a)

Dissociation Constant Not determined

Method OECD TG 112 Dissociation Constants in Water.
Remarks No determination of dissociation constant was possible by the above method, as the notified chemical contained no routes of dissociation. However, it is expected to be ionized in the environmental pH range of 4 – 9, given the presence of ionic moieties in the notified chemical.
Test Facility SafePharm Laboratories (2007a)

Particle Size

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

Range (μm)	*Mass (%)*
< 100	18.6
< 10	0.404
< 5.5	8.27×10^{-2}

Remarks Too few particles were of a size less than 10.0 μm to allow accurate assessment of mass median aerodynamic diameter.
Test Facility SafePharm Laboratories (2007a)

Flammability Not highly flammable

Method	EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks	Determined to be not highly flammable as it did not propagate combustion over the 200 mm of the preliminary screening test.
Test Facility	SafePharm Laboratories (2008a)

Autoignition Temperature No self-ignition temperature below its melting temperature (229ºC).

Method	EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks	The notified chemical was subjected to increasing temperature from ambient to 239°C (approximately 10°C higher than the melting temperature).
Test Facility	SafePharm Laboratories (2008a)

Explosive Properties Not explosive

Method	EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks	Result was determined based on lack of structural groups associated with explosivity.
Test Facility	SafePharm Laboratories (2008a)

Surface Tension 42.1 mN/m at 22 ± 0.5ºC and concentration of 1.07 g/L

Method	OECD TG 115 Surface Tension of Aqueous Solutions. EC Directive 92/69/EEC A.5 Surface Tension.
Remarks	The test result is consistent with the structure typical of a surfactant (polar head with carbon tail).
Test Facility	SafePharm Laboratories (2007a)

Oxidizing Properties Not oxidising

Method	EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Remarks	Predicted based on the lack of the chemical groups on the notified chemical that would imply oxidising properties.
Test Facility	SafePharm Laboratories (2008a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure EC Directive 2004/73/EC B.1bis Acute Toxicity (Oral)
Species/Strain	Rat/Sprague-Dawley CD (Crl:CD (SD) IGS BR)
Vehicle	Distilled water

RESULTS

Sighting Study

Dose mg/kg bw	*Administered*	*Evident Toxicity*	*Mortality*
2000	Oral gavage	Yes	1/1*
300	Oral gavage	No	0/1

* Animal was killed *in extremis* two days after dosing due to severe signs of systemic toxicity

Signs of Toxicity	There were no signs of systemic toxicity in the animal treated at 300 mg/kg. The animal treated at a dose of 2000 mg/kg showed the following signs of systemic toxicity 1-2 days after dosing: hunched posture, lethargy, pilo-erection, decreased respiratory rate, laboured respiration, diarrhoea, hypothermia, ataxia, pallor of the extremities, ptosis, body weight loss and dehydration.
Effects in Organs	No abnormalities were noted at necropsy in animals dosed at 300 or 2000 mg/kg bw.

Main Study

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	4, female	300	0/4

LD_{50}	> 300, < 2000 mg/kg bw
Signs of Toxicity	No signs of systemic toxicity were noted during the observation period.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks - Results	All animals showed expected gains in bodyweight.

CONCLUSION	The notified chemical is harmful via the oral route.
TEST FACILITY	SafePharm Laboratories (2007b)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal) – Limit Test.
Species/Strain	Rat/Sprague-Dawley CD (Crl:CD (SD) IGS BR)
Vehicle	Test material was moistened with distilled water prior to application
Type of dressing	Semi-occlusive.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 Male, 5 Female	2000	0/10

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Well-defined erythema was noted at all treatment sites one day after dosing, which reduced thereafter, clearing in all animals by day 5. Between days 2-12, animals showed other signs of dermal irritation including crust formation, small superficial scattered scabs and superficial cracking of the epidermis. There were no signs of oedema throughout the study in any animal. All treatment sites appeared normal by day 13 after dosing.
Signs of Toxicity - Systemic	There were no signs of toxicity.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks - other	All animals showed expected gains in bodyweight over the study period.

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

TEST FACILITY SafePharm Laboratories (2008b)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

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

Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	Moistened with 0.5 ml distilled water
Observation Period	7 days
Type of Dressing	Semi-occlusive.
Remarks - Method	In the absence of data on the potential for the notified chemical to produce corrosion, an *ex vivo* pre-screen test was performed using a rat skin disc preparation in a Transcutaneous Electrical Resistance Assay (TER). Result: the test material was considered unlikely to have the potential to cause corrosion *in vivo*; therefore an acute dermal irritation test in rabbits was conducted to determine its irritancy potential.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	1	1	0.33	1	< 7 days	0
Oedema	0	0	0	0	0	0

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

Remarks - Results	Very slight erythema was noted at all treatment skin sites one hour and 24 hours after patch removal. One treated skin site appeared normal at the 48 hour observation. Two animals continued to show very slight erythema at the 48 and 72 hour observations and all treated skin sites appeared normal by day 7.

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY Safepharm Laboratories (2007c)

B.4. Irritation – eye

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 2004/73/EC B.5 Acute Toxicity (Eye Irritation).

Species/Strain: Rabbit/New Zealand White
Number of Animals: 1
Observation Period: 21 days
Remarks - Method: No additional animals were treated due to the severe ocular responses produced in a single animal.

RESULTS

Lesion	*Mean Score**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Conjunctiva: redness	2	2	> 21 days	2
Conjunctiva: chemosis	2.67	3	> 21 days	2
Conjunctiva: discharge	2.67	3	> 21 days	2
Corneal opacity	1	1	> 21 days	1
Iridial inflammation	1	1	> 21 days	1

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

Remarks - Results: Iridial inflammation and scattered or diffuse corneal opacity affecting the majority of the corneal area (three-quarters to the whole) were noted in the treated eye one hour after treatment and at each subsequent observation. At day 14 and 21, vascularisation with a generalised ingrowth of blood vessels (approximately 3 mm length) was noted in the treated eye.

Diffuse, deep redness of the conjunctiva was noted at one hour after treatment and at each subsequent observation. Swelling of the eyelids (about half-closed) and conjunctival discharge (moistening of the lid and hairs a considerable area around the eye) was observed at 1, 24 and 48-hours. Obvious swelling (with partial lid eversion) and conjunctival discharge (moistening of the lid and hairs just adjacent to the lid) was observed at every observation after 48 hours. Other ocular signs include pale area covering the nictitating membrane from 24 hours onwards and small area of haemorrhage on the upper and lower area of the nictitating membrane from 72 hours and every subsequent observation. Ectropion (eversion of lower eyelid) was noted in the treated eye at the 14 and 21-day observations. All reactions persisted at the 21-day observation and were considered to be indicative of irreversible ocular damage.

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

TEST FACILITY: Safepharm Laboratories (2007d)

B.5. Skin sensitisation

TEST SUBSTANCE: Notified chemical

METHOD: Skin sensitisation study: Guinea Pig Maximisation Test (GPMT), similar to OECD TG 406 Skin Sensitisation – Guinea Pig Maximisation Test (GPMT).

Species/Strain: Guinea pig/CrJ:Hartley
PRELIMINARY STUDY: Maximum Non-irritating Concentration:
intradermal: < 0.005% (lowest tested concentration)
topical: 0.5%

	Maximum Slight-Moderately Irritating Concentration: Intradermal: 0.01% Topical: 10%
Signs of Irritation	*Intradermal injections*: At the 24, 48 and 72-hour observations, all treatment skin sites showed slight-moderate erythema after injections at 0.005% and 0.01%. Blackish skin, ulcer or necrosis was observed in all animals following injections at concentrations from 0.05% - 3%. *Topical application*: No sign of irritation was noted after application with 0.5%. Treated skin sites showed slight erythema after exposure to 1, 3, 5, and 10% in water and 10% in 50% ethanol solution at the 24, 48 and 72-hour observation point.
MAIN STUDY	
Number of Animals	Test Group: 10 Control Group: 5
INDUCTION PHASE	Induction Concentration: intradermal: 0.01% topical: 10%
CHALLENGE PHASE	
1st challenge	topical: 0.01, 0.03, 0.05, 0.3, 0.5%
Remarks - Method	A translucent solution was noted at 0.01 – 0.05% of test material in water, a translucent suspension at 0.1%, and a white-coloured suspension was observed at concentrations greater than 0.3% in water. The test material dissolved fully in water at 0.005% and 10% in a 50% ethanol solution. No positive control studies were conducted.

RESULTS

Animal	*Challenge Concentration (%)*	*Number of Animals Showing Skin Reactions after: 1st challenge*	
		24 h	*48 h*
Test Group	0.01	0	0
	0.03	0	0
	0.05	0	0
	0.3	0	0
	0.5	0	0
Control Group	0	0	0

CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical at the concentrations tested and under the study conditions.
TEST FACILITY	JBS (2000)

B.6. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)
Species/Strain	Mouse/CBA/CaBkl
Vehicle	Ethanol/distilled water (7:3)
Remarks - Method	The vehicle was chosen as it produced the highest concentration that was suitable for dosing. A preliminary toxicity screening test was performed using a single mouse exposed to 25% w/w in vehicle for three consecutive days. The test material did not produce systemic toxicity or excessive local irritation at 25% and this was chosen as the maximum concentration for the main study.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	688.8	
5	1912.3	2.78
10	5524.7	8.02
25	6255.0	9.08
Positive Control (HCA*)		
5	-	1.38
10	-	2.03
25	-	8.04

* α-Hexylcinnamaldehyde

Remarks - Results	There were no deaths and no signs of systemic toxicity in the test or control animals throughout the study. Slight desquamation on the base of the ears and head was noted on day 6 of the preliminary study. Fur loss and/or slight redness to the base of ears were noted on days 4 to 6 in animals treated with a test material concentration of 25%.
CONCLUSION	There was evidence of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Safepharm Laboratories (2008c)

B.7. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test.
Species/Strain	Rat/Sprague-Dawley Crl:CD (SD) IGS BR
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: up to 45 days Dose regimen: 7 days per week Post-exposure observation period: None
Vehicle	Arachis oil BP
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10 per sex	0	1/20
low dose	10 per sex	1	0/20
mid dose (I)	10 per sex	2	0/20
mid dose (II)	10 per sex	5	0/20
high dose	10 per sex	25	1/20

Mortality and Time to Death

One control female died during routine blood sampling procedures following two weeks of treatment. One male in the high dose group was killed on welfare grounds after three weeks of treatment.

Clinical Observations

Respiratory abnormalities with or without excessive transient salivation developed in animals of either sex in the high dose group during the first two weeks of dosing. One male in the high dose group showed marked deterioration in condition including respiratory distress, diarrhoea and distended abdomen during the third week of treatment and was therefore terminated for humane reasons. Other animals showed sporadic signs of

pallor of the extremities, hunched posture and staining of the fur around the snout and mouth. Generalised fur loss was observed among females of all treatment groups, including controls, but this was attributed to the commonly seen use of fur as a nesting material by pregnant rats.

Lower bodyweight gain and lower food intake was evident for males treated at 25 mg/kg/day compared with the controls. Females treated at 2, 5 and 25 mg/kg/day showed slightly lower weight gain and lower food intake than controls during maturation, but no significant difference from controls during gestation and lactation.

Laboratory Findings – Blood Chemistry, Haematology
No statistically significant changes were detected.

Effects in Organs
One male in the high dose (25 mg/kg/day) group that was killed *in extremis* during the study was found to have gaseous distension of the stomach and lower gastrointestinal tract and small spleen at necropsy. Of the remaining nine males that survived to the end of the study, there were two isolated cases of small seminal vesicles and testes in the high dose group.

Trachea
Flattening and deciliation of the tracheal epithelium was seen in four males, including the premature death male and one female in the high dose group. One female rat in the mid-dose (5 mg/kg/day) group was similarly affected. These changes were considered to be the result of accidental instillation of the test material into the respiratory tract during dosing.

Oesophagus
Mononuclear cell infiltration of the peripheral musculature of the oesophagus was observed in two female control rats and all males and one female from each of the dose groups. Although this is a common finding in animals dosed by gavage, the disparity between the numbers of treated rats compared to the controls indicated the possibility that the test material exacerbated the condition.

Stomach
Hyperkeratosis and/or acanthosis of the forestomach were observed in two male and two female rats in the high dose group. Focal ulceration was also noted in one high dose male rat.

Thymus
Lymphoid atrophy of minimal severity was observed for three high dose females and for one mid-dose female (5 mg/kg/day).

Effects on reproduction
No adverse effects were detected on mating performance, fertility or gestation length. Nine of ten females from the control and high dose (25 mg/kg/day) groups and all females from 1, 2 and 5 mg/kg/day dose groups gave birth to a live litter and successfully reared young to the end of the study period (Day 5 after birth). One high dose female showed positive evidence of mating but did produce a litter and had not achieved pregnancy, but this was determined to be a result of biological variability and not considered toxicologically significant.

Effects on growth and development
No toxicologically significant findings were observed in offspring throughout the study and at necropsy.

Remarks – Results
There were no treatment-related changes indicative of systemic toxicity. Abnormalities occurred in isolated animals without clear dose-related effects. Respiratory abnormalities, increased salivation, stained fur and reduced food intake were prominent in high dose animals but the effects were considered to be associated with local irritation of the test material on the respiratory and gastrointestinal tract, rather than an indication of systemic toxicity.

The cause of lymphoid atrophy in treated female rats was uncertain. The study authors indicate that such effects are occasionally observed in control animals and may be associated with stress responses to treatment. As such, the observation of this effect in one mid-dose female (5 mg/kg bw/day) is not considered adverse. However, the incidence of this effect in the high dose group suggests that it should be considered adverse at this dose level.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 5 mg/kg bw/day in this study based on the lack of changes at this dose level that are considered to be adverse.
The No Observed Effect Level (NOEL) was established as 2 mg/kg bw/day in this study.
The NOEL for reproductive toxicity was considered to be 25 mg/kg/day based on the absence of systemic toxicity.

TEST FACILITY Safepharm Laboratories (2008f)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

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

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA$^-$
Metabolic Activation System	S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 0-5000 µg/plate b) Without metabolic activation: 0-5000 µg/plate
Vehicle	Sterile distilled water
Remarks - Method	No significant protocol deviations.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*		
	*Cytotoxicity in Preliminary Test**	*Cytotoxicity in Main Test**	*Genotoxic Effect*
Absent			
Test 1	≥ 150	≥ 50	Negative
Test 2	-	≥ 50	Negative
Present			
Test 1	≥ 500	≥ 150	Negative
Test 2	-	≥ 150	Negative

* Considering reductions in revertant colonies and bacterial background lawn.

Remarks - Results
No test material precipitate was observed on plates at any dose either in the presence or absence of S9-mix.

No significant increase in the frequency of revertant colonies was recorded for any bacterial strain at any dose either with or without metabolic activation compared to the controls.

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

TEST FACILITY Safepharm Laboratories (2008c)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

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

Cell Type/Cell Line	Human peripheral lymphocytes
Metabolic Activation System	Phenobarbitone and β-naphthoflavone-induced rat liver S9 preparation

Vehicle	Eagles minimal essential medium with HEPES buffer (MEM)
Remarks - Method	The test substance concentrations chosen for metaphase analysis were based on dose levels that achieved close to 50% toxicity in the absence (12 µg/ml) and presence of metabolic activation (16 µg/ml). Vehicle and positive control tests were performed in parallel to the test material.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 4*, 8*, 12*, 16, 24, 32	4 hours	20 hours
Test 2	0*, 1, 2, 4*, 8*, 12*, 16	24 hours	24 hours
Present			
Test 1	0*, 8*, 12*, 16*, 24, 32, 48	4 hours	20 hours
Test 2	0*, 4*, 8*, 16*, 20, 24, 28	4 hours	20 hours

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation in Main Test*	*Genotoxic Effect*
Absent				
Test 1	≥ 15.86	≥ 16	None observed	Negative
Test 2	≥ 7.93	≥ 12	None observed	Negative
Present				
Test 1	≥ 7.93	≥ 24	None observed	Negative
Test 2	-	≥ 15	None observed	Negative

Remarks - Results	Haemolysis was observed at concentrations ≥ 24 µg/ml in the absence of S9 and ≥ 32 µg/ml in the presence of S9. It was not observed during the 24 hr continuous exposure experiment. There were no statistically significant increases in the frequency of cells with chromosome aberrations at any dose either in the absence or presence of metabolic activation. In test 1, a small but statistically significant increase in the numbers of polyploid cells at 12 µg/ml was observed in the absence of metabolic activation. In test 2, the test material induced a modest and statistically significant increase in the numbers of polyploid cells at 16 µg/ml in the presence of metabolic activation. As the polyploidy was observed at dose levels close to cytotoxic concentrations, the study authors suggest that their biological relevance is questionable. They attributed the polyploidy to a cytotoxic mode of action on the nuclear spindle or cell-cycle checkpoint molecules and not on any genotoxic mechanisms involving aberrations or mutations.
CONCLUSION	The notified chemical was not clastogenic to human peripheral lymphocytes treated *in vitro* under the conditions of the test.
TEST FACILITY	Safepharm Laboratories (2008d)

B.10. Genotoxicity – in vivo

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 474 Mammalian Erythrocyte Micronucleus Test. EC Directive 2000/32/EC B.12 Mutagenicity - Mammalian Erythrocyte Micronucleus Test.

Species/Strain	Mouse/Crl:CD-1(ICR)BR
Route of Administration	Oral – gavage
Vehicle	Distilled water
Remarks - Method	A preliminary study was used to determine appropriate dose levels for the main test. One of two animals died when tested at 1200 mg/kg and thus 1000 mg/kg was the maximum tolerated dose. There were no differences between effects in males and females and thus only males were used for the main test. No significant protocol deviations.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control, distilled water)	7M (group 1); 7M (group 2)	0	48 (group 1); 24 (group 2)
II (low dose)	7, male	250	24
III (mid dose)	7, male	500	24
IV (high dose)	7M (group 1); 7M (group 2)	1000	48 (group 1); 24 (group 2)
V (positive control, CP)	7M	50	24

CP=cyclophosphamide

RESULTS

Doses Producing Toxicity	One animal in the 48-hour 1000 mg/kg group died prematurely during the study period. At doses ≥ 500 mg/kg in the 24 and 48-hour groups, clinical signs included: hunched posture, ptosis, diarrhoea, noisy respiration, lethargy, ataxia and splayed gait.
Genotoxic Effects	There was no statistically significant increase in the frequency of micronucleated polychromatic erythrocytes (PCE) in any dose group when compared to concurrent vehicle control groups. The positive control group showed an expected increase in the frequency of micronucleated PCEs.
Remarks - Results	There was a small, but statistically significant decrease in PCE/NCE (polychromatic erythrocytes/normochromatic erythrocytes) ratio in the 24 hour 1000 mg/kg test group compared to controls. This reduction, together with the observed clinical signs suggest that systemic absorption occurred and the bone marrow was reached by the test substance.

CONCLUSION The notified chemical was not clastogenic under the conditions of this *in vivo* micronucleus test in the mouse.

TEST FACILITY Safepharm Laboratories (2008e)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO2 Evolution Test. EC Directive 92/69/EEC C.4-C Biodegradation: Determination of the " Ready" Biodegradability: Carbon Dioxide Evolution Test
Inoculum	Activated sewage sludge
Exposure Period	28 Days
Auxiliary Solvent	None
Analytical Monitoring	CO_2 Analysis
Remarks - Method	Following preliminary range-finding tests, the notified chemical of a concentration of 5 mg C/L was exposed to activated sewage sludge micro-organisms in the dark at 21˚C for 28 days. Sodium benzoate was used as the reference substance at 10 mg C/L in the reference control to verify the validation of the test system. All the test group, blank control and the reference control tests were conducted in duplicates. The toxicity control containing the reference substance (10 mg C/L) and the notified chemical (5 mg C/L) was conducted in one vessel only.

RESULTS

Test substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
2	5	2	52
14	8	14	97
22	38	22	110
29	44	29	113

Remarks - Results	All the validation criteria for the test were satisfied. The toxicity control test attained 31% degradation by day 14 and 71% degradation by day 28 of the test confirming that the notified chemical is not toxic to the inoculum. The notified chemical reached a degradation of 44% by day 29 of the test indicating it is not readily biodegradation under strict terms and conditions of the OECD test guideline.
CONCLUSION	The notified chemical can not be classified as readily biodegradable.
TEST FACILITY	SafePharm Laboratories (2007f)

C.1.2. Bioaccumulation

TEST SUBSTANCE	Notified chemical
Remarks - Method	Due to the difficulties in determining the water solubility, log Pow, and adsorption/desorption coefficient, the potential of the notified chemical to bioaccumulate cannot be accurately assessed. Based on the surfactant properties, molecular weight of 406.14, and not being readily biodegradable, the notified chemical is expected to have potential for bioaccumulation. However, the ionic nature of the notified chemical would mitigate any bioaccumulation potential.
CONCLUSION	The notified chemical has potential for bioconcentrating.

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-static. EC Directive 92/69/EEC C.1 Acute Toxicity for Fish – Semi-static.
Species	Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	140 mg $CaCO_3$/L
Analytical Monitoring	HPLC-MS for determination of test concentrations
Remarks – Method	Following preliminary range-finding test, fish were exposed to the notified chemical, in groups of seven, over a range of concentrations of 0.010, 0.018, 0.032, 0.056 and 0.10 mg/L for a period of 96 hours at approximately 14˚C. The medium used for the test was laboratory tap water dechlorinated by passage through an activated carbon filter and partly softened.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
0.01	0.0087	7	0	0	0	0	0
0.018	0.014	7	0	0	0	0	0
0.032	0.02	7	0	0	0	0	0
0.056	0.043	7	0	0	0	0	0
0.1	0.061	7	0	0	7	7	7

LC50	0.051 mg/L at 96 hours.
NOEC	0.020 mg/L at 96 hours.
Remarks – Results	Overall inspection of the data showed a general trend for a decline in measured concentrations, which was considered due to the insolubility or adherence of the test substance to the glass vessels. Given this decline in measured test concentrations it was considered justifiable to base the results on the time weighted mean measured test concentrations of test media to give a 'worst case' analysis of the data. The 96-hour LC50 based on the time weighted mean measured concentrations of the test media was 0.051 mg/L with 95% confidence limits of 0.043 – 0.061 mg/L. The NOEC was 0.020 mg/L. Sub-lethal effects of exposure were observed at the nominal test concentration of 0.056 mg/L. The response was loss of equilibrium.
CONCLUSION	The notified chemical is very toxic to fish.
TEST FACILITY	SafePharm Laboratories (2008g)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test – semi-static. EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia – semi-static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	HPLC-MS for determination of test concentrations

Remarks - Method: Following preliminary range-finding tests, 10 daphnids were exposed in duplicate to an aqueous solution of the notified chemical at concentrations of 0.0018, 0.0032, 0.0056, 0.010, 0.018, 0.032, 0.056, 0.10 and 0.18 mg/L at 21 ˚C for a period of 48 hours. Positive control tests were also conducted in duplicates using potassium dichromate as the reference substance at concentrations of 0.32, 0.56, 1.0, 1.8 and 3.2 mg/L.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24*	*48 h*
0.0018	0.0015	20	0	0
0.0032	0.0014	20	0	0
0.0056	0.0026	20	0	0
0.010	0.0053	20	0	2
0.018	0.010	20	0	9
0.032	0.017	20	1	20
0.056	0.033	20	17	20
0.10	0.055	20	20	20
0.18	0.098	20	20	20

EC50: 0.0096 mg/L at 48 hours
NOEC: 0.0026 mg/L at 48 hours
Remarks - Results: Given that all test concentrations were analysed throughout the study measured concentrations were determined for all concentrations and it was considered appropriate to base the results on the time weighted mean measured test concentrations.

The 48-hour EC50 for the reference substance to daphnia based on nominal concentrations was 0.75 mg/L with 95% confidence limits of 0.56 – 1.0 mg/L and the NOEC was 0.56 mg/L.

Based on the time weighted mean measured test concentrations of the test medium the 48-hour EC50 was 0.0096 mg/L with 95% confidence limits of 0.0080 – 0.011 mg/L and the NOEC was determined to be 0.0026 mg/L.

CONCLUSION: The notified chemical is very toxic to *Daphnia magna*.

TEST FACILITY: SafePharm Laboratories (2008h)

C.2.3. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 211 Daphnia magna, Reproduction Test – 21 Day, semi-static
Species: *Daphnia magna*
Exposure Period: 21 days
Auxiliary Solvent: None
Water Hardness: 140 mg/L as $CaCO_3$
Analytical Monitoring: HPLC-MS for determination of test concentrations
Remarks - Method: Based on the results of an acute toxicity test, 1st instar *Daphnia magna* were exposed (10 replicates of a single daphnia per group) to an aqueous solution of the notified chemical at concentrations of 0.00017, 0.00054, 0.0017, 0.0054 and 0.017 mg/L for a period of 21 days at 20˚C. The numbers of adult and young daphnids (of live and dead for both) were recorded daily. Laboratory tap water was used for the test after being dechlorinated by passage through an activated carbon filter and partly softened. The control group was maintained under identical conditions but not exposed to the notified chemical.

The analysis of the test concentrations showed a concentration dependent increase in the measured concentration with increase in nominal concentration throughout the duration of the test in both the fresh and old test concentration samples. However, given the general trend for decline in measured concentration over each media renewal period, it was considered justifiable to base the result on the time-weighted mean measured test concentrations of the test media to give a "worst case" analysis of the data.

Survival of parental daphnids, mean adult body length and cumulative mean number of offspring released per female daphnid (*Daphnia magna*) at Day 21

Nominal loading rate (mg/L)	Time weighted mean measured concentration (mg/L)	Number of adult daphnids immobilized	Mean percent survival of adult daphnids	Mean number of live offspring released per female	Mean number of dead offspring released per female	Mean total body length (mm, SD)
Control	Control	0	100	104	0	4.2 ± 0.3
0.00017	0.00019	0	100	109	0	4.2 ± 0.1
0.00054	0.00028	1	90	114	< 1	4.2 ± 0.1
0.0017	0.00066	0	100	46	6	4.1 ± 0.1
0.0054	0.0019	10	0	0	0	
0.017	0.0062	10	0	0	0	
Day 21 EC50 (mg/L)		0.0010 (immobilization); 0.00063 (reproduction)				
NOELR (mg/L)		0.00028				

Remarks - Results

Results from the control and 0.00017, 0.00054 and 0.0017 mg/L test groups were compared using one way analysis of variance incorporating Bartlettt's test for homogeneity of variance and Dunnett's multiple comparison procedure for comparing several treatments with a control.

The Day 21 EC50 (immobilization) value for the parental Daphnia magna was calculated to be 0.0010 mg/L with 95% confidence limits of 0.00088 – 0.0012 mg/L based on the time weighted mean measured concentration of the test medium.

The Day 21 EC50 (reproduction) value for the parental Daphnia magna was calculated to be 0.00063 mg/L with 95% confidence limits of 0.00047 – 0.00085 mg/L based on the time weighted mean measured concentration of the test medium.

CONCLUSION The notified chemical is very toxic to *Daphnia magna* on a chronic basis.

TEST FACILITY SafePharm Laboratories (2008j)

C.2.4. 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	*Desmodesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 0.0625, 0.125, 0.25, 0.5 and 1.0 mg/L Actual: 0.017, 0.028, 0.061, 0.12 and 0.51 mg/L
Auxiliary Solvent	None
Water Hardness	Not stated
Analytical Monitoring	HPLC-MS for determination of test concentrations. Coulter® Multisizer Particle Counter was used for determination of cell density.
Remarks - Method	Following preliminary range-finding tests, *Desmodesmus subspicatus* was exposed in triplicates to an aqueous solution of the test material at a

series of five nominal concentrations ranging 0.0625 – 1.0 mg/L for 72 hours, under constant illumination and shaking at a temperature of 24 ± 1˚C. A blank control was conducted in 6 replicates under identical conditions to the main test but not exposed to the notified chemical. A positive control was conducted in triplicates using potassium dichromate as the reference substance at concentrations of the same to the main test.

All statistical analyses were performed using the SAS computer software package (SAS 1999 - 2001).

RESULTS

Biomass		*Growth*	
E_bC50	*NOEC*	*E_rC50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L*	*mg/L at 72 h*
0.031	0.017	0.078	0.017

Remarks - Results

Analyses of the test concentrations showed low measured concentration at 0-hour and a decline at 72 hours, which were considered due to the absorption of the notified chemical to glassware and algal cell present. Thus it was considered justifiable to base the results on the geometric mean measured test concentrations in order to give a 'worst case' analysis of the data.

Based on the geometric measured concentrations the E_rC50 (0-72 h) value was 0.078 mg/L with 95% confidence limits of 0.067 – 0.092 mg/L, the E_bC50 (0-72 h) value was 0.031 mg/L with 95% confidence limits of 0.027 – 0.035 mg/L. The NOEC was 0.017 mg/L.

CONCLUSION

The notified chemical is very toxic to algae.

TEST FACILITY

SafePharm Laboratories (2008i)

C.2.5. Inhibition of microbial activity

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 209 Activated Sludge, Respiration Inhibition Test.
EC Directive 87/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test

Inoculum: Activated sewage sludge from domestic sewage treatment plant
Exposure Period: 3 hours
Concentration Range: Nominal: 10, 32, 100, 320 and 1000 mg/L
Remarks – Method: Following preliminary range-finding test, activated sewage sludge was exposed to the notified chemical at concentrations of 10 – 1000 mg/L. A negative control in duplicates and a reference control using 3,5-dichlorophenol at concentrations of 3.2, 10 and 32 mg/L were conducted. Test water used was laboratory tap water dechlorinated by passage through an activated carbon filter and partly softened giving water with total hardness of 140 mg/L as CaO_3. The test preparations were dark brown dispersions with no visible undissolved test material through out the test period. Foam on the surface was observed for the 100, 320 and 1000 mg/L at 0-hour and the 1000 mg/L at 3-hour of the test.

RESULTS

IC50: 440 mg/L
NOEC: 10 mg/L
Remarks – Results: The 3-Hour IC50 for the reference substance for effect on the respiration of activated sewage sludge micro-organisms was determined to be 9.3 mg/L with 95% confidence limits of 7.6 – 11 mg/L.

	The 3-Hour IC50 for the notified chemical for effect on the respiration of activated sewage sludge micro-organisms was determined to be 440 mg/L with 95% confidence limits of 330 – 590 mg/L. The NOEC after 3 hour exposure was 10 mg/L.
CONCLUSION	The notified chemical is not considered harmful to sewage treatment bacteria.
TEST FACILITY	SafePharm Laboratories (2007g)

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