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January 2011

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

1-Dodecanaminium, N-(2-hydroxy-3-sulfopropyl)-N,N-dimethyl-, inner salt (INCI: Lauryl hydroxysultaine)

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

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

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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NICNAS

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Unilever Australia Limited (ABN 66 004 050 828)
20 Cambridge Street
Epping, NSW 2121

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
No details are claimed exempt from publication.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: melting point, boiling point, vapour pressure, hydrolysis as a function of pH, adsorption/desorption, dissociation constant, particle size, flash point, flammability limits, autoignition temperature, explosive properties, acute inhalation toxicity, repeat dose toxicity, bioaccumulation and inhibition of microbial activity.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Amphitol 20HD-L (contains 28-32% notified chemical)
Betadet S-20 (contains 28-32% notified chemical)

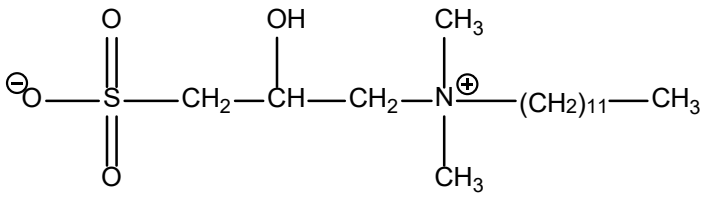
CAS NUMBER
13197-76-7

CHEMICAL NAME
1-Dodecanaminium, N-(2-hydroxy-3-sulfopropyl)-N,N-dimethyl-, inner salt

OTHER NAME(S)
Lauryl hydroxysultaine (INCI name)

MOLECULAR FORMULA
$C_{17}H_{37}NO_4S$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
351.54 g.mol^{-1}

ANALYTICAL DATA
Reference IR spectra were provided.

3. COMPOSITION

DEGREE OF PURITY 28-32%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS

Chemical Name	Heavy metals (including Pb)		
CAS No.	Varies	*ppm*	20
Chemical Name	Arsenic		
CAS No.	7440-38-2	*ppm*	2
Hazardous Properties	T: R23, R25 N: R50, R53		

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS

Chemical Name	Quaternary ammonium salts		
CAS No.	Varies	*Weight %*	< 4
Chemical Name	Free amine		
CAS No.	Varies	*Weight %*	< 1
Chemical Name	Sodium chloride		
CAS No.	7647-14-5	*Weight %*	< 14

ADDITIVES/ADJUVANTS

Chemical Name	Water		
CAS No.	7732-18-5	*Weight %*	50-57

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: The imported aqueous solution containing the notified chemical at a concentration of 28-32% is a colourless to light yellow liquid.

Property	Value	Data Source/Justification
Melting Point	272°C	Estimated (EPISuite)
Boiling Point	627°C at 101.3 kPa	Estimated (EPISuite)
Density	1108 kg/m^3 at 25°C (aqueous solution containing 28-32% notified chemical)	MSDS
Viscosity	29 mPa at 25°C (aqueous solution containing 28-32% notified chemical)	MSDS
pH	6 – 8 (1% Amphitol 20HD-L)	MSDS
Vapour Pressure	1.1×10^{-18} kPa at 25°C	Estimated (EPISuite) Modified Grain method
Water Solubility	Not determined	The notified chemical is water dispersible, as it is a surfactant and is expected to form micelles in concentrated solutions.
Hydrolysis as a Function	Not determined	The notified chemical does not contain any

Property	Value	Data Source/Justification
of pH		readily hydrolysable groups and is therefore expected to be hydrolytically stable.
Partition Coefficient (n-octanol/water)	log K_{OW} ≤1.65	Analogue data. The notified chemical is a surfactant and will tend to accumulate at the phase interface of octanol and water.
Adsorption/Desorption	Not determined	The notified chemical is expected to partition to surfaces from water in the environment based on its surface activity
Dissociation Constant	Not determined	The notified chemical is a zwitterion and will be ionised under environmental conditions.
Particle Size	Not determined	Imported in an aqueous solution
Flash Point	Not determined	Imported in an aqueous solution
Flammability	Not determined	Imported in an aqueous solution
Autoignition Temperature	Not determined	Imported in an aqueous solution
Explosive Properties	Not expected to be explosive	The structural formula contains no explosophores.

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

Reactivity
Stable under normal conditions of use.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured within Australia. The notified chemical will be imported as a raw material for local blending (aqueous solution containing 28-32% notified chemical) and in finished cosmetic products at concentrations up to 10% for rinse off products and 5% for leave on products.

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

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

PORT OF ENTRY
Sydney

TRANSPORTATION AND PACKAGING
When the notified chemical is introduced as a raw material (aqueous solution containing 28-32% notified chemical) it will be imported in 180 L plastic drums. When imported as finished cosmetic products the packaging will vary but the maximum size is expected to be 400 mL. Transportation of the products containing the notified chemical will predominantly be by road.

USE
The notified chemical will be used as a component of cosmetic products at concentrations up to 10% for rinse off products and 5% for leave on products. The notified chemical will also be used as a component of cleaning products such as acid, bleach, bathroom and glass cleaners at concentrations up to 10%.

OPERATION DESCRIPTION
Reformulation
When imported as a raw material (28-32% in an aqueous solution) the notified chemical will undergo quality assurance tests prior to being reformulated into cosmetic and cleaning products. The notified chemical will then be weighed before being manually added to the mixing tank. The mixing facilities are expected to be automated, well ventilated (local exhaust ventilation) and closed systems. After being reformulated, the mixture containing the notified chemical at concentrations up to 10% will undergo further quality assurance tests before being packaged into containers.

End use
The finished cosmetic products containing the notified chemical will be used occupationally by beauticians. Depending on the nature of the product these could be applied in a number of ways such as by hand or using an applicator. Cleaning products containing the notified chemical may also be used by commercial cleaners in both domestic and industrial environments.

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	12
Professional compounder	1	8	12
Chemist	1	3	12
Packers (dispensing and capping)	2	8	12
Store personnel	2	4	12
End users	300,000	8	365

EXPOSURE DETAILS
Transport and warehousing
It is expected that transport and warehouse workers handling the imported aqueous solution containing up to 28-32% notified chemical or finished products containing up to 10% notified chemical will only be exposed to the notified chemical in the event of spills due to an accident or as a result of leaking a container. The main route of exposure in these situations will be dermal.

Reformulation
During reformulation, dermal and ocular exposure to the notified chemical (at 28-32%) may occur when weighing and transfer to the mixing tank. It is expected that negligible exposure will occur during the automated and closed blending process. Workers involved in the reformulation process are expected to wear safety glasses with shields, gloves and an apron or coveralls to further minimise exposure. Exposure to the notified chemical at concentrations up to 10% during transfer of the formulated product to packaging is expected to be low due to the largely automated processes.

Inhalation exposure during reformulation is expected to be negligible given the very low estimated vapour pressure of the notified chemical (1.1×10^{-18} kPa at 25°C). In addition, blending and packaging facilities are expected to be well ventilated. Inhalation exposure to the notified chemical as a solid particulate is not expected as it will be imported as a 28-32% aqueous solution.

End use
Beauticians and hairdressers will be exposed to cosmetic products containing the notified chemical (≤ 10%)

during application of the products to their clients. The main route of exposure is expected to be dermal, although ocular exposure to splashes is possible. PPE is not expected to be worn, however good hygiene practices are expected to be in place.

Cleaners will be exposed to the notified chemical at concentrations up to 10% during the use of cleaning products. The main route of exposure is expected to be dermal, although inhalation exposure to aerosols formed through the spray application of cleaning products is also possible. The level of PPE used by cleaners is likely to vary but would include gloves in many cases.

6.1.2. Public exposure

The general public will be repeatedly exposed to the notified chemical via a number of different cosmetic and cleaning products at concentrations up to 5% in leave on cosmetic products and up to 10% in rinse off cosmetic products and cleaning products. Exposure to the notified chemical will vary depending on individual use patterns. Although, the principal route of exposure will be dermal, accidental ocular exposure may also occur. Inhalation exposure is also possible if products are applied by spray. Accidental ingestion from the use of these types of products is also possible from oral care and facial products.

Public exposure to the notified chemical in cosmetics has been estimated using the Scientific Committee on Consumer Products' (SCCP's) Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation and applying the following assumptions (SCCP, 2006):

- Bodyweight of 60 kg for females;
- 100% dermal absorption

Total systemic exposure estimated is presented below.

Product type	**mg/event**	**events/day**	**C** (%)	**RF**	**Daily exposure** (mg/day)	**Daily systemic exposure**** (mg/kg bw/day)
Leave on (max. conc. 5%)						
Body lotion	7820	1	5	1	391	6.5
Face cream	1540	1	5	1	77	1.3
General purpose cream	1200	2	5	1	120	2
Rinse off (max. conc. 10%)						
Facial cleansers	4060	1-2*	10	0.01	4	0.068
Make up remover	2500	2	10	0.1	50	0.83
Shower gel	5000	2	10	0.01	10	0.17
Shampoo	10460	1	10	0.01	10	0.17
Hair conditioner	14000	0.28	10	0.01	4	0.065
Hair styling products	5000	2	10	0.1	100	1.7

C = concentration; RF = retention factor; Daily exposure = mg/event x events/day x C(%) x RF; Daily systemic exposure = daily exposure x dermal absorption (50%) /bw (60 kg)

* 1 Used in calculation

** Using 100% dermal adsorption

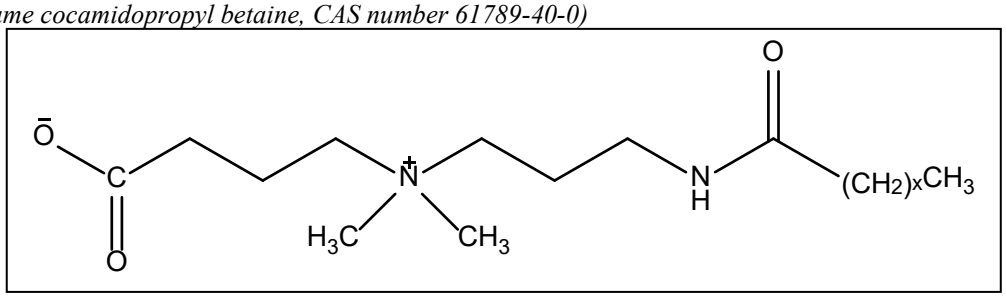
The above exposure estimates were calculated using conservative assumptions and is expected to reflect a worst case scenario.

Public exposure from transport, storage, reformulation or disposal is considered to be negligible.

6.2. Human health effects assessment

Toxicological data were provided for various concentrations of the notified chemical. In addition, toxicological data were provided for the following analogues of the notified chemical.

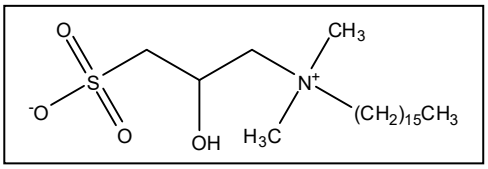
Analogue 1 - *1-Propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-coco acyl derivs., inner salts (INCI name cocamidopropyl betaine, CAS number 61789-40-0)*



x = 4-16 (predominantly 10)

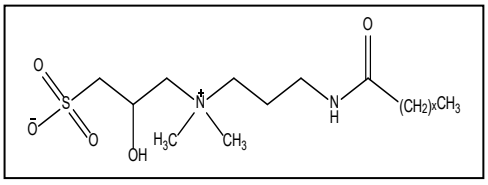
Analogue 1 is essentially the betaine version (comprised of a quarternary ammonium and carboxylate group) of Analogue 3 which is a sulfobetaine (comprised of a quarternary ammonium and sulfonate group) like the notified chemical. Given the physicochemical and toxicity properties of betaines and sulfobetaines are expected to be similar and Analogue 3 is considered an acceptable analogue (see justification for Analogue 3 below), Analogue 1 is considered an acceptable analogue of the notified chemical.

Analogue 2 - *Cetyl hydroxysultaine*



Like the notified chemical, Analogue 2 is a hydroxysultaine that only differs from the notified chemical in the carbon chain length (i.e. C16 for the analogue versus C12 for the notified chemical). It is therefore considered an acceptable analogue of the notified chemical.

Analogue 3 – *1-Propanaminium, N-(3-aminopropyl)-2-hydroxy-N,N-dimethyl-3-sulfo-, N-coco acyl derives., inner salts (CAS number 68139-30-0) (cocoamidopropyl hydroxysultaine)*



x = 4-16 (predominantly 10)

Like the notified chemical, Analogue 3 is a hydroxysultaine that differs from the notified chemical in the composition (i.e. contains an amide linkage) and length of the carbon chain (equivalent to C16 for the analogue versus C12 for the notified chemical). Given the amide linkage is not expected to contribute to toxicity and the carbon chain lengths are comparable, Analogue 3 is considered an acceptable analogue of the notified chemical.

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	LD50 > 2000 mg/kg bw LD50 > 560-640 mg/kg bw for the notified chemical
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	irritating
Guinea pig, skin sensitisation – adjuvant test	inadequate evidence; no conclusion
Mutagenicity – bacterial reverse mutation	non mutagenic

* 28-32% notified chemical

Toxicokinetics, metabolism and distribution.
Absorption of the notified chemical across biological membranes is likely given its moderately low molecular weight 351.54 $g.mol^{-1}$ and because it is a surfactant (EC, 2003).

Acute toxicity.
The LD50 for the notified chemical was > 560-640 mg/kg bw, based on a study conducted in rats where at a concentration of 28-32% it was found to be of low acute oral toxicity (LD50 > 2000 mg/kg bw). The analogue chemical cocamidopropyl betaine (Analogue 1) when tested at a concentration of 30% showed oral LD50 values of 4.91 g/kg bw in mice and 7.45, 8.55 and 4.9 g/kg bw in rats in three different studies (CIR, 2010). In a further acute oral study at a concentration of 35.61% cocamidopropyl betaine (Analogue 1) had a LD50 of > 1.8 g/kg bw for male rats but the LD50 for females could not be determined as at this only dose level all the female rats died (CIR, 2010). Based on the weight of evidence from the above studies the notified chemical is likely to be of low toxicity via the oral route.

No acute dermal or inhalation toxicity data were provided for the notified chemical. An acute dermal toxicity study with the analogue cocamidopropyl betaine (Analogue 1) tested at a concentration of 31% gave an LD50 value of > 2 g/kg bw indicating low dermal toxicity for the notified chemical (CIR, 2010).

Irritation and Sensitisation.
Based on a test conducted in rabbits the notified chemical is considered to be slightly irritating to the skin and irritating to the eye when applied at a concentration of 28-32%. Severe eye irritation effects can not be ruled out at 100% concentration, considering the irritation effects seen at up to 32% concentration.

The notified chemical has a quarternary ammonium functional group which is a known structural alert for sensitisation (Barrett et al., 1994).

A GPMT sensitisation study provided for the notified chemical showed no indication of sensitisation after challenge at concentrations up to 0.1% following an induction phase of 0.3% of the notified chemical. However as signs of irritation after the induction phase were not reported it is not possible to determine if the test was valid. In addition the concentration tested was very low, compared to the intended concentration (up to 10%) in consumer products.

In a non-standard non-adjuvant GPMT, the analogous chemical cetyl hydroxysultaine (Analogue 2) produced no signs of sensitisation (Unilever, 1974). In the study, 10 animals were induced intradermally at Day 0 at a dose concentration of 0.15% (irritant dose based on preliminary test) and at Day 14 and Day 21 at a dose concentration of 0.06% (mildly irritant dose based on preliminary test). The challenge phase was conducted on Day 34 with a topical application at a dose concentration of 2.5%. The challenge dose was selected on the basis that irritation was observed at 5% the lowest dose tested in the preliminary test. It is not clear from the study report if irritation was elicited at the induction phase. Given this is a non-standard test it is difficult to draw any conclusions from this study.

In a GPMT adjuvant test conducted generally in line with OECD TG 406, the analogous chemical cocoamidopropyl hydroxysultaine (Analogue 3) produced no signs of sensitisation (Unilever, 1987). In the study, 10 animals were induced intradermally at a dose concentration of 0.05% (mildly irritating dose based on preliminary test) at Day 0 and then topically at Day 7 at a dose concentration of 10%. A topical application at a dose concentration of 10% was used for the challenge phase at Day 21. A second challenge was made one week after the first. As irritation was not observed at 10%, the highest dose concentration tested in the preliminary test, sodium lauryl sulphate (concentration not specified) was applied at Day 6 to create a local irritation. In the first

challenge, one animal elicited a very faint to mild erythema at both 24 and 48- hours post removal of the occluded patch. No animals elicited a reaction to the second challenge. As it is not clear from the study report if irritation occurred in the induction phase, it can only be concluded from this study that Analogue 3 is not a sensitiser at up to the concentration tested i.e. up to 10%.

The analogue cocamidopropyl betaine (Analogue 1) was reported to give a positive response in an LLNA study and gave evidence of sensitisation in maximisation tests at concentrations up to 3% (CIR, 2010). However, in conflict with the results of these studies, a further maximisation test with cocamidopropyl betaine at a concentration of 6% for the induction phase and 1% for the challenge phase produced no signs of sensitisation. Slight dermal reactions were reported at the induction phase. A test with cocamidopropyl betaine at 0.75% for the induction phase and 0.02% for the challenge phase also produced no sign of sensitisation (CIR, 2010). A HIRPT with 88 test subjects using a shampoo containing cocamidopropyl betaine at a concentration of 18.72% at a 10% dilution produced no signs of sensitisation, also a further three HRIPT studies at concentrations of 0.3, 0.93 and 3.0% produced no signs of sensitisation (CIR, 2010). In three patch test studies with cocamidopropyl betaine at 1% concentration on patients that were deemed likely to have contact dermatitis, 0.27, 3 and 7.2% were deemed to show a positive reaction (CIR, 2010). The authors of the CIR report note that the sensitisation effects are likely due to impurities present in the cocamidopropyl betaine, however they do not rule out the possibility of the chemical itself also being sensitising.

In summary, it cannot be definitively concluded from the studies available on the notified chemical and analogous chemicals that the notified chemical is not a sensitiser given it contains a structural alert for sensitisation. However, based on the result of the GPMT adjuvant test with the acceptable analogue cocoamidopropyl hydroxysultaine (Analogue 3) suggests that the notified chemical is not a skin sensitiser at concentrations up to 10%. Therefore the notified chemical, if at all, can only be at most a weak sensitiser.

Repeated Dose Toxicity (sub acute, sub chronic, chronic).
There is no repeated dose toxicity data on the notified chemical. In a 28 day oral repeat dose toxicity study in rats (8/sex/group) on a 30.6% aqueous solution of the analogue chemical cocamidopropyl betaine (Analogue 1) at doses tested of 100, 500 and 1000 mg/kg bw/day deaths were seen at all of the dose levels although there was no dose response relationship and the study authors suggest that some of the deaths may have been due to substance administration errors. Other effects in this study included stomach lesions in the rats in the high dose group (CIR, 2010). In a second 28 day oral repeat dose toxicity study on an aqueous solution of the analogue chemical cocamidopropyl betaine (concentration not stated) (Analogue 1) the NOEL was found to be 500 mg/kg bw/day in rats with oedema of the mucosa of the non-glandular stomach seen in the 1000 mg/kg bw/day dose group (CIR, 2010). These effects were considered to be the result of irritating properties of the notified chemical and not of systemic toxicity. Complete regeneration of the mucosa in the 1000 mg/kg bw/day dose group was observed. In a 90 day oral repeat dose toxicity study in rats on an aqueous solution of the analogue chemical cocamidopropyl betaine (concentration not stated) (Analogue 1) at concentrations of 250, 500 and 1000 mg/kg bw/day stomach ulcers were seen in 2/20 rats in the 1000 mg/kg bw/day dose group with non-glandular gastritis seen in 9/20 rats in the 1000 mg/kg bw/day dose group and 4/20 rats in the 500 mg/kg bw/day dose group leading to a NOEL of 250 mg/kg bw/day in this study (CIR, 2010).

Mutagenicity.
The notified chemical was found to be non-mutagenic using a bacterial reverse mutation test. The analogous chemical cocamidopropyl betaine (Analogue 1) was found to be non-mutagenic in 4 bacterial reverse mutation tests (CIR, 2010).

The analogous chemical cocamidopropyl betaine (Analogue 1) was found to be non-mutagenic in an *in vitro* test on mouse lymphoma L5178Y TK± cells and in an *in vivo* mouse micronucleus test (CIR, 2010).

Carcinogenicity.
No signs of carcinogenicity were found in a 20 month study in mice for a hair dye containing the analogous chemical cocamidopropyl betaine (Analogue 1) at a concentration of 0.09%.

Health hazard classification
Based on the results of the eye irritation study (at a concentration of 28-32% of the notified chemical), the notified chemical is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:
Xi: R36 Irritating to eyes

Severe eye irritation effects sufficient to classify the chemical as R41, cannot be ruled out at higher concentrations than those tested.

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
Based on data provided, the notified chemical is a slight skin irritant and an eye irritant and, although the sensitisation potential is inconclusive, it may not be a skin sensitiser at concentrations up to 10%. The risk of systemic effects is expected to be low based on the absence of effects in the repeat dose and acute toxicity tests on analogous chemicals. The notified chemical is also not mutagenic and unlikely to be genotoxic based on studies on analogous chemicals.

Although reformulation workers will handle the notified chemical at a concentration of 28-32%, exposure is expected to be low given the proposed use of PPE and largely enclosed, automated processes used in reformulation facilities, minimising the risk of irritation and dermal sensitisation. Overall, the risk to the occupational health and safety of reformulation workers is not considered unacceptable, due to the expected low exposure to the notified chemical from the use of PPE and enclosed and automated processes.

Beauticians and hairdressers will be exposed to cosmetic products containing the notified chemical (≤ 10%) during application of the products to their clients. As eye irritation and sensitisation effects are not expected at the proposed use concentrations, the risk to these workers from use of the notified chemical as described is not considered unacceptable.

Dermal, ocular and inhalation exposure of workers to the notified chemical at concentrations up to 10% may occur during use of cleaning products. As eye irritation and sensitisation effects are not expected at the proposed use concentrations, the risk to professional cleaners from use of the notified chemical as described is not considered unacceptable.

6.3.2. Public health
The general public will be repeatedly exposed to the notified chemical via a number of different consumer products, applied to the skin and also through the use of cleaning products.

Local effects
The notified chemical is a slight skin irritant and an eye irritant, and although the sensitisation potential is inconclusive, it is not expected to be a skin sensitiser at concentrations up to 10%. Given the notified chemical will be present in finished products at a maximum concentration of 10% for wash off cosmetic products and cleaning products and 5% for leave-on cosmetic products, the potential risk of irritation and dermal sensitisation to the public from use of the notified chemical is not considered unacceptable.

Systemic effects
A NOEL of 500 mg/kg bw/day and 250 mg/kg bw/day were found in a 28 day and 90 day oral repeat dose toxicity study in rats on an aqueous solution of the analogue chemical cocamidopropyl betaine (concentration not stated), respectively. The effects observed in both of the studies were due to the irritant effects of the notified chemical and there was no apparent systemic toxicity. The concentration of the analogue chemical cocamidopropyl betaine in the test substance was not stated in the two tests mentioned above but was stated as 30.6% in the 28 day oral repeat dose toxicity study where the NOEL could not be determined due to deaths at all doses. In this latter study the reported irritant effects were comparable to the two other studies, hence it could be assumed that the concentration of the analogue chemical in the former two studies was similar (i.e. 30.6%). Therefore, after adjusting for the concentration of the analogue chemical in the test substance a NOEL of 76.5 mg/kg bw/day (assuming a NOEL of 250 mg/kg bw/day for the analogue chemical at 30.6%) is estimated for the notified chemical using the 90-day rat study.

The NOEL of 75.6 mg/kg bw/day, established in the 90-day repeat dose oral toxicity study in rats for the

analogue cocamidopropyl betaine is used in the estimation of the margin of exposure (MOE) below. An MOE value greater than or equal to 100 is considered acceptable to account for intra- and inter-species differences. The MOE values for the notified chemical are estimated using the following:

$$MOE = \frac{\text{Estimated NOEL}}{\text{Estimated systemic exposure}}$$

Product type	C (%)	Daily systemic exposure* (mg/kg bw)	MOE**
Leave on			
Body lotion	5	6.5	13
Face cream	5	1.3	58
General purpose cream	5	2	38
Rinse off			
Facial cleansers (1of 2 events)	10	0.068	1103
Make up remover	10	0.83	91
Shower gel	10	0.17	441
Shampoo	10	0.17	441
Hair conditioner	10	0.065	1154
Hair styling products	10	1.7	44

* Based on 100% dermal absorption
** Using NOEL of 75.6 mg/kg bw/day

The above table indicates MOE values of less than 100 at the intended maximum use concentration for all leave on cosmetic products (body lotions, face creams and general purpose creams) and for some rinse-off cosmetics (make up remover and hair styling products). Although the MOE values for these products are less than 100 the calculation is based on a worse case scenario where the dermal absorption is assumed to be 100% and using an adjusted NOEL of 75.6 mg/kg bw/day. Also the effects seen in the repeat dose studies for the analogue cocamidopropyl betaine on which the NOEL was based were only due to the irritant effects of the chemical. There was no evidence of systemic toxicity even at the highest dose tested of 1000 mg/kg bw/day, hence the risk of systemic effects from repeated exposure to the notified chemical is not expected from topical use at a maximum concentration of 10%.

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 as a component of finished cosmetic and cleaning products and will also be imported as a raw material (28-32% aqueous solution) for blending. The notified chemical is expected to be released to landfill as residue in containers (estimated to be up to 1% of the annual import volume) and released to sewer from the cleaning of blending equipment (up to 3%).

Accidental spills during transport or reformulation are expected to be collected with inert material and disposed of to landfill.

RELEASE OF CHEMICAL FROM USE
The notified chemical is a component in cosmetic and cleaning products. Therefore, it is expected that the majority of the imported quantity of notified chemical will be released to sewer as a result of its use pattern.

RELEASE OF CHEMICAL FROM DISPOSAL
Expired wastes and residue of the notified chemical in empty containers (1%) are likely either to share the fate of the container and be disposed of to landfill, or to be washed to the sewer when containers are rinsed before recycling.

7.1.2 Environmental fate

The majority of the notified chemical will be disposed of to the sewer, with minor amounts disposed of to landfill. The notified chemical is expected to be largely removed from sewage treatment plant (STP) influent as the notified chemical is readily biodegradable and, due to its surface activity, is expected to partition to sludge in STPs. Notified chemical released to surface waters is expected to partition to suspended solids and organic matter, or to disperse and degrade. The potential for the notified chemical to bioaccumulate is low, based on its low octanol-water partition coefficient (log Pow ≤1.65) and the low bioconcentration factor, predicted by regression-based method for the protonated form of the notified chemical depicted by EPISuite (BCF <71, BCFBAF (v2.00); EPISuite (4.00)).

STP sludge containing the notified chemical may be disposed of to landfill or used for soil remediation. In soil and landfill, the notified chemical may leach due to its water dispersability, however sorption to soil or sediment will limit its mobility. It is expected to degrade through biotic or abiotic processes to form water and oxides of carbon, nitrogen and sulfur.

For the details of the environmental fate studies, refer to Appendix C.

7.1.3 Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) can be estimated as outlined below based on the hypothetical worst case assumptions of complete discharge of the total annual import of the notified chemical to receiving waters via sewage treatment works nationwide.

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:	13.70	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	21.161	million
Removal within STP	0%	
Daily effluent production:	4,232	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	3.24	µg/L
PEC - Ocean:	0.32	µg/L

The notified chemical is readily biodegradable and is expected to adsorb to sludge, thus, its removal from sewage treatment plants (STPs) is expected. However, for the worst case scenario, it is assumed that the notified chemical is not removed from influent and is released with STP effluent. STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 3.24 µg/L may potentially result in a soil concentration of approximately 21.58 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 107.9 µg/kg and 215.8 µg/kg, respectively. However, given the expected degradation and mobility of the notified chemical, these values should be considered as theoretical maximum concentrations only.

7.2. Environmental effects assessment

The results from ecotoxicological investigations conducted on the notified chemical are summarised in the table below. The tests were conducted on aqueous solutions containing the notified chemical and inseparable by-products. The results have been corrected to reflect the minimum median lethal concentration of the notified chemical. Fish studies (OECD TG 203, 96 h) were conducted on three different species: Zebra fish, LC50 ≥ 36.8 mg/L; red killifish, LC50 ≥ 9.2 mg/L; and rainbow trout, LC50 ≥ 2.6 mg/L. For the purposes of regulation, the minimum median lethal concentration of the most sensitive fish species (rainbow trout 96 h EC50 = 2.6

mg/L) is used for the classification of the notified chemical.

Details of these studies can be found in Appendix C.

Studies were not submitted for the toxic effect of the notified chemical on algae, however, published results are available for another zwitterionic surfactant of comparable carbon chain length, C_{12-14} alkyl betaine (Madsen et al., 2001). The C_{12-14} alkyl betaine and the notified chemical are both formally classified as harmful to zebra fish (96 h LC50 = 21.9 mg/L and ≥ 36 mg/L, respectively). The similar level of toxicity of the alkyl betaine towards zebra fish, combined with its structural similarity to the notified chemical, presents the alkyl betaine to be an acceptable analogue for the notified chemical. Therefore, the algal endpoint for the analogue chemical was read-across and used for the classification of the notified chemical.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	LC50 (96 h) ≥ 2.6 mg/L	Toxic to fish
Daphnia Toxicity	EC50 (48 h) ≥ 3.2 mg/L	Toxic to aquatic invertebrates
Algal Toxicity	EC50 (72 h) = 2.5 mg/L*	Toxic to algae

*Based on the toxicity of an analogue chemical, C_{12-14} alkyl betaine (Madsen et al., 2001).

Under the Globally Harmonised System of Classification and Labelling of Chemicals (United Nations, 2009) the notified chemical is classified as toxic to fish, aquatic invertebrates and algae. Based on the toxicity to aquatic biota the notified chemical is formally classified as "Acute category 2; Toxic to aquatic life". However, as the notified chemical is readily biodegradable and has a predicted log Pow of <4, it is not classified for long term effects.

7.2.1 Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) was calculated using the read-across of algal toxicity EC50 (72 h) of the conservative analogue and an assessment factor of 100, as the endpoints for three trophic levels, including three species of fish, are available.

EC50 (Invertebrates).	2.5	mg/L
Assessment Factor	100	
PNEC:	25	µg/L

7.3. Environmental risk assessment

The risk quotients (Q = PEC/PNEC) are calculated below:

Risk Assessment	***PEC µg/L***	***PNEC µg/L***	***Q***
Q - River:	3.24	25	0.129
Q - Ocean:	0.32	25	0.013

The notified chemical is a zwitterionic surfactant used in cosmetics and cleaning products. As a result of its use pattern, the majority of the total annual import volume is expected to be disposed of to the sewer. In sewage treatment plants the notified chemical is expected to sorb to sludge and degrade. Notified chemical released to surface waters has a low potential to bioaccumulate and is not expected to persist in the environment. As the risk quotient is below 1 for the unmitigated worst case treated effluent discharge scenario, the notified chemical is not expected to pose a risk to the environment on the basis of its reported use pattern and maximum annual importation volume.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

Based on the provided data, the notified chemical is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] with the following risk phrase:
Xi: R36 Irritating to eyes

and

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

	Hazard category	*Hazard statement*
Eye irritation	Category 2A	Causes serious eye irritation
Aquatic environment	Acute Category 2	Toxic to aquatic life

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

When used in the proposed manner at concentrations up to 10%, 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 reported use pattern, the notified chemical is not expected to pose a risk to the environment.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- Safe Work Australia, should consider the following hazard classification for the notified chemical:
  - Xi: R36 Irritating to eyes

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - ≥ 20%: Xi: R36

- The notified chemical is a quarternary ammonium compound included in the SUSMP under Schedule 5 or 6 based on its concentration/preparation. All preparations containing quarternary ammonium compounds at 20% or less are included in Schedule 5 of the SUSMP 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 SUSMP for quarternary ammonium compounds are applicable to the notified chemical.

CONTROL MEASURES
Occupational Health and Safety

- Employers at reformulation plants should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical:
  - Avoid contact with eyes and skin.

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure during handling of the notified chemical as introduced for formulation of products:
  - Safety glasses or face shield
  - Gloves
  - Overalls

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

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

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

Public Health

- The 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 imported into Australia exceeds 32%.

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from a component of cosmetic products at concentrations up to 10% for rinse off products and 5% for leave on products or a component of cleaning products at concentrations up to 10%, 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 products containing the notified chemical provided by the notifier were reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Water Solubility Not determined

Method A certificate stating the water solubility of the notified chemical to be >300 g/L was provided. Details of the test and analytical method were not provided.

Remarks Commercial aqueous solutions of the notified chemical (28-32%, ~800mM) are available, and these solutions are reported to be transparent and, therefore, soluble. However, the notified chemical is a surfactant and will aggregate in solution to form micelles at concentrations above its critical micelle concentration (CMC). The CMC of the notified chemical was not specified but, as the CMCs of other zwitterionic surfactants range from 0.01 to <400 mM (SigmaAldrich), it is likely that the notified chemical is forming micelles in solutions at concentrations of 28 - 32%. Whilst the notified chemical is not likely to be water soluble at high concentrations, it is expected to be water dispersable.

Test Facility Kao Corporation (2010)

Partition Coefficient (n-octanol/water) log K_{OW} ≤1.65

Method Equal volumes of water and n-octanol were stirred (24 h, 22 ± 1°C) to achieve saturation of each phase. The test substance (lauryl sulfobetaine; analogue) was added at a concentration below its critical micelle concentration (CMC) and after 24 h of continuous stirring the phases were separated. The concentration of the test substance in each phase was determined by HPLC with EIMS detection.

Remarks By the method summarised above, the analogue, lauryl sulfobetaine, was determined to have a log Pow of 1.65. Lauryl sulfobetaine is structurally identical to the notified chemical, except that it lacks the hydroxyl group that is present in the polar head of the notified chemical. The hydroxyl group is likely to result in increased water affinity of the notified chemical, and consequently the notified chemical is likely to have a lower log Pow than the analogue chemical. Therefore, the log K_{OW} for the notified chemical is expected to be ≤1.65. The notified chemical is a surfactant and will tend to accumulate at the phase interface of octanol and water.

Test Facility Davies et al. (2004)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Aqueous solution containing 28-32% notified chemical.

METHOD: OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure.
EC Directive 92/69/EEC B.1 bis Acute toxicity (oral) fixed dose method.

Species/Strain	Rat/Wistar Crl: (WI) BR
Vehicle	Water
Remarks - Method	GLP compliant. No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5 per sex	2000	0/10

LD50	> 2000 mg/kg bw for the test substance > 560-640 mg/kg bw for the notified chemical
Signs of Toxicity	There were no deaths. No signs of systemic toxicity were noted.
Effects in Organs	No abnormalities were noted at necroscopy
Remarks - Results	Body weight gains were as expected.

CONCLUSION: The test substance containing 28-32% notified chemical is of low toxicity via the oral route. This is an inconclusive result for the notified chemical as it was tested at < 2000 mg/kg bw and therefore could potentially be harmful.

TEST FACILITY: Centro de Investigacion y Desarrollo Aplicado S.A.L. (1995a).

B.2. Irritation – skin

TEST SUBSTANCE: Aqueous solution containing 28-32% notified chemical.

METHOD: OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 Male
Vehicle	Test substance administered as supplied
Observation Period	72 Hours
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations. GLP compliant.

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	0.3	1	1	1	< 7 days	0
Oedema	0	0	0	0	< 1 hour	0

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

Remarks - Results	A single 4-hour, semi-occluded application of the test material to the intact skin of the 3 rabbits produced very slight erythema at the 24 hour

observation in one animal and at the 24, 48 and 72 hour observations in the other two animals. All treated skin sites appeared normal at the 7 day observation.
No corrosive effects were noted.

CONCLUSION The notified chemical is slightly irritating to the skin at a concentration of 28-32%.

TEST FACILITY Centro de Investigacion y Desarrollo Aplicado S.A.L. (1995b).

B.3. Irritation – eye

TEST SUBSTANCE Aqueous solution containing 28-32% notified chemical.

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 Male
Observation Period	21 Days
Remarks - Method	Conjunctival discharge was not measured. GLP compliant.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	2	2	2	2	< 21 Days	0
Conjunctiva: chemosis	1	1.6	1	2	< 14 Days	0
Corneal opacity	1	1	1	1	21 Days	1
Iridial inflammation	1	1	1	1	< 7 Days	0

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

Remarks - Results A single application of the test material to the non-irrigated eye of three rabbits produced conjunctival irritation, corneal opacity and iridial inflammation in all test animals. One treated eye appeared normal at the 14 day observation with a second eye appearing normal at the 21 day observation. In one treated animal there was still opacity of the cornea present at the 21 day observation.

Although there were effects present at the end of the observation period, they were only present in one of the three treated animals and were of the lowest grade.

CONCLUSION The notified chemical is irritating to the eye at a concentration of 28-32%. The notified chemical may have severe eye irritant effects at 100% concentration.

TEST FACILITY Centro de Investigacion y Desarrollo Aplicado S.A.L. (1995c).

B.4. Skin sensitisation

TEST SUBSTANCE Aqueous solution containing 29% notified chemical.

METHOD Method similar to OECD TG 406 Skin Sensitisation – Guinea Pig

	Maximisation Test
Species/Strain	Guinea pig/Hartley
PRELIMINARY STUDY	No preliminary study conducted
MAIN STUDY	
Number of Animals	Test Group: 15 Control Group: 15
INDUCTION PHASE	Induction Concentration: intradermal: 0.2% aqueous solution of lauryl hydroxysulfobetaine and a 0.4% solution of lauryl hydroxysulfobetaine and Freund's complete adjuvant at a 1:1 ratio. topical: 1%
Signs of Irritation	No signs of irritation were reported.
CHALLENGE PHASE	
1st challenge	topical: 0.03, 0.1 and 0.3%
Remarks - Method	Adequate induction of the test substance could not be confirmed as scores from the induction phase were not reported. No positive control was used to confirm the validity of the study.

RESULTS

Remarks - Results	There were no deaths or substance-related signs of toxicity during the study. There were no signs of irritation noted in any of the test or control animals. As no irritation was reported during the induction phase it is not possible to confirm that the test was valid and therefore no conclusions about the sensitising potential can be drawn.
CONCLUSION	The notified chemical may have skin sensitising ability but the test conditions employed are not sufficiently documented. Therefore, on the basis of inadequate evidence, no conclusion is made.
TEST FACILITY	Hokkaido Pharmaceutical University (1985)

B.5. Genotoxicity – bacteria

TEST SUBSTANCE	Aqueous solution containing 29% notified chemical.
METHOD	Specific Procedure of the mutagenicity test using microorganisms in the Japanese Industrial Safety and Health Act. Test method similar to OECD TG 471 Bacterial Reverse Mutation Test. Pre incubation procedure
Species/Strain	*S. typhimurium*: TA1538, TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	Rat S9 fraction from phenobarbital/5,6-benzoflavone induced rat liver.
Concentration Range in Main Test	a) With metabolic activation: 0.5 – 1000 μg/plate b) Without metabolic activation: 0.05 – 100 μg/plate
Vehicle	Water
Remarks - Method	No preliminary test was reported. No positive control was reported.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				

Test 1	Not applicable	> 50	> 100	negative
Present				
Test 1	Not applicable	> 100	> 1000	negative

Remarks - Results

CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Tochigi Research institute (1985)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical (≥ 20% in aqueous solution)
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test.
Inoculum	Sewage treatment plant microorganisms
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	The chemical oxygen demand (COD) was determined by Hach Reactor Digestion Method, using a HACH DR/2000 spectrophotometer.
Remarks - Method	The test substance was added at a nominal concentration of 10 mg/L to inoculated mineral medium and incubated in the dark over a period of 28 days. A reference control (sodium benzoate, 3 mg/L) and toxicity control (test substance, 10 mg/L, and sodium benzoate, 1.5 mg/L) were run in parallel. Biodegradation was determined by measuring the oxygen depletion and expressed as a percentage of the calculated oxygen demand (COD: 4.3 mg/L).

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
6	6	6	79
15	61	15	92
21	70	21	93
28	78	28	93

Remarks - Results	As the percentage degradation of the reference compound surpassed the pass levels of 60% by 14 days, and the other validity criteria for these guidelines were met, the test is considered valid. The toxicity control attained 90% degradation after 28 days, confirming that the test substance is not inhibitory to the sewage treatment organisms used in the study. Analysis indicated that there was no oxygen depletion due to nitrification. The test substance reached the pass level of 60% degradation within a ten day window, and is therefore considered to be readily biodegradable.
CONCLUSION	The test substance and, by inference, the notified chemical is readily biodegradable
TEST FACILITY	SafePharm Laboratories (1995a)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Notified chemical (≥ 20% in aqueous solution)

METHOD
- Species: Zebra fish (*Brachydanio rerio*)
- Exposure Period: 96 hours
- Auxiliary Solvent: None
- Water Hardness: 195.8 – 231.4 mg $CaCO_3$/L
- Analytical Monitoring: None
- Remarks – Method: After a range finding test, a definitive test using nominal concentrations ranging from 100 – 400 mg test substance/L was conducted in accordance with the guidelines above. The test chambers were maintained under static conditions at 21-26°C, pH 8.1-8.5 and 65-90% dissolved oxygen. The fish were observed for mortality and sub-lethal effects over a period of 4 days. Statistical evaluation was conducted by graphic estimation.

RESULTS

Nominal Concentration mg test substance/L	*Number of Fish*	*Mortality*				
		3 h	*24 h*	*48 h*	*72 h*	*96 h*
0	20	0	0	0	0	0
100	20	0	0	0	0	0
141.4	10	0	0	0	0	0
168.2	20	0	0	0	0	0
200	20	0	14	3	2	0
400	10	10	-	-	-	-

- LC50: 184 mg test substance/L at 96 hours.
  ≥ 36.8 mg notified chemical/L at 96 hours.
- NOEC: Not reported
- Remarks – Results: The test substance was an aqueous solution containing the notified chemical (≥ 20%), therefore, the results for the test substance have been corrected to reflect the endpoint of the notified chemical.

  There was no observed sub-lethal effects or mortality of fish in the control thus validating the test. Whilst the temperature range variation was greater than 2°C over the course of the test, this is not expected to affect the outcome of the test. Sub-lethal effects, including inactivity, cessation of swimming, rapid respiration, flaccidity and surfacing and sounding in the aquarium, were observed in the fish exposed to the test substance at concentration of 100 and 168.2 mg/L.

CONCLUSION: The notified chemical is harmful to fish.

TEST FACILITY: Centro de Investigacion y Desarrollo Aplicado S.A.L. (1996)

C.2.2. Acute toxicity to fish

TEST SUBSTANCE: Notified chemical (≥ 20% in aqueous solution)

METHOD
- Species: Rainbow trout (*Oncorhynchus mykiss*)
- Exposure Period: 96 hours
- Auxiliary Solvent: None
- Water Hardness: ~100 mg $CaCO_3$/L
- Analytical Monitoring: Not reported

Remarks – Method	Following a preliminary range-finding test, fish were exposed, in groups of ten, to an aqueous dispersion of the test substance, in accordance with the guidelines above. The number of mortalities and adverse reactions were observed over the duration of the test. Test conditions were: 14 ± 1°C, pH 7.6-8.1, 9.7-10.3 mg O_2/L, 16 h/8 h light dark cycle. The data was statistically evaluated by the moving average method of Thompson (1947).

RESULTS

Nominal Concentration mg test substance/L	*Number of Fish*	*Mortality*				
		3 h	*24 h*	*48 h*	*72 h*	*96 h*
0	10	0	0	0	0	0
1.0	10	0	0	0	0	0
1.8	10	0	0	0	0	0
3.2	10	0	0	0	0	0
5.6	10	0	0	0	0	0
10	10	0	0	0	0	0
18	10	0	0	10	-	-

LC50	13 mg test substance/L at 96 hours (95% CI: 10–18 mg/L) ≥ 2.6 mg notified chemical/L at 96 hours
NOEC	1.8 mg test substance/L at 96 hours ≥ 0.4 mg/L at 96 hours
Remarks – Results	The test substance was an aqueous solution containing the notified chemical (≥ 20%), therefore, the results for the test substance have been corrected to reflect the endpoints of the notified chemical. There was no mortality observed in the control over the duration of the study, thereby validating the test. Sublethal effects, including increased pigmentation, loss of equilibrium and the presence of moribund fish, were observed in the fish exposed to the test substance at concentrations of 3.2 to 10 mg/L.
CONCLUSION	The notified chemical is toxic to fish
TEST FACILITY	SafePharm Laboratories (1996)

C.2.3. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical (≥ 20% active matter in aqueous solution)
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-static
Species	Red killifish (*Oryzias latipes*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	Not reported
Remarks – Method	Study summary provided only. The study was conducted according to the guidelines above, with groups of 8 fish exposed to the solution of test substance. The test media was exchanges at 48 hours after the exposure initiated. Deviations from protocol and statistical methods were not reported.

RESULTS

Nominal Concentration mg test substance/L	*Number of Fish*	*Mortality*				
		3 h	*24 h*	*48 h*	*72 h*	*96 h*
32.1	8	0	0	0	0	0
38.6	8	0	1	1	1	1
46.2	8	0	0	2	2	3
55.7	8	0	8	-	-	-
66.6	8	0	8	-	-	-
80.0	8	2	8	-	-	-

LC50	46 mg test substance/L at 96 hours (95% CI: 42.1–50.1 mg/L) ≥ 9.2 mg active matter/L at 96 hours.
NOEC (or LOEC)	Not reported
Remarks – Results	The test was conducted on an aqueous solution containing ≥ 20% active matter and, therefore, the results for the test substance have been corrected to reflect the endpoint of the active matter. The test cannot be considered valid as the use of a control, and the mortality of fish in the control, was not reported.
CONCLUSION	The test was not demonstrated to be valid and, therefore, these results have not been used to classify the notified chemical.
TEST FACILITY	Kao Corporation (1998)

C.2.4. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical (≥ 20% in aqueous solution)
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test - Static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	270 mg $CaCO_3$/L
Analytical Monitoring	Not reported
Remarks - Method	Study summary provided only. The study was conducted according to the guidelines above, with no amendments to protocol reported. Test conditions were: 21.0°C, pH 7.8 ± 0.2. Statistical analysis was conducted according to the method of Thompsen (1947).

RESULTS

Concentration mg/L Nominal	*Number of D. magna*	*Number Immobilised*	
		24 h	*48 h*
0	2 × 10	0	0
0.1	2 × 10	0	0
0.6	2 × 10	0	0
1.0	2 × 10	0	0
3.0	2 × 10	0	0
6.0	2 × 10	0	0
10	2 × 10	0	10
30	2 × 10	0	14

EC50	16 mg test substance/L at 48 hours (95% CI: 12–22 mg/L) ≥ 3.2 mg notified chemical/L at 48 hours
NOEC	6.0 mg test substance/L at 48 hours ≥ 1.2 mg notified chemical/L at 48 hours
Remarks - Results	The test substance was an aqueous solution containing the notified chemical (≥ 20%), therefore, the results for the test substance have been

	corrected to reflect the endpoints of the notified chemical. There was no immobilisation observed in daphnids in the control, thereby validating the test.
CONCLUSION	The notified chemical is toxic to aquatic invertebrates
TEST FACILITY	SafePharm Laboratories (1995b)

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