File No: STD/1354 and STD/1355

June 2010

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

STD/1354: Glycine, N-coco acyl derivs., potassium salts (INCI name: Potassium cocoyl glycinate) STD/1355: Fatty acids, coco, reaction products with glycine, potassium salts (INCI name: Potassium cocoyl glycinate)

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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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
STD/1354: Standard (Reduced fee notification): Chemical other than polymer (more than 1 tonne per year) – Similar to a chemical that has been previously assessed by NICNAS

STD/1355: Standard (Reduced fee notification): Chemical other than polymer (more than 1 tonne per year) – Chemical is being notified at the same time as a chemical which is similar

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, Density, Water Solubility, Hydrolysis as a Function of pH, Partition Coefficient, Adsorption/Desorption, Dissociation Constant, Particle Size, Flash Point, Flammability, Autoignition Temperature, Explosive Properties, Acute dermal toxicity, Acute inhalation toxicity, Repeat dose toxicity, In vitro genotoxicity, In vivo genotoxicity.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
STD/1354: Amilite GCK-12K, Amilite GCK-12, Amilite GCK-11 (each containing 26% notified chemical)
STD/1355: Amilite GCK-12H (containing 12% notified chemical)

CAS NUMBER
STD/1354: 301341-58-2
STD/1355: 1170699-53-2

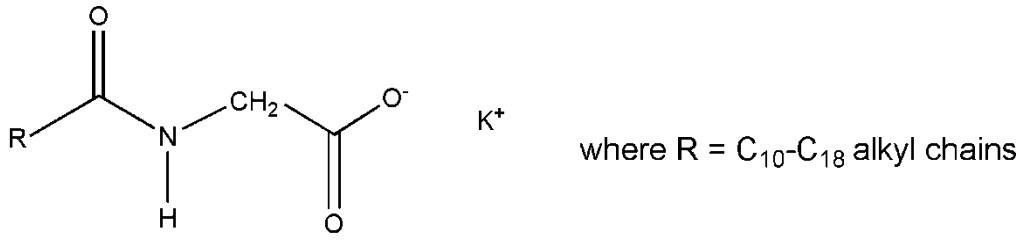
CHEMICAL NAME
STD/1354: Glycine, N-coco acyl derivs., potassium salts
STD/1355: Fatty acids, coco, reaction products with glycine, potassium salts

OTHER NAME(S)
STD/1354: Potassium cocoyl glycinate (International Nomenclature of Cosmetic Ingredients (INCI) name)
STD/1355: Potassium cocoyl glycinate (INCI name)

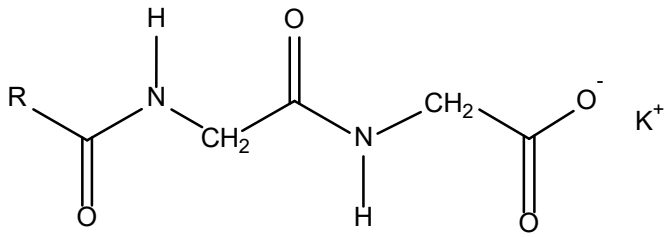
MOLECULAR FORMULA
STD/1354:
$C_{14}H_{26}O_3NK$ (as lauroyl derivative)
The notified chemical is a mixture of glycine N-acyl derivatives of fatty acids from coconut oil. The main component (47%) represents the derivative of lauric acid.

STD/1355:
$C_{14}H_{26}O_3NK$ and $C_{16}H_{29}O_4N_2K$ (based on lauroyl derivatives)
The notified chemical is a reaction product of fatty acids from coconut oil with glycine. The main component (47%) represents the derivative of lauric acid.

STRUCTURAL FORMULA
STD/1354:



STD/1355:
Approximately 80% has the structure shown for STD/1354, together with 20% of a compound of the following typical structure:



For both STD/1354 and STD/1355, the component derivatives in the cocoyl mixture (from coconut oil) are as follows:
47% lauroyl derivatives C12
18% myristoyl derivatives C14
9% palmitoyl derivatives C16
6% capryloyl derivatives C10
6% oleoyl derivatives C18
2% linoleoyl derivatives C18
3% stearoyl derivatives C18

NOTE: The notified chemicals are produced using similar starting materials though with different reaction conditions.

MOLECULAR WEIGHT
STD/1354:
267 – 379 Da
295 Da (as lauroyl derivative)

STD/1355:
267 – 436 Da
295 and 352 Da (as lauroyl derivatives)

ANALYTICAL DATA
Reference NMR, IR, HPLC, GC, GPC, UV spectra were provided.
STD/1354: Reference IR spectra were provided. Major peaks observed at 3310, 2920, 2850, 1550 and 1410 cm^{-1}

3. COMPOSITION

DEGREE OF PURITY STD/1354: 84%
STD/1355: 50%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (≥1% by weight)

Chemical Name	Potassium sulfate		
CAS No.	7778-80-5	*Weight %*	2 (STD/1354)
Chemical Name	Potassium chloride		
CAS No.	7447-40-7	*Weight %*	1 (STD/1354)
Chemical Name	Fatty acids, coco, potassium salts		
CAS No.	61789-30-8	*Weight %*	13 (STD/1354) 37 (STD/1355)
Chemical Name	Glycine		
CAS No.	56-40-6	*Weight %*	10 (STD/1355)
Chemical Name	Glycine, glycyl-		
CAS No.	556-50-3	*Weight %*	3 (STD/1355)

ADDITIVES/ADJUVANTS None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: White to light yellow solid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	Not determined	Imported as a mixture in water.
Boiling Point	Not determined	Imported as a mixture in water.
Density	Not determined	Imported as a mixture in water.
Vapour Pressure	$< 10^{-5}$ kPa	Estimated.
Water Solubility	>300 g/L at 20°C	Measured (visual observation)
Hydrolysis as a Function of pH	Not determined	Contains hydrolysable functionality, however, hydrolysis is expected to be slow in the environmental pH range (4–9) at ambient temperature. Hydrolytic stability in cosmetic formulations is a functional requirement.
Partition Coefficient (n-octanol/water)	log Pow = 0.158 – 3.89	Calculated using KOWWIN (v1.67) (US EPA, 2009) for the fully ionised and unionised forms of potassium lauryl glycinate. The notified chemicals are surfactants and are expected to concentrate at phase boundaries.
Adsorption/Desorption	log K_{oc} = 0.219 – 2.282	Calculated using the Kow method KOCWIN (v2.00) (US EPA, 2009) for the fully ionised and unionised forms of potassium lauryl glycinate. The notified chemicals are expected to adsorb to organic carbon soil and sediment because they are surfactants.
Dissociation Constant	Not determined	The notified chemicals are ionised in the environmental pH range (4–9)

Particle Size	Not determined	Imported as a mixture in water.
Flash Point	Not determined	Imported as a mixture in water.
Flammability	Not determined	Notified chemical is a solid.
Autoignition Temperature	Not determined	Not expected to autoignite.
Explosive Properties	Not determined	Not expected to be explosive based on absence of structural alerts for explosivity.

DISCUSSION OF PROPERTIES
The notified chemical of STD/1355 differs to the notified chemical of STD/1354 as the former contains up to 20% potassium cocoyl glycylglycinate in addition to the glycinate homologue series found in STD/1354. The glycylglycinate derivatives are deemed closely similar to the glycinate series as they are chemically comparable with common functional groups, have an identical structure-activity profile (OECD Toolbox; OECD, 2009), and have common precursors and breakdown products. In addition, the physico-chemical properties of the lauroyl derivatives are not significantly different: the predicted partition coefficients (log Pow) of potassium lauroyl glycinate and potassium lauroyl glycylglycinate are 0.158 and -0.671 respectively (KOWWIN (v1.67); US EPA, 2009), and the adsorption coefficients (log K_{oc}) are predicted to be 0.219 and -0.266, respectively (Kow method, KOCWIN (v2.00); US EPA, 2009). Thus, the notified chemical of STD/1354 is deemed to be an acceptable analogue for the notified chemical of STD/1355.

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

Reactivity
Expected to be stable under normal environmental and usage conditions. The CIR compendium and report (CIR 2001, 2004) raised concerns about the possible formation of potentially carcinogenic nitrosated derivatives of the analogue chemicals (acyl sarcosines) for which the precursor amine sarcosine is a secondary amine. Secondary amines are of more concern for nitrosamine formation than primary or tertiary amines. The nitrogen in the notified chemicals are secondary, however their functional group is an amide rather than amine. Therefore the possibility of nitrosamine formation in the notified chemicals is considered to be low.

Dangerous Goods classification
Based on the physical-chemical data in the above table the notified chemicals are not classified according to the Australian Dangerous Goods Code (NTC, 2007). However the data above does not address all Dangerous Goods endpoints. Therefore consideration of all endpoints should be undertaken before a final decision on the Dangerous Goods classification is made by the introducer of the chemicals.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemicals will be imported as ingredients in finished cosmetic products or at higher concentrations for local blending into cosmetic products.

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

	Year	*1*	*2*	*3*	*4*	*5*
STD/1354	*Tonnes*	10	15	15	15	15
STD/1355	*Tonnes*	5	7	10	10	10

PORT OF ENTRY
Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
Unilever Australia Ltd

TRANSPORTATION AND PACKAGING
The cosmetic products containing the notified chemicals will be imported in 200mL bottles or 200g tubes packaged in cardboard cartons. From the wharf they will be transported by road to a warehouse for storage, then to the central distribution centres of the principal retailers and subsequently to retail chains. When imported for formulation of cosmetics, the notified chemicals are expected to be contained within 15 kg drums.

USE
The notified chemicals will be used as components of finished cosmetic rinse off and leave on products. Typical rinse off products include cleansing products for skin and hair, whilst typical leave on products include skin lotions and creams.
For STD/1354, the notified chemical will be present at up to 15% in rinse off products and up to 5% in leave on products.
For STD/1355, the notified chemical will be present at up to 10% in rinse off products and up to 5% in leave on products.

OPERATION DESCRIPTION
During formulation of cosmetics, the notified chemicals will be quality control tested, subsequently manually weighed into a container and transferred into a mixing vessel where they will be blended with other ingredients whilst closed. The resulting blend (containing the notified chemicals at concentrations up to 15%) will then undergo further quality testing. The finished product will then be filled into retail containers using an automated filling machine.

The finished products containing the notified chemicals (either imported or formulated in Australia) will be used by consumers and professionals such as hairdressers or workers in beauty salons. Depending on the nature of the product these could be applied a number of ways such as by hand, using an applicator or sprayed.

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
QC personnel	1	3	12
Reformulation workers	1	8	12
Packaging workers	2	8	12
Store persons	2	4	12

EXPOSURE DETAILS
Reformulation
Dermal, ocular and inhalation (aerosol) exposure of workers to the notified chemicals as imported may occur during opening of the import containers, weighing and transferring the notified chemicals into a mixing vessel, and connecting and disconnecting transfer and filling lines. Dermal, ocular and inhalation exposure may also occur to concentrations of up to 15% of the notified chemicals during quality control operations, and dispensing of the reformulated product into end use containers. Exposure is expected to be lowered by the enclosed nature of the mixing vessel, the automated systems used for mixing and dispensing, the use of exhaust hoods, and the wearing of personal protective equipment (PPE), that may include overalls, face-mask or safety glasses, safety shoes, gloves and respiratory protection (if ventilation is inadequate).

End-Use
Dermal, ocular, and inhalation exposure to the notified chemicals (concentrations up to 15%) may occur in professions (e.g. hair dressers, workers in beauty salons) where the services provided involve the application of personal care products. Such professionals may use some personal protective equipment to minimise exposure, and good hygiene practices are expected to be in place. As such, exposure of these professionals is expected to be of either a similar or higher level than that experienced by consumers using products containing the notified chemicals.

6.1.2. Public exposure
Public exposure to the notified chemicals is expected to be widespread and frequent through daily use of personal care products containing the notified chemicals at concentrations up to 15%. The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible, particularly if products are applied by spray. Exposure to the notified chemicals will vary depending on individual use patterns. Data on

typical use patterns of a number of product categories in which the notified chemicals are proposed to be used are shown below (European Commission 2003, SCCP 2006, Loretz et al 2008). For the purposes of the exposure assessment, Australian use patterns for the various product categories are assumed to be similar to those in Europe.

Default dermal absorption of 100% was assumed for calculation purposes (European Commission, 2003). The actual level of dermal absorption may be lower than 100%. The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the below table. An adult bodyweight of 60kg has been used for calculation purposes.

Product type	mg/event	events/day	**RF**	**Daily exposure** based on 100% concentration (mg/day)
Leave on				
Body lotion	8000	1	1	8000
Face cream	1540	2	1	3080
General purpose cream	1200	2	1	2400
Leave on - total				**13480**
Rinse off				
Bath products	17000	0.29	0.001	4.93
Facial cleansers	4060	1-2 (1 used for calcs)	0.01	40.6
Facial masks	3700	0.1	0.1	37
Make up remover	2500	1	0.1	250
Shower gel	5000	1.07	0.01	53.5
Shampoo	10460	1	0.01	104.6
Hair conditioner	14000	0.28	0.01	39.2
Rinse off - total				**529.83**

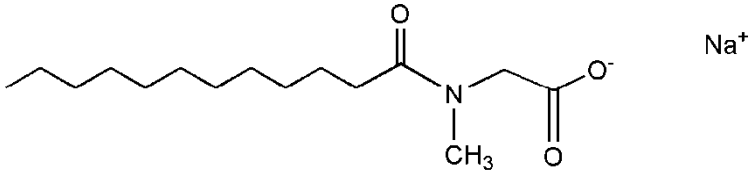
STD/1354:
Total exposure to the notified chemical at up to 15% in rinse off products and up to 5% in leave on products is 12.56 mg/kg bw/day.

STD/1355:
Total exposure to the notified chemical at up to 10% in rinse off products and up to 5% in leave on products is 12.12 mg/kg bw/day.

6.2. Human health effects assessment

The results from toxicological investigations conducted on the notified chemicals, and in some cases, an analogue chemical, which is the sodium salt of that assessed in STD/1354, are summarised in the table below. Details of these studies can be found in Appendix B.

In addition, some published information from the Cosmetic Ingredient Review (CIR) on modified fatty acids known as acyl sarcosines and sarcosinates that are structurally related to the notified chemicals is included in the health effects assessment, eg. Sodium Lauroyl Sarcosinate:



Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity[1]	Low toxicity LD50 >2000 mg/kg bw

Rat, acute oral toxicity[2]	Low toxicity LD50 >2000 mg/kg bw
Rabbit, skin irritation[1]	Slightly irritating at 5% concentration
Rabbit, skin irritation[2]	Irritating at tested concentration (assumed to be up to 30%)
Rabbit, eye irritation[1]	Irritating at 5% concentration
Rabbit, eye irritation[2]	Irritating at tested concentration
Guinea pig, skin sensitisation – adjuvant test[1]	no evidence of sensitisation up to 2.5% concentration
Guinea pig, skin sensitisation – adjuvant test[2]	no evidence of sensitisation up to 2.5% concentration
Mutagenicity – bacterial reverse mutation[1]	non mutagenic
Mutagenicity – bacterial reverse mutation[2]	non mutagenic
Genotoxicity – in vitro chromosome aberration test in hamster lung fibroblasts (CHL/IU)[3]	genotoxic in the presence of metabolic activation
Genotoxicity – in vivo mammalian erythrocyte micronucleus test[3]	non genotoxic

[1]Study conducted on notified chemical (STD/1354)
[2]Study conducted on notified chemical (STD/1355)
[3]Study conducted on analogue chemical (glycine, N-coco acyl derivs., sodium salts; CAS number 90387-74-9)

Toxicokinetics, metabolism and distribution

No information was provided on the notified chemicals. N-acyl derivatives of sarcosine (acyl sarcosines) and their salts (sarcosinates) are structurally similar to the notified chemicals and are also used as surfactant-cleansing agents in cosmetic products. A skin permeability test on rats revealed that acyl sarcosines and sarcosinates enhanced the skin absorption of other ingredients when applied together in the same formulation (CIR 2001). Due to this finding, cosmetic products containing the notified chemicals should be carefully formulated to avoid combining with other ingredients (including colourants and dyes) if transdermal absorption is a health concern. The structurally related chemical, Sodium Lauroyl Sarcosinate, is reported as not being hydrolysable by either gastric or intestinal enzymes *in vitro*. In a metabolism study in rats, 82%-89% of a 50 mg/kg oral dose of Sodium Lauroyl Sarcosinate was excreted in the urine and faeces within 24 hours, and 1%-2% was excreted over the next 24 hours (CIR 2001), suggesting that it is not readily absorbed through the gastrointestinal wall. In an oral dosing study in rats, radiolabelled Sodium Lauroyl Sarcosinate was administered and tissue samples (including urine and faeces) were analysed. At 24 hours after administration, 42% was present in the urine and less than 2% were found in organs such as the liver, kidneys, teeth and oral mucosa. Around 1% of the compound remained adhered to the teeth, oral mucosa and tongue and the radioactivity could not be washed out by physiological saline, indicating that Sodium Lauroyl Sarcosinate was absorbed into the blood. However, the uptake is not permanent according to a different study, which found that frequent application did not cause an accumulation of radiolabelled sarcosinate in bone or muscle (CIR 2001). The notified chemicals are likely to have similar absorption, metabolism and elimination kinetics to sarcosinates and are not likely to lead to bioaccumulation.

Acute toxicity

The oral LD_{50} of Amilite GCK-12 and Amilite GCK-12H was determined to be over 2000 mg/kg bw in tests conducted in rats. Based on this data, the notified chemicals are considered to be of low toxicity via the oral route.

No data was provided on the acute dermal toxicity of the notified chemicals. A study involving dermal application of Sodium Lauroyl Sarcosinate on the skin of rabbits for 14 days was reported to result in no signs of dermal toxicity in any animals. This further suggests that the notified chemicals are expected to be of low acute dermal toxicity.

The acute inhalation toxicity or potential for respiratory irritation of the notified chemicals is unknown. In addition, there is no data available on the inhalation toxicity of acyl sarcosines or sarcosinates.

Skin irritation

The notified chemical (STD/1354) caused slight irritation in the skin of rabbits when tested at 5%. These symptoms had resolved within one week, though all animals showed obvious scaling at day 7 (Ajinomoto Co Inc 1998a). Considering the effects at this tested concentration, the notified chemical at higher concentrations should be classified as at least a skin irritant. This is further supported by testing on the second notified chemical (STD/1355) at concentrations of 30% or lower (exact concentration uncertain). The study report suggested that scaling persisted in 2 of the 3 animals at the final 14 day observation. In the other animal, high severity erythema/eschar formation (grade 4) remained at 14 days (Ajinomoto Co Inc 2008b). Thus at the tested concentration, the notified chemical is irritating to the skin. At higher concentrations, the notified chemical is

expected to be at least a skin irritant, and may be corrosive at neat concentrations. Based on the results of these two studies, both of the notified chemicals are considered to be classified as irritating to the skin.

Eye irritation

In an eye irritation test in rabbits, 5% notified chemical (STD/1354) caused iridial inflammation, corneal opacity and signs of conjunctival irritation in all animals tested. Four out of 6 animals continued to show conjunctival redness (score 1) at the day 7 observation (Ajinomoto Co Inc 1998b). Considering the irritant effects observed at 5% concentration, the notified chemical at higher concentrations should be classified as a severe eye irritant. An eye irritation study was also provided for the other notified chemical (STD/1355) at concentrations of 30% or lower (exact concentration uncertain). In this study, significant eye irritation was observed, particularly conjunctival redness. Based on the severity of the effects, the study authors decided to test only one animal. Considering the effects observed at the tested concentration, the notified chemical at higher concentrations should be classified as a severe eye irritant (Ajinomoto Co Inc 2008c). Based on the results of these two studies, both of the notified chemicals are classified as severe eye irritants.

Sensitisation

The notified chemical (STD/1354) did not produce a reaction in a guinea pig maximisation test (Ajinomoto Co Inc 2004) when tested up to the maximum non-irritating concentration of 2.5%, and is therefore not considered to be a skin sensitiser. This is further supported by the results of an earlier guinea pig maximisation test on the notified chemical (STD/1354) (results not shown in Appendix B) (Ajinomoto Co Inc 1994) and a guinea pig maximisation test using the notified chemical (STD/1355) (Bozo Research Center Inc 2008a).

Subchronic and chronic toxicity

No information on repeat dose toxicity was available for the notified chemicals. The Cosmetic Ingredient Review reports that weanling rats given a diet containing 2% Sodium Lauroyl Sarcosinate for 6 months had no effect on weight gain, feeding, general health or behaviour. There were no abnormalities of the internal organs. Rats fed 0.5% Sodium Lauroyl Sarcosinate for 100 days also showed no signs of toxicity. In a chronic toxicity study, 200 albino Wistar rats were fed Sodium Lauroyl Sarcosinate ranging from 0.05% to 2.0% for a period of 2 years. There were no significant differences in lesions, fertility, mortality, haematology or body weight gain between the control and treated groups. The only significant change after 24 months was minor hyperplasia of the stratified squamous epithelium and excess keratin formation in the stomach mucosa of rats treated at the highest doses (1% and 2%) (CIR 2001). It is expected that the notified chemicals may have similar repeat dose toxicity to that described above for Sodium Lauroyl Sarcosinate.

Reproductive Effects

Information on Sodium Lauroyl Sarcosinate indicated that rats fed up to 1000 mg/kg/day did not experience adverse effects on fertility in a 2-year oral toxicity study (CIR 2001).

Mutagenicity

The notified chemicals were not mutagenic to bacteria in the presence or absence of metabolic activation in two separate Ames tests (BML Inc 1994, Bozo Research Center Inc 2008b).

No further information was provided on the genotoxicity of the notified chemicals. However, an analogue chemical, glycine, N-coco acyl derivs., sodium salts (CAS number 90387-74-9), has been tested for genotoxicity. It was found to not be clastogenic in a Chromosomal Aberration test using mammalian lung fibroblasts in the absence of metabolic activation but it increased the percentage of cells with aberrations in the presence of metabolic activation at the highest concentration tested. Based on this result the analogue chemical is considered to be clastogenic to mammalian cells in vitro in the presence of metabolic activation. However, the significance of the positive result is unclear as the increase of aberrations was only observed at the highest concentration and there was no repeat of the experiment at the selected or other concentrations of the analogue chemical. The analogue chemical was found to be non clastogenic in an in vivo micronucleus test in mice. Some cytotoxic effect was observed as determined by the decrease of the number of immature erythroblasts, indicating that the analogue chemical had reached the bone marrow. Due to cytotoxicity of the analogue chemical the test concentrations for all genotoxicity studies were low. The cytotoxicity of the analogue chemical is most likely due to the surfactant characteristics and interference with the cell membrane. Thus the analogue chemical does not appear to exhibit clastogenicity in vivo. Based on the available data, the analogue chemical is not considered mutagenic.

There was also some data available on the genotoxicity of Sodium Lauroyl Sarcosinate. In a comet assay using V79 Chinese hamster cells and human white blood cells it did not induce double-strand DNA breaks, though it

was cytotoxic (CIR 2001).

In summary, based on the available data on the notified chemicals and two structurally related chemicals, the notified chemicals are not considered to be genotoxic.

Carcinogenicity
No carcinogenicity data was available for the notified chemicals. The potential for formation of carcinogenic nitrosamines in formulations containing the notified chemicals was not expected to be significant due to the presence of amide functional groups in the notified chemicals rather than amines. Therefore the possibility of carcinogenicity due to nitrosamine formation is low.

Health hazard classification
Based on the results of the eye irritation and skin irritation tests conducted on the notified chemicals at concentrations of 5% or up to 30%, the notified chemicals are classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrases:

R38 Irritating to skin
R41 Risk of serious damage to eyes

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
Based on the available data, adverse effects associated with exposure to the notified chemicals may include eye and skin irritation. Exposure of workers to the notified chemicals may occur during reformulation processes (dermal, ocular, or inhalation).

Upon dermal contact with the notified chemicals irritation may occur and the severity of this is likely to be dependent on the concentration. Ocular contact with the notified chemicals at concentrations above 5% may cause significant eye irritation Appropriate use of exhaust hoods, automated systems and personal protective equipment, particularly safety glasses or face masks, overalls, impervious gloves, and safety shoes during reformulation operations is expected to reduce exposure levels to the notified chemicals and hence lower the incidence of irritation effects.

Overall, the notified chemicals are not considered to pose an unacceptable risk to cosmetic production workers, given the use of automated systems and personal protective equipment. Appropriate control measures to minimise dermal and ocular exposure are required to protect workers from irritation effects at higher concentrations.

The risk for beauty care professionals who regularly use products containing the notified chemicals (up to 15%) is expected to be of a similar or perhaps higher level than that experienced by members of the public who use such products on a regular basis, in light of the duration of exposure. Skin irritation effects from formulated products containing up to 15% of the notified chemicals are not expected. However, it is noted that accidental eye contact of beauty care professionals using such products is expected to occur less frequently than that of members of the public.

6.3.2. Public health
The public will have widespread dermal exposure to the notified chemicals, which are proposed to be used at a level of up to 15% in rinse off and 5% in leave on cosmetic products. Eye exposure is also a possibility due to accidental contact.

Eye contact with the notified chemicals in rinse off products at concentrations of up to 10-15% may lead to serious eye damage. If the product were diluted with water when eye contact occurs, eye irritation may still occur, though the dilution and reduced contact time generally associated with use of rinse off products is expected to minimise this possibility.

When used in leave on products at concentrations up to 5%, the potential for eye irritation still exists. However, intentional ocular exposure is not expected, and rinsing of the eyes is recommended in the event of accidental exposure.

When using leave-on products, some skin irritation may occur, but is expected to be limited by the low

proposed concentrations (up to 5%). Significant skin irritation effects are also not expected when rinse off products containing the notified chemicals (up to 15%) are used due to dilution and the reduced skin contact time.

Though information was not available on the effects of long term repeated exposure to the notified chemicals, information on sodium lauroyl sarcosinate (2-year rat oral toxicity study resulting in no adverse effects on fertility at feeding dose of 1000 mg/kg/day) suggested that the notified chemicals are likely to be of low repeated dose toxicity. Thus it was not considered necessary to calculate the margin of exposure for repeated exposure to the notified chemicals.

In summary, use of products containing the notified chemicals at concentrations up to 15% may lead to eye irritation. The risk is not expected to be significant when the notified chemicals are present in rinse off products (up to 15%) due to the dilution and reduced skin/eye contact time. In the previous NICNAS assessment of the notified chemical for STD/1354, it was assessed for use in rinse off cosmetic products at concentrations up to 23%. In addition, the risk of irritation effects due to the notified chemicals in leave on products (up to 5%) is expected to be limited by the relatively low concentrations at which they are present. The eye and any possible skin irritation risk associated with use of the notified chemicals in cosmetic products may be further minimised by the inclusion of appropriate labelling and directions for use to warn against eye contact and of the possibility of skin irritation reactions. Packaging directions, should recommend that use be discontinued if irritation occurs. When used in the proposed manner, with appropriate safety information on the packaging, the risk to the public associated with eye and skin contact with the notified chemicals at the proposed concentrations is not considered to be unacceptable.

In addition, the risk associated with repeated exposure to the notified chemicals is not considered to be unacceptable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemicals will be imported as components of finished cosmetic products and will also be imported as raw materials in aqueous solutions for blending. The notified chemicals are expected to be released to landfill as residue remaining in containers (estimated to be up to 1% of the annual import volumes) and released to sewer from the cleaning of blending equipment (3%).

Accidental spills during transport or reformulation are expected to involve minimal amounts of the notified chemicals and will be collected with inert material and disposed of to landfill.

RELEASE OF CHEMICAL FROM USE

The majority of the notified chemicals are expected to be washed to sewer as a result of their use pattern (as rinse-off and leave-on cosmetic products).

RELEASE OF CHEMICAL FROM DISPOSAL

Residue of the notified chemicals in empty containers (1%) will share the fate of the container and will either be disposed of to landfill, or washed to sewer when containers are rinsed before recycling. Waste and expired material is expected to be disposed of to landfill.

7.1.2 Environmental fate

The notified chemicals are readily biodegradable and are expected to be largely degraded by sewage treatment processes. Approximately 33% of the total annual import of the notified chemicals (calculated by SimpleTreat; European Commission, 2003) may be discharged to receiving waters in treated effluent as the notified chemicals are water soluble, yet the notified chemicals are expected to disperse and degrade. Bioaccumulation is not likely as the notified chemicals are water soluble and readily biodegradable. In landfill the notified chemicals are expected to biodegrade, and will degrade biotically or abiotically to form water, oxides of carbon and nitrogen, and inorganic salts. 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 chemicals in STD/1354 and STD/1355 to receiving waters via sewage treatment works nationwide.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	25,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	25,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	68.49	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:	16.18	µg/L
PEC - Ocean:	1.62	µg/L

The notified chemicals were found to be readily biodegradable, thus, their removal from influent by sewage treatment plant (STP) processes is expected. A mitigated PEC is presented below, based on the same assumptions as above and taking into account degradation of up to 67% in STPs, as calculated by the SimpleTreat Model (European Commission, 2003):

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	25,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	25,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	68.49	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	21.161	million
Removal within STP	67%	**Mitigation**
Daily effluent production:	4,232	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	5.34	µg/L
PEC - Ocean:	0.53	µg/L

The SimpleTreat Model estimates that 33% of the notified chemicals may remain in the effluent after STP processes, however the SimpleTreat Model may overestimate environmental concentrations for water soluble and highly adsorptive substances (European Commission, 2003). Thus, it is possible that the environmental concentration of the notified chemicals may be even lower than calculated.

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 chemicals in this volume are 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 5.341 µg/L may potentially result in a soil concentration of approximately 35.60 µg/kg. Assuming accumulation of the notified chemicals in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemicals in the applied soil in 5 and 10 years may be approximately 178.0 µg/kg and 356.0 µg/kg, respectively. However, given the expected rapid degradation and the adsorptive nature of the notified chemicals, 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 (STD/1354) for daphnia and algal toxicity are summarised in the table below. A modelled estimate (ECOSAR (v1.00), surfactants, anionic; US EPA, 2009) for the fish toxicity of the notified chemicals is also included. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	LC50 (96 h) = 2.59 mg/L	Predicted to be toxic to fish
Daphnia Toxicity	EC50 (48 h) >80 mg/L	Not toxic to aquatic invertebrates
Algal Toxicity	E_rC50 (72 h) =16.3 mg/L	Harmful to algae

The anionic surfactant ECOSAR estimate is derived from the carbon chain length of the hydrophobic component of anionic surfactants, in this case using the chain length of the most abundant component of the notified chemicals (i.e. the lauroyl derivatives, C12) to estimate the endpoint for fish toxicity. The ECOSAR calculation was used since the method has been validated for fish and produces a conservative endpoint which is similar in magnitude to the measured fish test data for comparable classes of surfactants. Further discussion about the use of QSARs for estimating the fish toxicity of anionic surfactants can be found in Appendix C.
The notified chemical of STD/1354 is deemed to be an acceptable analogue for the notified chemical of STD/1355, hence the results from the ecological investigations on the former are suitable for the environmental assessment and classification of the notified chemical of STD/1355. Under the Globally Harmonised System of Classification and Labelling of Chemicals (United Nations, 2009) the notified chemicals are toxic to fish, harmful to algae and not toxic to aquatic invertebrates.

7.2.1 Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) has been calculated from the estimated fish toxicity of the notified chemicals using an assessment factor of 100, as experimental endpoints for two trophic levels and a conservative estimated endpoint for a third trophic level are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50 (Fish).	2.59	mg/L
Assessment Factor	100	
PNEC:	25.95	µg/L

7.3. Environmental risk assessment

Based on the above mitigated PEC and PNEC, the following Risk Quotient (Q) has been calculated:

Risk Assessment	***PEC µg/L***	***PNEC µg/L***	***Q***
Q - River:	5.34	25.95	0.206
Q - Ocean:	0.53	25.95	0.021

Based on the above calculations for the Risk Quotients (Q), the notified chemicals are not expected to pose an unacceptable risk to the environment from the proposed use of the cosmetics containing the notified chemicals at the maximum importation volumes.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available data the notified chemicals are classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] with the following risk phrases:

R38 Irritating to skin
R41 Risk of serious damage to eyes

and

As a comparison only, the classification of the notified chemicals using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations 2009) 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***
Skin irritation	2	Causes skin irritation
Irritant	2A	Causes serious eye irritation
Acute hazards to the aquatic environment	2	Toxic to aquatic life

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

When used in the proposed manner with appropriate labelling, the notified chemicals are 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 chemicals are not expected to pose a risk to the environment.

Recommendations
REGULATORY CONTROLS
Hazard Classification and Labelling

- Safe Work Australia should consider the following health hazard classification for the notified chemicals:
  - R38 Irritating to skin
  - R41 Risk of serious damage to eyes

- The following risk phrases are recommended in the workplace on products/mixtures containing the notified chemicals:
  - ≥5% Concentration <10%: R36
  - ≥10% Concentration <20%: R41
  - Concentration ≥ 20%: R38, R41

- The National Drugs and Poisons Standing Committee (NDPSC) should consider the notified chemicals for listing on the SUSDP based on the results of skin and eye irritation tests. The Full Public Report will be provided to the NDPSC.

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemicals for formulation of cosmetics:
  - Avoid contact with skin and eyes

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemicals for formulation of cosmetics:
  - Protective eye wear
  - Impermeable gloves
  - Coveralls

  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 chemicals 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

- Consumer products containing the notified chemicals at concentrations ≥ 5% should be labelled with a warning against eye contact, and directions on first aid measures if the product contacts the eye (e.g. avoid contact with eyes, in case of contact with eyes, rinse immediately with plenty of water and seek medical advice).

- Precautionary warning on possible skin irritation is also recommended for leave on products and for rinse-off products containing ≥ 20% notified chemicals, including direction to discontinue use if skin irritation occurs.

- The following measures should be taken to minimise public exposure to the notified chemicals:
  - the notified chemical should not be used in spray products for consumer/domestic use.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

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

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 notified chemical is imported for use in spray products;
  - the concentration of the notified chemicals used in rinse-off products has increased above 23%;
  - the concentration of the notified chemicals used in leave-on products has increased above 5%.

or

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

- the function or use of the chemicals has changed from a component in rinse-off and leave-on cosmetic products, or is likely to change significantly;
- the amount of each of the chemicals being introduced has increased from 15 tonnes per annum, or is likely to increase, significantly;
- the chemicals have begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemicals on occupational health and safety, public health, or the environment.

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

No additional secondary notification conditions are stipulated.

Material Safety Data Sheet

The MSDS of the notified chemicals and products containing the notified chemicals 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 >300 g/L at 22ºC

Method	In house method. Solutions of 15%, 20% and 30% w/w were prepared from the neat notified chemical (STD 1354), purity 85%, by manual stirring (pH 7–9). All solutions were clear with a thick layer of foam on the top.
Remarks	Summary report provided. The notified chemical of STD/1354 is a suitable analogue for the mixture of STD/1355 and therefore, the water solubility of the notified chemicals is >300 g/L.
Test Facility	Unilever (2009)

Partition Coefficient log Pow = 0.158 – 3.89

Method	KOWWIN (v1.67)
Remarks	Calculated for potassium lauroyl glycinate (i.e. the ionised form) and lauroyl glycine (i.e. the unionised form), respectively.
Test Facility	US EPA (2009)

Adsorption Coefficient log K_{oc} = 0.219 – 2.282

Method	KOCWIN (v2.00)
Remarks	Calculated using the Kow method for potassium lauroyl glycinate (i.e. ionised form) and lauroyl glycine (i.e. unionised form), respectively. The log K_{oc} for both forms is estimated to be 2.251 when calculated using the MCI method.
Test Facility	US EPA (2009)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Amilite GCK-12 (STD/1354)
METHOD	In-house test similar to OECD TG 401 – Limit Test.
Species/Strain	Mice/ICR
Vehicle	30% test solution was diluted with water to 20% solution at time of use.
Remarks - Method	10 animals (5 female, 5 male) were given 2000 mg/kg of the notified chemical as a 20% test substance solution by oral gavage after being deprived of food for approximately 16 hours. 10 mice (5 female, 5 male) were given water only and served as the control. The animals were observed for 14 days after administration. The concentration at which the notified chemical was present in the test substance was unclear from the study report.
RESULTS	
LD50	> 2000 mg/kg bw
Signs of Toxicity	None were observed.
Effects in Organs	No changes in organs at necropsy.
Remarks - Results	No deaths occurred and there were no abnormal clinical signs. There was no significant difference in body weights of the animals in the treatment group compared with that of the control group.
CONCLUSION	The test substance is of low toxicity via the oral route.
TEST FACILITY	Bozo Research Center Inc (1997)

B.2. Acute toxicity – oral

TEST SUBSTANCE	Amilite GCK-12H (STD/1355)
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Sprague-Dawley (Crl:CD(SD))
Vehicle	30% test solution was diluted with water to 20% solution at time of use.
Remarks - Method	Three male and three female rats were administered with the test substance at a dose of 2000 mg/kg bw. The concentration at which the notified chemical was present in the test substance was unclear from the study report. No significant protocol deviations.
RESULTS	
LD50	> 2000 mg/kg bw
Signs of Toxicity	None
Effects in Organs	In one male rat, multiple whitish grey areas were observed in the spleen. These observations were not considered to be related to test article administration.
Remarks - Results	No deaths occurred and there were no abnormal clinical signs. There was no significant difference in body weights of the animals in the treatment group compared with that of the control group.
CONCLUSION	The test substance is of low toxicity via the oral route.
TEST FACILITY	Ajinomoto Co Inc (2008a)

B.3. Irritation – skin

TEST SUBSTANCE	Notified chemical (STD/1354) at 5% in aqueous solution

METHOD
- Species/Strain: Rabbit/New Zealand White
- Number of Animals: 4 Males
- Vehicle: Distilled water
- Observation Period: 7 days
- Type of Dressing: Occlusive
- Remarks - Method: 0.3 ml of the test substance solution was placed on a patch with adhesive plaster and applied to previously clipped area of skin. The area was covered with a torso cover and left for 24 hours. Skin irritation was assessed according to the Draize scale at 24, 48 and 72 hours and 1 week after the application.

RESULTS

Lesion	*Mean Score**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Erythema/Eschar	1.08	2 (at 24 and 48 hr)	< 7 days	0
Oedema	0	0	0	0

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

Remarks - Results: At the end of the observation period, all animals showed scaling of the skin. An erythema score of 2 (well defined erythema) was observed in 1 animal at 24 and 48 hours. This had reduced to score 1 (very slight erythema) by 72 hours. Erythema of score 1 was observed in the other 3 animals at 24 and 48 hours and remained at this level in 2 of the animals at 72 hours. Erythema resolved by day 7.

CONCLUSION: The notified chemical is slightly irritating to the skin at 5% concentration based on the erythema/eschar observed and the persistence of scaling in all animals up to day 7.
Considering the effects at 5% concentration, the notified chemical at higher concentrations should be classified as a skin irritant.

TEST FACILITY: Ajinomoto Co Inc (1998a)

B.4. Irritation – skin

TEST SUBSTANCE: Amilite GCK-12H (STD/1355); 30% purity
Note: the concentration at which the notified chemical is present in the test substance is unclear from the test report.

METHOD: OECD TG 404 Acute Dermal Irritation/Corrosion.
- Species/Strain: Rabbit/New Zealand White
- Number of Animals: 3 males
- Vehicle: None
- Observation Period: 14 days
- Type of Dressing: Occlusive
- Remarks – Method: No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	1.7	1.0	2.7	4	14 days	4
Oedema	0	0	1.3	2	< 72 hr	0

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

Remarks – Results	Slight oedema was observed in one of the animals at 24 and 48 hours after patch removal. Erythema was observed in all animals. In two of the animals the erythema was very slight to well-defined and had resolved by the 7 day observation, leaving scaling. It is unclear from the test report whether the scaling remained at the completion of the study. In the other animal, the erythema was well-defined at the 24 and 48 hour observation and then increased in severity to grade 4 (severe erythema (beet redness) to eschar formation preventing grading of erythema). The test report suggests that this was due to eschar formation. This persisted at the same severity at subsequent observations and remained at the final observation time (14 days).
CONCLUSION	The notified chemical at the tested concentration is irritating to the skin based on the assumed persistence of scaling in two animals and erythema/eschar at the end of the observation period in the remaining animal. Considering the effects observed at the tested concentration, the notified chemical at higher concentrations should be classified as at least a skin irritant, though may be corrosive at neat concentrations.
TEST FACILITY	Ajinomoto Co Inc (2008b)

B.5. Irritation – eye

TEST SUBSTANCE	Notified chemical (STD/1354) at 1% and 5%, in aqueous solution.
METHOD	In-house modified Draize test
Species/Strain	Rabbit/New Zealand White
Number of Animals	6 Males
Observation Period	7 days
Remarks - Method	The observation period is 7 days, which is a shorter period than the 21 days recommended in the OECD test method. The report using 5% test substance did not provide individual scores for conjunctival symptoms at the 48 and 72 hour observation points, therefore the mean overall scores could not be calculated for conjunctival redness, chemosis and discharge. SLS (5%) was used as a positive control.

RESULTS

1% test substance

Lesion	*Mean Score**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Conjunctiva: redness	0.33	1	< 72 hours	0
Conjunctiva: chemosis	0	0	0	0
Conjunctiva: discharge	0	0	0	0
Corneal opacity	0	0	0	0
Iridial inflammation	0	0	0	0

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

5% test substance

Lesion	*Mean Score at 24 hours**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Conjunctiva: redness	2.83	3.0	Present after 7 days	1
Conjunctiva: chemosis	1.83	3.0	< 7 days	0
Conjunctiva: discharge	1.67	3.0	< 7 days	0
	*Mean Score^*			
Corneal opacity	0.08	1.0	< 48 hours	0
Iridial inflammation	0.33	1.0	< 48 hours	0

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

Remarks - Results	*1% test substance*: Only slight irritation effects observed at this concentration, which cleared within 72 hours. *5% test substance*: Corneal opacity and iridial inflammation were only observed in one animal at the 24 hour observation. Conjunctival irritation slowly improved over time. However, redness of the conjunctiva (score 1 – blood vessels normal) was still present after 7 days in four of the six animals.
CONCLUSION	The notified chemical is irritating to the eye at 5% concentration based on the persistence of irritation effects at the end of the 7 day observation period and mean conjunctival redness score of > 2.5 at 24 hours. Considering the effects observed at 5% concentration, the notified chemical at higher concentrations should be classified as a severe eye irritant.
TEST FACILITY	Ajinomoto Co Inc (1998b)

B.6. Irritation – eye

TEST SUBSTANCE	Amilite GCK-12H (STD/1355); 30% purity Note: the concentration at which the notified chemical is present in the test substance is unclear from the test report.
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	1 male
Observation Period	10 days
Remarks – Method	Only one animal was tested due to the severity of the effects observed in this animal.

RESULTS

Lesion	*Scores**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Conjunctiva: redness	2.66	3 (at 24, 48 hr)	< 10 days	0
Conjunctiva: chemosis	1.66	4 (at 1 hr)	< 96 hr	0
Conjunctiva: discharge	0	3 (at 5 min, 1hr)	< 24 hr	0
Cornea opacity	1.66	2 (up to 48 hr)	< 10 days	0
Iridial inflammation	0.66	1 (up to 48 hr)	< 72 hr	0

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

Remarks – Results

The following observations were made of the treated eye.

- Corneal opacity with slightly obscured iris details, which persisted up until the 48 hour observation. After this the severity of the corneal opacity decreased and cleared by the 10 day observation.
- Iris congestion was observed but had resolved by 72 hours.
- Redness of the conjunctivae increased in severity with beefy red conjunctivae observed at the 24 and 48 hour observations. After this time the severity decreased and had cleared by the 10 day observation.
- Conjunctival swelling with about half or more of the eye lid closed was observed at the 5 minute and one hour time point. This decreased to obvious swelling at 24 and 48 hours and less again by 72 hours. By the 96 hour observation the chemosis had cleared.
- Considerable discharge from the eye was observed up to the 1 hour time point, after which it cleared.

CONCLUSION	The notified chemical at the tested concentration is irritating to the eye. Considering the effects observed at the tested concentration, the notified chemical at higher concentrations should be classified as a severe eye irritant.
TEST FACILITY	Ajinomoto Co Inc (2008c)

B.7. Skin sensitisation

TEST SUBSTANCE	Notified chemical (STD/1354)
METHOD	OECD TG 406 Skin Sensitisation – Guinea Pig Maximisation Test EC Directive 96/54/EC B.6 Skin Sensitisation.
Species/Strain	Guinea pig/Hartley
VEHICLE	Physiological saline for intradermal and water for topical application.
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: 0.1% topical: 2.5% Maximum concentration to cause mild-moderate irritation: intradermal: 0.1% (no irritation, but necrosis observed at 0.25% and 0.5%) topical: 5%
MAIN STUDY	
Number of Animals	Test Group: 10 Control Group: 5
INDUCTION PHASE	Induction Concentration: intradermal: 0.1% topical: 5%
CHALLENGE PHASE	
1st challenge	topical: 2.5, 1%
Remarks - Method	No observations of irritation during the induction phase were included in the study.

RESULTS	
Remarks - Results	No skin reactions (score 0) were observed at any site of application on any animal when challenged with 2.5% or 1% test substance solution. No skin reactions were observed at any site of application on any animal in the control group. There were no deaths and no signs of systemic toxicity in any group during the observation period. Six animals in the test group and 4 animals in the control group at the challenge observation period (day 24) showed body weight loss but all of these animals were recovered at the end of the study on day 25. The reason for the temporary weight loss was unclear.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Ajinomoto Co Inc (2004)

B.8. Skin sensitisation

TEST SUBSTANCE	Notified chemical (STD/1355) (30% aqueous solution)
METHOD	OECD TG 406 Skin Sensitisation – Guinea Pig Maximisation Test.
Species/Strain	Guinea pig/ Hartley strain albino.
Vehicle	Physiological saline for intradermal induction (10%, 5%, 2.5% and 1% solution) and water for topical induction (10%, 5%, 2.5% solution).
PRELIMINARY STUDY	intradermal: 1% solution – maximum concentration not causing necrosis, however, intense erythema and oedema was observed. topical: 10% solution.- maximum concentration showing mild

	irritation. 2.5% was the maximum concentration inducing no irritation.
MAIN STUDY	
Number of Animals	Test Group: 10 Control Group: 5
INDUCTION PHASE	Induction Concentration: intradermal injection: 1% solution topical application 10% solution
Signs of Irritation	Signs of irritation (means score 3.0 – intense erythema and swelling) were noted in test animals observed following intradermal induction. There were no signs of irritation following topical induction.
CHALLENGE PHASE	
1st challenge	topical application: 2.5% and 1%
Remarks – Method	No significant protocol deviations.

RESULTS

Remarks – Results	There were no deaths or abnormal clinical signs and no decreases in body weight in any animal in any group during the observation period.

CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Bozo Research Center Inc (2008a).

B.9. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical (STD/1354) (30% aqueous solution)
METHOD	Study in compliance with Japanese regulatory standards - Standards for Mutagenicity Tests using Microorganism (Ministry of Labour, Japan) and GLP standards.
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 fraction from Phenobarbital and 5,6-benzoflavone activated rat liver
Concentration Range in Main Test	a) With metabolic activation: Salmonella typhimurium: 10-313 µg/plate; *E. coli*: 156-5000 µg/plate b) Without metabolic activation: *Salmonella typhimurium*: 1.2-78 µg/plate (TA100, TA1535, TA1537); 1.2-313 µg/plate (TA98) *E. coli*: 156-5000 µg/plate
Vehicle	Water
Remarks - Method	Concentration of the tested material was 30% and the measured weights of test substance were corrected accordingly. The positive controls used were (2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, sodium azide, 2-methoxy-6-chloro-9-[3-(2-chloroethyl) aminopropylamino] acridine.2HCl, 2-aminoanthracene, Benzo(a)pyrene. The preincubation method was used. A second test (Test 2) was run for the strains TA100, TA1535 and TA 1537 due to the low number of doses without growth inhibition in the preliminary toxicity test.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:* *Cytotoxicity in Preliminary Test*	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 78 for *S. typhimurium**	≥ 39 for *S. typhimurium*	> 313	Negative
	5000 for *E.coli*	≥ 2500 for *E.coli*	> 5000	Negative
Test 2	-	≥ 39 for *S. typhimurium*	> 313	Negative
Present				

Test 1	≥ 313 for *S. typhimurium* 5000 for *E.coli*	≥ 156 for *S. typhimurium* ≥ 2500 for *E.coli*	> 313 > 5000	Negative Negative

* Except for TA98, where cytotoxicity was observed at ≥ 313 µg/plate.

Remarks - Results: There was no significant increase in the number of revertant colonies with or without metabolic activation compared to the negative control, and there was no dose-related effect observed in any strain. The revertant colonies of the positive control showed an increase of more than twice that of the negative controls indicating that the study was performed correctly.

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

TEST FACILITY: BML Inc (1994)

B.10. Genotoxicity – bacteria

TEST SUBSTANCE: Notified chemical (STD/1355) (30% aqueous solution)

METHOD: OECD TG 471 Bacterial Reverse Mutation Test.
Pre-incubation procedure

Species/Strain: *S. typhimurium*: TA1535, TA1537, TA98, TA100
E. coli: WP2uvrA

Metabolic Activation System: S9 fraction from Phenobarbital and 5,6-benzoflavone activated rat liver

Concentration Range in Main Test:
a) With metabolic activation:
S. typhimurium: 9.77 - 313 µg/plate;
E. coli: 39.1 - 1250 µg/plate
b) Without metabolic activation:
S. typhimurium: 0.61 - 313 µg/plate
E. coli: 39.1 - 1250 µg/plate

Vehicle: 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*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 19.5	≥ 19.5	Not observed	Negative
Test 2	-	≥ 19.5	Not observed	Negative
Present				
Test 1	≥ 78.1	≥ 78.1	Not observed	Negative
Test 2	-	≥ 78.1	Not observed	Negative

* Varied for different test strains.

Remarks - Results: There was no significant increase in the number of revertant colonies with or without metabolic activation compared to the negative control, and there was no dose-related effect observed in any strain.

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

TEST FACILITY: Bozo Research Center Inc (2008b)

B.11. Genotoxicity – in vitro

TEST SUBSTANCE: Analogue chemical (glycine, N-coco acyl derivs., sodium salts; CAS number 90387-74-9)

METHOD

Study in compliance with Japanese regulatory standards for Toxicity testing of Pharmaceutical products and GLP standards.

Species/Strain: Chinese hamster

Cell Type/Cell Line: Lung fibroblasts (CHL/IU) cells

Metabolic Activation System: S9 fraction from Phenobarbital and 5,6-benzoflavone activated Sprague-Dawley Rat liver at 5%

Vehicle: Saline

Remarks - Method: Concentration of the chemical in the test is stated to be ten times higher than in the table below. However, the dilution in the cell medium was not taken into account.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	4*; 8*; 12*; 16*	24h	24h
Test 2	4*; 8*; 12*; 16*	48h	48h
Test 3	7.8*; 15.6*; 31.3*; 62.5*	6h	24h
Present			
Test 3	7,8*; 15,6*; 31,3*; 62.5*	6h	24h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1[a]	10.5	Not determined	Not observed	no
Test 2[a]	-	16	Not observed	no
Test 3[b]				no
Present				
Test 1[a]	42	-	-	-
Test 3[b]	-	Not determined	Not observed	yes

[a] Positive control – Mitomycin C (MMC)

[b] Positive control - N-nitrosodimethylamine (DMN)

Remarks - Results

Absence of metabolic activation

In Test 2 there was a small increase of the percentage of cells with aberrations including gaps in cultures treated with 8 and 12 µg/mL of chemical (2% and 1.5%, respectively) compared with the solvent control (0%). However, this is not considered to be significant as there were also some aberrant cells (0.5%) in the non-treated control while the positive control using treatment with Mitomycin C (MMC) generated significantly higher increase. At the highest concentration tested in the Main test 1, the cytotoxicity was very high and did not allow for examination of sufficient number of cells to determine genotoxicity.

Presence of metabolic activation

The percentage of cells with aberrations including and excluding gaps was increased to 23% in the cultures treated with 62.5 µg/mL of chemical in the presence of metabolic activation. This increase was assessed as a positive genotoxic effect even though concentration dependent trend was not observed at the lower concentrations. In Tests 1 and 2, the incidence of structural aberrations was increased with the positive control MMC. In Test 3 the percentage of cells with structural aberrations tested with the positive control DMN was increased in the presence of metabolic activation, but was not increased in the absence of metabolic activation. A possible reason for the result is that this control requires metabolic activation.

CONCLUSION	The analogue chemical was clastogenic to hamster lung fibroblasts (CHL/IU) treated in vitro in the presence of metabolic activation.
TEST FACILITY	BML (1998)

B.12. Genotoxicity – in vivo

TEST SUBSTANCE	Analogue chemical (glycine, N-coco acyl derivs., sodium salts; CAS number 90387-74-9)
METHOD	In house method similar to OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
Species/Strain	Mouse/ICR (Crj:CD-1) SPF
Route of Administration	Intraperitoneal twice within 24h
Vehicle	Saline
Remarks - Method	In a preliminary, range finding study, the LD50 for intraperitoneal administration was determined to be between 250 and 500 mg/kg bw.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	6 male	0	24h
II (low dose)	6 male	50	24h
III (mid dose 1)	6 male	100	24h
IV (mid dose 2)	6 male	200	24h
V (high dose)	6 male	400	24h
VI (positive control - M)	6 male	2	24h

M=mitomycin C

RESULTS	
Doses Producing Toxicity	In the main test four deaths were observed in the 400 mg/kg bw group (4/6) and one death was observed in the 200 mg/kg bw group (1/6). A decrease in locomotor activity and bradypnea were observed in the 50mg/kg bw or higher concentration groups, piloerection was observed in the 100 mg/kg bw or higher concentration groups, hypothermia, lacrimation and prone position were observed in the 200 mg/kg bw or higher concentration groups.
Genotoxic Effects	None observed in the animals treated with the solvent control. No increase in the frequency of micronucleated polychromatic erythrocytes at any dose level or exposure time was observed in the dose range finding study or the main study. The positive control showed a marked increase in the frequency of micronucleated polychromatic erythrocytes, indicating that the test system responded appropriately.
Remarks - Results	The ratio of polychromatic erythrocytes to total erythrocytes was significantly decreased in the mid dose I (group III) and above. This finding suggests that the notified chemical has reached the bone marrow after intraperitoneal administration and it is toxic to erythroblasts.
CONCLUSION	The analogue chemical was not clastogenic under the conditions of this test.
TEST FACILITY	JBC (1998)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical (STD 1354) (30% aqueous solution)
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I).
Inoculum	Standard activated sludge (30 mg/L dry weight)
Exposure Period	28 Days
Auxiliary Solvent	None
Analytical Monitoring	Biological oxygen demand (BOD) and dissolved organic carbon (DOC) were determined using a Central Kagaku D unit BOD measuring apparatus and Shimadzu TOC-500 total organic carbon measuring apparatus, respectively.
Remarks - Method	The test was conducted according to the guidelines above at a test substance concentration of 340 mg/L (i.e. ~100 mg/L notified chemical). A non-culture vessel (containing test substance, at 340 mg/L, and deionised water) and reference control (aniline, 100 mg/L) were run in parallel. Test conditions: 25 ± 1°C, pH not reported. Biodegradability was calculated from the BOD data, corrected by an inoculum blank, and the theoretical oxygen demand (ThOD), assuming nitrification.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	65.8	7	56.7
14	73.2	14	63.6
21	75.2	21	66.2
28	79.8	28	69.5

Remarks - Results	Biodegradability of the test substance after 28 days was 79.8%, and reached >60% in a 10-day window. Oxygen consumption of the reference material in the control was >40% after 7 days, and 63.6% after 14 days. A test is considered valid if consumption is >40% after 7 days, and >65% after 14 days. Although the Day 14 result is slightly lower than 65%, it is not expected to affect the result of the test substance. Biodegradability of the test substance based on DOC was >90% after 28 days. Degradation also occurred in the non-culture vessel, reaching 43% after 28 days based on BOD. The notified chemical (STD 1354) is considered to be an acceptable analogue of the notified chemicals of STD 1355.
CONCLUSION	The notified chemicals are readily biodegradable.
TEST FACILITY	Japan Food Research Laboratory (1995)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemicals (STD/1354, STD 1355)
METHOD	QSAR estimation methods

RESULTS

ECOSAR (v1.00) *Anionic surfactant class*	
potassium lauroyl glycinate *LC50 (96 h) mg/L*	*potassium lauroyl glycylglycinate* *LC50 (96 h) mg/L*
2.59	2.59

REMARKS - RESULTS

Surfactant toxicity has been found to depend on carbon chain length (Nabholz et al., 1993) and consequently, QSARs based on chain length have been derived and validated for fish (e.g. ECOSAR (v1.00), anionic surfactant class; US EPA, 2009). If the toxicity of a mixture is to be estimated, usually the weighted average carbon chain length (WACCL) is used for the calculation. For the notified chemicals of STD/1354 and STD/1355 the cocoyl acid profile depends on the source coconuts and, as a result, the WACCL is variable. As such, the chain length of the most abundant component (i.e. the lauroyl derivatives, C12) is used for the estimation of fish toxicity to give an endpoint of LC50 (96 h) of 2.59 mg/L (ECOSAR (v1.00), anionic surfactant class). This estimation value is similar in magnitude to measured fish test data for comparable classes of surfactants.

CONCLUSION The notified chemicals are toxic to fish

TEST FACILITY US EPA (2009)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical (STD/1354)

METHOD OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Static.

Species	*Ceriodaphnia dubia*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	91 mg $CaCO_3$/L
Analytical Monitoring	None
Remarks - Method	The test was conducted according to the guidelines above at test substance concentrations of 80.0, 40.0, 19.7, 10.0 and 5.1 mg/L. The test substance did not completely dissolve upon addition of dilution water. The solutions were stirred for 24 h, and allowed to settle for 5.5 h. All solutions were observed to contain suspended material, and the 80 mg/L solution contained precipitate on the bottom of the vessel. The solutions were siphoned off into clean vessels to remove the suspended matter. A control and toxicant reference control were run in parallel. Test conditions: 25 ± 1°C, 16 h/8 h light dark cycle, pH 7.9–8.2, 97.4–100.4 mg O_2/L.

RESULTS

Concentration mg/L		*Number of C. dubia*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	Not tested	20	0	0
5.1	Not tested	20	0	0
10.0	Not tested	20	0	0
19.7	Not tested	20	0	0
40.0	Not tested	20	0	2
80.0	Not tested	20	0	6

EC50	>80 mg/L at 48 hours
NOEC	40 mg/L at 48 hours
Remarks - Results	After siphoning, all the solutions still contained suspended material. The 80.0 mg/L solution was also cloudy in appearance.

There were no immobilised daphnia in the control after 48 h, and the reference toxicant endpoint was between the acceptable limits 179.1–268.7 mg KCl/L (260.8 mg KCl/L), thus validating the test.
The notified chemical (STD/1354) is considered to be an acceptable analogue of the notified chemicals of STD/1355.

CONCLUSION The notified chemicals are not toxic to aquatic invertebrates

TEST FACILITY Ecotox (2009)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical (STD/1354)

METHOD OECD TG 201 Alga, Growth Inhibition Test.
Species *Pseudokirchneriella subcaptiata*
Exposure Period 72 hours
Concentration Range Nominal: 0–32.0 mg/L
Actual: Not reported
Auxiliary Solvent None
Water Hardness Not reported
Analytical Monitoring A spectrophotometer was used to measure algal density
Remarks - Method The test was conducted according to the guidelines above at test substance concentrations of 32.0, 15.9, 8.0, 4.4, 2.4, 1.2, and 0.6 mg/L in triplicate. A blank and reference toxicant control (potassium chloride) were run in parallel. Test conditions: 25 ± 2°C, pH 7.7–8.9, continuous illumination. The endpoints and confidence limits were determined by linear interpolation, and Dunnett's Test (Toxcalc v5.0.31).

RESULTS

Biomass		*Growth*	
E_bC_{50} *mg/L at 72 h*	*NOEC mg/L*	E_rC_{50} *mg/L at 72 h*	*NOEC mg/L*
5.7 (4.3–6.4)	1.2	16.3 (14.5–18.3)	1.2

Remarks - Results After mixing, all solutions contained a small amount of suspended matter, and the 32.0 mg/L solution appeared cloudy.
Negative inhibition (i.e. stimulation) was observed for the test substance at concentration 1.2 mg/L at 72 hours.
Cell density of the control increased 195-fold, and the reference toxicant endpoint was between the acceptable limits 0.9–4.2 g KCl/L (2.6 g KCl/L), thus validating the test.
The notified chemical (STD/1354) is considered to be an acceptable analogue of the notified chemicals of STD/1355.

CONCLUSION The notified chemicals are harmful to algae

TEST FACILITY Ecotox (2009)

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