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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Protein hydrolyzates, silk, lauroyl, sodium salts (INCI: Sodium lauroyl silk amino acids)

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

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

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

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Director
NICNAS

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Neways International (Australia) Pty Ltd (ABN 11 065 366 458)
Level 1, 200 East Terrace
Adelaide SA 5000

NOTIFICATION CATEGORY
Limited-small volume: Chemical other than polymer (1 tonne or less 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, Vapour pressure, Hydrolysis as a function of pH, Dissociation constant, Particle size, Flash point, Flammability, Auto-ignition temperature.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

CHEMICAL NAME
Protein hydrolyzates, silk, lauroyl, sodium salts

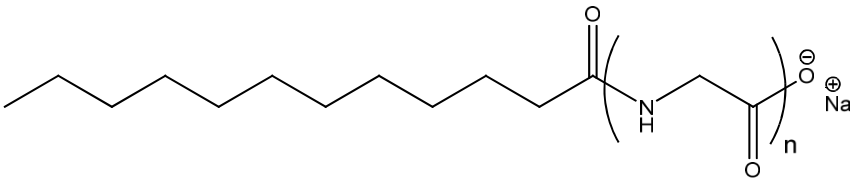
MARKETING NAME(S)
Keratonic Shampoo (contains up to 3.2% notified chemical)
Promois EFLS-C (contains 20% notified chemical)

OTHER NAME(S)
Sodium lauroyl silk amino acids (INCI name)

CAS NUMBER
169590-94-7

MOLECULAR FORMULA
Unspecified. The notified chemical is the sodium salt of condensation of silk amino acids and lauric acid. Some peptide segments may also be present.

STRUCTURAL FORMULA *A representative structure is depicted*



R = side chain of various amino acids e.g. —H (glycine)

—CH_3 (alanine)

— CH_2—OH (serine)

n = degree of amino acid polymerisation

Amino acid	*Composition (Mol %)*
Glycine	42.9
Alanine	30.6
Serine	9.7
Tyrosine	4.9
Valine	2.6
Phenylalanine	2.3
Aspartic acid	2.1
Glutamic acid	1.5
Isoleucine	1.0
Threonine	0.9
Leucine	0.6
Lysine	0.5
Proline	0.3
Arginine	0.1

AVERAGE MOLECULAR WEIGHT
300 Da

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

3. COMPOSITION

DEGREE OF PURITY 100% (reaction mixture)
Promois EFLS-C contains not more than 20 ppm of heavy metals and not more than 2 ppm of arsenic.

ADDITIVES

Chemical Name	Propanol, oxybis-		
CAS No.	25265-71-8	*Weight %*	3 (in Promois EFLS-C)
Chemical Name	Ethanol, 2-phenoxy-		
CAS No.	122-99-6	*Weight %*	1 (in Promois EFLS-C)

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Yellow-light brown, transparent to slightly opaque liquid (Promois EFLS-C containing 20% notified chemical in aqueous solution with additives).

Property	Value	Data Source/Justification
Melting Point	~350°C	Estimated using MPBPVP (v1.43) for the sodium lauroyl derivatives of glycine, alanine and serine.
Boiling Point	> 700°C	Estimated using MPBPVP (v1.43) for the sodium lauroyl derivatives of glycine, alanine and serine.
Density	1035 kg/m^3 at 20°C	Promois EFLS-C Technical data sheet
Vapour Pressure	< 1 x 10^{-24} kPa at 25°C	Estimated using MPBPVP (v1.43) for the sodium lauroyl derivatives of glycine, alanine and serine.
Water Solubility	Not determined	The notified chemical is claimed to be 'freely soluble' by the notifier. The notified chemical is a surfactant.
Hydrolysis as a Function of pH	Not determined	Expected to be very slow in the environmental pH range of 4-9. Hydrolytic stability is a functional requirement in cosmetic/personal-care formulations.
Partition Coefficient (n-octanol/water)	log P_{OW} = -3.27 – -2.21 at 20°C	Calculated using KOWWIN (v1.67) for the sodium lauroyl derivatives of glycine, alanine and serine. The notified chemical is a surfactant which will concentrate at the phase boundaries.
Adsorption/Desorption	log K_{OC} = -2.55 – -1.55 at 20°C	Calculated using KOCWIN (v2.00) for the sodium lauroyl derivatives of glycine, alanine and serine, based on the calculated log K_{OW}. The notified chemical can be expected to adsorb to organic carbon, soil and sediment because it is a surfactant.
Dissociation Constant	Not determined	The notified chemical is a sodium salt of the condensation reaction of lauric acid chloride and hydrolysed silk protein and is, therefore, expected to remain dissociated under ambient environmental conditions.
Particle Size	Not determined	Imported in finished product
Flash Point	Not determined	Low vapour pressure solid
Autoignition Temperature	Not determined	Not expected to autoignite as imported
Explosive Properties	Not explosive	Does not contain structural groups associated with explosive properties

DISCUSSION OF PROPERTIES

Reactivity

Stable under normal environmental and usage conditions.

5. INTRODUCTION AND USE INFORMATION

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

Imported in a finished and packaged shampoo product.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	0.2	0.1	0.1	0.11	0.12

PORT OF ENTRY
Adelaide or Sydney

IDENTITY OF RECIPIENTS
Neways International (Australia) Pty Ltd

TRANSPORTATION AND PACKAGING
The shampoo will be imported in PET plastic bottles and transported by road from the port to the distribution centre and subsequently to retail stores.

USE
Surfactant in shampoo at concentrations up to 3.2%.

OPERATION DESCRIPTION
The notified chemical will not be reformulated or repackaged in Australia. The finished product will be sold as hair shampoo at retail stores. End users will be members of the public who will apply the shampoo to hair and rinse off with water.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

The shampoo containing the notified chemical will not be used in an occupational setting, therefore no worker exposure will occur.

6.1.2. Public exposure

There will be widespread and frequent dermal exposure to the hair shampoo containing up to 3.2% notified chemical through deliberate application of the products to the hair. The predominant areas of exposure are the scalp and hands but ocular exposure is also possible through accidental eye contact.

An estimate of exposure dosage to the notified chemical is as follows:

Product	*Quantity (g/application)**	*Application Frequency **	*Retention Factor**	*% Notified Chemical*	*Systemic Exposure Dosage (mg/kg bw/day)***
Shampoo	8.0	1/day	0.01	3.2	**0.043**

*data from SCCP (2006)
** assuming 60kg body weight and 100% dermal absorption (in the absence of absorption data).

6.2. Human health effects assessment

No toxicological data was available on the neat chemical. The results from toxicological investigations conducted on the product, Promois EFLS-C (containing 20% notified chemical in aqueous solution) 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, low toxicity
Rabbit, skin irritation	slightly irritating
Human epicutaneous patch test, skin irritation	slightly irritating at 3.3%
HET-CAM, eye irritation	slightly irritating at 2.5%
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Mutagenicity – bacterial reverse mutation	non mutagenic

Absorption and Metabolism
The notified chemical is an anionic surfactant which is able to reduce the surface tension of water and remove lipids on the skin during the washing process (Mehling et al 2007). Generally, percutaneous absorption of anionic detergents are low (Black & Howes 1992), therefore the likelihood of systemic effects from skin

absorption is low. However, due to the ability of surfactants to disrupt the natural skin barrier, increased duration of exposure on the skin and higher concentrations can promote dermal absorption

Acute toxicity
There was no mortality and no sign of systemic toxicity in an oral study in rats. The notified chemical at 20% is of low toxicity via the oral route.

Irritation
Slight to well-defined erythema and slight oedema was observed in all animals when tested with 20% notified chemical. Crust formation and moderate desquamation of two treated skin sites were observed at 7 days after exposure. All skin lesions and signs of irritation resolved by the end of the study therefore the notified chemical is considered slightly irritating to the skin of rabbits at a concentration of 20%.

In an *in vivo* human patch test, a single occlusive application of 6% notified chemical (3.3% of Promois EFLS-C) over a period of 24 hours resulted in slight irritation at the treated skin site in 4 out of 20 volunteers when observed 24 hours after removal of the patches. At 48-hours after patch removal, 1 out of 20 volunteers showed slight skin irritation. Based on the cutaneous irritation scoring system used in this test, the test substance (6% notified chemical) was considered 'safe' or 'acceptable'.

There was evidence of slight eye irritation in an *in vitro* Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) test using a concentration of 2.5% Promois EFLS-C (0.5% notified chemical). Hyperaemia and minimal haemorrhage was observed at each reading. The HET-CAM assay has not yet been validated as a replacement test for the *in vivo* Draize test, however validation of this assay is currently being considered by the US National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods and the Interagency Coordinating Committee on the Validation of Alternative Methods (NICEATM-ICCVAM). The draft ICCVAM recommendations from this validation process were released in April 2009 and recommended that the Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) not be used for regulatory hazard classification purposes based on a lack of adequate data (ICCVAM, 2009a).

Based on the chemical structure and the slight eye irritation seen at very low concentrations (0.5%) the notified chemical should be considered to be at least an eye irritant. The potential for severe eye irritation at higher concentrations cannot be ruled out.

Sensitisation
When tested in a murine Local Lymph Node Assay the test substance (20% notified chemical) did not induce lymphocyte proliferation indicative of sensitisation. Therefore this test substance is considered to be non-sensitising. Due to a diluted test substance being used the notified chemical itself was not tested at concentrations above 20%. However given the results from this test and that there are no structural alerts for sensitisation, the notified chemical is unlikely to be a significant skin sensitiser.

Mutagenicity
The product containing the notified chemical at 20% concentration was found to be non-mutagenic to bacteria in a reverse mutation assay. Therefore the notified chemical is not expected to be mutagenic.

Health hazard classification
Based on the available studies/information, the notified chemical cannot be classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
As shampoos containing the notified chemical are not expected to be used in occupational settings, there is expected to be no risk to workers associated with exposure to the notified chemical.

6.3.2. Public health

The notified chemical is expected to be at least irritating to the eyes and may also be irritating to the skin at neat concentrations.

The public will encounter dermal exposure and occasional ocular exposure to the notified chemical at concentrations up to 3.2% during use of hair shampoo products.

At the proposed use concentration of up to 3.2%, skin irritation is unlikely to occur; additionally, the rinse off nature of the product and corresponding relatively short skin contact times are likely to further reduce the potential for skin irritancy effects.

The potential for eye irritation effects during accidental ocular exposure cannot be ruled out at the concentrations proposed to be used (up to 3.2%). However, the rinse-off nature of the products is expected to reduce the contact time with the eyes and thus the potential for eye irritation.

In summary, the risk to the public associated with eye and skin contact with the notified chemical when used in the proposed manner 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

Release to the environment may occur in the unlikely event of an accident during transport or an accidental spill during handling. The notified chemical will be transported to Australia by ship in packaged ready-to-use PET consumer bottles (typically < 1 L). Formulation or repackaging is not expected in Australia.

RELEASE OF CHEMICAL FROM USE

As the notified chemical is used in shampoo it is expected that effectively the entire annual volume will be released to sewer via consumer use. A small proportion (estimated to be ≤ 2%) may remain as residual within the end-use containers.

RELEASE OF CHEMICAL FROM DISPOSAL

It is expected that end use containers will be disposed of as domestic garbage and end up in landfill sites.

7.1.2 Environmental fate

A single ready biodegradability test report was submitted for an acceptable analogue of the notified chemical. The test report indicates that the analogue is readily biodegradable. Based on the result for the analogue, and considering the structure of the notified chemical, it is considered that the notified chemical is also readily biodegradable. For the details of the environmental fate study please refer to Appendix C.

The notified chemical is a readily biodegradable anionic surfactant and is therefore not expected to bioaccumulate.

7.1.3 Predicted Environmental Concentration (PEC)

Since most of the notified chemical will be washed into the sewer, under a worst case scenario, with no removal of the notified chemical in the sewage treatment plant, the resultant Predicted Environmental Concentration (PEC) in sewage effluent on a nationwide basis is estimated as follows:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import Volume	120	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	120	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	0.33	kg/day

Water use	200	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:	0.08	µg/L
PEC - Ocean:	0.01	µg/L

7.2. Environmental effects assessment

No ecotoxicity data were submitted for the notified chemical. Three ecotoxicity endpoints were submitted for an acceptable analogue, as shown below. However, test reports were not submitted to verify these endpoints, and thus these are considered indicative only.

Endpoint	*Result*	*Assessment Conclusion (GHS)*
Fish Toxicity (Acute - 96 h)	LC50 >1000 mg/L	Not harmful to fish
Daphnia Toxicity (Acute – 48 h)	EC50 45 mg/L	Harmful to aquatic invertebrates
Algal Toxicity (Acute – 72 h)	EC50 76 mg/L	Harmful to algae

7.2.1 Predicted No-Effect Concentration

Based on the most sensitive endpoint for the analogue, the following PNEC has been derived using a highly conservative assessment factor of 1000 for indicative purposes.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Invertebrates)	45.00	mg/L
Assessment Factor	1,000.00	
PNEC:	45.00	µg/L

7.3. Environmental risk assessment

Based on the conservative PEC and indicative PNEC, the Q values (Risk Quotient, PEC/PNEC) have been calculated as follows for river and ocean receiving environments.

Risk Assessment	***PEC µg/L***	***PNEC µg/L***	***Q***
Q - River:	0.08	45	0.0017
Q - Ocean:	0.01	45	0.0002

Based on the above Q values, the notified chemical is not considered to pose a risk to aquatic ecosystems under the proposed use pattern and volume.

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 0.078 µg/L may potentially result in a soil concentration of approximately 5.179×10^{-4} mg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 2.589×10^{-3} mg/kg and 5.179×10^{-3} mg/kg, respectively. However, given the expected rapid degradation of the notified chemical, these values should be considered as theoretical maximum concentrations only.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available studies, the notified chemical cannot be classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

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 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***
Environment	Acute Category 3	Harmful to aquatic life

Human health risk assessment
When used in the proposed manner, the notified chemical is not considered to pose an unacceptable risk to the health of workers.

When used in the proposed manner, 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

CONTROL MEASURES
Occupational Health and Safety

- 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 imported product containing the notified chemical and available to the public should carry the following safety direction on the label:
  - Avoid contact with eyes

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 importation volume exceeds one tonne per annum notified chemical;
- the concentration of the notified chemical in hair shampoo products exceeds 5%;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component of hair shampoo, or is likely to change significantly;
- the amount of chemical being introduced has increased from 0.12 tonnes per annum, 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 was reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Promois EFLS-C (20% notified chemical in aqueous solution)
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Sprague-Dawley CD
Vehicle	None
Remarks - Method	6 female rats were administered a single dose of 2000 mg/kg bw by oral gavage.
RESULTS	
LD50	> 2000 mg/kg bw
Signs of Toxicity	There were no signs of systemic toxicity.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks - Results	There were no deaths and all animals showed expected bodyweight gains during the study period.
CONCLUSION	The test substance is of low toxicity via the oral route.
TEST FACILITY	SafePharm Laboratories (2004a)

B.2. Irritation – skin

TEST SUBSTANCE	Promois EFLS-C (20% notified chemical in aqueous solution)
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation). EC Directive 2004/73/EC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	None
Observation Period	14 days
Type of Dressing	Semi-occlusive.
Remarks - Method	The pH of the undiluted test material was determined to be 7.2

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	1.33	0.67	1.67	2	< 14 days	0
Oedema	0.67	0.67	1	1	< 7 days	0

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

Remarks - Results	Slight to well-defined erythema was observed at all treated sites one hour after patch removal and at the 24 and 48-hour observation. Slight erythema was noted in two animals at 72 hours and in one animal at 7 days. Crust formation was evident in one skin site and moderate desquamation was noted at a separate skin site at 7 days. Slight oedema was noted at all treated sites one hour after patch removal, at the 24 and 48-hour observation and persisted in one animal at 72 hours.
CONCLUSION	The test substance is slightly irritating to the skin.
TEST FACILITY	Safepharm Laboratories (2004b)

B.3. Skin irritation – human volunteers

TEST SUBSTANCE: Promois EFLS-C (diluted to 6% notified chemical in aqueous solution)

METHOD: Epicutaneous 24-hour Patch Test (similar to epicutaneous plaster test method, COLIPA Standard Ref. 16)

Study Group	20 volunteers (2 males and 18 females) aged 18-60 years
Vehicle	None
Type of Dressing	Occlusive
Negative Control Substances	Distilled water, white petrolatum and normal saline.
Remarks - Method	Four test substances (notified chemical plus 3 negative controls) weighing ~ 30 mg each, were applied by means of epicutaneous plaster (using Finn chambers and Scanpor tape) for a period of 24 hours. Cutaneous examinations were performed at 30-60 minutes, 24 hours and 48 hours after removal of patches.

Cutaneous reactions were evaluated for each volunteer according to the following scale:

Examination	Cutaneous Reaction	Score
-	no reaction	0
±	slight erythema	0.5
+	clear erythema	1.0
++	erythema+edema, papulae	2.0
+++	erythema+edema・papulae+vesicle	3.0
++++	bullous reaction	4.0

$$\text{Cutaneous Irritation index} = \frac{\text{Sum of the scores at each times point}}{\text{Number of volunteers}} \times 100$$

Index of cutaneous irritation and classification:

Cutaneous Irritation index	Classification (1985)	Classification (1995)
< 5.0	safe	safe
5.0 - 15.0	safe	acceptable
15.0 - 30.0	acceptable	requires improvement
30.0 - 60.0	requires improvement	unsafe
> 60.0	unsafe	unsafe

RESULTS

Type of test article	Promois EFLS-C (Active 6% water solution)		Distilled water for injection		White petrolatum		Normal saline	
After	24 hrs.	48 hrs.	24 hrs.	24 hrs.	48 hrs.	48 hrs.	24 hrs.	48 hrs.
-	16/20	19/20	20/20	20/20	20/20	20/20	20/20	20/20
±	4/20	1/20	0/20	0/20	0/20	0/20	0/20	0/20
+	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
++	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Cutaneous Irritation index	10.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0

REMARKS - RESULTS: According to the classification scheme used in this test the test substance is considered to be 'acceptable'.

CONCLUSION: A single-application human patch test was conducted using the product Promois EFLS-C (diluted to 6% notified chemical in aqueous solution) under occlusive dressing. The test substance was slightly irritating under the conditions of the test.

TEST FACILITY: Dermis Research Centre (2004)

B.4. Irritation – eye

TEST SUBSTANCE: Promois EFLS-C 20% notified chemical diluted to 1.25% (i.e. 0.25% notified chemical)

METHOD: Hen's Egg Test – Utilising the Chrioallantoic Membrane (HET-CAM). Modification of that described by Kemper and Luepke (1986).

Species/Strain: White Leghorn chicken eggs

Number of Animals: 4 for each test substance

Observation Period: Readings taken at 0.5, 2 and 5 mins after exposure

Method: After 10 days of incubation at 37.2°C in a Kuhl incubator, the shell covering the air sack of each egg was removed. Forceps were then used to remove the shell down to the shell-membrane junction. The inner egg membrane was hydrated with warm, physiological saline for 2-5 minutes. Subsequently, the inner egg membrane was removed to reveal the CAM. A 0.3 ml test solution was added to each CAM for a period of 20 secs. The test or control solution was rinsed from each CAM with 5 ml of physiological saline. Effects of hyperaemia, haemorrhage (including minimal haemorrhage) and coagulation were observed over a period of 5 mins and scored according to the maximum scores shown in the following table.

Effect	*Scores at time (min):*		
	0.5	*2*	*5*
Hyperaemia	5	3	1
Minimal Haemorrhage ("Feathering")	7	5	3
Haemorrhage (Obvious leakage)	9	7	5
Coagulation and/or Thrombosis	11	9	7

Each reaction type can be recorded only once for each CAM, therefore the maximum score per CAM is 32. The mean score was determined for all CAM's similarly tested.

Each test substance was classified according to the following:

Mean Score	*Irritation Potential*
0.0-4.9	Practically none
5.0-9.9	Slight
10.0-14.9	Moderate
15.0-32.0	Severe

Remarks - Method: No details of test substance preparation were included. Johnson's Baby Shampoo (50%) and Prell Shampoo Concentrate (50%) were the reference articles included in the study. Johnson's Baby Shampoo has historically been categorised as being moderately irritating and Prell has previously been categorised as being severely irritating according to the scoring system above.

The study authors state that previous studies have shown that the CAM of the hen's egg is more sensitive to liquid irritants than the rabbit eye, therefore Johnson's Baby Shampoo and the Prell Shampoo concentrate were diluted and tested at 50% concentration to equate to the Draize results for those substances at 100% concentration. The study authors state that the sponsor requested the irritation potential of Promois EFLS-C at 2.5% (this equates to 0.5% notified chemical). Due to it being a liquid irritant a dilution of Promois EFLS-C to 1.25% was used in the actual test.

The Draft Updated ICCVAM Recommended HET-CAM Test Method Protocol (available online [20 July 2009]:

http://iccvam.niehs.nih.gov/methods/ocutox/mildmod/HET-CAMProtocol11May09FD.pdf) recommends testing solutions undiluted unless dilution is justified.

RESULTS

Test Solution	*Average Irritation score*
Promois EFLS–C (1.25%)	**7.75**
Johnson's Baby Shampoo (50 %)	11.00
Prell Shampoo Concentrate (50%)	24.25

Remarks - Results: At 30 seconds after exposure, one CAM showed hyperaemia and another CAM showed hyperaemia and minimal haemorrhage. At the 2-min reading, hyperaemia was observed in two CAMs and minimal haemorrhage was present in one CAM. At the 5-minute reading, three CAMs appeared normal and one CAM continued to show hyperaemia.

CONCLUSION: Under the conditions of this test, the test substance is predicted to be slightly irritating to the eye at a concentration of 2.5% (0.5% notified chemical).

TEST FACILITY: Consumer Product Testing (2004)

B.5. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE: Promois EFLS-C (20% notified chemical in aqueous solution)

METHOD: OECD TG 429 Skin Sensitisation: Local Lymph Node Assay

Species/Strain: Mouse/CBA/Ca (CBA/CaBkl) Female

Vehicle: Butanone

Remarks - Method: A preliminary screening test was conducted on one mouse with 25 µl of undiluted test substance applied to the dorsal surface of each ear for three consecutive days. No signs of systemic toxicity were noted. Based on this 25, 50 and 100% were selected as dose levels for the main test. The result of a positive control study with α-Hexylcinnamaldehyde in acetone/olive oil (4:1) which was conducted prior to the main study has been included.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	447.74	
25	646.76	1.44
50	796.12	1.78
100	1166.66	2.61
Positive Control (% v/v)		
5	-	1.741
10	-	2.20
25	-	8.89

Remarks - Results: There were no deaths and no signs of systemic toxicity were noted in the test or control animals. Body weight changes of the test animals were comparable to those seen in the control animals. A stimulation index of less than 3 was observed for all concentrations of the test material.

CONCLUSION: There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to up to 100% test substance (20% notified chemical).

TEST FACILITY: Safepharm (2004c)

B.6. Genotoxicity – bacteria

TEST SUBSTANCE	Promois EFLS-C (20% notified chemical in aqueous solution)
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA$^-$
Metabolic Activation System	Phenobarbitone/β-naphthoflavone-induced rat liver S9 preparation
Concentration Range in Main Test	a) With metabolic activation: 50-5000 µg/plate b) Without metabolic activation: 50-5000 µg/plate
Vehicle	Sterile distilled water
Remarks - Method	No significant protocol deviations

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5000	> 5000	> 5000	Negative
Present				
Test 1	>5000	> 5000	> 5000	Negative

Remarks - Results	No significant increases in the frequency of revertant colonies were recorded for any bacterial strain at any dose level either with or without metabolic activation. All positive controls induced expected increases in revertant colony frequency.
CONCLUSION	The test substance was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	SafePharm Laboratories (2004d)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Sodium cocoyl apple amino acids (acceptable analogue)
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test.
Inoculum	Activated sludge from the aeration pool of an STP receiving predominantly domestic sewage.
Exposure Period	29 d
Auxiliary Solvent	None reported
Analytical Monitoring	Elemental analysis, CO_2 measurement made by IC measurement using a TOC 5050A (Fa, Shimadzu).
Remarks - Method	No significant protocol deviations were reported.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
2	21.6	2	-0.3
5	48.7	5	40.5
7	62.2	7	62.05
9	71.2	9	72.75
13	79.2	13	78.6
20	88.1	20	82.45
28	94.5	28	85.05
29	93.2	29	83.25

Remarks - Results	The notified chemical met the 10 d window criterion for ready biodegradability. A significant quantity of CO_2 was emitted from a poisoned flask containing the test substance, which served as an abiotic control. Although this was interpreted by the study authors as evidence of abiotic degradation of the test substance, no rationalisation for the degradation of this surfactant to CO_2 under nominally sterile conditions was presented. All test validity criteria were satisfied.
CONCLUSION	The test substance is considered ready biodegradable.
TEST FACILITY	LAUS GmbH (2004)

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