GREEN

# Safety Assessment of Amino Acid Alkyl Amides Ingredients as Used in Cosmetics

CIR EXPERT PANEL MEETING

JUNE 10-11, 2013

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Christina L. Burnett
Scientific Writer/Analyst
Date: May 17, 2013
Subject: Draft Report on Amino Acid Alkyl Amides

In February 2013, CIR issued the Scientific Literature Review (SLR) for amino acid alkyl amides, which mainly function as skin and hair conditioning agents and as surfactants-cleansing agents in personal care products. There are 115 ingredients included in this report.

Unpublished data were provided by the Personal Care Products Council (Council) during the preparation of the SLR, and, since the February announcement, the Council has provided comments on the SLR which have been considered. The data have been incorporated into the report, and both the data and the comments can be found in this report's package.

As noted in the report's introduction, the Panel has recently reviewed the safety of α-amino acids and previously has reviewed several related fatty acid constituents. These supporting materials have not been included in this report's package; however, if they are needed during the course of your review of the materials, please contact me and I will gladly provide them to you.

According to the FDA's VCRP database, lauroyl lysine has the most reported uses in cosmetic and personal care products, with a total of 649; most uses are in leave-on eye and facial makeup. Sodium cocoyl glutamate has the second greatest number of overall uses reported, with a total of 178; more than half of those uses are in rinse-off products. In the Council's survey of use concentrations, lauroyl lysine is used up to concentrations of 45%, with the maximum concentration reported in lipsticks.

If no further data are needed, the Panel should issue a Tentative Report.

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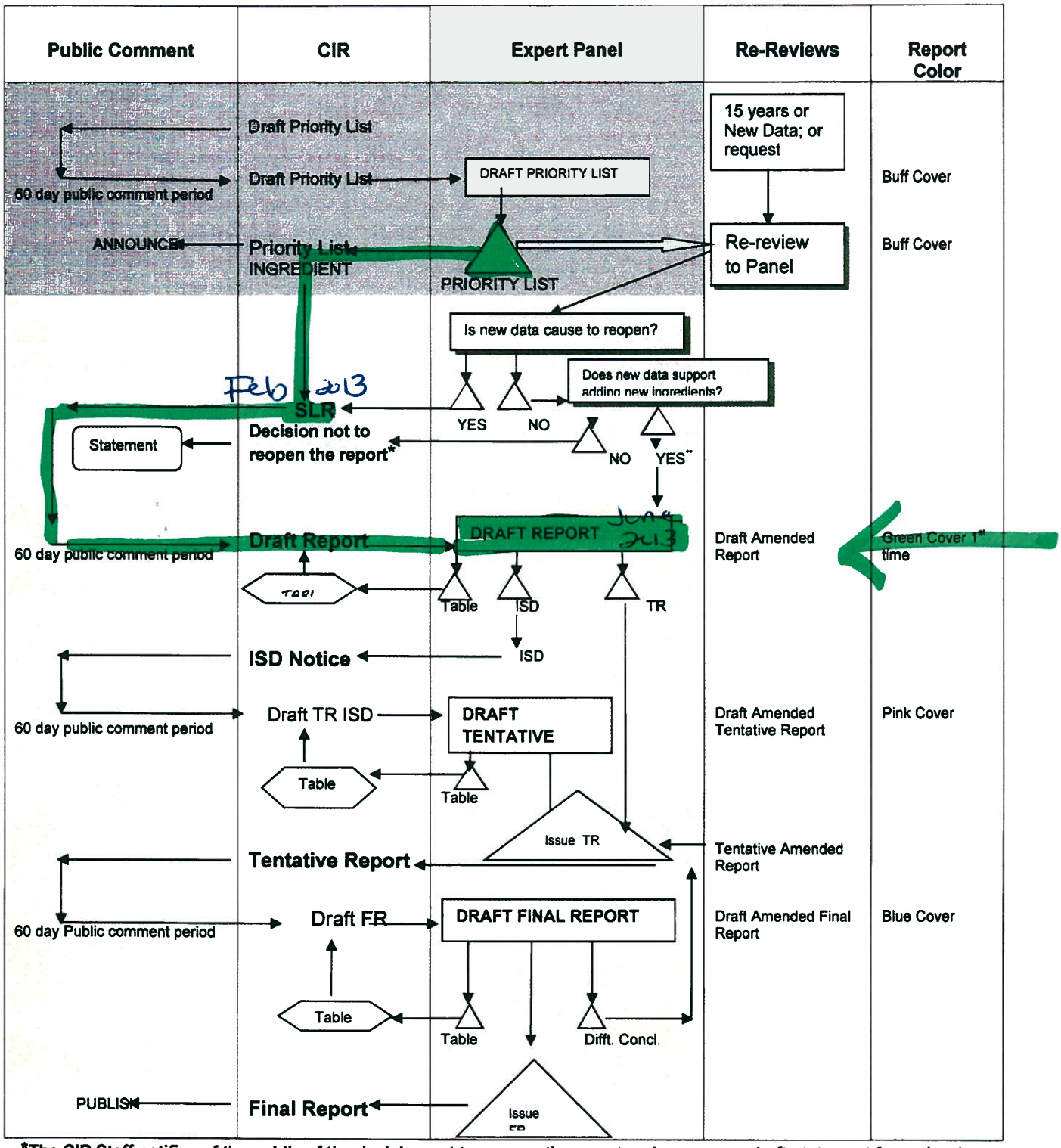
Amino Acid Alkyl Amides



June 2013

# SAFETY ASSESSMENT FLOW CHART




*The CIR Staff notifies of the public of the decision not to re-open the report and prepares a draft statement for review by the Panel. After Panel review, the statement is issued to the Public.

**If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.

Expert Panel Decision

Document for Panel Review

Option for Re-review

# Amino Acids Alkyl Amides History

**February 2013** – Scientific Literature Review announced.

| Amino Acid Alkyl Amides Data Profile* – June 2013 – Writer, Christina Burnett | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Reported Use | Chemical Properties | Irritation/ Sensitization - Animal | Irritation/ Sensitization - Clinical | Ocular/ Mucousal | Phototoxicity/ Photosensitization | Genotoxicity |
| **Acetyl Arginine** | | X | | | | | |
| **Acetyl Cysteine** | X | X | | | | | |
| **Acetyl Glutamic Acid** | | X | | | | | X |
| **Acetyl Glutamine** | X | X | | | | | |
| **Acetyl Methionine** | X | X | | | | | |
| **Acetyl Proline** | | | X | X | X | | X |
| **Acetyl Tyrosine** | X | X | | X | X | X | X |
| **Capryloyl Glycine** | X | X | | | | | |
| **Cocoyl Glutamic Acid** | X | | | | | | |
| **Dipalmitoyl Cystine** | | X | | | | | |
| **Disodium Capryloyl Glutamate** | X | X | X | X | X | | X |
| **Disodium Cocoyl Glutamate** | X | | | | | | |
| **Disodium Hydrogenated Tallow Glutamate** | X | | | | | | |
| **Disodium Lauroyl Glutamate** | X | | | | | | |
| **Disodium Malyl Tyrosinate** | X | | | | | | |
| **Disodium Stearoyl Glutamate** | X | | | | | | |
| **Lauroyl Arginine** | X | X | | | X | | |
| **Lauroyl Collagen Amino Acids** | X | | | | | | |
| **Lauroyl Glutamic Acid** | | X | | | | | |
| **Lauroyl Lysine** | X | | | | | | |
| **Lauroyl Proline** | X | X | | | | | |
| **Lauroyl Silk Amino Acids** | X | | | | | | |
| **Magnesium Palmitoyl Glutamate** | X | | | | | | |
| **Oleoyl Tyrosine** | X | | | | | | |
| **Palmitoyl Alanine** | | X | | | | | |
| **Palmitoyl Arginine** | | X | | | | | |
| **Palmitoyl Collagen Amino Acids** | X | | | | | | |
| **Palmitoyl Glutamic Acid** | | X | | | | | |
| **Palmitoyl Glycine** | X | X | | | | | |
| **Palmitoyl Isoleucine** | | X | | | | | |
| **Palmitoyl Keratin Amino Acids** | X | | | | | | |
| **Palmitoyl Proline** | X | X | | | | | |
| **Palmitoyl Silk Amino Acids** | X | | | | | | |
| **Potassium Cocoyl Glutamate** | X | | | | | | |
| **Potassium Cocoyl Glycinate** | X | | | | | | |
| **Potassium Lauroyl Wheat Amino Acids** | X | | | | | | |
| **Potassium Myristoyl Glutamate** | X | | | | | | |
| **Sodium Cocoyl Alaninate** | X | | | | | | |
| **Sodium Cocoyl Amino Acids** | X | | | | | | |
| **Sodium Cocoyl Apple Amino Acids** | X | | | | | | |
| **Sodium Cocoyl Collagen Amino Acids** | X | | | | | | |
| **Sodium Cocoyl Glutamate** | X | | | X | X | X | X |
| **Sodium Cocoyl Glycinate** | X | | | | | | |
| **Sodium Hydrogenated Tallowoyl Glutamate** | X | | | | | | |
| **Sodium Lauroyl Aspartate** | X | | | | | | |
| **Sodium Lauroyl Glutamate** | X | X | | X | X | X | X |
| **Sodium Lauroyl Oat Amino Acids** | X | | | | | | |
| **Sodium Lauroyl Wheat Amino Acids** | X | | | | | | |

| Amino Acid Alkyl Amides Data Profile* – June 2013 – Writer, Christina Burnett | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Reported Use | Chemical Properties | Irritation/ Sensitization - Animal | Irritation/ Sensitization - Clinical | Ocular/ Mucousal | Phototoxicity/ Photosensitization | Genotoxicity |
| **Sodium Myristoyl Glutamate** | X | | | | | | |
| **Sodium Palmitoyl Proline** | X | | | | | | |
| **Sodium Palmoyl Glutamate** | X | | | | | | |
| **Sodium Stearoyl Glutamate** | X | | | | | | |
| **Stearoyl Glutamic Acid** | | X | | | | | |
| **Stearoyl Leucine** | | X | | | | | |
| **TEA-Cocoyl Alaninate** | X | | | | | | |
| **TEA-Cocoyl Glutamate** | X | | | | | | |
| **TEA-Lauroyl Collagen Amino Acids** | X | | | | | | |
| **TEA-Lauroyl Glutamate** | X | | | | | | |
| **Undecylenoyl Collagen Amino Acids** | X | | | | | | |
| **Undecylenoyl Glycine** | X | | | | | | |
| **Undecylenoyl Phenylalanine** | X | X | | | | | |
| **NO USES OR DATA WERE AVAILABLE FOR THE REMAINING AMINO ACID ALKYL AMIDES IN TABLE 1.** | | | | | | | |

*"X" indicates that data were available in a category for the ingredient

**SEARCH STRATEGY FOR Amino Acid Alkyl Amides**

September 2012-November 2012: SCIFINDER search for Amino Acid Alkyl Amides:

- Using CAS #, 49 chemicals identified.
- Using name, 143 chemicals identified.
- Using properties, 50 chemicals identified.

Combined answer sets and deleted duplicates, limited to adverse reactions = 3095.
Limiting this list to document type and specific exposure types = 35.

November 2012 Merck search of CAS #/name yielded 5 hits

Searches also performed using TOXLINE and PUBMED using CAS # and names.

Searches difficult to perform due to nomenclature (amino acid name base).
Limits were not productive.
Little relevant data were discovered.

## All searches limited to dermal exposures.

**Total references ordered: 34**

# Safety Assessment of Amino Acid Alkyl Amides as Used in Cosmetics

Status: Draft Report for CIR Expert Panel Review
Release Date: May 17, 2013
Panel Meeting Date: June 10-11, 2013

The 2013 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Christina Burnett, Scientific Analyst/Writer, and Bart Heldreth, Ph.D., Chemist CIR.

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**Table of Contents**

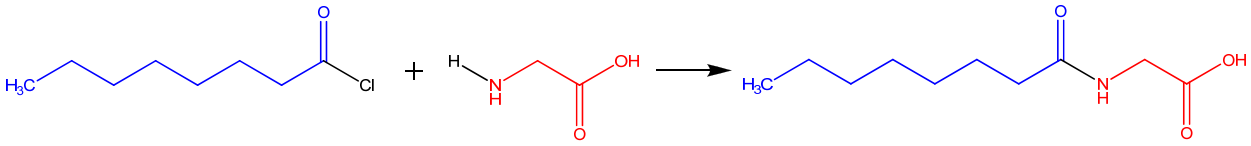
## INTRODUCTION

This safety assessment summarizes the available data relevant to assessing the safety of 115 amino acid alkyl amides as used in cosmetics. These ingredients mainly function as skin and hair conditioning agents and as surfactants-cleansing agents in personal care products. The list of ingredients in this report is found in Table 1.

By and large, the ingredients in this report will not rapidly dissociate (beyond zwitterion formation) in the presence of water, but action by amidases is the most likely first step of metabolism if dermal penetration occurs. The relative exposure, hence, would also include amino acids and fatty acids. Concurrent reviews of the safety of animal- and plant-derived amino acids and hydrolyzed protein ingredients as they are used in cosmetics are being performed by the Cosmetic Ingredient Review (CIR) Expert Panel. The Panel previously has reviewed the safety of α-amino acids and concluded that these ingredients are safe for use in cosmetic ingredients.[1] The Panel also reviewed the following fatty acid constituents and concluded that these fatty acids are safe for use as cosmetic ingredients: coconut acid, olive acid, sunflower seed acid, palm acid, acetic acid, lauric acid, oleic acid, palmitic acid, stearic acid, and myristic acid.[2-8] The Panel concluded that malic acid was safe for use as a pH adjuster but the data were insufficient to determine safety for any other functions.[9] The maximum concentrations of use along with summaries of the data included in those existing safety assessments are provided in Table 2.

## CHEMISTRY

The amino acid alkyl amides in this report are comprised of amino acids acylated with acids or acid chlorides at the amino acid nitrogen, to form amides. For example, capryloyl glycine is the *N*-acylation product of glycine with caprylic acid chloride.




Caprylic Acid Chloride (a.k.a. Capryloyl Chloride) | Glycine | Capryloyl Glycine

Figure 1. Synthesis of the amino acid alkyl amide, Capryloyl Glycine.

A likely metabolic pathway for these ingredients is to be acted upon by amidases, should they penetrate the skin. The net result would be the release of the amino acid (glycine in the above case) and a fatty acid (caprylic acid in the above case).

The definitions of the amino acid alkyl amides can be found in Table 1 and the structures can be found in Table 3.

### Physical and Chemical Properties

The ingredients in this report are typically water soluble, waxy solids. Available chemical properties can be found in Table 4.

### Method of Manufacturing

As shown in Figure 1, the ingredients in this report are most commonly manufactured by the acylation of a free amine of an amino acid with an acyl chloride, a reaction known as the Schotten-Baumann reaction.[10-12] The major side product for this reaction is hydrochloric acid, which can be easily removed.

Disodium Capryloyl Glutamate, Sodium Cocoyl Glutamate and Sodium Lauroyl Glutamate

According to a supplier, disodium capryloyl glutamate, sodium cocoyl glutamate, and sodium lauroyl glutamate are produced via the Schotten-Baumann reaction.[13-15] The supplier also described the origin of starting materials: glutamic acid is obtained through formation of glucose/molasses or from wheat and capryloyl chloride, cocoyl chloride, and lauroyl chloride are obtained from caprylic acid, coconut acid and lauric acid that come from cleavage and distillation of coconut oil. The respective resultant materials are aqueous solutions comprised of 37%-41% disodium capryloyl glutamate, 32.6%-38% sodium cocoyl glutamate, and 36%-40% sodium lauroyl glutamate.

### Impurities

Disodium Capryloyl Glutamate

A supplier has reported that disodium capryloyl glutamate may contain 4%-6% propylene glycol, 3% caprylic acid (max.), 5% disodium glutamate (max.), and 6-8% sodium chloride.[13] Disodium capryloyl glutamate

may also contain < 2 ppm arsenic, < 5 ppm antimony, < 1 ppm lead, < 2 ppm cadmium, < 2 ppm mercury, < 1 ppm nickel, < 2 ppm chromium, and < 10 ppm total heavy metals (as iron).

Sodium Cocoyl Glutamate

The same supplier has reported that sodium cocoyl glutamate may contain 4%-6% propylene glycol, 5% (max.) sodium glutamate, 3% coconut acid, and 4%-5.5% sodium chloride.[15] Sodium cocoyl glutamate may also contain < 2 ppm arsenic, < 5 ppm antimony, < 1 ppm lead, < 2 ppm cadmium, < 2 ppm mercury, < 1 ppm nickel, < 2 ppm chromium, and < 10 ppm total heavy metals (as iron).

Sodium Lauroyl Glutamate

A supplier has reported that sodium lauroyl glutamate may contain 4%-6% propylene glycol, 5% (max.) glutamic acid, 3% (max.) lauric acid, and 3%-4.5% sodium chloride.[14] Sodium lauroyl glutamate may also contain < 2 ppm arsenic, < 5 ppm antimony, < 1 ppm lead, < 2 ppm cadmium, < 2 ppm mercury, < 1 ppm nickel, < 2 ppm chromium, and < 10 ppm total heavy metals (as iron).

## USE

### Cosmetic

Table 5a presents the current product-formulation data for amino acid alkyl amides. These ingredients function primarily as skin and hair conditioning agents and surfactants.[16] According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), lauroyl lysine has the most reported uses in cosmetic and personal care products, with a total of 649; most uses are in leave-on eye and facial makeup.[17] Sodium cocoyl glutamate has the second greatest number of overall uses reported, with a total of 178; more than half of those uses are in rinse-off products.

In the Personal Care Products Council's use concentration survey, lauroyl lysine had a wide maximum use concentration range of 0.001% to 45% with the 45% reported in lipsticks. Sodium lauroyl glutamate also had a wide maximum use concentration range of 0.003% to 40%, with the 40% reported in skin cleansing agents. All other use concentrations that were reported had similar ranges

In some cases, reports of uses were received from the VCRP, but no concentration of use data were available. For example, palmitoyl keratin amino acids are reported to be used in 5 formulations, but no use concentration data were available. In other cases, no reported uses were received from the VCRP, but a use concentration was provided in the industry survey. For example, cocoyl glutamic acid was not reported in the VCRP database to be in use, but the industry survey indicated that it is used in leave-on formulations at maximum concentrations ranging from 24%. It should be presumed that cocoyl glutamic acid is used in at least one cosmetic formulation.

Those ingredients with no reported uses or use concentrations are listed in Table 5b.

In the European Union, trialkylamines, trialkanolamines, and their salts (ingredients containing TEA) may only be used up to 2.5%, must be at least 99% pure, are not to be used with nitrosating systems, must have a maximum secondary amine content of 0.5%, must have a maximum nitrosamine content of 50 μg/kg, and must be kept in nitrite-free containers.[18] The remaining ingredients are not restricted from use in any way under the rules governing cosmetic products in the European Union.

### Non-Cosmetic

Amino acid alkyl amides are used in household detergents.[19]

Acetyl cysteine has been approved by the FDA to treat acetaminophen overdose and as a mucolytic therapy.[20] Acetyl methionine is an approved direct food additive (21 CFR §172.372).

## TOXICOKINETICS

### Absorption, Distribution, Metabolism, Excretion

A percutaneous absorption study of 3 formulations containing 1.75% acetyl tyrosinamide was performed in vitro on human trunk skin using the finite dose technique and Franz diffusion cells.[21] The formulations were a gel, a cream, and a water solution in silicone. Each formulation was evaluated on 3 replicate sections from 2 different ex vivo human trunk skin donors. At dosing, 10 mg formulation/$cm^2$/skin-section equivalent volume was dispensed by pipette and a glass rod was used to evenly distribute the formulation into the skin. The percutaneous absorption of the test material was determined over a 48-h dose period. At 6, 12, 32, and 48 h after application, the dermal receptor solution was removed in its entirety, replaced with stock receptor solution, and 4 ml aliquot was saved for subsequent analysis. After the last receptor solution collection, the skin surface was washed twice with

50:50 methanol:water to collect unabsorbed formulation from the skin. The glass rod used for dosing, the surface wash, stratum corneum, epidermis, and dermis were recovered and evaluated for compound content. The samples were analyzed for test material content using high performance liquid chromatography (HPLC) method.

In the formulation with water, the test material was found in the following mean distribution: 0.479% in receptor solution, 0.038% in dermis, 1.252% in epidermis, 4.639% in stratum corneum, and 83.15% in surface wash (total recovery was 89.554%). For the gel formulation, the test material was found in the following mean distribution: 1.031% in receptor solution, 0.066% in dermis, 1.149% in epidermis, 0.695% in stratum corneum, and 88.59% in surface wash (total recovery was 91.532%). Finally, in the cream formulation, the test material was found in the following mean distribution: 2.702% in the receptor solution, 0.386% in the dermis, 15.963% in the epidermis, 11.909% in the stratum corneum, and 54.336% in the surface wash (total recovery was 85.296%). The authors of the study concluded that acetyl tyrosinamide does penetrate into and through ex vivo human skin using the in vitro finite dose model in all 3 formulations evaluated. The authors noted that one donor skin exhibited greater permeability to acetyl tyrosinamide than the other.[21]

## TOXICOLOGICAL STUDIES

The amino acids alkyl amides in this assessment most likely dissociate into amino acids and fatty acids in the presence of water. Because most of these amino acids and fatty acids are found in the foods we consume daily, oral toxicity is not expected. In turn, dermal toxicity would not be expected to be different from oral exposures. Irritation and sensitization are of concern, and the focus in this report. Data from the previous safety assessments on α-amino acids and fatty acids support that these ingredients would not likely be irritants or sensitizers.

## REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No published reproductive and developmental toxicity studies on amino acid alkyl amides were discovered and no unpublished data were submitted.

## GENOTOXICITY

In vitro and in vivo genotoxicity studies are presented in Table 6. In in vitro studies, acetyl glutamic acid, acetyl proline, acetyl tyrosinamide, disodium capryloyl glutamate, sodium cocoyl glutamate, and sodium lauroyl glutamate were negative for genotoxicity. Acetyl glutamic acid was negative in an in vivo study.

## CARCINOGENICITY

No published carcinogenicity studies on amino acid alkyl amides were discovered and no unpublished data were submitted.

## IRRITATION AND SENSITIZATION

*[From the CIR Safety Assessment of α-amino acids][1]: Cysteine HCl and methionine were used as negative controls in in vitro assays to predict potential skin irritants. In separate efficacy studies, arginine, cysteine, and glycine did not produce any adverse effects in rats, guinea pigs, or mouse skin models. Glutamic acid was used as a negative control in an in vitro study to identify skin sensitizers. HRIPT studies of many products containing amino acid ingredients concluded that products containing these ingredients were not dermal irritants or sensitizers. In several validation studies for in vitro phototoxicity assays, histidine was used as a negative control. Magnesium aspartate up to 0.5% and 1% tyrosine were not phototoxic in assays using yeast.*

### Irritation

In vitro and human dermal irritation studies are presented in Table 7. No irritation was observed in in vitro studies with disodium capryloyl glutamate. Acetyl proline was a mild irritant in another in vitro study. In human studies, acetyl proline, acetyl tyrosinamide, disodium capryloyl glutamate, sodium cocoyl glutamate, and sodium lauroyl glutamate were not dermal irritants.

***Ocular***

Non-human and human ocular irritation studies are presented in Table 8. No ocular irritation was observed in in vitro studies of acetyl tyrosinamide, disodium capryloyl glutamate, and sodium lauroyl glutamate. Severe irritation was observed in 1 study of sodium cocoyl glutamate at 5%, but was not irritating in another study with an unknown concentration. No adverse effects were observed during in-use studies of acetyl hydroxyproline and acetyl tyrosinamide in human subjects.

### Sensitization

Non-human and human dermal sensitization studies are presented in Table 9. No sensitization was observed in human studies with acetyl hydroxyproline, acetyl proline, acetyl tyrosinamide, disodium capryloyl glutamate, sodium cocoyl glutamate, and sodium lauroyl glutamate.

Phototoxicity

Non-human and human phototoxicity studies are presented in Table 10. In non-human and human studies, acetyl tyrosinamide was not phototoxic. Sodium cocoyl glutamate and sodium lauroyl glutamate were not phototoxic in human studies.

## CLINICAL USE

No relevant published clinical use studies on amino acid alkyl amides were discovered and no unpublished data were submitted.

## SUMMARY

The 115 amino acid alkyl amides mainly function as skin and hair conditioning agents and as surfactants-cleansing agents in personal care products. These ingredients are comprised of amino acids acylated with acids or acid chlorides at the amino acid nitrogen to form amides. By and large, the ingredients in this report will not rapidly dissociate (beyond zwitterion formation) in the presence of water, but action by amidases is the most likely first step of metabolism if dermal penetration occurs. The relative exposure, hence, would also include amino acids and fatty acids.

Lauroyl lysine has the most reported uses in cosmetic and personal care products, with a total of 649; most uses are in leave-on eye and facial makeup. Sodium cocoyl glutamate has the second greatest number of overall uses reported, with a total of 178; more than half of those uses are in rinse-off products. Lauroyl lysine is used up to concentrations of 45%, with the maximum concentration reported in lipsticks.

In the European Union, trialkylamines, trialkanolamines, and their salts (ingredients containing TEA) may only be used up to 2.5%, must be at least 99% pure, are not to be used with nitrosating systems, must have a maximum secondary amine content of 0.5%, must have a maximum nitrosamine content of 50 μg/kg, and must be kept in nitrite-free containers. The remaining ingredients are not restricted from use in any way under the rules governing cosmetic products in the European Union.

Amino acid alkyl amides are used in household detergents. The FDA has approved acetyl cysteine in drug therapies. Acetyl methionine is an approved direct food additive.

In a study of 3 formulations containing 1.75% acetyl tyrosinamide, the test material was found to penetrate into and through ex vivo human skin.

In in vitro studies, acetyl glutamic acid, acetyl proline, acetyl tyrosinamide, disodium capryloyl glutamate, sodium cocoyl glutamate, and sodium lauroyl glutamate were negative for genotoxicity. Acetyl glutamic acid was negative in an in vivo study.

No dermal irritation was observed in in vitro studies with disodium capryloyl glutamate. Acetyl proline was a mild irritant in another in vitro study. In human studies, acetyl proline, acetyl tyrosinamide, disodium capryloyl glutamate, sodium cocoyl glutamate, and sodium lauroyl glutamate were not dermal irritants.

No ocular irritation was observed in in vitro studies of acetyl tyrosinamide, disodium capryloyl glutamate, and sodium lauroyl glutamate. Severe irritation was observed in 1 study of sodium cocoyl glutamate at 5%, but was not irritating in another study with an unknown concentration. No adverse effects were observed during in-use studies of acetyl hydroxyproline and acetyl tyrosinamide in human subjects.

No sensitization was observed in human studies with acetyl hydroxyproline, acetyl proline, acetyl tyrosinamide, disodium capryloyl glutamate, sodium cocoyl glutamate, and sodium lauroyl glutamate.

In non-human and human studies, acetyl tyrosinamide was not phototoxic. Sodium cocoyl glutamate and sodium lauroyl glutamate were not phototoxic in human studies.

No published reproductive and development toxicity, carcinogenicity, nor relevant clinical use studies on amino acid alkyl amides were discovered and no unpublished data were submitted.

## DISCUSSION

To be determined.

## CONCLUSION

To be determined.

## TABLES AND FIGURES

**Table 1.** Definitions and functions of the Amino Acid Alkyl Amides in this safety assessment.[16] (Any italicized text below represents additions made by CIR staff.)

| Ingredient CAS No. | Definition | Function |
|---|---|---|
| Acetyl Arginine<br>210545-23-6 | Acetyl Arginine is the substituted amino acid that conforms to the formula. *Acetyl Arginine is the amide formed from the reaction of acetic acid chloride and arginine.* | humectants; skin-conditioning agents - emollient |
| Acetyl Cysteine<br>616-91-1 | Acetyl Cysteine is the organic compound that conforms to the formula. *Acetyl Cysteine is the amide formed from the reaction of acetic acid chloride and cysteine.* | antioxidants; skin-conditioning agents – misc. |
| Acetyl Glutamic Acid<br>1188-37-0 | Acetyl Glutamic Acid is the substituted amino acid that conforms to the formula. *Acetyl Glutamic Acid is the amide formed from the reaction of acetic acid chloride and glutamic acid.* | skin-conditioning agents – misc. |
| Acetyl Glutamine<br>2490-97-3<br>35305-74-9 | Acetyl Glutamine is the organic compound that conforms to the formula. *Acetyl Glutamine is the amide formed from the reaction of acetic acid chloride and glutamine.* | skin-conditioning agents – misc. |
| Acetyl Histidine<br>39145-52-3 | Acetyl Histidine is the organic compound that conforms to the formula. *Acetyl Histidine is the amide formed from the reaction of acetic acid chloride and histidine.* | skin-conditioning agents - emollient; skin-conditioning agents - humectant |
| Acetyl Methionine<br>1115-47-5<br>65-82-7 | Acetyl Methionine is the substituted amino acid that conforms to the formula. *Acetyl Methionine is the amide formed from the reaction of acetic acid chloride and methionine.* | skin-conditioning agents-misc. |
| Acetyl Proline<br>68-95-1 | Acetyl Proline is the substituted amino acid that conforms to the formula. *Acetyl Proline is the amide formed from the reaction of acetic acid chloride and proline.* | skin-conditioning agents-emollient |
| Acetyl Tyrosine<br>537-55-3 | Acetyl Tyrosine is the organic compound that conforms to the formula. *Acetyl Tyrosine is the amide formed from the reaction of acetic acid chloride and tyrosine.* | skin-conditioning agents-misc. |
| Capryloyl Collagen Amino Acids | Capryloyl Collagen Amino Acids is the condensation product of caprylic acid chloride with Collagen Amino Acids. | hair conditioning agents; surfactants-cleansing agents |
| Capryloyl Glycine<br>14246-53-8 | Capryloyl Glycine is the acylation product of glycine with caprylic acid chloride. | hair conditioning agents; surfactants-cleansing agents |
| Capryloyl Gold of Pleasure Amino Acids | Capryloyl Gold of Pleasure Amino Acids is the condensation product of caprylic acid chloride and the amino acids derived from the complete hydrolysis of the protein fraction obtained from the seeds of *Camelina sativa*. | cosmetic biocides; deodorant agents |
| Capryloyl Keratin Amino Acids | Capryloyl Keratin Amino Acids is the condensation product of caprylic acid chloride with Keratin Amino Acids. | hair conditioning agents; surfactants-cleansing agents |
| Capryloyl Pea Amino Acids | Capryloyl Pea Amino Acids is the product obtained by the condensation of caprylic acid chloride and pea amino acids. | hair conditioning agents; skin-conditioning agents-misc. |
| Capryloyl Quinoa Amino Acids | Capryloyl Quinoa Amino Acids is the condensation product of caprylic acid chloride and amino acids obtained from the complete hydrolysis of the protein obtained from the seeds of *Chenopodium quinoa*. | hair conditioning agents; skin-conditioning agents-misc. |
| Capryloyl Silk Amino Acids | Capryloyl Silk Amino Acids is the product obtained by the condensation of caprylic acid chloride with Silk Amino Acids. | hair conditioning agents; surfactants-cleansing agents |
| Cocoyl Glutamic Acid | Cocoyl Glutamic Acid is the Coconut Acid amide of Glutamic Acid that conforms to the formula. | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Dipalmitoyl Cystine<br>17627-10-0 | Dipalmitoyl Cystine is the product obtained by acylation of cystine with palmitoyl chloride. | hair conditioning agents |
| Dipotassium Capryloyl Glutamate | Dipotassium Capryloyl Glutamate is the organic compound that conforms to the formula. *Dipotassium Capryloyl Glutamate is the dipotassium salt of the amide formed from the reaction of capryloyl chloride and glutamic acid.* | deodorant agents; surfactants-cleansing agents |
| Dipotassium Undecylenoyl Glutamate | Dipotassium Undecylenoyl Glutamate is the substituted amino acid that conforms to the formula. *Dipotassium Undecylenoyl Glutamate is the dipotassium salt of the amide formed from the reaction of undecenoyl chloride and glutamic acid.* | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Disodium Capryloyl Glutamate | Disodium Capryloyl Glutamate is the organic compound that conforms to the formula. *Disodum Capryloyl Glutamate is the disodium salt of the amide formed from the reaction of capryloyl chloride and glutamic acid.* | deodorant agents; surfactants-cleansing agents |

**Table 1.** Definitions and functions of the Amino Acid Alkyl Amides in this safety assessment.[16] (Any italicized text below represents additions made by CIR staff.)

| Ingredient CAS No. | Definition | Function |
|---|---|---|
| Disodium Cocoyl Glutamate 68187-30-4 | Disodium Cocoyl Glutamate is the disodium salt of the coconut acid amide of glutamic acid. It conforms generally to the formula. | surfactants-cleansing agents |
| Disodium Hydrogenated Tallow Glutamate | Disodium Hydrogenated Tallow Glutamate is the disodium salt of the hydrogenated tallow acid amide of Glutamic Acid. It conforms generally to the formula. | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Disodium N-Lauroyl Aspartate | Disodium N-Lauroyl Aspartate is the organic compound that conforms to the formula. *Disodium N-Lauroyl Aspartate is the disodium salt of the amide formed from the reaction of lauroyl chloride and aspartic acid.* | surfactants-cleansing agents |
| Disodium Lauroyl Glutamate | Disodium Lauroyl Glutamate is the organic compound that conforms to the formula. *Disodium Lauroyl Glutamate is the disodium salt of the amide formed from the reaction of lauroyl chloride and glutamic acid.* | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Disodium Malyl Tyrosinate 126139-79-5 | Disodium Malyl Tyrosinate is the organic compound that conforms to the formula. *Disodium Malyl Tyrosinate is the disodium salt of the amide formed from the reaction of malyl chloride and tyrosine.* | skin-conditioning agents-misc. |
| Disodium Stearoyl Glutamate 38079-62-8 | Disodium Stearoyl Glutamate is the organic compound that conforms to the formula. *Disodium Stearoyl Glutamate is the disodium salt of the amide formed from the reaction of stearoyl chloride and glutamic acid.* | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Disodium Undecylenoyl Glutamate | Disodium Undecylenoyl Glutamate is the substituted amino acid that conforms to the formula. *Disodium Undecenoyl Glutamate is the disodium salt of the amide formed from the reaction of undecenoyl chloride and glutamic acid.* | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Lauroyl Arginine 42492-22-8 | Lauroyl Arginine is the substituted amino acid that conforms to the formula. *Lauroyl Arginine is the amide formed from the reaction of lauroyl chloride and arginine.* | hair conditioning agents; skin-conditioning agents-emollient |
| Lauroyl Collagen Amino Acids 68920-59-2 | Lauroyl Collagen Amino Acids is the product obtained by the condensation of lauric acid chloride with Collagen Amino Acids. | hair conditioning agents; surfactants-cleansing agents |
| Lauroyl Glutamic Acid 3397-65-7 | Lauroyl Glutamic Acid is the substituted amino acid that conforms to the formula. *Lauroyl Glutamic Acid is the amide formed from the reaction of lauroyl chloride and glutamic acid.* | skin-conditioning agents-misc. |
| Lauroyl Lysine 52315-75-0 | Lauroyl Lysine is the lauroyl derivative of Lysine that conforms to the formula. *Lauroyl Lysine is the amide formed from the reaction of lauroyl chloride and lysine.* | hair conditioning agents; skin-conditioning agents-misc. |
| Lauroyl Proline 58725-39-6 | Lauroyl Proline is the organic compound that conforms to the formula. *Lauroyl Proline is the amide formed from the reaction of lauroyl chloride and proline.* | hair conditioning agents; skin-conditioning agents – misc. |
| Lauroyl Silk Amino Acids | Lauroyl Silk Amino Acids is the product obtained by the condensation of lauric acid chloride and Silk Amino Acids. | hair conditioning agents; surfactants-cleansing agents |
| Magnesium Palmitoyl Glutamate 57539-47-6 | Magnesium Palmitoyl Glutamate is the substituted amino acid that conforms to the formula. *Magnesium Palmitoyl Glutamate is the magnesium salt of the amide formed from the reaction of palmitoyl chloride and glutamic acid.* | skin-conditioning agents - misc. |
| Myristoyl Glutamic Acid | Myristoyl Glutamic Acid is the substituted amino acid that conforms to the formula. *Myristoyl Glutamic Acid is the amide formed from the reaction of myristoyl chloride and glutamic acid.* | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Oleoyl Tyrosine | Oleoyl Tyrosine is the organic compound that conforms to the formula. *Oleoyl Tyrosine is the amide formed from the reaction of oleoyl chloride and tyrosine.* | skin-conditioning agents-misc. |
| Palmitoyl Alanine 56255-31-3 | Palmitoyl Alanine is the substituted amino acid that conforms to the formula. *Palmitoyl Alanine is the amide formed from the reaction of palmitoyl chloride and alanine.* | skin protectants |
| Palmitoyl Arginine 58725-47-6 | Palmitoyl Arginine is the organic compound that conforms to the formula. *Palmitoyl Arginine is the amide formed from the reaction of palmitoyl chloride and arginine..* | hair conditioning agents; skin-conditioning agents-emollient |
| Palmitoyl Collagen Amino Acids | Palmitoyl Collagen Amino Acids is the condensation product of palmitic acid chloride and Collagen Amino Acids. | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |

**Table 1.** Definitions and functions of the Amino Acid Alkyl Amides in this safety assessment.[16] (Any italicized text below represents additions made by CIR staff.)

| Ingredient CAS No. | Definition | Function |
|---|---|---|
| Palmitoyl Glutamic Acid 38079-66-2 | Palmitoyl Glutamic Acid is the substituted amino acid that conforms to the formula. *Palmitoyl Glutamic Acid is the amide formed from the reaction of palmitoyl chloride and glutamic acid.* | skin-conditioning agents-misc. |
| Palmitoyl Glycine 2441-41-0 | Palmitoyl Glycine is the acylation product of glycine with palmitic acid chloride. | hair conditioning agents; surfactants-cleansing agents |
| Palmitoyl Gold of Pleasure Amino Acids | Palmitoyl Gold of Pleasure Amino Acids is the condensation product of palmitic acid chloride and the amino acids obtained from the complete hydrolysis of the protein fraction derived from the seeds of gold of pleasure. | hair conditioning agents; skin-conditioning agents-emollient |
| Palmitoyl Isoleucine 54617-29-7 | Palmitoyl Isoleucine is the substituted amino acid that conforms to the formula. *Palmitoyl Isoleucine is the amide formed from the reaction of palmitoyl chloride and isoleucine.* | skin protectants |
| Palmitoyl Keratin Amino Acids | Palmitoyl Keratin Amino Acids is the condensation product of palmitic acid chloride and Keratin Amino Acids. | hair conditioning agents;skin-conditioning agents-misc.; surfactants-cleansing agents |
| Palmitoyl Millet Amino Acids | Palmitoyl Millet Amino Acids is the condensation product of palmitic acid chloride and the amino acids obtained from the complete hydrolysis of the protein fraction of *Panicum miliaceum.* | hair conditioning agents; skin-conditioning agents-emollient |
| Palmitoyl Oat Amino Acids | Palmitoyl Oat Amino Acids is the condensation product of palmitic acid chloride and the amino acids obtained from the complete hydrolysis of the protein fraction of *Avena sativa* (Oat). | hair conditioning agents; skin-conditioning agents-emollient |
| Palmitoyl Pea Amino Acids | Palmitoyl Pea Amino Acids is the condensation product of palmitic acid chloride and pea amino acids. | hair conditioning agents;skin-conditioning agents-misc. |
| Palmitoyl Proline 59441-32-6 | Palmitoyl Proline is the product obtained by the condensation of palmitic acid chloride with Proline. | none reported |
| Palmitoyl Quinoa Amino Acids | Palmitoyl Quinoa Amino Acids is the condensation product of palmitic acid chloride and the amino acids obtained from the complete hydrolysis of the protein fraction derived from the seeds of *Chenopodium quinoa.* | hair conditioning agents; skin-conditioning agents-misc. |
| Palmitoyl Silk Amino Acids | Palmitoyl Silk Amino Acids is the condensation product of palmitic acid chloride and Silk Amino Acids. | hair conditioning agents; surfactants-cleansing agents |
| Potassium Caproyl Tyrosine | Potassium Caproyl Tyrosine is the organic compound that conforms to the formula. *Potassium Caproyl Tyrosine is the potassium salt of the amide formed from the reaction of caproyl chloride and tyrosine.* | skin-conditioning agents - misc |
| Potassium Capryloyl Glutamate | Potassium Capryloyl Glutamate is the substituted amino acid that conforms to the formula. *Potassium Capryloyl Glutamate is the potassium salt of the amide formed from the reaction of capryloyl chloride and glutamic acid.* | deodorant agents; surfactants-cleansing agents |
| Potassium Cocoyl Glutamate | Potassium Cocoyl Glutamate is the mixed potassium salts of the coconut acid amide of glutamic acid. It conforms generally to the formula. | hair conditioning agents; surfactants-cleansing agents |
| Potassium Cocoyl Glycinate 301341-58-2 | Potassium Cocoyl Glycinate is the organic compound that conforms to the formula. *Potassium Cocoyl Glycinate is the potassium salt of the amide formed from the reaction of coconut acid chloride and glycine.* | hair conditioning agents; surfactants-cleansing agents |
| Potassium Cocoyl Rice Amino Acids | Potassium Cocoyl Rice Amino Acids is the potassium salt of the product obtained by the reaction of coconut acid chloride with Rice Amino Acids. | skin-conditioning agents - emollient; skin-conditioning agents – misc.; surfactants - emulsifying agents; surfactants - foam boosters |
| Potassium Lauroyl Collagen Amino Acids | Potassium Lauroyl Collagen Amino Acids is the potassium salt of the condensation product of lauric acid chloride and Collagen Amino Acids. | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Potassium Lauroyl Glutamate 89187-78-0 (L-) | Potassium Lauroyl Glutamate is the substituted amino acid that conforms to the formula. *Potassium Lauroyl Glutamate is the potassium salt of the amide formed from the reaction of lauroyl chloride and glutamic acid.* | hair conditioning agents; surfactants-cleansing agents |
| Potassium Lauroyl Oat Amino Acids | Potassium Lauroyl Oat Amino Acids is the potassium salt of the product obtained by the reaction of lauroyl chloride and Oat Amino Acids. | hair conditioning agents |
| Potassium Lauroyl Pea Amino Acids | Potassium Lauroyl Pea Amino Acids is the potassium salt of the reaction product of lauric acid chloride with the amino acids derived from the seeds of *Pisum sativum.* | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |

**Table 1.** Definitions and functions of the Amino Acid Alkyl Amides in this safety assessment.[16] (Any italicized text below represents additions made by CIR staff.)

| Ingredient CAS No. | Definition | Function |
|---|---|---|
| Potassium Lauroyl Silk Amino Acids | Potassium Lauroyl Silk Amino Acids is the potassium salt of the condensation product of lauric acid chloride and Silk Amino Acids. | hair conditioning agents; skin-conditioning agents - misc.; surfactants - cleansing agents |
| Potassium Lauroyl Wheat Amino Acids | Potassium Lauroyl Wheat Amino Acids is the potassium salt of the condensation product of lauric acid chloride and Wheat Amino Acids. | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Potassium Myristoyl Glutamate | Potassium Myristoyl Glutamate is the potassium salt of the myristic acid amide of glutamic acid. It conforms to the formula. | hair conditioning agents; surfactants-cleansing agents |
| Potassium Olivoyl/Lauroyl Wheat Amino Acids | Potassium Olivoyl/Lauroyl Wheat Amino Acids is the potassium salt of the condensation product of olivoyl chloride, lauroyl chloride, and Wheat Amino Acids. | surfactants-cleansing agents |
| Potassium Stearoyl Glutamate | Potassium Stearoyl Glutamate is the potassium salt of Stearoyl Glutamic Acid. *Potassium Stearoyl Glutamate is the potassium salt of the amide formed from the reaction of stearoyl chloride and glutamic acid.* | hair conditioning agents; skin-conditioning agents-misc. |
| Potassium Undecylenoyl Glutamate | Potassium Undecylenoyl Glutamate is the substituted amino acid that conforms to the formula. *Potassium Undecylenoyl Glutamate is the potassium salt of the amide formed from the reaction of undecylenoyl chloride and glutamic acid.* | abrasives; hair conditioning agents |
| Propionyl Collagen Amino Acids | Propionyl Collagen Amino Acids is the condensation product of propionic acid chloride with Collagen Amino Acids. | skin-conditioning agents-occlusive |
| Sodium Caproyl Prolinate 1364318-34-2 | Sodium Caproyl Prolinate is the organic compound that conforms to the formula. *Sodium Caproyl Prolinate is the sodium salt of the amide formed from the reaction of caproyl chloride and proline.* | hair conditioning agents; skin-conditioning agents - humectant; surfactants - cleansing agents |
| Sodium Capryloyl Glutamate | Sodium Capryloyl Glutamate is the substituted amino acid that conforms to the formula. *Sodium Capryloyl Glutamate is the sodium salt of the amide formed from the reaction of capryloyl chloride and glutamic acid.* | deodorant agents; surfactants-cleansing agents |
| Sodium Cocoyl Alaninate 90170-45-9 | Sodium Cocoyl Alaninate is the organic compound that conforms to the formula. *Sodium Cocoyl Alaninate is the sodium salt of the amide formed from the reaction of coconut acid chloride and alanine.* | hair conditioning agents; surfactants-cleansing agents |
| Sodium Cocoyl Amino Acids | Sodium Cocoyl Amino Acids is the sodium salt of a mixture of amino acids acylated by cocoyl chloride. | surfactants-cleansing agents |
| Sodium Cocoyl Apple Amino Acids | Sodium Cocoyl Apple Amino Acids is the sodium salt of the condensation product of coconut acid chloride and the amino acids obtained by the complete hydrolysis of the protein fraction isolated from the seeds of *Pyrus malus.* | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Sodium Cocoyl Barley Amino Acids | Sodium Cocoyl Barley Amino Acids is the sodium salt of the condensation product of coconut acid chloride and the amino acids derived from barley protein. | emulsion stabilizers; skin-conditioning agents – misc.; surfactants - emulsifying agents |
| Sodium Cocoyl Collagen Amino Acids | Sodium Cocoyl Collagen Amino Acids is the sodium salt of the condensation product of coconut acid chloride and Collagen Amino Acids. | hair conditioning agents; surfactants-cleansing agents |
| Sodium Cocoyl Glutamate 68187-32-6 | Sodium Cocoyl Glutamate is the sodium salt of Cocoyl Glutamic Acid. It conforms generally to the formula. *Sodium Cocoyl Glutamate is the sodium salt of the amide formed from the reaction of coconut acid chloride and glutamic acid.* | surfactants-cleansing agents |
| Sodium Cocoyl Glutaminate | Sodium Cocoyl Glutaminate is the organic compound that conforms to the formula. *Sodium Cocoyl Glutaminate is the sodium salt of the amide formed from the reaction of coconut acid chloride and glutamine.* | surfactants- cleansing agents |
| Sodium Cocoyl Glycinate 90387-74-9 | Sodium Cocoyl Glycinate is the organic compound that conforms generally to the formula. *Sodium Cocoyl Glycinate is the sodium salt of the amide formed from the reaction of coconut acid chloride and glycine.* | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Sodium Cocoyl/Hydrogenated Tallow Glutamate | Sodium Cocoyl/Hydrogenated Tallow Glutamate is the organic compound that conforms generally to the formula. *Sodium Cocoyl/Hydrogenated Tallow Glutamate is the sodium salt of the mixture of cocoyl acid amides and hydrogenated tallow acid amides of glutamic acid.* | surfactants-cleansing agents |
| Sodium Cocoyl Oat Amino Acids | Sodium Cocoyl Oat Amino Acids is the sodium salt of the condensation product of coconut acid chloride and the amino acids derived from Avena Sativa (Oat) Protein. | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |

**Table 1.** Definitions and functions of the Amino Acid Alkyl Amides in this safety assessment.[16] (Any italicized text below represents additions made by CIR staff.)

| Ingredient CAS No. | Definition | Function |
|---|---|---|
| Sodium Cocoyl/Palmoyl/Sunfloweroyl Glutamate | Sodium Cocoyl/Palmoyl/Sunfloweroyl Glutamate is the sodium salt of the product formed by the reaction of Glutamic Acid with a mixture of Coconut Acid, Palm Acid and Sunflower Seed Acid. | surfactants-cleansing agents; surfactants-emulsfying agents |
| Sodium Cocoyl Proline | Sodium Cocoyl Proline is the substituted amino acid that conforms to the formula. *Sodium Cocoyl Proline is the sodium salt of the amide formed from the reaction of coconut acid chloride and proline.* | surfactants-cleansing agents; surfactants-solubilizing agents |
| Sodium Cocoyl Threoninate | Sodium Cocoyl Threoninate is the organic compound that conforms to the formula. *Sodium Cocoyl Threoninate is the sodium salt of the amide formed from the reaction of coconut acid chloride and threonine.* | surfactants-cleansing agents; surfactants-emulsfying agents |
| Sodium Cocoyl Wheat Amino Acids | Sodium Cocoyl Wheat Amino Acids is the sodium salt of the condensation product of coconut acid chloride and the amino acids derived from Triticum Vulgare (Wheat) Protein. | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Sodium Hydrogenated Tallowoyl Glutamate | Sodium Hydrogenated Tallowoyl Glutamate is the sodium salt of the hydrogenated tallow acid amide of glutamic acid. It conforms generally to the formula. | surfactants-cleansing agents |
| Sodium Lauroyl Aspartate<br>41489-18-3 | Sodium Lauroyl Aspartate is the organic compound that conforms to the formula. *Sodium Lauroyl Aspartate is the sodium salt of the amide formed from the reaction of lauroyl chloride and aspartic acid.* | hair conditioning agents; surfactants-cleansing agents |
| Sodium Lauroyl Collagen Amino Acids | Sodium Lauroyl Collagen Amino Acids is the sodium salt of the condensation product of lauric acid chloride and Collagen Amino Acids. | hair conditioning agents; surfactants-cleansing agents |
| Sodium Lauroyl Glutamate<br>29923-31-7 (L-)<br>29923-34-0 (DL-)<br>42926-22-7 (L-)<br>98984-78-2 | Sodium Lauroyl Glutamate is the sodium salt of the lauric acid amide of glutamic acid. It conforms generally to the formula. | hair conditioning agents |
| Sodium Lauroyl Millet Amino Acids | Sodium Lauroyl Millet Amino Acids is the sodium salt of the condensation product of lauric acid chloride and the amino acids obtained by the complete hydrolysis of the protein fraction of *Panicum miliaceum*. | surfactangs-cleansing agents |
| Sodium Lauroyl/Myristoyl Aspartate | Sodium Lauroyl/Myristoyl Aspartate is the sodium salt of the substituted amino acid that conforms generally to the formula. *Sodium Lauroyl/Myristoyl Aspartate is the sodium salt of the amide formed from the reaction of a mixture of lauroyl chloride and myristoyl chloride with aspartic acid.* | hair conditioning agents; surfactants-cleansing agents |
| Sodium Lauroyl Oat Amino Acids | Sodium Lauroyl Oat Amino Acids is the sodium salt of the condensation product of lauric acid chloride with the amino acids derived from Avena Sativa (Oat) Kernel Protein. | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Sodium Lauroyl Silk Amino Acids | Sodium Lauroyl Silk Amino Acids is the sodium salt of the condensation product of lauric acid chloride and Silk Amino Acids. | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Sodium Lauroyl Wheat Amino Acids | Sodium Lauroyl Wheat Amino Acids is the sodium salt of the condensation product of lauric acid chloride and Wheat Amino Acids. | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Sodium Myristoyl Glutamate<br>38517-37-2<br>38754-83-5 (DL-)<br>71368-20-2 | Sodium Myristoyl Glutamate is the sodium salt of the myristic acid amide of glutamic acid. It conforms generally to the formula. | surfactants-cleansing agents |
| Sodium Olivoyl Glutamate | Sodium Olivoyl Glutamate is the sodium salt of olivoyl glutamic acid. It conforms generally to the formula. *Sodium Olivoyl Glutamate is the sodium salt of the amide formed from the reaction of olivoyl chloride and glutamic acid.* | surfactants-cleansing agents |
| Sodium Palmitoyl Proline<br>58725-33-0 | Sodium Palmitoyl Proline is the substituted amino acid that conforms to the formula. *Sodium Palmitoyl Proline is the sodium salt of the amide formed from the reaction of palmitoyl chloride and proline.* | skin-conditioning agents-misc. |
| Sodium Palmoyl Glutamate | Sodium Palmoyl Glutamate is the sodium salt of palmoyl glutamic acid. It conforms generally to the formula. *Sodium Palmoyl Glutamate is the sodium salt of the amide formed from the reaction of palm acid chloride and glutamic acid.* | surfactants-cleansing agents |

**Table 1.** Definitions and functions of the Amino Acid Alkyl Amides in this safety assessment.[16] (Any italicized text below represents additions made by CIR staff.)

| Ingredient CAS No. | Definition | Function |
|---|---|---|
| Sodium Stearoyl Glutamate<br>38517-23-6<br>79811-24-8 (L-) | Sodium Stearoyl Glutamate is the organic compound that conforms to the formula. *Sodium Stearoyl Glutamate is the sodium salt of the amide formed from the reaction of stearoyl chloride and glutamic acid.* | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Sodium/TEA-Lauroyl Collagen Amino Acids | Sodium/TEA-Lauroyl Collagen Amino Acids is a mixture of sodium and triethanolamine salts of the condensation product of lauric acid chloride and Collagen Amino Acids. | hair conditioning agents; surfactants-cleansing agents |
| Sodium/TEA-Lauroyl Keratin Amino Acids | Sodium/TEA-Lauroyl Keratin Amino Acids is a mixture of sodium and triethanolamine salts of the condensation product of lauric acid chloride and Keratin Amino Acids. | hair conditioning agents; surfactants-cleansing agents |
| Sodium/TEA-Undecylenoyl Collagen Amino Acids | Sodium/TEA-Undecylenoyl Collagen Amino Acids is a mixture of sodium and triethanolamine salts of the condensation product of undecylenic acid chloride and Collagen Amino Acids. | hair conditioning agents; surfactants-cleansing agents |
| Sodium Undecylenoyl Glutamate | Sodium Undecylenoyl Glutamate is the substituted amino acid that conforms generally to the formula. *Sodium Undecenoyl Glutamate is the sodium salt of the amide formed from the reaction of undecenoyl chloride and glutamic acid.* | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Stearoyl Glutamic Acid<br>3397-16-8 | Stearoyl Glutamic Acid is the substituted amino acid that conforms to the formula. *Stearoyl Glutamic Acid is the amide formed from the reaction of stearoyl chloride and glutamic acid.* | hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents |
| Stearoyl Leucine<br>14379-43-2 | Stearoyl Leucine is the stearoyl derivative of leucine that conforms to the formula. *Stearoyl Leucine is the amide formed from the reaction of stearoyl chloride and leucine.* | hair conditioning agents; skin-conditioning agents-misc.; surfactants-emulsifying agents |
| TEA-Cocoyl Alaninate | TEA-Cocoyl Alaninate is the triethanolamine salt of the coconut acid amide of alanine. It conforms generally to the formula. | hair conditioning agents; surfactants-cleansing agents |
| TEA-Cocoyl Glutamate<br>68187-29-1 | TEA-Cocoyl Glutamate is the triethanolamine salt of the coconut acid amide of glutamic acid. It conforms generally to the formula. | hair conditioning agents; surfactants-cleansing agents |
| TEA-Cocoyl Glutaminate | TEA-Cocoyl Glutaminate is the organic compound that conforms to the formula.*TEA-Cocoyl Glutaminate is the triethanolamine salt of the coconut acid amide of glutamine.* | surfactants-cleansing agents |
| TEA-Hydrogenated Tallowoyl Glutamate | TEA-Hydrogenated Tallowoyl Glutamate is the triethanolamine salt of the hydrogenated tallow acid amide of glutamic acid. It conforms generally to the formula. | hair conditioning agents; surfactants-cleansing agents |
| TEA-Lauroyl Collagen Amino Acids | TEA-Lauroyl Collagen Amino Acids is the triethanolamine salt of the condensation product of lauric acid chloride and Collagen Amino Acids. | hair conditioning agents; surfactants-cleansing agents |
| TEA-Lauroyl Glutamate<br>31955-67-6<br>53576-49-1 | TEA-Lauroyl Glutamate is the triethanolamine salt of the lauric acid amide of glutamic acid. It conforms generally to the formula. | hair conditioning agents; surfactants-cleansing agents |
| TEA-Lauroyl Keratin Amino Acids | TEA-Lauroyl Keratin Amino Acids is the triethanolamine salt of the condensation product of lauric acid chloride and Keratin Amino Acids. | hair conditioning agents; surfactants-cleansing agents |
| TEA-Lauroyl/Myristoyl Aspartate | TEA-Lauroyl/Myristoyl Aspartate is the triethanolamine salt of the substituted amino acid that conforms generally to the formula | hair conditioning agents; surfactants-cleansing agents |
| Undecylenoyl Collagen Amino Acids | Undecylenoyl Collagen Amino Acids is the condensation product of undecylenoyl acid chloride and Collagen Amino Acids. | surfactants-cleansing agents |
| Undecylenoyl Glycine | Undecylenoyl Glycine is the acylation product of glycine with undecylenic acid chloride. It conforms to the formula. | hair conditioning agents; surfactants-cleansing agents |
| Undecylenoyl Phenylalanine<br>175357-18-3 | Undecylenoyl Phenylalanine is the substituted amino acid that conforms to the formula. *Undecylenoyl Phenylalanine is the amide formed from the reaction of undecylenoyl chloride and phenylalanine.* | skin protectants; skin-conditioning agents-misc. |
| Undecylenoyl Wheat Amino Acids | Undecylenoyl Wheat Amino Acids is the condensation product of undecylenic acid chloride and Wheat Amino Acids. | hair conditioning agents; surfactants-cleansing agents |
| Zinc Lauroyl Aspartate<br>899426-42-7 | Zinc Lauroyl Aspartate is the organic compound that conforms to the formula. *Zinc Lauroyl Aspartate is the zinc salt of the amide formed from the reaction of lauroyl chloride and aspartic acid.* | binders; surface modifiers |

**Table 2.** Constituent acids with CIR conclusions

| Constituent | Conclusion (year issued; maximum use concentration reported) | Summary of Findings | Reference |
|---|---|---|---|
| Acetic Acid | Safe as used (2012; 0.0004% in leave-ons; 0.3% in rinse-offs) | Central nervous system depression has been documented in animals exposed to acetic acid. Acetic acid has been labeled as a minor skin irritant, at low concentrations, in animal and human studies, and a severe ocular irritant in a rabbit ocular irritation test. The sodium salt of acetic acid has a more than 2-fold higher toleration level than the pure free acid, and acetic acid is not mutagenic when buffered to physiological pH. | [7] |
| Coconut Acid, Olive Acid, Palm Acid, Sunflower Seed Acid | safe as used (2011; coconut acid no reported uses in leave-ons, 14% in rinse-offs; olive acid no reported uses; palm acid no reported uses in leave-ons, 17% in rinse-offs; sunflower seed acid no reported uses) | The safety focus of use of the plant-derived fatty acid oils was on the potential for irritation and sensitization since the cosmetic ingredients reviewed were also found in the foods that are consumed daily. 5% aq. solutions of a bar soap containing 13% sodium cocoate had irritation scores of 1.6-4.0/8 in animal studies. However, the remaining animal and clinical irritation and/or sensitization studies conducted on a large number of the oils included in this report, primarily in formulation, did not report any significant irritation or sensitization reactions, indicating that refined oils derived from plants are not dermal irritants or sensitizers. | [4,5,8] |
| Lauric Acid, Oleic Acid, Stearic Acid | safe as used (1987; reaffirmed in 2006; lauric acid 10%, oleic acid 25% and stearic acid > 50% in leave-ons; lauric acid 25% and oleic and stearic acid 50% in rinse-offs)) | Oleic, lauric, palmitic, and stearic acids are fatty acids with hydrocarbon chains ranging in length from 12 to 18 carbons with a terminal carboxyl group. These fatty acids are absorbed, digested, and transported in animals and humans. Little acute toxicity was observed when oleic, lauric, palmitic, or stearic acid or cosmetic formulations containing these fatty acids were given to rats orally at doses of 15-19 g/kg body weight. Feeding of 15% dietary oleic acid to rats in a chronic study resulted in normal growth and health, but reproductive capacity of female rats was impaired. Results from topical application of oleic, palmitic, and stearic acid to the skin of mice, rabbits, and guinea pigs produced little or no apparent toxicity. Studies using product formulations containing oleic and stearic acids indicate that neither is a sensitizer or photosensitizing agent. Animal studies also indicate that these fatty acids are not eye irritants. Lauric, stearic, and oleic acids were noncarcinogenic in separate animal tests. In primary and cumulative irritation clinical studies, oleic and stearic acids at high concentrations were nonirritating. Cosmetic product formulations containing oleic, lauric, palmitic, and stearic acids at concentrations ranging up to 13% were not primary or cumulative irritants, nor sensitizers. | [2,6] |
| Malic Acid | Safe for use as a pH adjuster, insufficient data for any other functions (2001; 1% in leave-ons and rinse-offs) | Malic acid is a direct food additive. In oral and IP tests with radioactive malic acid, most of the radioactivity was excreted as carbon dioxide. Oral $LD_{50}$values for mice, rats, and rabbits ranged from 2.66 to > 3.2, 1.60 to 3.5, and 3 to 5 g/kg, respectively. The intravenous $LD_{50}$ value in rabbits was 2.4 g/kg and the intraperitoneal $LD_{50}$ values in mice and rats were 50 to 100 and 100 to 200 mg/kg, respectively. In repeated dose oral studies, rats fed malic acid had some changes in body weight gains and feed consumption, but no compound-related lesions were observed. No significant changes or lesions were observed in dogs fed malic acid repeatedly. Malic acid did not cause reproductive toxicity in mice, rats, or rabbits. Malic acid was moderately irritating to rabbit skin and was a strong irritant in guinea pigs. It also caused severe ocular irritation in rabbit eyes. Malic acid was not mutagenic in plate test, an Ames test, a suspension test, or a chromosomal aberration assay. In one study, pyrolyzates of malic acid were not mutagenic, but in another study they were. Products formed from treatment of malic acid with aqueous solutions of chorine were mutagenic. In a test determining the subjective skin irritation potential, the average irritation scores over a 15-min period were 39.4, 37.1, and 23.1 for malic acid at pH 3, 5, and 7, respectively. In predictive testing using patients with atopic dermatitis, 18 of 34 patients reacted to a diet high in malic and citric acids, and 6 reacted to a diet high in malic acid. In assessing the effect of malic acid on cell renewal, an 18%, 10%, and 5% increase was observed at pH 3, 5, and 7, respectively. Malic acid was not toxic in a clinical efficacy and safety test. | [9] |

**Table 2.** Constituent acids with CIR conclusions

| Constituent | Conclusion (year issued; maximum use concentration reported) | Summary of Findings | Reference |
|---|---|---|---|
| Myristic Acid | safe as used (2010; 15% in leave-ons; 50% in rinse-offs) | Myristic acid is approved as a food reagent and additive. Myristic acid enhanced the dermal penetration of several drugs. The acute oral $LD_{50}$ and acute dermal $LD_{50}$ of salts of myristic acid were >8 g/kg and >16 mL/kg, respectively, in rats. Acute dermal application of butyl myristate (2 g/kg) was nontoxic and nonirritating to rabbits. When 10 rabbits were treated with a single dermal dose of ethyl myristate (5 g/kg) resulted in the death of 2 over 7 days. The intraperitoneal and subcutaneous $LD_{50}$ for isopropyl myristate exceeded 79.5 mL/kg in rats and the intraperitoneal $LD_{50}$ was >50.2 mL/kg in mice. No death occurred, and no evidence of systemic toxicity was found at necropsy when the rats were exposed to aerosolized isopropyl myristate. Myristic acid, isopropyl myristate, and myristyl myristate were minimally irritating to the eyes of rabbits. Butyl myristate was nonirritating to the rabbit eye. Myristic acid was nonirritating in a single insult occlusive patch test and slightly irritating in a repeat open patch test on rabbits. Butyl myristate was a moderate skin irritant in rabbits and guinea pigs. Isopropyl myristate and myristyl myristate were minimally irritating in several formulations in rabbits and mice. Isopropyl myristate was nonirritating when injected parenterally in albino rabbits. Butyl myristate and myristyl myristate were nonsensitizing to guinea pigs. Isopropyl myristate and myristyl myristate were comedogenic to rabbit ears. Isopropyl myristate tested negative in the Salmonella/microsome test, with and without activation. In clinical primary and cumulative irritation studies, myristic acid was nonirritating. Isopropyl myristate can produce slight irritation but is not a human sensitizer at up to 50%. | [3] |

**Table 3.** Idealized structures of the ingredients in this safety assessment.[16] (The asterisk marked structures below represent additions made by CIR staff.)

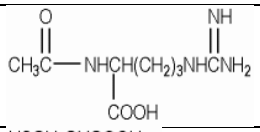
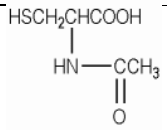
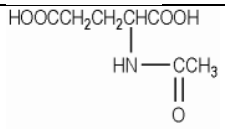
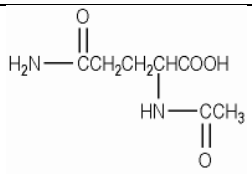
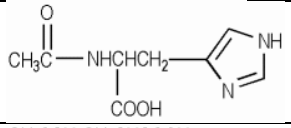
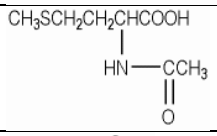
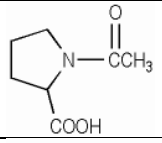
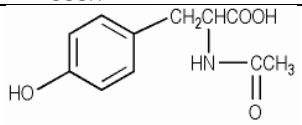
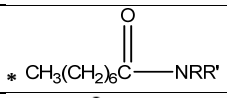
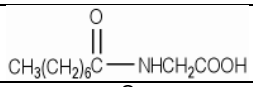
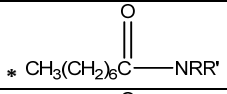
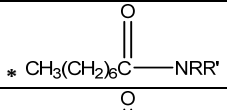
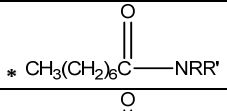
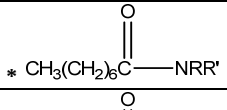
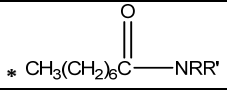
| | |
|---|---|
| Acetyl Arginine |  |
| Acetyl Cysteine |  |
| Acetyl Glutamic Acid |  |
| Acetyl Glutamine |  |
| Acetyl Histidine |  |
| Acetyl Methionine |  |
| Acetyl Proline |  |
| Acetyl Tyrosine |  |
| Capryloyl Collagen Amino Acids |  where NRR' represents the amino acid residues from collagen |
| Capryloyl Glycine |  |
| Capryloyl Gold of Pleasure Amino Acids |  where NRR' represents the amino acid residues from gold of pleasure |
| Capryloyl Keratin Amino Acids |  where NRR' represents the amino acid residues from keratin |
| Capryloyl Pea Amino Acids |  where NRR' represents the amino acid residues from pea |
| Capryloyl Quinoa Amino Acids |  where NRR' represents the amino acid residues from quinoa |
| Capryloyl Silk Amino Acids |  where NRR' represents the amino acid residues from silk |

**Table 3.** Idealized structures of the ingredients in this safety assessment.[16] (The asterisk marked structures below represent additions made by CIR staff.)

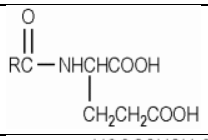
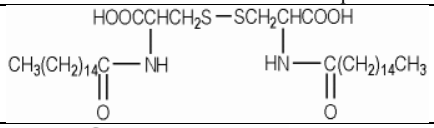
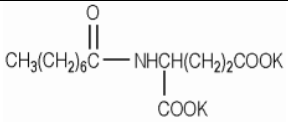
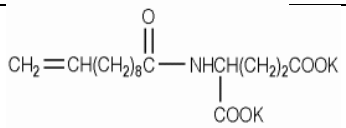
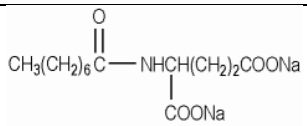
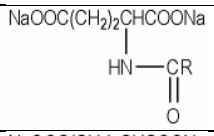
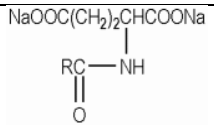
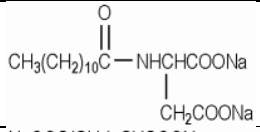
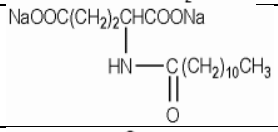
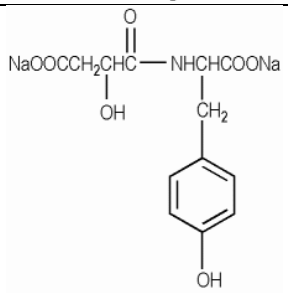
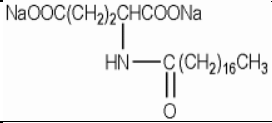
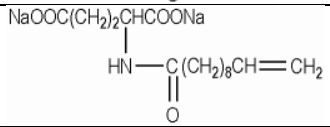
| | |
|---|---|
| Cocoyl Glutamic Acid |  where RCO- represents the fatty acids derived from coconut oil. |
| Dipalmitoyl Cystine |  |
| Dipotassium Capryloyl Glutamate |  |
| Dipotassium Undecylenoyl Glutamate |  |
| Disodium Capryloyl Glutamate |  |
| Disodium Cocoyl Glutamate |  where RCO- represents the fatty acids derived from coconut oil. |
| Disodium Hydrogenated Tallow Glutamate |  where RCO- represents the fatty acids derived from hydrogenated tallow. |
| Disodium N-Lauroyl Aspartate |  |
| Disodium Lauroyl Glutamate |  |
| Disodium Malyl Tyrosinate |  |
| Disodium Stearoyl Glutamate |  |
| Disodium Undecylenoyl Glutamate |  |

**Table 3.** Idealized structures of the ingredients in this safety assessment.[16] (The asterisk marked structures below represent additions made by CIR staff.)

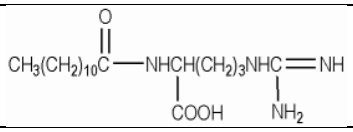
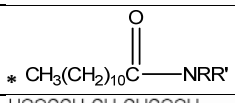
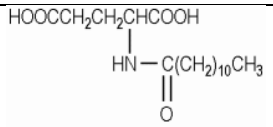
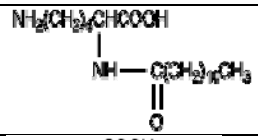
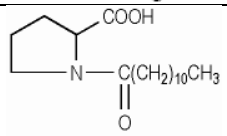
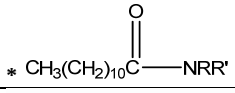
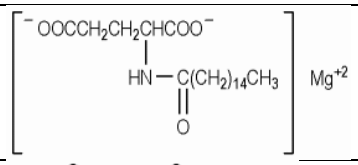
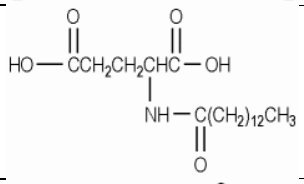
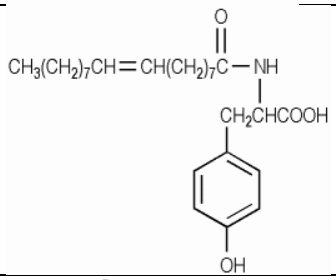
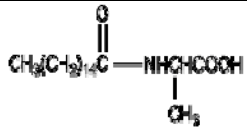
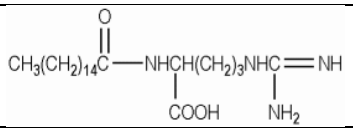
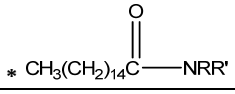
| | |
|---|---|
| Lauroyl Arginine |  |
| Lauroyl Collagen Amino Acids |  where NRR' represents the amino acid residues from collagen |
| Lauroyl Glutamic Acid |  |
| Lauroyl Lysine |  |
| Lauroyl Proline |  |
| Lauroyl Silk Amino Acids |  where NRR' represents the amino acid residues from silk |
| Magnesium Palmitoyl Glutamate |  |
| Myristoyl Glutamic Acid |  |
| Oleoyl Tyrosine |  |
| Palmitoyl Alanine |  |
| Palmitoyl Arginine |  |
| Palmitoyl Collagen Amino Acids |  where NRR' represents the amino acid residues from collagen |

**Table 3.** Idealized structures of the ingredients in this safety assessment.[16] (The asterisk marked structures below represent additions made by CIR staff.)

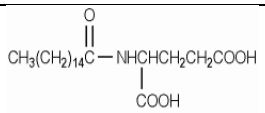
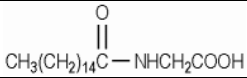
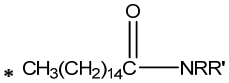
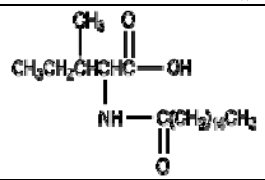
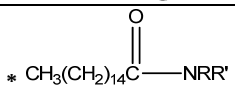
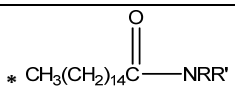
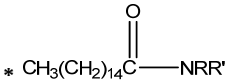
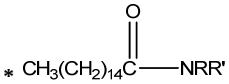
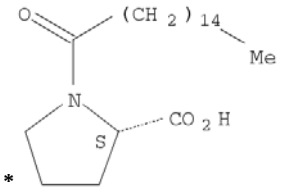
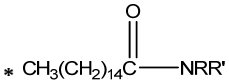
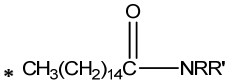
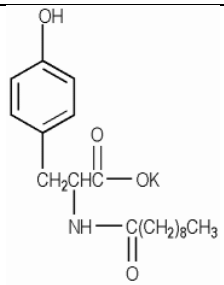
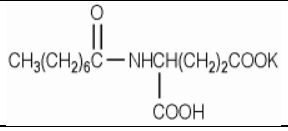
| | |
|---|---|
| Palmitoyl Glutamic Acid |  |
| Palmitoyl Glycine |  |
| Palmitoyl Gold of Pleasure Amino Acids |  where NRR' represents the amino acid residues from gold of pleasure |
| Palmitoyl Isoleucine |  |
| Palmitoyl Keratin Amino Acids |  where NRR' represents the amino acid residues from keratin |
| Palmitoyl Millet Amino Acids |  where NRR' represents the amino acid residues from millet |
| Palmitoyl Oat Amino Acids |  where NRR' represents the amino acid residues from oat |
| Palmitoyl Pea Amino Acids |  where NRR' represents the amino acid residues from pea |
| Palmitoyl Proline |  |
| Palmitoyl Quinoa Amino Acids |  where NRR' represents the amino acid residues from quinoa |
| Palmitoyl Silk Amino Acids |  where NRR' represents the amino acid residues from silk |
| Potassium Caproyl Tyrosine |  |
| Potassium Capryloyl Glutamate |  |

**Table 3.** Idealized structures of the ingredients in this safety assessment.[16] (The asterisk marked structures below represent additions made by CIR staff.)

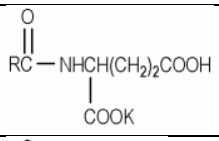
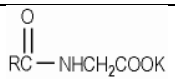
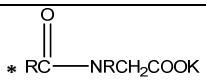
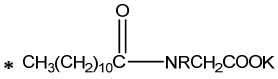
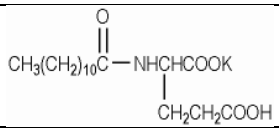
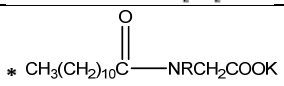
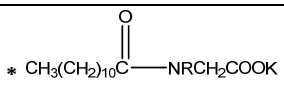
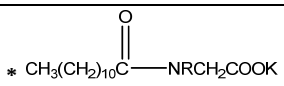
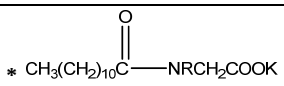
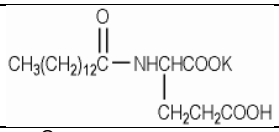
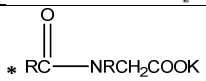
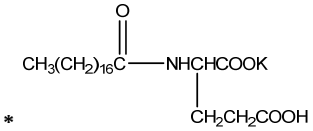
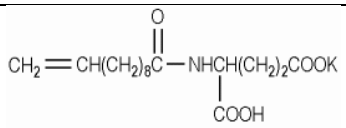
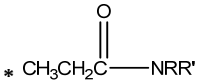
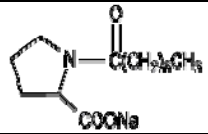
| | |
|---|---|
| Potassium Cocoyl Glutamate |  where RCO- represents the fatty acids derived from coconut oil. |
| Potassium Cocoyl Glycinate |  where RCO- represents the cocoyl moiety. |
| Potassium Cocoyl Rice Amino Acids | *  where RCO- represents the cocoyl moiety and $NRCH_2COOK$ represents the salt of the rice amino acid residues |
| Potassium Lauroyl Collagen Amino Acids | *  where $NRCH_2COOK$ represents the salt of the collagen amino acid residues |
| Potassium Lauroyl Glutamate |  |
| Potassium Lauroyl Oat Amino Acids | *  where $NRCH_2COOK$ represents the salt of the oat amino acid residues |
| Potassium Lauroyl Pea Amino Acids | *  where $NRCH_2COOK$ represents the salt of the pea amino acid residues |
| Potassium Lauroyl Silk Amino Acids | *  where $NRCH_2COOK$ represents the salt of the silk amino acid residues |
| Potassium Lauroyl Wheat Amino Acids | *  where $NRCH_2COOK$ represents the salt of the wheat amino acid residues |
| Potassium Myristoyl Glutamate |  |
| Potassium Olivoyl/Lauroyl Wheat Amino Acids | *  where RCO- represents the olivoyl/lauroyl moiety and $NRCH_2COOK$ represents the salt of the wheat amino acid residues |
| Potassium Stearoyl Glutamate |  |
| Potassium Undecylenoyl Glutamate |  |
| Propionyl Collagen Amino Acids | *  where NRR' represents the amino acid residues from collagen |
| Sodium Caproyl Prolinate |  |

**Table 3.** Idealized structures of the ingredients in this safety assessment.[16] (The asterisk marked structures below represent additions made by CIR staff.)

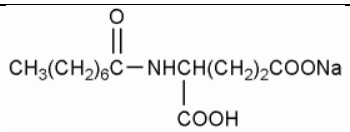
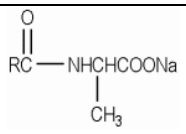
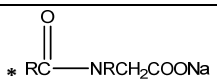
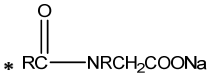
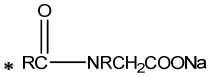
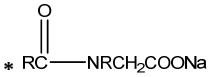
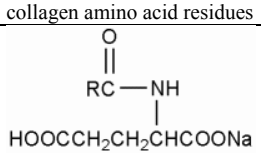
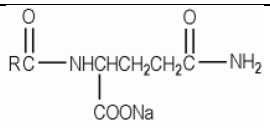
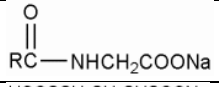
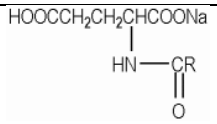
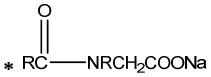
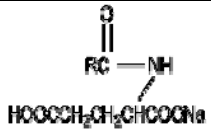
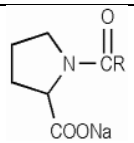
| | |
|---|---|
| Sodium Capryloyl Glutamate |  |
| Sodium Cocoyl Alaninate |  where RCO- represents the fatty acids derived from coconut oil. |
| Sodium Cocoyl Amino Acids |  where RCO- represents the cocoyl moiety and $NRCH_2COONa$ represents the salt of amino acid residues |
| Sodium Cocoyl Apple Amino Acids |  where RCO- represents the cocoyl moiety and $NRCH_2COONa$ represents the salt of apple amino acid residues |
| Sodium Cocoyl Barley Amino Acids |  where RCO- represents the cocoyl moiety and $NRCH_2COONa$ represents the salt of barley amino acid residues |
| Sodium Cocoyl Collagen Amino Acids |  where RCO- represents the cocoyl moiety and $NRCH_2COONa$ represents the salt of collagen amino acid residues |
| Sodium Cocoyl Glutamate |  where RCO- represents the fatty acids derived from coconut oil. |
| Sodium Cocoyl Glutaminate |  where RCO- represents the fatty acids derived from coconut oil. |
| Sodium Cocoyl Glycinate |  where RCO- represents the cocoyl moiety. |
| Sodium Cocoyl/Hydrogenated Tallow Glutamate |  where RCO- represents a mixture of fatty acids derived from coconut oil and hydrogenated tallow. |
| Sodium Cocoyl Oat Amino Acids |  where RCO- represents the cocoyl moiety and $NRCH_2COONa$ represents the salt of oat amino acid residues |
| Sodium Cocoyl/Palmoyl/Sunfloweroyl Glutamate |  where RCO- represents the mixture of fatty acids. |
| Sodium Cocoyl Proline |  where RCO- represents the fatty acids derived from coconut oil. |

**Table 3.** Idealized structures of the ingredients in this safety assessment.[16] (The asterisk marked structures below represent additions made by CIR staff.)

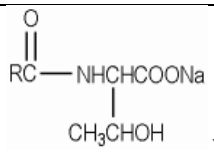
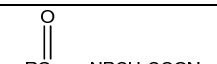
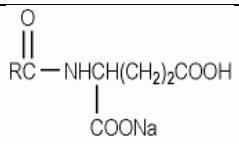
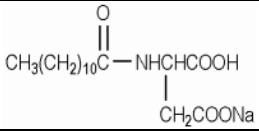
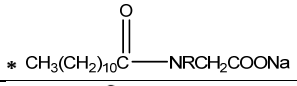
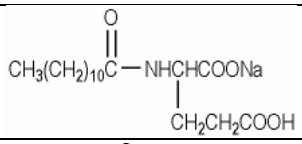
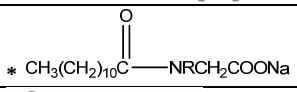
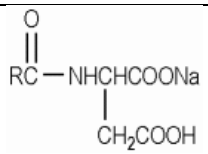
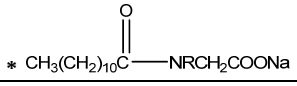
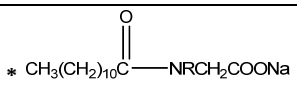
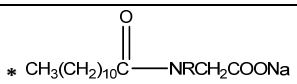
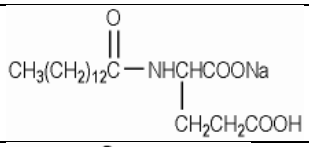
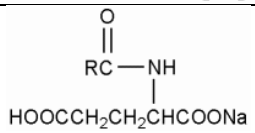
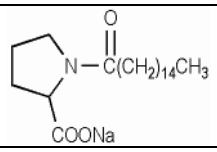
| | | |
|---|---|---|
| Sodium Cocoyl Threoninate |  | where RCO- represents the fatty acids derived from Cocos Nucifera (Coconut) Oil |
| Sodium Cocoyl Wheat Amino Acids |  | where RCO- represents the cocoyl moiety and $NRCH_2COONa$ represents the salt of wheat amino acid residues |
| Sodium Hydrogenated Tallowoyl Glutamate |  | where RCO- represents the fatty acids derived from hydrogenated tallow. |
| Sodium Lauroyl Aspartate |  | |
| Sodium Lauroyl Collagen Amino Acids |  | where $NRCH_2COONa$ represents the salt of the collagen amino acid residues |
| Sodium Lauroyl Glutamate |  | |
| Sodium Lauroyl Millet Amino Acids |  | where $NRCH_2COONa$ represents the salt of the millet amino acid residues |
| Sodium Lauroyl/Myristoyl Aspartate |  | where RCO- represents the lauroyl/myristoyl grouping. |
| Sodium Lauroyl Oat Amino Acids |  | where $NRCH_2COONa$ represents the salt of the oat amino acid residues |
| Sodium Lauroyl Silk Amino Acids |  | where $NRCH_2COONa$ represents the salt of the silk amino acid residues |
| Sodium Lauroyl Wheat Amino Acids |  | where $NRCH_2COONa$ represents the salt of the wheat amino acid residues |
| Sodium Myristoyl Glutamate |  | |
| Sodium Olivoyl Glutamate |  | where RCO- represents the fatty acids derived from olive oil. |
| Sodium Palmitoyl Proline |  | |

**Table 3.** Idealized structures of the ingredients in this safety assessment.[16] (The asterisk marked structures below represent additions made by CIR staff.)

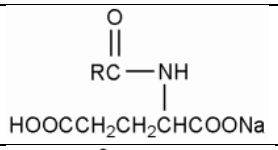
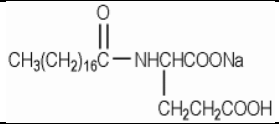
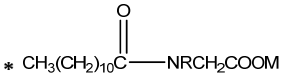
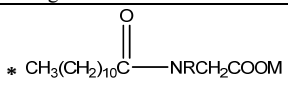
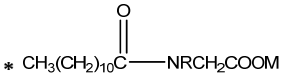
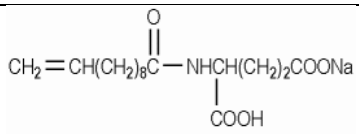
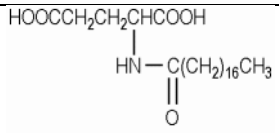
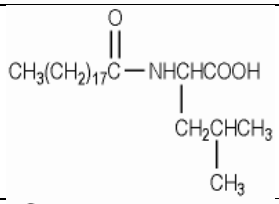
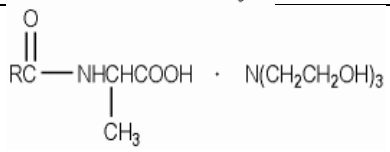
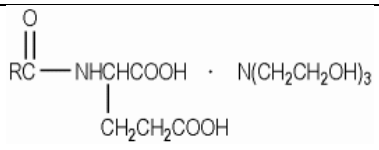
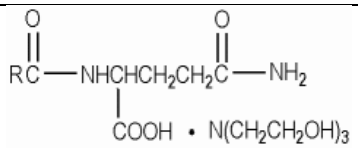
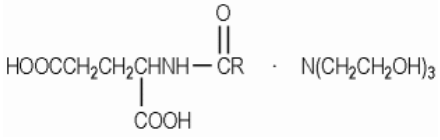
| | | |
|---|---|---|
| Sodium Palmoyl Glutamate |  | where RCO- represents the palmoyl radical. |
| Sodium Stearoyl Glutamate |  | |
| Sodium/TEA-Lauroyl Collagen Amino Acids |  | where $NRCH_2COOM$ represents the mixture of sodium and TEA salts of the collagen amino acid residues |
| Sodium/TEA-Lauroyl Keratin Amino Acids |  | where $NRCH_2COOM$ represents the mixture of sodium and TEA salts of the keratin amino acid residues |
| Sodium/TEA-Undecylenoyl Collagen Amino Acids |  | where $NRCH_2COOM$ represents the mixture of sodium and TEA salts of the keratin amino acid residues |
| Sodium Undecylenoyl Glutamate |  | |
| Stearoyl Glutamic Acid |  | |
| Stearoyl Leucine |  | |
| TEA-Cocoyl Alaninate |  | where RCO- represents the fatty acids derived from coconut oil. |
| TEA-Cocoyl Glutamate |  | where RCO- represents the fatty acids derived from coconut oil. |
| TEA-Cocoyl Glutaminate |  | where RCO- represents the coconut acid moiety. |
| TEA-Hydrogenated Tallowoyl Glutamate |  | where RCO- represents the fatty acids derived from hydrogenated tallow. |

**Table 3.** Idealized structures of the ingredients in this safety assessment.[16] (The asterisk marked structures below represent additions made by CIR staff.)

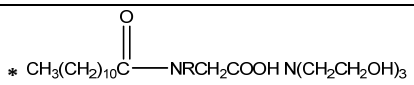
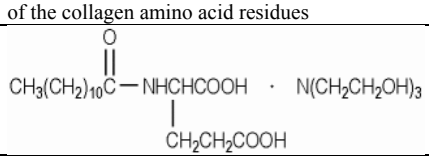
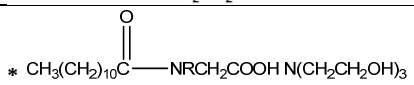
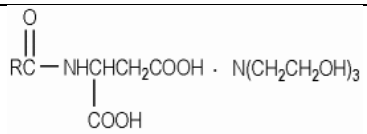
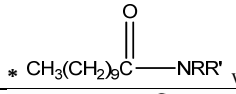
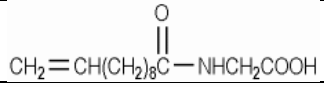
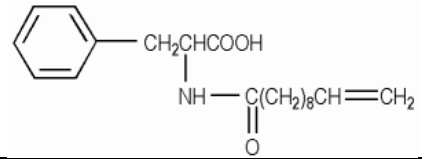
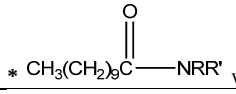
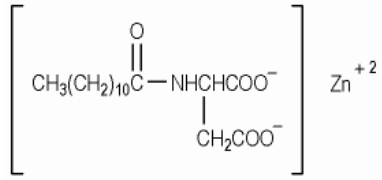
| | | |
|---|---|---|
| TEA-Lauroyl Collagen Amino Acids |  | where $NRCH_2COOH\ N(CH_2CH_2OH)_3$ represents the TEA salt of the collagen amino acid residues |
| TEA-Lauroyl Glutamate |  | |
| TEA-Lauroyl Keratin Amino Acids |  | where $NRCH_2COOH\ N(CH_2CH_2OH)_3$ represents the TEA salt of the keratin amino acid residues |
| TEA-Lauroyl/Myristoyl Aspartate |  | where RCO- represents the lauroyl/myristoyl grouping. |
| Undecylenoyl Collagen Amino Acids |  | where NRR' represents the amino acid residues from collagen |
| Undecylenoyl Glycine |  | |
| Undecylenoyl Phenylalanine |  | |
| Undecylenoyl Wheat Amino Acids |  | where NRR' represents the amino acid residues from wheat |
| Zinc Lauroyl Aspartate |  | |

**Table 4.** Chemical properties of amino acids alkyl amides

| Property | Value | Reference |
|---|---|---|
| ***Acetyl Arginine*** | | |
| Molecular Weight g/mol | 216.24 | PubChem |
| ***Acetyl Cysteine*** | | |
| Physical Form | Crystals in water | Merck |
| Odor | Slight acetic | Merck |
| Molecular Weight g/mol | 163.19 | [22] |
| Molecular Volume $cm^3$/mol @ 20 ºC | 126.0 | [22] |
| Density/Specific Gravity @ 20 ºC | 1.294 | [22] |
| Vapor pressure mmHg@ 25 ºC | $8.68 \times 10^{-8}$ | [22] |
| Melting Point ºC | 109-110 | Merck |
| Boiling Point ºC | 407.7 | [22] |
| Solubility | Freely sol in water, alcohol. Practically insol in chloroform, ether | Merck |
| logP @ 25 ºC | -0.696 | [22] |
| Disassociation constants (pKa, pKb) @ 25 ºC | 3.25 most acidic; -0.91 most basic | [22] |
| ***Acetyl Glutamic Acid*** | | |
| Molecular Weight g/mol | 189.17 | [22] |
| Molecular Volume $cm^3$/mol @ 20 ºC | 139.6 | [22] |
| Density/Specific Gravity @ 20 ºC | 1.354 | [22] |
| Vapor pressure mmHg@ 25 ºC | $3.48 \times 10^{-11}$ | [22] |
| Boiling Point ºC | 495.9 | [22] |
| logP @ 25 ºC | -2.131 | [22] |
| Disassociation constants (pKa) @ 25ºC | 3.45 most acidic; -0.86 most basic | [22] |
| ***Acetyl Glutamine*** | | |
| Physical Form | Crystals from ethanol | Merck |
| Molecular Weight g/mol | 188.18 | [22] |
| Molecular Volume $cm^3$/mol @ 20 ºC | 145.8 | [22] |
| Density/Specific Gravity @ 20 ºC | 1.290 | [22] |
| Vapor pressure mmHg@ ºC | $1.28 \times 10^{-8}$ | [22] |
| Melting Point ºC | 197 | Merck |
| Boiling Point ºC | 430.5 | [22] |
| logP @ 25 ºC | -2.215 | [22] |
| Disassociation constants (pKa) @ 25ºC | 2.19 most acidic; 9.19 most basic | [22] |

**Table 4.** Chemical properties of amino acids alkyl amides

| | | |
|---|---|---|
| ***Acetyl Methionine*** | | |
| Physical Form | Crystals; large prisms from water (DL-); plates from water or ethyl acetate (D-) | Merck |
| Molecular Weight g/mol | 191.25 | [22] |
| Molecular Volume $cm^3$/mol @ 20 °C | 158.9 | [22] |
| Density/Specific Gravity @ 20 °C | 1.202 | [22] |
| Vapor pressure mmHg@ °C | $1.72 \times 10^{-9}$ | [22] |
| Melting Point °C | 102-104; 114-115 (DL-); 104-105 (D-) | Merck |
| Boiling Point °C | 453.6 | [22] |
| Water Solubility g/100 ml @ 25 °C | 9.12 (DL-); 30.7 (D-) | Merck |
| Other Solubility g/100 ml @ 25 °C | Acetone 10.0 (DL-) and 29.6 (D-); Ethyl acetate 2.29 (DL-) and 7.04 (D-); chloroform 1.33 (DL-) and 6.43 (D-) | Merck |
| logP @ 25 °C | -0.885 | [22] |
| Disassociation constants (pKa) @ 25°C | 3.50 most acidic; -0.84 most basic | [22] |
| ***Acetyl Tyrosine*** | | |
| Molecular Weight g/mol | 223.23 | [22] |
| Molecular Volume $cm^3$/mol @ 20 °C | 171.1 | [22] |
| Density/Specific Gravity @ 20 °C | 1.304 | [22] |
| Vapor pressure mmHg@ °C | $4.07 \times 10^{-12}$ | [22] |
| Boiling Point °C | 531.3 | [22] |
| logP @ 25 °C | -1.676 | [22] |
| Disassociation constants (pKa) @ 25°C | 3.15 most acidic; -0.83 most basic | [22] |
| ***Capryloyl Glycine*** | | |
| Molecular Weight g/mol | 201.26 | [22] |
| Molecular Volume $cm^3$/mol @ 20 °C | 194.1 | [22] |
| Density/Specific Gravity @ 20 °C | 1.036 | [22] |
| Vapor pressure mmHg@ °C | $1.19 \times 10^{-7}$ | [22] |
| Boiling Point °C | 403.9 | [22] |
| logP @ 25 °C | 1.065 | [22] |
| Disassociation constants (pKa) @ 25°C | 3.62 most acidic; -0.98 most basic | [22] |
| ***Dipalmitoyl Cystine*** | | |
| Molecular Weight g/mol | 717.12 | [22] |
| Molecular Volume $cm^3$/mol @ 20 °C | 685.6 | [22] |
| Density/Specific Gravity @ 20 °C | 1.045 | [22] |
| Vapor pressure mmHg@ 25 °C | $3.93 \times 10^{-32}$ | [22] |
| Boiling Point °C | 852.2 | [22] |
| logP @ 25 °C | 12.988 | [22] |
| Disassociation constants (pKa) @ 25°C | 2.93 most acidic; -0.63 most basic | [22] |

**Table 4.** Chemical properties of amino acids alkyl amides

| | | |
|---|---|---|
| ***Disodium Capryloyl Glutamate*** | | |
| Physical Form @ 20 °C | Clear to light turbid liquid | [23] |
| Color | Colorless to light yellow | [23] |
| pH @ 20 °C | 9.0-10.5 | [23] |
| ***Lauroyl Arginine*** | | |
| Molecular Weight g/mol | 356.50 | [22] |
| Molecular Volume $cm^3$/mol @ 20 °C | 316.2 | [22] |
| Density/Specific Gravity @ 20 °C | 1.12 | [22] |
| logP @ 25 °C | 2.547 | [22] |
| Disassociation constants (pKa) @ 25°C | 3.60 most acidic; 13.84 most basic | [22] |
| ***Lauroyl Glutamic Acid*** | | |
| Molecular Weight g/mol | 329.43 | [22] |
| Molecular Volume $cm^3$/mol @ 20 °C | 304.7 | [22] |
| Density/Specific Gravity @ 20 °C | 1.081 | [22] |
| Vapor pressure mmHg@ °C | $2.95 \times 10^{-13}$ | [22] |
| Melting Point °C | 95-96 | [10] |
| Boiling Point °C | 543.6 | [22] |
| logP @ 25 °C | 2.964 | [22] |
| Disassociation constants (pKa) @ 25°C | 3.46 most acidic; -0.88 most basic | [22] |
| ***Lauroyl Proline*** | | |
| Molecular Weight g/mol | 297.43 | [22] |
| Molecular Volume $cm^3$/mol @ 20 °C | 288.3 | [22] |
| Density/Specific Gravity @ 20 °C | 1.031 | [22] |
| Vapor pressure mmHg@ °C | $6.01 \times 10^{-10}$ | [22] |
| Boiling Point °C | 465.3 | [22] |
| logP @ 25 °C | 5.356 | [22] |
| Disassociation constants (pKa) @ 25°C | 3.70 most acidic; -2.37 most basic | [22] |
| ***Palmitoyl Alanine*** | | |
| Molecular Weight g/mol | 327.50 | [22] |
| Molecular Volume $cm^3$/mol @ 20 °C | 343.1 | [22] |
| Density/Specific Gravity @ 20 °C | 0.954 | [22] |
| Vapor pressure mmHg@ °C | $2.73 \times 10^{-11}$ | [22] |
| Boiling Point °C | 498.4 | [22] |
| logP @ 25 °C | 5.495 | [22] |
| Disassociation constants (pKa) @ 25°C | 3.69 most acidic; -0.81 most basic | [22] |

**Table 4.** Chemical properties of amino acids alkyl amides

| ***Palmitoyl Arginine*** | | |
|---|---|---|
| Molecular Weight g/mol | 412.61 | [22] |
| Molecular Volume $cm^3$/mol @ 20 °C | 380.5 | [22] |
| Density/Specific Gravity @ 20 °C | 1.08 | [22] |
| logP @ 25 °C | 4.585 | [22] |
| Disassociation constants (pKa) @ 25°C | 3.60 most acidic; 13.84 most basic | [22] |

| ***Palmitoyl Glutamic Acid*** | | |
|---|---|---|
| Molecular Weight g/mol | 385.54 | [22] |
| Molecular Volume $cm^3$/mol @ 20 °C | 370.7 | [22] |
| Density/Specific Gravity @ 20 °C | 1.039 | [22] |
| Vapor pressure mmHg@ °C | $5.17 \times 10^{-15}$ | [22] |
| Boiling Point °C | 581.1 | [22] |
| logP @ 25 °C | 5.002 | [22] |
| Disassociation constants (pKa) @ 25°C | 3.46 most acidic; -0.88 most basic | [22] |

| ***Palmitoyl Glycine*** | | |
|---|---|---|
| Molecular Weight g/mol | 313.48 | [22] |
| Molecular Volume $cm^3$/mol @ 20 °C | 326.2 | [22] |
| Density/Specific Gravity @ 20 °C | 0.960 | [22] |
| Vapor pressure mmHg@ °C | $5.13 \times 10^{-11}$ | [22] |
| Melting Point °C | 122-125 | [10] |
| Boiling Point °C | 491.8 | [22] |
| logP @ 25 °C | 5.141 | [22] |
| Disassociation constants (pKa) @ 25°C | 3.59 most acidic; -1.01 most basic | [22] |

| ***Palmitoyl Isoleucine*** | | |
|---|---|---|
| Molecular Weight g/mol | 369.58 | [22] |
| Molecular Volume $cm^3$/mol @ 20 °C | 392.9 | [22] |
| Density/Specific Gravity @ 20 °C | 0.940 | [22] |
| Vapor pressure mmHg@ °C | $1.44 \times 10^{-12}$ | [22] |
| Boiling Point °C | 528.2 | [22] |
| logP @ 25 °C | 6.867 | [22] |
| Disassociation constants (pKa) @ 25°C | 3.67 most acidic; -0.81 most basic | [22] |

| ***Palmitoyl Proline*** | | |
|---|---|---|
| Molecular Weight g/mol | 353.54 | [22] |
| Molecular Volume $cm^3$/mol @ 20 °C | 354.3 | [22] |
| Density/Specific Gravity @ 20 °C | 0.997 | [22] |
| Vapor pressure mmHg@ °C | $7.58 \times 10^{-12}$ | [22] |

**Table 4.** Chemical properties of amino acids alkyl amides

| | | |
|---|---|---|
| Boiling Point ºC | 511.6 | [22] |
| logP @ 25 ºC | 7.394 | [22] |
| Disassociation constants (pKa) @ 25ºC | 3.69 most acidic; -2.37 most basic | [22] |
| | | |
| ***Sodium Lauroyl Glutamate*** | | |
| Physical Form @ 20 ºC | Clear to slightly turbid liquid | [19] |
| Color | Colorless to slightly yellow | [19] |
| | | |
| ***Stearoyl Glutamic Acid*** | | |
| Molecular Weight g/mol | 413.594 | [24] |
| Molecular Volume $cm^3$/mol @ 20 ºC | 403.7 | [22] |
| Density/Specific Gravity @ 20 ºC | 1.024 | [22] |
| Vapor pressure mmHg@ ºC | $5.85 \times 10^{-16}$ | [22] |
| Melting Point ºC | 154.75 | [24] |
| Boiling Point ºC | 600.3 | [22] |
| logP @ 25 ºC | 6.021 | [22] |
| Disassociation constants (pKa) @ 25ºC | 3.46 most acidic; -0.88 most basic | [22] |
| | | |
| ***Stearoyl Leucine*** | | |
| Molecular Weight g/mol | 397.63 | [22] |
| Molecular Volume $cm^3$/mol @ 20 ºC | 426.0 | [22] |
| Density/Specific Gravity @ 20 ºC | 0.933 | [22] |
| Vapor pressure mmHg@ ºC | $1.41 \times 10^{-13}$ | [22] |
| Melting Point ºC | 64-65 | [12] |
| Boiling Point ºC | 550.6 | [22] |
| logP @ 25 ºC | 7.886 | [22] |
| Disassociation constants (pKa) @ 25ºC | 3.67 most acidic; -0.81 most basic | [22] |
| | | |
| ***Undecylenoyl Phenylalanine*** | | |
| Molecular Weight g/mol | 331.45 | [22] |
| Molecular Volume $cm^3$/mol @ 20 ºC | 316.3 | [22] |
| Density/Specific Gravity @ 20 ºC | 1.047 | [22] |
| Vapor pressure mmHg@ ºC | $1.70 \times 10^{-12}$ | [22] |
| Boiling Point ºC | 540.0 | [22] |
| logP @ 25 ºC | 3.155 | [22] |
| Disassociation constants (pKa) @ 25ºC | 3.63 most acidic; -0.82 most basic | [22] |

**Table 5a.** Frequency and concentration of use (2012-2013) according to duration and type of exposure for Amino Acid Alkyl Amides.[25]

| | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* |
|---|---|---|---|---|---|---|
| | **Acetyl Cysteine** | | **Acetyl Glutamine** | | **Acetyl Methionine** | |
| **Totals*** | **23** | **0.0005-0.1** | **8** | **0.01-1** | **9** | **0.00001** |
| ***Duration of Use*** | | | | | | |
| Leave-On | 14 | 0.0005-0.1 | 2 | 0.01-1 | 7 | 0.00001 |
| Rinse-Off | 9 | NR | 6 | 0.1 | 2 | NR |
| Diluted for (Bath) Use | NR | NR | NR | NR | NR | NR |
| ***Exposure Type*** | | | | | | |
| Eye Area | 4 | NR | NR | NR | 4 | NR |
| Incidental Ingestion | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Spray | NR | NR | NR | 1[a] | NR | NR |
| Incidental Inhalation-Powder | NR | NR | NR | NR | NR | NR |
| Dermal Contact | 13 | 0.0005-0.03 | 2 | 0.01-1 | 4 | NR |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | 10 | 0.1 | 6 | NR | 4 | 0.00001 |
| Hair-Coloring | NR | NR | NR | NR | NR | NR |
| Nail | NR | NR | NR | NR | 1 | NR |
| Mucous Membrane | NR | NR | NR | NR | NR | NR |
| Baby Products | NR | NR | NR | NR | NR | NR |

| | **Acetyl Tryosine** | | **Capryloyl Glycine** | | **Cocoyl Glutamic Acid** | |
|---|---|---|---|---|---|---|
| **Totals*** | **29** | **0.03-0.3** | **75** | **0.05-2** | **NR** | **24** |
| ***Duration of Use*** | | | | | | |
| Leave-On | 23 | 0.08-0.3 | 46 | *0.09-2* | NR | NR |
| Rinse Off | 6 | 0.03 | 28 | *0.05-2* | NR | 24 |
| Diluted for (Bath) Use | NR | NR | 1 | NR | NR | NR |
| ***Exposure Type*** | | | | | | |
| Eye Area | 2 | 0.3 | 3 | 0.4-2 | NR | NR |
| Incidental Ingestion | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Spray | 3 | NR | 4 | 0.1 | NR | NR |
| Incidental Inhalation-Powder | NR | NR | NR | NR | NR | NR |
| Dermal Contact | 21 | 0.03-0.3 | 62 | 0.05-2 | NR | 24 |
| Deodorant (underarm) | NR | NR | 2 | 0.1 | NR | NR |
| Hair - Non-Coloring | 8 | 0.3 | 10 | 0.4-2 | NR | NR |
| Hair-Coloring | NR | NR | NR | NR | NR | NR |
| Nail | NR | NR | NR | NR | NR | NR |
| Mucous Membrane | 1 | NR | 6 | NR | NR | NR |
| Baby Products | NR | NR | NR | NR | NR | NR |

| | **Disodium Capryloyl Glutamate** | | **Disodium Cocoyl Glutamate** | | **Disodium Hydrogenated Tallow Glutamate** | |
|---|---|---|---|---|---|---|
| **Totals*** | **2** | **0.4** | **76** | **0.02-3** | NR | **0.1-1** |
| ***Duration of Use*** | | | | | | |
| *Leave-On* | 2 | NR | 9 | *0.02-0.3* | NR | 0.1 |
| *Rinse-Off* | NR | 0.4 | 67 | *0.6-3* | NR | 1 |
| *Diluted for (Bath) Use* | NR | NR | NR | NR | NR | NR |
| ***Exposure Type*** | | | | | | |
| Eye Area | NR | NR | 1 | 0.02-0.05 | NR | NR |
| Incidental Ingestion | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Spray | NR | NR | NR | 0.3[b] | NR | NR |
| Incidental Inhalation-Powder | NR | NR | 2 | 0.1 | NR | NR |
| Dermal Contact | 2 | 0.4 | 31 | 0.02-3 | NR | 0.1-1 |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | NR | NR | 15 | NR | NR | NR |
| Hair-Coloring | NR | NR | 30 | NR | NR | NR |
| Nail | NR | NR | NR | 0.05 | NR | NR |
| Mucous Membrane | NR | NR | 7 | 0.6-2[c] | NR | NR |
| Baby Products | NR | NR | NR | NR | NR | NR |

**Table 5a.** Frequency and concentration of use (2012-2013) according to duration and type of exposure for Amino Acid Alkyl Amides.[25]

| | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* |
|---|---|---|---|---|---|---|
| | **Disodium Lauroyl Glutamate** | | **Disodium Malyl Tyrosinate** | | **Disodium Stearoyl Glutamate** | |
| **Totals** | **1** | NR | **1** | NR | **135** | **0.000006-6** |
| ***Duration of Use*** | | | | | | |
| Leave-On | NR | NR | NR | NR | 135 | 0.000006-6 |
| Rinse Off | 1 | NR | NR | NR | NR | 0.1-0.3 |
| Diluted for (Bath) Use | NR | NR | NR | NR | NR | NR |
| ***Exposure Type*** | | | | | | |
| Eye Area | NR | NR | NR | NR | 15 | 0.05-1 |
| Incidental Ingestion | NR | NR | NR | NR | 3 | 0.000006-0.02 |
| Incidental Inhalation-Spray | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Powder | NR | NR | NR | NR | 6 | 0.2-6 |
| Dermal Contact | 1 | NR | 1 | NR | 130 | 0.03-6 |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | NR | NR | NR | NR | NR | NR |
| Hair-Coloring | NR | NR | NR | NR | NR | NR |
| Nail | NR | NR | NR | NR | NR | NR |
| Mucous Membrane | NR | NR | NR | NR | 3 | 0.000006-0.02 |
| Baby Products | NR | NR | NR | NR | NR | NR |

| | **Lauroyl Arginine** | | **Lauroyl Collagen Amino Acids** | | **Lauroyl Lysine** | |
|---|---|---|---|---|---|---|
| **Totals*** | **1** | NR | **1** | NR | **649** | **0.001-45** |
| ***Duration of Use*** | | | | | | |
| Leave-On | NR | NR | NR | NR | 643 | 0.001-45 |
| Rinse-Off | 1 | NR | 1 | NR | 6 | 0.001-0.3 |
| Diluted for (Bath) Use | NR | NR | NR | NR | NR | NR |
| ***Exposure Type*** | | | | | | |
| Eye Area | NR | NR | NR | NR | 265 | 0.005-10.2 |
| Incidental Ingestion | NR | NR | NR | NR | 24 | 0.2-45 |
| Incidental Inhalation-Spray | NR | NR | NR | NR | 7 | NR |
| Incidental Inhalation-Powder | NR | NR | NR | NR | 173 | 0.005-12 |
| Dermal Contact | NR | NR | NR | NR | 583 | 0.005-14 |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | 1 | NR | 1 | NR | 4 | 0.001-0.3 |
| Hair-Coloring | NR | NR | NR | NR | NR | NR |
| Nail | NR | NR | NR | NR | 1 | 0.001 |
| Mucous Membrane | NR | NR | NR | NR | 24 | 0.2-45 |
| Baby Products | NR | NR | NR | NR | NR | NR |

| | **Lauroyl Proline** | | **Lauroyl Silk Amino Acids** | | **Magnesium Palmitoyl Glutamate** | |
|---|---|---|---|---|---|---|
| **Totals*** | **1** | NR | **2** | NR | **15** | **0.0006-0.2** |
| ***Duration of Use*** | | | | | | |
| Leave-On | 1 | NR | 1 | NR | 15 | 0.0006-0.2 |
| Rinse-Off | NR | NR | 1 | NR | NR | NR |
| Diluted for (Bath) Use | NR | NR | NR | NR | NR | NR |
| ***Exposure Type*** | | | | | | |
| Eye Area | NR | NR | NR | NR | NR | NR |
| Incidental Ingestion | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Spray | NR | NR | NR | NR | NR | 0.2[d] |
| Incidental Inhalation-Powder | NR | NR | NR | NR | NR | NR |
| Dermal Contact | 1 | NR | 1 | NR | 14 | 0.0006-0.2 |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | NR | NR | 1 | NR | NR | 0.2 |
| Hair-Coloring | NR | NR | NR | NR | NR | NR |
| Nail | NR | NR | NR | NR | 1 | 0.001-0.002 |
| Mucous Membrane | NR | NR | NR | NR | NR | NR |
| Baby Products | NR | NR | NR | NR | NR | NR |

**Table 5a.** Frequency and concentration of use (2012-2013) according to duration and type of exposure for Amino Acid Alkyl Amides.[25]

| | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* |
|---|---|---|---|---|---|---|
| | **Oleoyl Tyrosine** | | **Palmitoyl Collagen Amino Acids** | | **Palmitoyl Glycine** | |
| **Totals*** | **3** | NR | **1** | NR | **5** | **1** |
| ***Duration of Use*** | | | | | | |
| Leave-On | 3 | NR | 1 | NR | 5 | 1 |
| Rinse-Off | NR | NR | NR | NR | NR | NR |
| Diluted for (Bath) Use | NR | NR | NR | NR | NR | NR |
| ***Exposure Type*** | | | | | | |
| Eye Area | NR | NR | NR | NR | 3 | NR |
| Incidental Ingestion | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Spray | 3 | NR | 1 | NR | NR | NR |
| Incidental Inhalation-Powder | NR | NR | NR | NR | NR | NR |
| Dermal Contact | 3 | NR | 1 | NR | 5 | 1 |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | NR | NR | NR | NR | NR | NR |
| Hair-Coloring | NR | NR | NR | NR | NR | NR |
| Nail | NR | NR | NR | NR | NR | NR |
| Mucous Membrane | NR | NR | NR | NR | NR | NR |
| Baby Products | NR | NR | NR | NR | NR | NR |

| | **Palmitoyl Keratin Amino Acids** | | **Palmitoyl Proline** | | **Palmitoyl Silk Amino Acids** | |
|---|---|---|---|---|---|---|
| **Totals*** | **5** | NR | **15** | NR | **2** | NR |
| ***Duration of Use*** | | | | | | |
| Leave-On | 4 | NR | 15 | NR | 2 | NR |
| Rinse-Off | 1 | NR | NR | NR | NR | NR |
| Diluted for (Bath) Use | NR | NR | NR | NR | NR | NR |
| ***Exposure Type*** | | | | | | |
| Eye Area | NR | NR | NR | NR | NR | NR |
| Incidental Ingestion | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Spray | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Powder | NR | NR | NR | NR | NR | NR |
| Dermal Contact | 4 | NR | 14 | NR | 2 | NR |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | 1 | NR | NR | NR | NR | NR |
| Hair-Coloring | NR | NR | NR | NR | NR | NR |
| Nail | NR | NR | 1 | NR | NR | NR |
| Mucous Membrane | NR | NR | NR | NR | NR | NR |
| Baby Products | NR | NR | NR | NR | NR | NR |

| | **Potassium Cocoyl Glutamate** | | **Potassium Cocoyl Glycinate** | | **Potassium Lauroyl Wheat Amino Acids** | |
|---|---|---|---|---|---|---|
| **Totals*** | **6** | **0.03-12** | **16** | **1-39** | **4** | **0.7** |
| ***Duration of Use*** | | | | | | |
| Leave-On | NR | 0.03 | NR | 2 | NR | NR |
| Rinse Off | 6 | 3-12 | 15 | 1-39 | 4 | 0.7 |
| Diluted for (Bath) Use | NR | 6 | 1 | NR | NR | NR |
| ***Exposure Type*** | | | | | | |
| Eye Area | NR | NR | NR | NR | NR | NR |
| Incidental Ingestion | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Spray | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Powder | NR | NR | NR | NR | NR | NR |
| Dermal Contact | 6 | 0.03-12 | 16 | 1-39 | 4 | 0.7 |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | NR | 8 | NR | NR | NR | NR |
| Hair-Coloring | NR | NR | NR | NR | NR | NR |
| Nail | NR | NR | NR | NR | NR | NR |
| Mucous Membrane | NR | 3-6 | 4 | 1 | 1 | NR |
| Baby Products | NR | NR | NR | NR | NR | NR |

**Table 5a.** Frequency and concentration of use (2012-2013) according to duration and type of exposure for Amino Acid Alkyl Amides.[25]

| | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* |
|---|---|---|---|---|---|---|
| | **Potassium Myristoyl Glutamate** | | **Sodium Cocoyl Alaninate** | | **Sodium Cocoyl Amino Acids** | |
| **Totals*** | **5** | **11-27** | **8** | NR | **21** | **0.4-2.8** |
| ***Duration of Use*** | | | | | | |
| Leave-On | NR | NR | 4 | NR | 10 | 0.4-1 |
| Rinse-Off | 5 | 11-27 | 4 | NR | 11 | 0.4-2.8 |
| Diluted for (Bath) Use | NR | NR | NR | NR | NR | NR |
| ***Exposure Type*** | | | | | | |
| Eye Area | NR | NR | 2 | NR | NR | NR |
| Incidental Ingestion | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Spray | NR | NR | NR | NR | NR | 0.4[e] |
| Incidental Inhalation-Powder | NR | NR | NR | NR | NR | NR |
| Dermal Contact | 5 | 11-27 | 6 | NR | 8 | 2.8 |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | NR | NR | 2 | NR | 12 | 0.4-1 |
| Hair-Coloring | NR | NR | NR | NR | 1 | NR |
| Nail | NR | NR | NR | NR | NR | NR |
| Mucous Membrane | NR | NR | NR | NR | 1 | 2.8 |
| Baby Products | NR | NR | 2 | NR | NR | NR |

| | **Sodium Cocoyl Apple Amino Acids** | | **Sodium Cocoyl Collagen Amino Acids** | | **Sodium Cocoyl Glutamate** | |
|---|---|---|---|---|---|---|
| **Totals*** | **21** | **0.3-3** | **13** | **0.02** | **178** | **0.004-10** |
| ***Duration of Use*** | | | | | | |
| Leave-On | 10 | 0.3 | 3 | 0.02 | 66 | 0.004-3 |
| Rinse-Off | 11 | *0.5-3* | 10 | 0.02 | 110 | 0.01-10 |
| Diluted for (Bath) Use | NR | NR | NR | NR | 2 | NR |
| ***Exposure Type*** | | | | | | |
| Eye Area | 7 | 0.3 | 1 | NR | 8 | 0.004-0.6 |
| Incidental Ingestion | NR | NR | NR | NR | 7 | NR |
| Incidental Inhalation-Spray | NR | NR | NR | NR | NR | 0.03%[f] |
| Incidental Inhalation-Powder | NR | NR | NR | NR | 1 | NR |
| Dermal Contact | 18 | 0.3-3 | 2 | NR | 114 | 0.004-9 |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | 3 | 0.5 | 11 | 0.02 | 27 | 0.2-10 |
| Hair-Coloring | NR | NR | NR | NR | 30 | 3 |
| Nail | NR | NR | NR | NR | NR | NR |
| Mucous Membrane | 4 | NR | 1 | NR | 31 | 0.2-3 |
| Baby Products | NR | NR | NR | NR | NR | NR |

| | **Sodium Cocoyl Glycinate** | | **Sodium Hydrogenated Tallowoyl Glutamate** | | **Sodium Lauroyl Aspartate** | |
|---|---|---|---|---|---|---|
| **Totals*** | **32** | **0.2-20** | **2** | **0.8** | **4** | **0.005-2** |
| ***Duration of Use*** | | | | | | |
| Leave-On | 1 | NR | 1 | 0.8 | 4 | 0.005-0.2 |
| Rinse Off | 31 | *0.2-20* | 1 | NR | NR | 2 |
| Diluted for (Bath) Use | NR | NR | NR | NR | NR | NR |
| ***Exposure Type*** | | | | | | |
| Eye Area | NR | NR | NR | NR | 2 | 0.1 |
| Incidental Ingestion | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Spray | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Powder | NR | NR | NR | NR | NR | 0.2 |
| Dermal Contact | 32 | 0.2-20 | 2 | 0.8 | 4 | 0.005-2 |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | NR | NR | NR | NR | NR | 2 |
| Hair-Coloring | NR | NR | NR | NR | NR | NR |
| Nail | NR | NR | NR | NR | NR | NR |
| Mucous Membrane | 10 | 0.2-3 | NR | NR | NR | NR |
| Baby Products | NR | NR | NR | NR | NR | NR |

**Table 5a.** Frequency and concentration of use (2012-2013) according to duration and type of exposure for Amino Acid Alkyl Amides.[25]

| | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* |
|---|---|---|---|---|---|---|
| | **Sodium Lauroyl Glutamate** | | **Sodium Lauroyl Oat Amino Acids** | | **Sodium Lauroyl Wheat Amino Acids** | |
| **Totals*** | **75** | **0.003-40** | **98** | **0.04-5** | **1** | NR |
| ***Duration of Use*** | | | | | | |
| Leave-On | 7 | 0.03-4 | 14 | 0.4-0.8 | NR | NR |
| Rinse-Off | 63 | 0.003-40 | 79 | 0.04-5 | 1 | NR |
| Diluted for (Bath) Use | 5 | 4 | 5 | 0.9 | NR | NR |
| ***Exposure Type*** | | | | | | |
| Eye Area | 1 | NR | NR | 5 | NR | NR |
| Incidental Ingestion | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Spray | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Powder | 1 | NR | NR | NR | NR | NR |
| Dermal Contact | 54 | 0.003-40 | 71 | 0.09-5 | 1 | NR |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | 21 | 3 | 27 | 0.04-0.4 | NR | NR |
| Hair-Coloring | NR | NR | NR | NR | NR | NR |
| Nail | NR | NR | NR | NR | NR | NR |
| Mucous Membrane | 20 | 4 | 38 | 0.09-5 | NR | NR |
| Baby Products | 2 | NR | 1 | NR | NR | NR |

| | **Sodium Myristoyl Glutamate** | | **Sodium Palmitoyl Proline** | | **Sodium Palmoyl Glutamate** | |
|---|---|---|---|---|---|---|
| **Totals*** | **51** | **0.1-31** | **7** | NR | NR | **2-22** |
| ***Duration of Use*** | | | | | | |
| Leave-On | 44 | 0.1-5 | 6 | NR | NR | NR |
| Rinse-Off | 7 | 0.1-31 | 1 | NR | NR | 2-22 |
| Diluted for (Bath) Use | NR | NR | NR | NR | NR | NR |
| ***Exposure Type*** | | | | | | |
| Eye Area | 10 | 0.1 | NR | NR | NR | NR |
| Incidental Ingestion | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Spray | NR | NR | 1 | NR | NR | NR |
| Incidental Inhalation-Powder | NR | NR | NR | NR | NR | NR |
| Dermal Contact | 50 | 0.1-31 | 7 | NR | NR | 2-22 |
| Deodorant (underarm) | NR | NR | 1 | NR | NR | NR |
| Hair - Non-Coloring | NR | NR | NR | NR | NR | NR |
| Hair-Coloring | NR | NR | NR | NR | NR | NR |
| Nail | 1 | 0.5 | NR | NR | NR | NR |
| Mucous Membrane | NR | 31 | NR | NR | NR | NR |
| Baby Products | NR | NR | NR | NR | NR | NR |

| | **Sodium Stearoyl Glutamate** | | **TEA-Cocoyl Alaninate** | | **TEA-Cocoyl Glutamate** | |
|---|---|---|---|---|---|---|
| **Totals*** | **120** | **0.03-2** | **2** | **0.8** | **65** | **2-10.5** |
| ***Duration of Use*** | | | | | | |
| Leave-On | 106 | 0.2-2 | NR | NR | 9 | 2 |
| Rinse Off | 14 | 0.03-1.1 | 2 | 0.8 | 56 | 2-10.5 |
| Diluted for (Bath) Use | NR | NR | NR | NR | NR | NR |
| ***Exposure Type*** | | | | | | |
| Eye Area | 5 | 1 | NR | NR | NR | NR |
| Incidental Ingestion | NR | 1 | NR | NR | NR | NR |
| Incidental Inhalation-Spray | 6 | 0.2-0.3[g] | NR | NR | 1 | NR |
| Incidental Inhalation-Powder | NR | NR | NR | NR | NR | NR |
| Dermal Contact | 114 | 0.2-2 | 2 | 0.8 | 63 | 2.1-10.5 |
| Deodorant (underarm) | 3 | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | 6 | 0.03-0.2 | NR | NR | 2 | 2-10 |
| Hair-Coloring | NR | NR | NR | NR | NR | NR |
| Nail | NR | NR | NR | NR | NR | NR |
| Mucous Membrane | 3 | 1 | NR | NR | 36 | 2.1-3 |
| Baby Products | NR | NR | NR | NR | 1 | NR |

**Table 5a.** Frequency and concentration of use (2012-2013) according to duration and type of exposure for Amino Acid Alkyl Amides.[25]

| | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* |
|---|---|---|---|---|---|---|
| | **TEA-Lauroyl Collagen Amino Acids** | | **TEA-Lauroyl Glutamate** | | **Undecylenoyl Collagen Amino Acids** | |
| **Totals*** | **3** | **0.4** | **1** | **NR** | **2** | NR |
| ***Duration of Use*** | | | | | | |
| Leave-On | 3 | 0.4 | NR | NR | NR | NR |
| Rinse-Off | NR | NR | 1 | NR | 2 | NR |
| Diluted for (Bath) Use | NR | NR | NR | NR | NR | NR |
| ***Exposure Type*** | | | | | | |
| Eye Area | NR | NR | NR | NR | NR | NR |
| Incidental Ingestion | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Spray | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Powder | NR | NR | NR | NR | NR | NR |
| Dermal Contact | NR | NR | 1 | NR | NR | NR |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | 3 | 0.4 | NR | NR | 2 | NR |
| Hair-Coloring | NR | NR | NR | NR | NR | NR |
| Nail | NR | NR | NR | NR | NR | NR |
| Mucous Membrane | NR | NR | 1 | NR | NR | NR |
| Baby Products | NR | NR | NR | NR | NR | NR |

| | **Undecylenoyl Glycine** | | **Undecylenoyl Phenylalanine** | |
|---|---|---|---|---|
| **Totals*** | **10** | **0.3** | **18** | **0.5-2** |
| ***Duration of Use*** | | | | |
| Leave-On | 6 | 0.3 | 17 | 0.5-2 |
| Rinse-Off | 4 | NR | 1 | NR |
| Diluted for (Bath) Use | NR | NR | NR | NR |
| ***Exposure Type*** | | | | |
| Eye Area | 1 | 0.3 | NR | NR |
| Incidental Ingestion | NR | NR | NR | NR |
| Incidental Inhalation-Spray | 3 | NR | NR | NR |
| Incidental Inhalation-Powder | NR | NR | NR | NR |
| Dermal Contact | 4 | 0.3 | 18 | 0.5-2 |
| Deodorant (underarm) | NR | NR | NR | NR |
| Hair - Non-Coloring | 4 | NR | NR | NR |
| Hair-Coloring | NR | NR | NR | NR |
| Nail | 2 | NR | NR | NR |
| Mucous Membrane | NR | NR | NR | NR |
| Baby Products | NR | NR | NR | NR |

[a]1% in a face and neck spray.

[b]0.3% in a foundation spray.

[c]0.6% in hand soap categorized as "other personal cleanliness product".

[d]0.2% in a pump hair spray; 0.2% in a spray tonic, dressing, and other hair grooming aids; and 0.2% in a body and hand spray.

[e]0.4% in pump hair spray.

[f]0.03% in a foundation spray.

[g]0.2% in an indoor tanning product, 0.3% in a body and hand spray

**Table 5b**. Amino acid alkyl amides not reported in use.

Acetyl arginine
Acetyl glutamic acid
Acetyl histidine
Acetyl proline
Capryloyl collagen amino acids
Capryloyl gold of pleasure amino acids
Capryloyl keratin amino acids
Capryloyl pea amino acids
Capryloyl quinoa amino acids
Capryloyl silk amino acids
Dipalmitoyl cystine
Dipotassium capryloyl glutamate
Dipotassium undecylenoyl glutamate
Disodium N-lauroyl aspartate
Disodium undecylenoyl glutamate
Lauroyl glutamic acid
Myristoyl glutamic acid
Palmitoyl alanine
Palmitoyl arginine
Palmitoyl glutamic acid
Palmitoyl gold of pleasure amino acids
Palmitoyl isoleucine
Palmitoyl millet amino acids
Palmitoyl oat amino acids
Palmitoyl pea amino acids
Palmitoyl quinoa amino acids
Potassium caproyl tyrosine
Potassium capryloyl glutamate
Potassium cocoyl rice amino acids
Potassium lauroyl collagen amino acids
Potassium lauroyl glutamate
Potassium lauroyl oat amino acids
Potassium lauroyl pea amino acids
Potassium lauroyl silk amino acids
Potassium olivoyl/lauroyl wheat amino acids
Potassium stearoyl glutamate
Potassium undecylenoyl glutamate
Propionyl collagen amino acids
Sodium capryloyl prolinate
Sodium capryloyl glutamate
Sodium cocoyl barley amino acids
Sodium cocoyl glutaminate
Sodium cocoyl/hydrogenated tallow glutamate
Sodium cocoyl oat amino acids
Sodium cocoyl/palmoyl/sunfloweroyl glutamate
Sodium cocoyl proline
Sodium cocoyl threoninate
Sodium cocoyl wheat amino acids
Sodium lauroyl collagen amino acids
Sodium lauroyl millet amino acids
Sodium lauroyl/myristoyl aspartate
Sodium lauroyl silk amino acids
Sodium lauroyl/myristoyl aspartate
Sodium lauroyl silk amino acids
Sodium olivoyl glutamate
Sodium/TEA-lauroyl collagen amino acids
Sodium/TEA-lauroyl keratin amino acids
Sodium/TEA-undecylenoyl collagen amino acids
Sodium undecylenoyl glutamate
Stearoyl glutamic acid
Stearoyl leucine
TEA cocoyl glutaminate
TEA-hydrogenated tallowyl glutamate
TEA-lauroyl keratin amino acids
TEA-lauroyl/myristoyl aspartate
Undecylenoyl wheat amino acids
Zinc lauroyl asparate

**Table 6.** Genotoxicity

| Concentration/Dose | Method | Results | Reference |
|---|---|---|---|
| *In Vitro* | | | |
| **ACETYL GLUTAMIC ACID** | | | |
| 333 to 5000 µg/plate with and without S9 metabolic activation | Bacterial reverse mutation assay in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and *Escherichia coli* strain WP2uvrA | Not mutagenic | [26] |
| **ACETYL PROLINE** | | | |
| 0.4%, 0.2%, 0.1%, 0.05%, 0.025%, and 0.0125% with S9 metabolic activation | Ames II assay in *S.typhimurium* strains TA 98 and mixed strains | Not mutagenic | [27] |
| **ACETYL TYROSINAMIDE** | | | |
| 0, 313, 625, 1250, 2500, and 5000 µg/plate with and without S9 metabolic activation | Bacterial reverse mutation assay in *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and *E.coli* strain WP2uvrA | Negative | [28] |
| Up to 2230 µg/mL under 3 h and 22 h treatment with and without metabolic activation | Chromosomal aberration assay in cultured peripheral blood lymphocytes | Negative | [29] |
| **DISODIUM CAPRYLOYL GLUTAMATE** | | | |
| Details not provided | Ames test (details not provided) | Not mutagenic | [23] |
| **SODIUM COCOYL GLUTAMATE** | | | |
| Details not provided | Ames test (details not provided) | Not mutagenic | [15] |
| **SODIUM LAUROYL GLUTAMATE** | | | |
| Details not provided | Ames test (details not provided) | Not mutagenic | [30] |
| *In Vivo* | | | |
| **ACETYL GLUTAMIC ACID** | | | |
| 500, 1000, or 2000 mg/kg | Bone marrow micronucleus assay in groups of 5 male and 5 female ICR mice. | No increased incidence of micronucleated polychromatic erythrocytes | [26] |

**Table 7.** Dermal irritation studies.

| Ingredient | Concentration | Method | Results | Reference |
|---|---|---|---|---|
| ***Non-Human*** | | | | |
| Acetyl Proline | 8% in a cream tested neat | MatTek EpiDerm assay | Very mild irritant | [31] |
| Disodium Capryloyl Glutamate | 5% of a solution containing 37%-41% test material | MTT Viability assay | Not irritating | [23] |
| ***Human*** | | | | |
| Acetyl Proline | 10% in a cream evaluated for treatment of eczema or active atopic dermatitis | Double-blind, randomized controlled usage study in 15 subjects where test material was applied to target lesion twice/day for 14 days | 1subject had an acute chronic dermatitis reaction that was considered related to the test material | [32] |
| Acetyl Tyrosinamide | 2% in a gel formulation | 48-h patch test in 53 volunteers; semi-occluded | Not irritating | [33] |
| Acetyl Tyrosinamide | 1.25%-2% in several gel and skin plumping cream formulations | 48-h patch test in 51 volunteers; semi-occluded | 1 subject had moderate erythema and edema post-application that became mild at the 72-h observation to the skin plumping cream containing 1.25% test material, another subject had mild erythema and edema 48-h to the same skin plumping cream formulation, which was barely perceptible at 72-h – this same subject had a barely perceptible erythema at 48-h to the skin plumping cream containing 2% of the test material, no reaction was observed at 72-h. The study concluded that the test material was not irritating in all formulations tested. | [34] |
| Disodium Capryloyl Glutamate | 18% of a solution containing 37%-41% test material | Patch test with Finn Chambers in 20 volunteers; occluded | Not irritating | [23] |
| Sodium Cocoyl Glutamate | 10% active matter | Flex Wash Test | Not irritating | [15] |
| Sodium Lauroyl Glutamate | 10% active matter | Flex Wash Test in 20 volunteers | Irritation index below 0.5, not irritating | [30] |
| Sodium Lauroyl Glutamate | A 1% solution and in mixtures with SLS at 0.75%, 0.50% and 0.25% | 15 volunteers received test material on test sites with polypropylene chambers for 24 h. Application sites were measured for transepidermal water loss (TEWL) and graded for irritation reactions. | TEWL values of 1% SLG were significantly higher than those of the deionized water control. | [35] |

**Table 8.** Ocular irritation studies.

| Ingredient | Concentration | Method | Results | Reference |
|---|---|---|---|---|
| ***Non-Human – In Vitro*** | | | | |
| Acetyl Tyrosinamide | 1.25% neat | EpiOcular irritation study | Not irritating | [36] |
| Disodium Caprylolyl Glutamate | 2% as received | HET-CAM method | Not irritating | [23] |
| Sodium Cocoyl Glutamate | Not reported | Red Blood Cell test | Not irritating | [15] |
| Sodium Cocoyl Glutamate | 5% | HET-CAM method | Score = 13, strong or severe irritation | [37,38] |
| Sodium Lauroyl Glutamate | 5% active matter | HET-CAM method | Not irritating | [30] |
| Sodium Lauroyl Glutamate | Not reported | Red Blood Cell test | Not irritating | [14] |
| Sodium Lauroyl Glutaminate | Up to 1% | Rabbit corneal epithelium model by measurement of viability with MTT assay | Viability at concentration 0.5% was 32.7%. The 50% inhibitory concentration (IC50) was 0.934%. | [39] |
| ***Non-Human – In Vivo*** | | | | |
| Lauroyl Arginine + mixture of collagen polypeptides with MW < 1000 Da | 10%, pH adjusted to 7.0 | Draize method in 6 male albino rabbits | Mean score was 7.5, not irritating | [40] |
| | | | | |
| ***Human*** | | | | |
| Acetyl Hydroxyproline | 2% in a gel under eye treatment | 4 week in-use study in 33 women; half contact lens wearers and half non-contact lens wearers | No adverse events during the study and no ophthalmic irritation potential | [41] |
| Acetyl Tyrosinamide | 2% in a gel under eye treatment | 4 week in-use study in 33 women; half contact lens wearers and half non-contact lens wearers | No adverse events during the study and no ophthalmic irritation potential | [42] |

**Table 9.** Dermal sensitization studies.

| Ingredient | Concentration | Method | Results | Reference |
|---|---|---|---|---|
| ***Human*** | | | | |
| Acetyl Hydroxyproline | 2% in a plumper gel | HRIPT in 109 volunteers; semi-occluded | Not irritating or sensitizing | [43] |
| Acetyl Proline | 10% in a cream | HRIPT in 107 volunteers; semi-occluded | Not irritating or sensitizing | [44] |
| Acetyl Tyrosinamide | 1% neat | HRIPT to a sodium lauryl sulfate pre-treated site with 26 volunteers; occluded | Non-sensitizing | [45] |
| Acetyl Tyrosinamide | 2% in a plumper gel | HRIPT in 109 volunteers; semi-occluded | Not irritating or sensitizing | [46] |
| Disodium Capryloyl Glutamate | 18% of a solution containing 37%-41% test material | Patch test with Finn Chambers in 20 volunteers; occluded | Non- sensitizing | [23] |
| Sodium Cocoyl Glutamate | 5% active matter | Method not reported, but test was occluded | Non-sensitizing | [15] |
| Sodium Lauroyl Glutamate | 5% active matter | Patch test with Finn Chambers in 20 volunteers; occluded | Non-sensitizing | [30] |

**Table 10.** Phototoxicity and photosensitization

| Ingredient | Concentration | Method | Results | Reference |
|---|---|---|---|---|
| ***Non-Human – In Vitro*** | | | | |
| Acetyl Tyrosinamide | Eight doses up to 1000 µg/mL with and without UVA | Neutral red uptake assay in BALB/c 3T3 mouse fibroblasts | Not predicted to have phototoxic potential | [47] |
| ***Human*** | | | | |
| Acetyl Tyrosinamide | 1% neat | Human photocontact allergenicty assay with 25 volunteers; occluded | No photocontact-sensitizing potential | [48] |
| Sodium Cocoyl Glutamate | 0.1%-5% aq. solutions | Not reported | No abnormality observed | [15] |
| Sodium Lauroyl Glutamate | 0.1%-5% aq. solutions | Not reported | No abnormality observed | [14] |

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2013 FDA VCRP RAW DATA

| | | | |
|---|---|---|---|
| 03B - Eyeliner | 616911 | ACETYL CYSTEINE | 1 |
| 03G - Other Eye Makeup Preparations | 616911 | ACETYL CYSTEINE | 3 |
| 05A - Hair Conditioner | 616911 | ACETYL CYSTEINE | 3 |
| 05E - Rinses (non-coloring) | 616911 | ACETYL CYSTEINE | 1 |
| 05F - Shampoos (non-coloring) | 616911 | ACETYL CYSTEINE | 4 |
| 05G - Tonics, Dressings, and Other Hair Grooming Aids | 616911 | ACETYL CYSTEINE | 1 |
| 05I - Other Hair Preparations | 616911 | ACETYL CYSTEINE | 1 |
| 07C - Foundations | 616911 | ACETYL CYSTEINE | 1 |
| 12F - Moisturizing | 616911 | ACETYL CYSTEINE | 3 |
| 12G - Night | 616911 | ACETYL CYSTEINE | 2 |
| 12H - Paste Masks (mud packs) | 616911 | ACETYL CYSTEINE | 1 |
| 12I - Skin Fresheners | 616911 | ACETYL CYSTEINE | 1 |
| 12J - Other Skin Care Preps | 616911 | ACETYL CYSTEINE | 1 |
| | | | |
| 05A - Hair Conditioner | 2490973 | ACETYL GLUTAMINE | 2 |
| 05F - Shampoos (non-coloring) | 2490973 | ACETYL GLUTAMINE | 3 |
| 05G - Tonics, Dressings, and Other Hair Grooming Aids | 2490973 | ACETYL GLUTAMINE | 1 |
| 11G - Other Shaving Preparation Products | 2490973 | ACETYL GLUTAMINE | 1 |
| 12F - Moisturizing | 2490973 | ACETYL GLUTAMINE | 1 |
| | | | |
| 03B - Eyeliner | 1115475 | ACETYL METHIONINE | 1 |
| 03G - Other Eye Makeup Preparations | 1115475 | ACETYL METHIONINE | 3 |
| 05A - Hair Conditioner | 1115475 | ACETYL METHIONINE | 1 |
| 05F - Shampoos (non-coloring) | 1115475 | ACETYL METHIONINE | 1 |
| 05G - Tonics, Dressings, and Other Hair Grooming Aids | 1115475 | ACETYL METHIONINE | 1 |
| 05I - Other Hair Preparations | 1115475 | ACETYL METHIONINE | 1 |
| 08A - Basecoats and Undercoats | 1115475 | ACETYL METHIONINE | 1 |
| | | | |
| 03D - Eye Lotion | 537553 | ACETYL TYROSINE | 1 |
| 03G - Other Eye Makeup Preparations | 537553 | ACETYL TYROSINE | 1 |
| 05A - Hair Conditioner | 537553 | ACETYL TYROSINE | 1 |
| 05E - Rinses (non-coloring) | 537553 | ACETYL TYROSINE | 1 |
| 05F - Shampoos (non-coloring) | 537553 | ACETYL TYROSINE | 1 |
| 05G - Tonics, Dressings, and Other Hair Grooming Aids | 537553 | ACETYL TYROSINE | 2 |
| 05I - Other Hair Preparations | 537553 | ACETYL TYROSINE | 3 |
| 10E - Other Personal Cleanliness Products | 537553 | ACETYL TYROSINE | 1 |
| 12A - Cleansing | 537553 | ACETYL TYROSINE | 2 |
| 12C - Face and Neck (exc shave) | 537553 | ACETYL TYROSINE | 2 |
| 12D - Body and Hand (exc shave) | 537553 | ACETYL TYROSINE | 3 |
| 12F - Moisturizing | 537553 | ACETYL TYROSINE | 4 |
| 12G - Night | 537553 | ACETYL TYROSINE | 3 |
| 12I - Skin Fresheners | 537553 | ACETYL TYROSINE | 1 |
| 13A - Suntan Gels, Creams, and Liquids | 537553 | ACETYL TYROSINE | 1 |

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| 13B - Indoor Tanning Preparations | 537553 | ACETYL TYROSINE | 1 |
| 13C - Other Suntan Preparations | 537553 | ACETYL TYROSINE | 1 |
| | | | |
| 02D - Other Bath Preparations | 14246538 | CAPRYLOYL GLYCINE | 1 |
| 03C - Eye Shadow | 14246538 | CAPRYLOYL GLYCINE | 1 |
| 03D - Eye Lotion | 14246538 | CAPRYLOYL GLYCINE | 1 |
| 03G - Other Eye Makeup Preparations | 14246538 | CAPRYLOYL GLYCINE | 1 |
| 04B - Perfumes | 14246538 | CAPRYLOYL GLYCINE | 1 |
| 04E - Other Fragrance Preparation | 14246538 | CAPRYLOYL GLYCINE | 1 |
| 05A - Hair Conditioner | 14246538 | CAPRYLOYL GLYCINE | 1 |
| 05F - Shampoos (non-coloring) | 14246538 | CAPRYLOYL GLYCINE | 8 |
| 05I - Other Hair Preparations | 14246538 | CAPRYLOYL GLYCINE | 1 |
| 07A - Blushers (all types) | 14246538 | CAPRYLOYL GLYCINE | 1 |
| 07C - Foundations | 14246538 | CAPRYLOYL GLYCINE | 1 |
| 07I - Other Makeup Preparations | 14246538 | CAPRYLOYL GLYCINE | 1 |
| 10A - Bath Soaps and Detergents | 14246538 | CAPRYLOYL GLYCINE | 1 |
| 10B - Deodorants (underarm) | 14246538 | CAPRYLOYL GLYCINE | 2 |
| 10C - Douches | 14246538 | CAPRYLOYL GLYCINE | 3 |
| 10E - Other Personal Cleanliness Products | 14246538 | CAPRYLOYL GLYCINE | 1 |
| 12A - Cleansing | 14246538 | CAPRYLOYL GLYCINE | 12 |
| 12C - Face and Neck (exc shave) | 14246538 | CAPRYLOYL GLYCINE | 9 |
| 12D - Body and Hand (exc shave) | 14246538 | CAPRYLOYL GLYCINE | 6 |
| 12F - Moisturizing | 14246538 | CAPRYLOYL GLYCINE | 14 |
| 12G - Night | 14246538 | CAPRYLOYL GLYCINE | 1 |
| 12H - Paste Masks (mud packs) | 14246538 | CAPRYLOYL GLYCINE | 2 |
| 12I - Skin Fresheners | 14246538 | CAPRYLOYL GLYCINE | 1 |
| 12J - Other Skin Care Preps | 14246538 | CAPRYLOYL GLYCINE | 4 |
| | | | |
| 07C - Foundations | 999002859 | DISODIUM CAPRYLOYL GLUTAMATE | 1 |
| 12C - Face and Neck (exc shave) | 999002859 | DISODIUM CAPRYLOYL GLUTAMATE | 1 |
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| 03C - Eye Shadow | 68187304 | DISODIUM COCOYL GLUTAMATE | 1 |
| 05F - Shampoos (non-coloring) | 68187304 | DISODIUM COCOYL GLUTAMATE | 15 |
| 06A - Hair Dyes and Colors (all types requiring caution statements and patch tests) | 68187304 | DISODIUM COCOYL GLUTAMATE | 30 |
| 07A - Blushers (all types) | 68187304 | DISODIUM COCOYL GLUTAMATE | 1 |
| 07B - Face Powders | 68187304 | DISODIUM COCOYL GLUTAMATE | 2 |
| 07C - Foundations | 68187304 | DISODIUM COCOYL GLUTAMATE | 4 |
| 10A - Bath Soaps and Detergents | 68187304 | DISODIUM COCOYL GLUTAMATE | 5 |
| 10E - Other Personal Cleanliness Products | 68187304 | DISODIUM COCOYL GLUTAMATE | 2 |
| 11E - Shaving Cream | 68187304 | DISODIUM COCOYL GLUTAMATE | 1 |

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| 12A - Cleansing | 68187304 | DISODIUM COCOYL GLUTAMATE | 14 |
| 12D - Body and Hand (exc shave) | 68187304 | DISODIUM COCOYL GLUTAMATE | 1 |
| | | | |
| 12A - Cleansing | 999001859 | DISODIUM LAUROYL GLUTAMATE | 1 |
| | | | |
| 12C - Face and Neck (exc shave) | 126139795 | DISODIUM MALYL TYROSINATE | 1 |
| | | | |
| 03C - Eye Shadow | 20716307 | DISODIUM STEAROYL GLUTAMATE | 3 |
| 03F - Mascara | 20716307 | DISODIUM STEAROYL GLUTAMATE | 2 |
| 03G - Other Eye Makeup Preparations | 20716307 | DISODIUM STEAROYL GLUTAMATE | 10 |
| 07A - Blushers (all types) | 20716307 | DISODIUM STEAROYL GLUTAMATE | 4 |
| 07B - Face Powders | 20716307 | DISODIUM STEAROYL GLUTAMATE | 6 |
| 07C - Foundations | 20716307 | DISODIUM STEAROYL GLUTAMATE | 56 |
| 07D - Leg and Body Paints | 20716307 | DISODIUM STEAROYL GLUTAMATE | 1 |
| 07E - Lipstick | 20716307 | DISODIUM STEAROYL GLUTAMATE | 3 |
| 07F - Makeup Bases | 20716307 | DISODIUM STEAROYL GLUTAMATE | 4 |
| 07H - Makeup Fixatives | 20716307 | DISODIUM STEAROYL GLUTAMATE | 2 |
| 07I - Other Makeup Preparations | 20716307 | DISODIUM STEAROYL GLUTAMATE | 12 |
| 12C - Face and Neck (exc shave) | 20716307 | DISODIUM STEAROYL GLUTAMATE | 9 |
| 12D - Body and Hand (exc shave) | 20716307 | DISODIUM STEAROYL GLUTAMATE | 1 |
| 12F - Moisturizing | 20716307 | DISODIUM STEAROYL GLUTAMATE | 15 |
| 12J - Other Skin Care Preps | 20716307 | DISODIUM STEAROYL GLUTAMATE | 7 |
| | | | |
| 05F - Shampoos (non-coloring) | 42492228 | LAUROYL ARGININE | 1 |
| | | | |
| 05A - Hair Conditioner | 68920592 | LAUROYL COLLAGEN AMINO ACIDS | 1 |
| | | | |
| 03A - Eyebrow Pencil | 52315750 | LAUROYL LYSINE | 8 |
| 03B - Eyeliner | 52315750 | LAUROYL LYSINE | 10 |
| 03C - Eye Shadow | 52315750 | LAUROYL LYSINE | 201 |
| 03D - Eye Lotion | 52315750 | LAUROYL LYSINE | 4 |
| 03F - Mascara | 52315750 | LAUROYL LYSINE | 37 |
| 03G - Other Eye Makeup Preparations | 52315750 | LAUROYL LYSINE | 5 |

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| 04B - Perfumes | 52315750 | LAUROYL LYSINE | 2 |
| 04C - Powders (dusting and talcum, excluding aftershave talc) | 52315750 | LAUROYL LYSINE | 1 |
| 04E - Other Fragrance Preparation | 52315750 | LAUROYL LYSINE | 2 |
| 05A - Hair Conditioner | 52315750 | LAUROYL LYSINE | 3 |
| 05E - Rinses (non-coloring) | 52315750 | LAUROYL LYSINE | 1 |
| 07A - Blushers (all types) | 52315750 | LAUROYL LYSINE | 65 |
| 07B - Face Powders | 52315750 | LAUROYL LYSINE | 172 |
| 07C - Foundations | 52315750 | LAUROYL LYSINE | 55 |
| 07D - Leg and Body Paints | 52315750 | LAUROYL LYSINE | 1 |
| 07E - Lipstick | 52315750 | LAUROYL LYSINE | 24 |
| 07F - Makeup Bases | 52315750 | LAUROYL LYSINE | 3 |
| 07G - Rouges | 52315750 | LAUROYL LYSINE | 9 |
| 07H - Makeup Fixatives | 52315750 | LAUROYL LYSINE | 4 |
| 07I - Other Makeup Preparations | 52315750 | LAUROYL LYSINE | 10 |
| 08E - Nail Polish and Enamel | 52315750 | LAUROYL LYSINE | 1 |
| 12C - Face and Neck (exc shave) | 52315750 | LAUROYL LYSINE | 8 |
| 12D - Body and Hand (exc shave) | 52315750 | LAUROYL LYSINE | 1 |
| 12F - Moisturizing | 52315750 | LAUROYL LYSINE | 14 |
| 12H - Paste Masks (mud packs) | 52315750 | LAUROYL LYSINE | 2 |
| 12J - Other Skin Care Preps | 52315750 | LAUROYL LYSINE | 3 |
| 13B - Indoor Tanning Preparations | 52315750 | LAUROYL LYSINE | 2 |
| 13C - Other Suntan Preparations | 52315750 | LAUROYL LYSINE | 1 |
| | | | |
| 12J - Other Skin Care Preps | 58725396 | LAUROYL PROLINE | 1 |
| | | | |
| 05F - Shampoos (non-coloring) | 999002635 | LAUROYL SILK AMINO ACIDS | 1 |
| 12F - Moisturizing | 999002635 | LAUROYL SILK AMINO ACIDS | 1 |
| | | | |
| 07C - Foundations | 999001497 | MAGNESIUM PALMITOYL GLUTAMATE | 1 |
| 08G - Other Manicuring Preparations | 999001497 | MAGNESIUM PALMITOYL GLUTAMATE | 1 |
| 12C - Face and Neck (exc shave) | 999001497 | MAGNESIUM PALMITOYL GLUTAMATE | 4 |
| 12F - Moisturizing | 999001497 | MAGNESIUM PALMITOYL GLUTAMATE | 5 |
| 12G - Night | 999001497 | MAGNESIUM PALMITOYL GLUTAMATE | 2 |
| 12I - Skin Fresheners | 999001497 | MAGNESIUM PALMITOYL GLUTAMATE | 1 |
| 12J - Other Skin Care Preps | 999001497 | MAGNESIUM PALMITOYL GLUTAMATE | 1 |
| | | | |
| 13B - Indoor Tanning Preparations | 999002305 | OLEOYL TYROSINE | 1 |
| 13C - Other Suntan Preparations | 999002305 | OLEOYL TYROSINE | 2 |
| | | | |
| 12E - Foot Powders and Sprays | 977098820 | PALMITOYL COLLAGEN AMINO ACIDS | 1 |
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|---|---|---|---|
| 03D - Eye Lotion | 2441410 | PALMITOYL GLYCINE | 1 |
| 03G - Other Eye Makeup Preparations | 2441410 | PALMITOYL GLYCINE | 2 |
| 12G - Night | 2441410 | PALMITOYL GLYCINE | 2 |
| | | | |
| 05A - Hair Conditioner | 977158374 | PALMITOYL KERATIN AMINO ACIDS | 1 |
| 12D - Body and Hand (exc shave) | 977158374 | PALMITOYL KERATIN AMINO ACIDS | 2 |
| 12F - Moisturizing | 977158374 | PALMITOYL KERATIN AMINO ACIDS | 2 |
| | | | |
| 07C - Foundations | 59441326 | PALMITOYL PROLINE | 1 |
| 08G - Other Manicuring Preparations | 59441326 | PALMITOYL PROLINE | 1 |
| 12C - Face and Neck (exc shave) | 59441326 | PALMITOYL PROLINE | 4 |
| 12F - Moisturizing | 59441326 | PALMITOYL PROLINE | 5 |
| 12G - Night | 59441326 | PALMITOYL PROLINE | 2 |
| 12I - Skin Fresheners | 59441326 | PALMITOYL PROLINE | 1 |
| 12J - Other Skin Care Preps | 59441326 | PALMITOYL PROLINE | 1 |
| | | | |
| 07C - Foundations | 977169871 | PALMITOYL SILK AMINO ACIDS | 2 |
| | | | |
| 12A - Cleansing | 977100085 | POTASSIUM COCOYL GLUTAMATE | 6 |
| | | | |
| 02D - Other Bath Preparations | 999001480 | POTASSIUM COCOYL GLYCINATE | 1 |
| 10A - Bath Soaps and Detergents | 999001480 | POTASSIUM COCOYL GLYCINATE | 2 |
| 10E - Other Personal Cleanliness Products | 999001480 | POTASSIUM COCOYL GLYCINATE | 1 |
| 12A - Cleansing | 999001480 | POTASSIUM COCOYL GLYCINATE | 12 |
| | | | |
| 10E - Other Personal Cleanliness Products | 977166930 | POTASSIUM LAUROYL WHEAT AMINO ACIDS | 1 |
| 12A - Cleansing | 977166930 | POTASSIUM LAUROYL WHEAT AMINO ACIDS | 3 |
| | | | |
| 12A - Cleansing | 977185559 | POTASSIUM MYRISTOYL GLUTAMATE | 5 |
| | | | |
| 01C - Other Baby Products | 90170459 | SODIUM COCOYL ALANINATE | 2 |
| 03G - Other Eye Makeup Preparations | 90170459 | SODIUM COCOYL ALANINATE | 2 |
| 05F - Shampoos (non-coloring) | 90170459 | SODIUM COCOYL ALANINATE | 2 |
| 12A - Cleansing | 90170459 | SODIUM COCOYL ALANINATE | 2 |
| | | | |
| 05A - Hair Conditioner | 999001175 | SODIUM COCOYL AMINO ACIDS | 2 |
| 05F - Shampoos (non-coloring) | 999001175 | SODIUM COCOYL AMINO ACIDS | 4 |
| 05G - Tonics, Dressings, and Other Hair Grooming Aids | 999001175 | SODIUM COCOYL AMINO ACIDS | 5 |

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| 05H - Wave Sets | 999001175 | SODIUM COCOYL AMINO ACIDS | 1 |
| 06D - Hair Shampoos (coloring) | 999001175 | SODIUM COCOYL AMINO ACIDS | 1 |
| 07C - Foundations | 999001175 | SODIUM COCOYL AMINO ACIDS | 1 |
| 10A - Bath Soaps and Detergents | 999001175 | SODIUM COCOYL AMINO ACIDS | 1 |
| 11A - Aftershave Lotion | 999001175 | SODIUM COCOYL AMINO ACIDS | 1 |
| 12A - Cleansing | 999001175 | SODIUM COCOYL AMINO ACIDS | 2 |
| 12D - Body and Hand (exc shave) | 999001175 | SODIUM COCOYL AMINO ACIDS | 1 |
| 12F - Moisturizing | 999001175 | SODIUM COCOYL AMINO ACIDS | 1 |
| 12J - Other Skin Care Preps | 999001175 | SODIUM COCOYL AMINO ACIDS | 1 |
| | | | |
| 03B - Eyeliner | 999002683 | SODIUM COCOYL APPLE AMINO ACIDS | 7 |
| 05F - Shampoos (non-coloring) | 999002683 | SODIUM COCOYL APPLE AMINO ACIDS | 1 |
| 05G - Tonics, Dressings, and Other Hair Grooming Aids | 999002683 | SODIUM COCOYL APPLE AMINO ACIDS | 2 |
| 10E - Other Personal Cleanliness Products | 999002683 | SODIUM COCOYL APPLE AMINO ACIDS | 4 |
| 12A - Cleansing | 999002683 | SODIUM COCOYL APPLE AMINO ACIDS | 4 |
| 12H - Paste Masks (mud packs) | 999002683 | SODIUM COCOYL APPLE AMINO ACIDS | 2 |
| 12J - Other Skin Care Preps | 999002683 | SODIUM COCOYL APPLE AMINO ACIDS | 1 |
| | | | |
| 03B - Eyeliner | 977166054 | SODIUM COCOYL COLLAGEN AMINO ACID | 1 |
| 05A - Hair Conditioner | 977166054 | SODIUM COCOYL COLLAGEN AMINO ACID | 9 |
| 05G - Tonics, Dressings, and Other Hair Grooming Aids | 977166054 | SODIUM COCOYL COLLAGEN AMINO ACID | 1 |
| 05I - Other Hair Preparations | 977166054 | SODIUM COCOYL COLLAGEN AMINO ACID | 1 |
| 10E - Other Personal Cleanliness Products | 977166054 | SODIUM COCOYL COLLAGEN AMINO ACID | 1 |
| | | | |
| 02B - Bubble Baths | 68187326 | SODIUM COCOYL GLUTAMATE | 1 |
| 02D - Other Bath Preparations | 68187326 | SODIUM COCOYL GLUTAMATE | 1 |
| 03B - Eyeliner | 68187326 | SODIUM COCOYL GLUTAMATE | 2 |
| 03C - Eye Shadow | 68187326 | SODIUM COCOYL GLUTAMATE | 1 |
| 03D - Eye Lotion | 68187326 | SODIUM COCOYL GLUTAMATE | 2 |
| 03G - Other Eye Makeup Preparations | 68187326 | SODIUM COCOYL GLUTAMATE | 3 |
| 05A - Hair Conditioner | 68187326 | SODIUM COCOYL GLUTAMATE | 1 |
| 05F - Shampoos (non-coloring) | 68187326 | SODIUM COCOYL GLUTAMATE | 22 |

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| 05G - Tonics, Dressings, and Other Hair Grooming Aids | 68187326 | SODIUM COCOYL GLUTAMATE | 1 |
| 05I - Other Hair Preparations | 68187326 | SODIUM COCOYL GLUTAMATE | 3 |
| 06A - Hair Dyes and Colors (all types requiring caution statements and patch tests) | 68187326 | SODIUM COCOYL GLUTAMATE | 30 |
| 07A - Blushers (all types) | 68187326 | SODIUM COCOYL GLUTAMATE | 1 |
| 07B - Face Powders | 68187326 | SODIUM COCOYL GLUTAMATE | 1 |
| 07C - Foundations | 68187326 | SODIUM COCOYL GLUTAMATE | 9 |
| 09A - Dentifrices | 68187326 | SODIUM COCOYL GLUTAMATE | 6 |
| 09C - Other Oral Hygiene Products | 68187326 | SODIUM COCOYL GLUTAMATE | 1 |
| 10A - Bath Soaps and Detergents | 68187326 | SODIUM COCOYL GLUTAMATE | 12 |
| 10E - Other Personal Cleanliness Products | 68187326 | SODIUM COCOYL GLUTAMATE | 10 |
| 11E - Shaving Cream | 68187326 | SODIUM COCOYL GLUTAMATE | 1 |
| 12A - Cleansing | 68187326 | SODIUM COCOYL GLUTAMATE | 26 |
| 12C - Face and Neck (exc shave) | 68187326 | SODIUM COCOYL GLUTAMATE | 9 |
| 12D - Body and Hand (exc shave) | 68187326 | SODIUM COCOYL GLUTAMATE | 7 |
| 12F - Moisturizing | 68187326 | SODIUM COCOYL GLUTAMATE | 11 |
| 12G - Night | 68187326 | SODIUM COCOYL GLUTAMATE | 4 |
| 12H - Paste Masks (mud packs) | 68187326 | SODIUM COCOYL GLUTAMATE | 1 |
| 12I - Skin Fresheners | 68187326 | SODIUM COCOYL GLUTAMATE | 4 |
| 12J - Other Skin Care Preps | 68187326 | SODIUM COCOYL GLUTAMATE | 8 |
| | | | |
| 07C - Foundations | 90387749 | SODIUM COCOYL GLYCINATE | 1 |
| 10E - Other Personal Cleanliness Products | 90387749 | SODIUM COCOYL GLYCINATE | 10 |
| 12A - Cleansing | 90387749 | SODIUM COCOYL GLYCINATE | 21 |
| | | | |
| 12A - Cleansing | 977067450 | SODIUM HYDROGENATED TALLOW GLUTAMATE | 1 |
| 12C - Face and Neck (exc shave) | 977067450 | SODIUM HYDROGENATED TALLOW GLUTAMATE | 1 |
| | | | |
| 03B - Eyeliner | 41489183 | SODIUM LAUROYL ASPARTATE | 1 |
| 03C - Eye Shadow | 41489183 | SODIUM LAUROYL ASPARTATE | 1 |
| 07G - Rouges | 41489183 | SODIUM LAUROYL ASPARTATE | 2 |
| | | | |
| 01A - Baby Shampoos | 29923317 | SODIUM LAUROYL GLUTAMATE | 2 |
| 02B - Bubble Baths | 29923317 | SODIUM LAUROYL GLUTAMATE | 5 |
| 03E - Eye Makeup Remover | 29923317 | SODIUM LAUROYL GLUTAMATE | 1 |
| 04C - Powders (dusting and talcum, excluding aftershave talc) | 29923317 | SODIUM LAUROYL GLUTAMATE | 1 |
| 05F - Shampoos (non-coloring) | 29923317 | SODIUM LAUROYL GLUTAMATE | 19 |
| 10A - Bath Soaps and Detergents | 29923317 | SODIUM LAUROYL GLUTAMATE | 12 |
| 10E - Other Personal Cleanliness Products | 29923317 | SODIUM LAUROYL GLUTAMATE | 3 |

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| 11G - Other Shaving Preparation Products | 29923317 | SODIUM LAUROYL GLUTAMATE | 1 |
| 12A - Cleansing | 29923317 | SODIUM LAUROYL GLUTAMATE | 24 |
| 12D - Body and Hand (exc shave) | 29923317 | SODIUM LAUROYL GLUTAMATE | 1 |
| 12F - Moisturizing | 29923317 | SODIUM LAUROYL GLUTAMATE | 2 |
| 12H - Paste Masks (mud packs) | 29923317 | SODIUM LAUROYL GLUTAMATE | 1 |
| 12J - Other Skin Care Preps | 29923317 | SODIUM LAUROYL GLUTAMATE | 3 |
| | | | |
| 01A - Baby Shampoos | 167074675 | SODIUM LAUROYL OAT AMINO ACIDS | 1 |
| 02B - Bubble Baths | 167074675 | SODIUM LAUROYL OAT AMINO ACIDS | 4 |
| 02D - Other Bath Preparations | 167074675 | SODIUM LAUROYL OAT AMINO ACIDS | 1 |
| 05F - Shampoos (non-coloring) | 167074675 | SODIUM LAUROYL OAT AMINO ACIDS | 23 |
| 05G - Tonics, Dressings, and Other Hair Grooming Aids | 167074675 | SODIUM LAUROYL OAT AMINO ACIDS | 3 |
| 10A - Bath Soaps and Detergents | 167074675 | SODIUM LAUROYL OAT AMINO ACIDS | 17 |
| 10E - Other Personal Cleanliness Products | 167074675 | SODIUM LAUROYL OAT AMINO ACIDS | 16 |
| 12A - Cleansing | 167074675 | SODIUM LAUROYL OAT AMINO ACIDS | 22 |
| 12C - Face and Neck (exc shave) | 167074675 | SODIUM LAUROYL OAT AMINO ACIDS | 1 |
| 12D - Body and Hand (exc shave) | 167074675 | SODIUM LAUROYL OAT AMINO ACIDS | 3 |
| 12F - Moisturizing | 167074675 | SODIUM LAUROYL OAT AMINO ACIDS | 3 |
| 12J - Other Skin Care Preps | 167074675 | SODIUM LAUROYL OAT AMINO ACIDS | 4 |
| | | | |
| 12A - Cleansing | 999002314 | SODIUM LAUROYL WHEAT AMINO ACIDS | 1 |
| | | | |
| 03B - Eyeliner | 38517372 | SODIUM MYRISTOYL GLUTAMATE | 1 |
| 03C - Eye Shadow | 38517372 | SODIUM MYRISTOYL GLUTAMATE | 1 |
| 03G - Other Eye Makeup Preparations | 38517372 | SODIUM MYRISTOYL GLUTAMATE | 8 |
| 07C - Foundations | 38517372 | SODIUM MYRISTOYL GLUTAMATE | 26 |
| 07F - Makeup Bases | 38517372 | SODIUM MYRISTOYL GLUTAMATE | 2 |
| 07G - Rouges | 38517372 | SODIUM MYRISTOYL GLUTAMATE | 2 |
| 07I - Other Makeup Preparations | 38517372 | SODIUM MYRISTOYL | 2 |

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| | | GLUTAMATE | |
| 08B - Cuticle Softeners | 38517372 | SODIUM MYRISTOYL GLUTAMATE | 1 |
| 12A - Cleansing | 38517372 | SODIUM MYRISTOYL GLUTAMATE | 7 |
| 12C - Face and Neck (exc shave) | 38517372 | SODIUM MYRISTOYL GLUTAMATE | 1 |
| 12F - Moisturizing | 38517372 | SODIUM MYRISTOYL GLUTAMATE | 1 |
| | | | |
| 10B - Deodorants (underarm) | 999002008 | SODIUM PALMITOYL PROLINE | 1 |
| 12A - Cleansing | 999002008 | SODIUM PALMITOYL PROLINE | 1 |
| 12F - Moisturizing | 999002008 | SODIUM PALMITOYL PROLINE | 1 |
| 12G - Night | 999002008 | SODIUM PALMITOYL PROLINE | 1 |
| 12J - Other Skin Care Preps | 999002008 | SODIUM PALMITOYL PROLINE | 3 |
| | | | |
| 03D - Eye Lotion | 38517236 | SODIUM STEAROYL GLUTAMATE | 4 |
| 03G - Other Eye Makeup Preparations | 38517236 | SODIUM STEAROYL GLUTAMATE | 1 |
| 05A - Hair Conditioner | 38517236 | SODIUM STEAROYL GLUTAMATE | 4 |
| 05I - Other Hair Preparations | 38517236 | SODIUM STEAROYL GLUTAMATE | 2 |
| 07C - Foundations | 38517236 | SODIUM STEAROYL GLUTAMATE | 3 |
| 07I - Other Makeup Preparations | 38517236 | SODIUM STEAROYL GLUTAMATE | 1 |
| 10B - Deodorants (underarm) | 38517236 | SODIUM STEAROYL GLUTAMATE | 3 |
| 10E - Other Personal Cleanliness Products | 38517236 | SODIUM STEAROYL GLUTAMATE | 3 |
| 11E - Shaving Cream | 38517236 | SODIUM STEAROYL GLUTAMATE | 1 |
| 12A - Cleansing | 38517236 | SODIUM STEAROYL GLUTAMATE | 6 |
| 12C - Face and Neck (exc shave) | 38517236 | SODIUM STEAROYL GLUTAMATE | 18 |
| 12D - Body and Hand (exc shave) | 38517236 | SODIUM STEAROYL GLUTAMATE | 15 |
| 12F - Moisturizing | 38517236 | SODIUM STEAROYL GLUTAMATE | 48 |
| 12G - Night | 38517236 | SODIUM STEAROYL GLUTAMATE | 5 |
| 12J - Other Skin Care Preps | 38517236 | SODIUM STEAROYL GLUTAMATE | 3 |
| 13B - Indoor Tanning Preparations | 38517236 | SODIUM STEAROYL GLUTAMATE | 3 |
| | | | |
| 12A - Cleansing | 999002261 | TEA-COCOYL ALANINATE | 2 |
| | | | |
| 01C - Other Baby Products | 68187291 | TEA-COCOYL GLUTAMATE | 1 |
| 04E - Other Fragrance Preparation | 68187291 | TEA-COCOYL GLUTAMATE | 1 |

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|---|---|---|---|
| 05F - Shampoos (non-coloring) | 68187291 | TEA-COCOYL GLUTAMATE | 2 |
| 07I - Other Makeup Preparations | 68187291 | TEA-COCOYL GLUTAMATE | 2 |
| 10A - Bath Soaps and Detergents | 68187291 | TEA-COCOYL GLUTAMATE | 13 |
| 10E - Other Personal Cleanliness Products | 68187291 | TEA-COCOYL GLUTAMATE | 23 |
| 11E - Shaving Cream | 68187291 | TEA-COCOYL GLUTAMATE | 1 |
| 12A - Cleansing | 68187291 | TEA-COCOYL GLUTAMATE | 17 |
| 12C - Face and Neck (exc shave) | 68187291 | TEA-COCOYL GLUTAMATE | 5 |
| | | | |
| 05G - Tonics, Dressings, and Other Hair Grooming Aids | 977099301 | TEA-LAUROYL COLLAGEN AMINO ACID | 3 |
| | | | |
| 10E - Other Personal Cleanliness Products | 60239727 | TEA-LAUROYL GLUTAMATE | 1 |
| | | | |
| 05F - Shampoos (non-coloring) | 977169882 | UNDECYLENOYL COLLAGEN AMINO ACIDS | 2 |
| | | | |
| 03D - Eye Lotion | 999001119 | UNDECYLENOYL GLYCINE | 1 |
| 04E - Other Fragrance Preparation | 999001119 | UNDECYLENOYL GLYCINE | 1 |
| 05F - Shampoos (non-coloring) | 999001119 | UNDECYLENOYL GLYCINE | 4 |
| 08G - Other Manicuring Preparations | 999001119 | UNDECYLENOYL GLYCINE | 2 |
| 12E - Foot Powders and Sprays | 999001119 | UNDECYLENOYL GLYCINE | 2 |
| | | | |
| 07C - Foundations | 999001661 | UNDECYLENOYL PHENYLALANINE | 1 |
| 12A - Cleansing | 999001661 | UNDECYLENOYL PHENYLALANINE | 1 |
| 12C - Face and Neck (exc shave) | 999001661 | UNDECYLENOYL PHENYLALANINE | 1 |
| 12D - Body and Hand (exc shave) | 999001661 | UNDECYLENOYL PHENYLALANINE | 1 |
| 12F - Moisturizing | 999001661 | UNDECYLENOYL PHENYLALANINE | 8 |
| 12G - Night | 999001661 | UNDECYLENOYL PHENYLALANINE | 1 |
| 12J - Other Skin Care Preps | 999001661 | UNDECYLENOYL PHENYLALANINE | 5 |

Personal Care Products Council
Committed to Safety, Quality & Innovation

**Memorandum**

**TO:** F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

**FROM:** Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

**DATE:** July 18, 2012

**SUBJECT:** Information on Glutamate Alkyl Amides

Zschimmer & Schwarz Italiana S.p.A. 2011. Toxicological information: Disodium Capryloyl Glutamate (Protelan AG 8).

Zschimmer & Schwarz Italiana S.p.A. 2006. Toxicological data: Disodium Capryloyl Glutamate (Protelan AG 8).

Zschimmer & Schwarz Italiana S.p.A. 2011. Toxicological information: Sodium Lauroyl Glutamate (Protelan AGL 95).

Zschimmer & Schwarz Italiana S.p.A. 2007. Toxicological data: Sodium Lauroyl Glutamate (Protelan AGL 95).

Zschimmer & Schwarz Italiana S.p.A. 2010. Toxicological information: Sodium Cocoyl Glutamate (Protelan AGL 95/C).

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**ZSCHIMMER & SCHWARZ ITALIANA S.p.A.**

Zschimmer & Schwarz Italiana – 13038 – Tricerro (VC) / ITALY

# INFORMAZIONI TOSSICOLOGICHE
# *TOXICOLOGICAL INFORMATION*

***Revisione n°***
***Revision n° 03***

| | | |
|---|---|---|
| **1.** | **Informazioni generali**<br>***General information*** | |
| **1.1** | **Nome commerciale**<br>***Trade name*** | **PROTELAN AG 8** |
| **1.2** | **Produttore/Fornitore**<br>(indirizzo, telefono, fax, contatto)<br>***Manufacturer/Supplier***<br>*(address, phone no., fax no., contact person)* | ZSCHIMMER & SCHWARZ ITALIANA<br>Via A. Ariotto 1/C - 13038 Tricerro (VC)<br>Italy<br>Tel: +39 (0)161 80811<br>Fax: +39 (0)161 801002<br>elisabetta.merlo@zsi.it |
| **1.3** | **Categoria della material prima**<br>(es. tensioattivo anionico)<br>***Raw material category***<br>*(e.g. anionic surfactant)* | Anionic surfactant |
| **1.4** | **Nome chimico**<br>***Chemical name*** | L-glutamic acid, N-coco acyl derivs. monosodium salt |
| **1.5** | **Nome INCI (CTFA)**<br>**Composizione**<br>***INCI (CTFA) name***<br>**Composition** | Disodium Capryloyl Glutamate: 37% - 41% as dry matter<br>Aqua: to 100% |
| **1.6** | **N° EC (EINECS-/ELINCS)**<br>***EC (EINECS/ELINCS) no.*** | 269-085-1 |
| **1.7** | **N° CAS**<br>***CAS no.*** | 167888-81-5 |

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| | | |
|---|---|---|
| 1.8 | **Registrazioni** (es. UE, USA, Giappone)<br>***Registration status*** *(e.g. EU, USA, Japan)* | CAS (Europe) |

| | | |
|---|---|---|
| **2.** | **Informazioni sulla produzione**<br>**Information on production** | |
| **2.1** | **Origine della materia prima**<br>(vegetale, animale, sintetica)<br>**Origin of starting material**<br>(plant, animal, synthetic) | Vegetable, mineral and synthetic origin.<br>Glutamic acid is obtained through fermentation of glucose/molasses or from wheat.<br>Capryloyl chloride is obtained starting from caprylic acid that comes from cleavage and distillation of coconut oil from Cocos Nucifera (South East Asia and Philippines).<br>NaOH is mineral and Propylene Glycol is synthetic. |
| **2.2** | **La materia prima deriva da organismi geneticamente modificati (OGM)?**<br>**Is the starting material derived from genetically modified organisms (GMO)?** | No |
| **2.3** | **Informazioni sul processo di produzione**<br>(descrizione generale)<br>**Information on production process**<br>(general description) | Schotten-Baumann reaction |

| | | |
|---|---|---|
| **3.** | **Additivi**<br>***Additives*** | |
| **3.1** | **Conservanti**<br>***Preservatives*** | Not added |
| **3.2** | **Antiossidanti**<br>***Antioxidants*** | Not added |
| **3.3** | **Solventi**<br>***Solvents*** | 4.0% - 6.0% of Propylene Glycol |

| | | |
|---|---|---|
| 3.4 | **Sbiancanti**<br>***Bleaching agents*** | Not added |
| 3.5 | **Altri**<br>***Others*** | Not added |

| | | |
|---|---|---|
| 4. | **Specifiche microbiologiche**<br>**Microbiological specification** | |
| 4.1 | **Conta microbica totale (ufc/g)**<br>***Total viable count (colony-forming units/g)*** | less than 10 ufc/g |

| | | |
|---|---|---|
| 5. | **Residui del processo di lavorazione**<br>La presenza di tracce delle sostanze elencate in Annex II della Direttiva 76/768/CEE (incl. CMR cat. I - III sostanze contrassegnate con *) deve essere dimostrata come presenza tecnicamente inevitabile lavorando in GMP e deve essere conforme all'Articolo 2 della Direttiva 76/768/CEE.<br>***By-products***<br>*The presence of traces of the substances listed in Annex II of Directive 76/768/EEC (incl. cmr cat. I – III substances marked with *) shall be allowed provided that such presence is technically unavoidable in good manufacturing practice and that it conforms with Article 2 of Directive 76/768/EEC.* | |
| 5.1 | **1,4-Diossano**<br>***1,4-Dioxane*** | Not expected |
| 5.2 | **Ossido di etilene**<br>***Ethylene oxide*** | Not expected |
| 5.3 | **Solventi residui**<br>***Residual solvents*** | 4.0% - 6.0% of Propylene Glycol |
| 5.4 | **Monomeri residui**<br>***Residual monomers*** | Not expected |
| 5.5 | **Ammine**<br>***Amines*** | Not expected |

| | | |
|---|---|---|
| 5.6 | **Nitrosammine**<br>***Nitrosamines*** | Not expected |
| 5.7 | **Metalli pesanti**<br>***Heavy metals*** | Arsenic (As) < 2 ppm, Antimony (Sb) < 5 ppm, Lead (Pb) < 1 ppm, Cadmium (Cd) < 2 ppm, Mercury (Hg) < 2 ppm, Nickel (Ni) < 1 ppm, Chromium (Cr) < 2 ppm, Total heavy metals (as Fe) < 10 ppm |
| 5.8 | **Acido monocloroacetico**<br>**Monochloroacetic acid** | Absent |
| 5.9 | **Acido dicloroacetico**<br>***Dichloroacetic acid*** | Absent |
| 5.10 | **Allergeni**<br>**Allergens according to the 7th Amendment to the Cosmetics Directive (ppm)** | Absent |
| 5.11 | **Altri**<br>***Others*** | Caprylic acid: 3% maximum<br>Disodium glutamate: 5% maximum<br>Sodium chloride: 6% - 8% |

| | | |
|---|---|---|
| **6.** | **Tossicologia**<br>***Toxicology*** | |
| 6.1 | **Informazioni sulla tossicità acuta**<br>***Information on acute toxicity*** | LD50 on rats (OECD 401) > 2000 mg/kg (from literature on acylglutamates) |
| 6.2 | **Informazioni sull'irritazione cutanea**<br>***Information on skin irritation*** | Patch test, 7% a.m., 20 volunteers = Not irritating (ISPE, 21/11/2000) |
| 6.3 | **Informazioni sull'irritazione oculare**<br>***Information on irritation of the mucous membrane*** | Het Cam test, 2% as it is = Predicted not to be irritating (Biolab, 23/11/2000) |

| | | |
|---|---|---|
| 6.4 | **Informazioni sulla sensibilizzazione**<br>***Information on sensitisation potential*** | Finn Chamber test, 7% a.m., 20 volunteers = None sensitizing effect (ISPE, 21/11/2000) |
| 6.5 | **Informazioni sulla genotossicità**<br>***Information on gene toxicity*** | Presence of mutagenic agents:<br>None mutagenic effects (from literature on acylglutamates) |
| 6.6 | **Informazioni sull'assorbimento percutaneo**<br>***Information on percutaneous permeation*** | Not determined |
| 6.7 | **Dichiarazione test animali**<br>***Animal testing declaration*** | ZSCHIMMER & SCHWARZ ITALIANA has never made or commissioned animal tests on this product. |
| 6.8 | **Altri**<br>***Others*** | / |

| | | |
|---|---|---|
| 8. | **Informazioni aggiuntive**<br>(Per i dettagli sulle specifiche vedere il bollettino tecnico allegato; per i dettagli sull'etichettatura e la classificazione vedere la scheda di sicurezza allegata.)<br><br>***Additional information***<br>*(For details on specification see enclosed instruction sheet; for details on labelling and classification see enclosed safety data sheet.)* | |

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| | | |
|---|---|---|
| | **Dichiarazione BSE**<br>***BSE statement*** | The product is not from animal origin. Furthermore it doesn't contain any ingredient of animal origin, it is not produced using ingredients of animal origins and it doesn't come into contact with animal origin ingredients at any stage of its production. It is therefore BSE free. |
| | **Dichiarazione test animali**<br>***Non-animal testing declaration*** | ZSCHIMMER & SCHWARZ ITALIANA has never made or commissioned animal tests on this product. |
| | **Glicol eteri**<br>***Glycol ethers*** | Absent |
| | ***Ftalati***<br>***Phtalates*** | Absent |
| | ***Glutine***<br>***Gluten*** | Absent |
| | ***Formaldeide***<br>***Formaldehyde (Formol)*** | Absent |
| | ***VOC***<br>***VOC compounds*** | The product doesn't contain any of the substances that are classified as VOC according to "Ordonnance sur taxe d'incitation sur les composes organiques volatils (OCOV) du 12 novembre 1997". It contains 4% - 6% of Propylene Glycol. |
| | ***Pesticidi***<br>***Pesticides*** | The product doesn't contain any pesticides or pollutant substances (under detection limits). |
| | ***Grado cosmetico***<br>***Cosmetic grade*** | The product is of cosmetic grade and it can be used in cosmetic products |
| **8.1** | **Data di scadenza**<br>***Shelf life*** | The product, if well preserved and in its original containers, maintains its appearance and characteristics for at least one year from delivery date. |

Data / *Date* 22/03/11

**Queste informazioni si riferiscono solo al prodotto sopramenzionato** e non possono essere considerate valide per altri prodotti o in altri processi produttivi. Le informazioni sono corrette e complete secondo le nostre attuali conoscenze e sono date in buona fede ma senza garanzia. E' responsabilità dell'utilizzatore l'assicurarsi che le informazioni siano appropriate e complete per lo specifico uso del prodotto.

***This information refers only to the above mentioned product*** *and does not need to be valid if used with other product(s) or in any process. The information is to our best present knowledge correct and complete and is given in good faith but without warranty. It remains the user's own responsibility to make sure that the information is appropriate and complete for his specific use of this product.*

Trade name:
**PROTELAN AG 8**

INCI name:
**Disodium Capryloyl Glutamate**

| Characteristics | Values | Analytical methods |
|---|---|---|
| Aspect at 20°C | from clear to light turbid liquid | visual |
| Colour | from colourless to light yellow | visual |
| Odour | characteristic | olfactory |
| Dry matter | 37% - 41% | IR lamp |
| pH (tq at 20°C) | 9.0 - 10.5 | ISO 4316-1977 |
| Sodium Chloride | 6.0% - 8.0% | potenziometric titration |
| **Preservative** | **absent** | - |
| Compatibility | compatible with non ionic, anionic and amphoteric surfactants | - |

***Note:*** These values are here given as indication. The only guaranteed specifications are those appearing in the official specifications requested by the customer.

**PROTELAN AG 8** is proved to be mild and safe.

## Skin irritation

### In vivo evaluation of irritation potential

Skin irritation was evaluated through a patch-test on 20 volunteers (18). The method consists in an occlusive application of the product by means of Finn chamber (aluminium cells of 20 microliters volume) on volunteers' volar part of the forearm. The irritating potential was clinically evaluated by observing the erythema induced by the product with the following parameters:

- 30 minutes after application (T1, immediate irritative power)
- 48 hours later (T2, irritative power)

The substance was tested at a concentration of 18% as it is.

The immediate irritative power and the irritative power were separately evaluated.

The irritative power was evaluated considering:

- the number of reactions that product caused related to the total number of subjects
- the severity of the irritative reactions.

| | Irritation % |
|---|---|
| Immediate irritative power | 0 |
| Irritative power | 0 |

**PROTELAN AG 8 is free of any irritation potential**

### In vitro evaluation of irritation potential

In vitro methods are an interesting alternative system to traditional in vivo tests to evaluate biological properties of cosmetic ingredients and products, according to the current European cosmetic rules that ask manufacturers to assess the product safety, without employing animals.

In vitro tests can be carried out in order to evaluate the citotoxicity of cosmetic ingredients on keratinocytes coltures. The in vitro test on skin-derived cells is a simplified but yet very informative model of the reactions that may occur in vivo.

The test was performed on a 3D epidermis obtained from epidermal keratinocytes seeded on a collagen matrix and grown in a serum-free medium to reach a multilayer conformation with a differentiated stratum corneum at the surface.

The products to be tested are placed in contact with the 3D epidermis for 24 hours. Sodium Lauryl Sulfate is used as a reference.

At the end of the exposure period, the epidermis layers are disrupted with the enzyme trypsin and the cytotoxicity is evaluated trough the quantification of surviving cell percentage.

Surviving cells are able to cleave a dye (MTT) in order to give a coloured solution that can be spectrophotometrically evaluated.

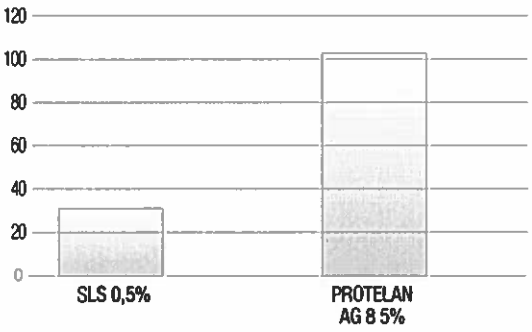
The absorbance is measured at 570 nm on a microplate, with background reading at 690 nm.

The results of the MTT test are expressed in terms of viability:

$$\text{\% Viability} = \frac{OD_{570} \text{ treated coltures x } 100}{OD_{570} \text{ untreated control coltures}}$$

Figure 8 shows the obtained results (19).

**Figure 8 - MTT** test results of **PROTELAN AG 8**
**% vitals cells**




**PROTELAN AG 8 gives 100% of viability**

Cytotoxicity data can be also obtained with the MTT assay performed on keratinocytes monolayer colture. In this case a dose response curve allows to obtain the theoretical $IC_{50}$ value (Inhibiting Concentration 50), i.e. the concentration of tested product which induce a decrease of cell survival by 50% as compared to untreated coltures.

The $IC_{50}$ value makes possible to evaluate the potential irritating effect as described:

$IC_{50} < 1$ mg/ml means
a cytotoxic/irritating effect

$IC_{50} > 1$ mg/ml means
the absence of cytotoxic/irritating effect

$IC_{50}$ values of **PROTELAN AG 8** is 1.1 mg/l ($IC_{50}$ of SLS is about 0.0097 mg/l) (19).

**PROTELAN AG 8** was tested at 5% as it is.

## Skin sensitization

### In vivo evaluation of sensitization power

Sensitization power (hypoallergenicity) of **PROTELAN AG 8** was evaluated through an occlusive application of the product by means of Finn chamber (alluminium cell of 20 microlitres volume) on 20 selected volunteers' volar part of the forearm for 48 hours (20). After the removal of the occlusion (T1) cutaneous reactions induced by the product were evaluated 24 (T2) and 48 (T3) hours later. The repeated control of the treated areas allows us to trace the presence and/or the activity in elicitation of cutaneous reactions of common allergens. The allergic potential is expressed as a percentage of allergic reactions and it is evaluated considering:

- the number of reactions observed
- severity, type and duration of reactions.

**PROTELAN AG 8** was tested at 18% as it is.

**TABLE 8 - Skin sensitization of PROTELAN AG 8**

N° total volunteers = 20

| Observed reaction | T1 | T2 | T3 |
|---|---|---|---|
| Doubtful reaction | 0 | 0 | 0 |
| Minimum reaction | 0 | 0 | 0 |
| Moderate reaction | 0 | 0 | 0 |
| Strong reaction | 0 | 0 | 0 |
| N° of allergic reactions | T1 0 | T2 0 | T3 0 |

**PROTELAN AG 8 can be defined as not sensitizing**

## Eye irritation

Eye irritation potential of **PROTELAN AG 8** was tested through the Het Cam Test.

**PROTELAN AG 8** at 2% as it is gives a score of 0.00 (the same as water).

**PROTELAN AG 8** can be considered not irritant to eyes (21).

## Mutagenicity

Mutagenicity of **PROTELAN AG 8** was tested trough the Ames test. **PROTELAN AG 8 is not mutagenic** (22).

# References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19. In Vitro Product Safety Study, rapporto ICZS00102 di I.Z.S di Lombardia ed Emilia Romagna (2002)
20. Ipoallergenicità, ISPE, rapporto 206/00/02 del 21.11.2000
21. HET CAM Test, Biolab, Rapporto n° 00/30178-1 del 23/11/2000
22. Ames Test, Biolab, 1999
23. Miti Test, Biolab, 2001


# Notes

- This brochure was printed in January 2006 and replaces all the previous data
- The only analytical specifications guaranteed are those mentioned on the certificate of analysis supplied with each delivery
- The information set forth herein is furnished free of charge and is based on technical data that Zschimmer & Schwarz believes to be reliable. It is intended for use by person having technical skill and at their own discretion and risk. Since conditions of use are outside our control, we make no warranties, express or implied, and assume no liability in connection with any use of this information. Nothing herein is to be taken as a licence to operate under or a recommendation to infringe any patents.

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**ZSCHIMMER & SCHWARZ ITALIANA S.p.A.**

Zschimmer & Schwarz Italiana – 13038 – Tricerro (VC) / ITALY

# INFORMAZIONI TOSSICOLOGICHE
# *TOXICOLOGICAL INFORMATION*

***Revisione n°***
***Revision n° 06***

| | | |
|---|---|---|
| **1.** | **Informazioni generali**<br>***General information*** | |
| **1.1** | **Nome commerciale**<br>***Trade name*** | **PROTELAN AGL 95** |
| **1.2** | **Produttore/Fornitore**<br>(indirizzo, telefono, fax, contatto)<br>***Manufacturer/Supplier***<br>*(address, phone no., fax no., contact person)* | ZSCHIMMER & SCHWARZ ITALIANA<br>Via A. Ariotto 1/C - 13038 Tricerro (VC)<br>Italy<br>Tel: +39 (0)161 808111<br>Fax: +39 (0)161 801002<br>elisabetta.merlo@zsi.it |
| **1.3** | **Categoria della materiai prima**<br>(es. tensioattivo anionico)<br>***Raw material category***<br>*(e.g. anionic surfactant)* | Anionic surfactant, moisturizing |
| **1.4** | **Nome chimico**<br>***Chemical name*** | Sodium hydrogen N-(1-oxododecyl)-L-glutamate |
| **1.5** | **Nome INCI (CTFA)**<br>**Composizione**<br>***INCI (CTFA) name***<br>**Composition** | Sodium Lauroyl Glutamate: 36% - 40%<br>as dry matter<br>Aqua: to 100% |
| **1.6** | **N° EC (EINECS-/ELINCS)**<br>***EC (EINECS/ELINCS) no.*** | 249-958-3 |
| **1.7** | **N° CAS**<br>***CAS no.*** | 29923-31-7 |

| | | |
|---|---|---|
| 1.8 | **Registrazioni** (es. UE, USA, Giappone)<br>***Registration status*** *(e.g. EU, USA, Japan)* | TSCA (USA), NDSL (Canada), ENCS (Japan), AICS (Australia), PICCS (Philippines), ASIA-PAC (Asia-Pacific), EINECS (Europe) and ECL (Korea).<br>Japanes have recently changed their system, so that publication in the Japanese list of approved ingredients is no longer necessary. Any cosmetic ingredient is now allowed in Japan with no prior approvation. |

| | | |
|---|---|---|
| **2.** | **Informazioni sulla produzione**<br>**Information on production** | |
| 2.1 | **Origine della materia prima**<br>(vegetale, animale, sintetica)<br>**Origin of starting material**<br>(plant, animal, synthetic) | Vegetable, mineral and synthetic origin.<br>PROTELAN AGL 95 is obtained by reaction between lauroyl chloride and glutamic acid.<br>Glutamic acid is obtained through fermentation of glucose/molasses or from wheat.<br>Lauroyl chloride is obtained starting from lauric acid that comes from cleavage and distillation of coconut oil from Cocos Nucifera (South East Asia and Philippines)<br>NaOH is mineral and Propylene Glycol is synthetic. |
| 2.2 | **La materia prima deriva da organismi geneticamente modificati (OGM)?**<br>**Is the starting material derived from genetically modified organisms (GMO)?** | No |
| 2.3 | **Informazioni sul processo di produzione**<br>(descrizione generale)<br>**Information on production process**<br>(general description) | Schotten-Baumann reaction |

| | | |
|---|---|---|
| **3.** | **Additivi**<br>***Additives*** | |
| 3.1 | **Conservanti**<br>***Preservatives*** | Not added |

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| | | |
|---|---|---|
| 3.2 | **Antiossidanti**<br>***Antioxidants*** | Not added |
| 3.3 | **Solventi**<br>***Solvents*** | 4.0% - 6.0% of Propylene Glycol |
| 3.4 | **Sbiancanti**<br>***Bleaching agents*** | Not added |
| 3.5 | **Altri**<br>***Others*** | Not added |

| | | |
|---|---|---|
| 4. | **Specifiche microbiologiche**<br>**Microbiological specification** | |
| 4.1 | **Conta microbica totale (ufc/g)**<br>***Total viable count (colony-forming units/g)*** | less than 10 ufc/g |

**5. Residui del processo di lavorazione**

La presenza di tracce delle sostanze elencate in Annex II della Direttiva 76/768/CEE (incl. CMR cat. I - III sostanze contrassegnate con *) deve essere dimostrata come presenza tecnicamente inevitabile lavorando in GMP e deve essere conforme all'Articolo 2 della Direttiva 76/768/CEE.

***By-products***

*The presence of traces of the substances listed in Annex II of Directive 76/768/EEC (incl. cmr cat. I – III substances marked with *) shall be allowed provided that such presence is technically unavoidable in good manufacturing practice and that it conforms with Article 2 of Directive 76/768/EEC.*

| | | |
|---|---|---|
| 5.1 | **1,4-Diossano**<br>***1,4-Dioxane*** | Not expected |
| 5.2 | **Ossido di etilene**<br>***Ethylene oxide*** | Not expected |
| 5.3 | **Solventi residui**<br>***Residual solvents*** | 4.0% - 6.0% of Propylene Glycol |

| | | |
|---|---|---|
| 5.4 | **Monomeri residui**<br>***Residual monomers*** | Not expected |
| 5.5 | **Ammine**<br>***Amines*** | Not expected |
| 5.6 | **Nitrosammine**<br>***Nitrosamines*** | Not expected but as product contains residue of glutamic acid, it should not be used in cosmetic products in which N-nitroso compounds may be formed. |
| 5.7 | **Metalli pesanti**<br>***Heavy metals*** | Arsenic (As) < 2 ppm, Antimony (Sb) < 5 ppm, Lead (Pb) < 1 ppm, Cadmium (Cd) < 2 ppm, Mercury (Hg) < 2 ppm, Nickel (Ni) < 1 ppm, Chromium (Cr) < 2 ppm, Total heavy metals (as Fe) < 10 ppm |
| 5.8 | **Acido monocloroacetico**<br>**Monochloroacetic acid** | Absent |
| 5.9 | **Acido dicloroacetico**<br>***Dichloroacetic acid*** | Absent |
| 5.10 | **Allergeni**<br>**Allergens according to the 7th Amendment to the Cosmetics Directive (ppm)** | Absent |
| 5.11 | **Altri**<br>***Others*** | Glutamic acid: 5% maximum<br>Lauric acid: 3% maximum<br>Sodium chloride: 3% - 4.5% |

| | | |
|---|---|---|
| **6.** | **Tossicologia**<br>***Toxicology*** | |
| **6.1** | **Informazioni sulla tossicità acuta**<br>***Information on acute toxicity*** | LD50 on mice (male) = 5.5/kg (from literature) |

| | | |
|---|---|---|
| **6.2** | **Informazioni sull'irritazione cutanea**<br>***Information on skin irritation*** | 10% a.m. = Not irritant (our Flex Wash Test n° 990354FWT) |
| **6.3** | **Informazioni sull'irritazione oculare**<br>***Information on irritation of the mucous membrane*** | Not irritant (our test, Red Blood Cell n° cmrbc) |
| **6.4** | **Informazioni sulla sensibilizzazione**<br>***Information on sensitisation potential*** | Occlusive applications, 5% a.m. = The product hasn't any sensitization danger (our test n° 37/99/02) |
| **6.5** | **Informazioni sulla genotossicità**<br>***Information on gene toxicity*** | Ames test = Not mutagenic (from literature) |
| **6.6** | **Informazioni sull'assorbimento percutaneo**<br>***Information on percutaneous permeation*** | Not determined |
| **6.7** | **Altri**<br>***Others*** | Phototoxicity and photosensitization:<br>40 healthy males, 0.1%-0.5%-1%-3%-5% aqueous solutions = No abnormality was found (from literature) |

| | |
|---|---|
| | / |

# ZSCHIMMER & SCHWARZ ITALIANA S.p.A.

| | | |
|---|---|---|
| **8.** | **Informazioni aggiuntive**<br>(Per i dettagli sulle specifiche vedere il bollettino tecnico allegato; per i dettagli sull'etichettatura e la classificazione vedere la scheda di sicurezza allegata.)<br><br>***Additional information***<br>*(For details on specification see enclosed instruction sheet; for details on labelling and classification see enclosed safety data sheet.)* | |
| | **Dichiarazione BSE**<br>***BSE statement*** | The product is not from animal origin. Furthermore it doesn't contain any ingredient of animal origin, it is not produced using ingredients of animal origins and it doesn't come into contact with animal origin ingredients at any stage of its production. It is therefore BSE free. |
| | **Dichiarazione test animali**<br>***Non-animal testing declaration*** | ZSCHIMMER & SCHWARZ ITALIANA has never made or commissioned animal tests on this product. |
| | **Glicol eteri**<br>***Glycol ethers*** | Absent |
| | ***Ftalati***<br>***Phtalates*** | Absent |
| | ***Glutine***<br>***Gluten*** | Absent |
| | ***Formaldeide***<br>***Formaldehyde (Formol)*** | Absent |
| | ***VOC***<br>***VOC compounds*** | The product doesn't contain any of the substances that are classified as VOC according to "Ordonnance sur taxe d'incitation sur les composes organiques volatils (OCOV) du 12 novembre 1997". It contains 4% - 6% of Propylene Glycol. |
| | ***Pesticidi***<br>***Pesticides*** | The product doesn't contain any pesticides or pollutant substances (under detection limits). |
| | ***Grado cosmetico***<br>***Cosmetic grade*** | The product is of cosmetic grade and it can be used in cosmetic products |
| **8.1** | **Data di scadenza**<br><br>***Shelf life*** | The product, if well preserved and in its original containers, maintains its appearance and characteristics for at least one year from delivery date. After this time, product can be used but it must be rechecked. |

**ZSCHIMMER & SCHWARZ**
**ITALIANA S.p.A.**

Data / *Date* 22/03/11

**Queste informazioni si riferiscono solo al prodotto sopramenzionato** e non possono essere considerate valide per altri prodotti o in altri processi produttivi. Le informazioni sono corrette e complete secondo le nostre attuali conoscenze e sono date in buona fede ma senza garanzia. E' responsabilità dell'utilizzatore l'assicurarsi che le informazioni siano appropriate e complete per lo specifico uso del prodotto.

***This information refers only to the above mentioned product*** *and does not need to be valid if used with other product(s) or in any process. The information is to our best present knowledge correct and complete and is given in good faith but without warranty. It remains the user's own responsibility to make sure that the information is appropriate and complete for his specific use of this product.*

# Toxicological data

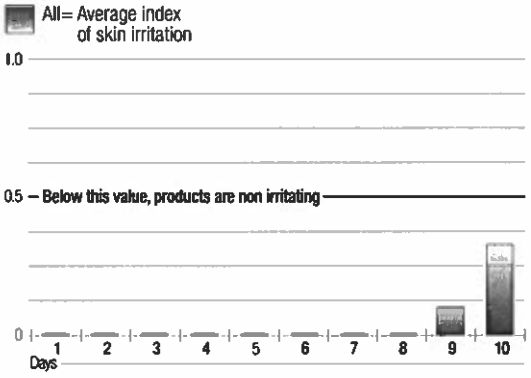
**ACYLGLUTAMATES** are proved to be mild and safe

## Skin irritation

Skin irritation of **PROTELAN AGL 95** was evaluated through a Flex Wash Test (10% active matter) (10).

20 volunteers, both males and females, aged between 18 and 65 years had to put known quantities of product on anticubital fold of the arm, massage gentle for 30 seconds and wash. The test was repeated three times a day for ten consecutive days. The index of average irritation according to the amended Draize classification was lower than 0.5. Figure 3 shows the obtained results.

**Figure 3** -Flex Wash Test (10% active matter) of **PROTELAN AGL 95**




**PROTELAN AGL 95 is non irritant for the skin**

## Eye irritation

Eye irritation of **PROTELAN AGL 95** was tested through the Het Cam Test.

**PROTELAN AGL 95** at 5% of active matter gave a score of 0.00 (the same as water).

**PROTELAN AGL 95** can be considered as non irritant to eyes (11).

**RBC-Test** (Red Blood Cell Test), an in vitro test for determination of eye irritation, was made on **PROTELAN AGL 95**, comparing it also with other surfactants. This test evaluates the potential eye irritation of surfactants by the determination of haemolysis (destruction of red blood cell membrane) and denaturation (destruction of the native structure of haemoglobin) of red blood cells.

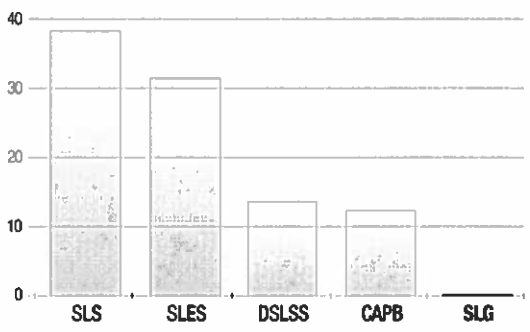
The **MIOI** value (Mean Index of Ocular Irritation) has been derived from a correlation of the data of haemolysis and denaturation in the RBC test with existing data of the eye irritation in the rabbit according to Draize.

The **MIOI** value allows a prediction of the eye irritation by the following correlation scheme:

| | |
|---|---|
| MIOI<4.7: non irritant | MIOI<41.4: irritant |
| MIOI<9.4: slightly irritant | MIOI>41.4: strongly irritant |
| MIOI<19.6: moderately irritant | |

**Figure 4 - RBC** test results

**SLS** Sodium Lauryl Sulfate
**SLES** Sodium Laureth Sulfate
**DSLSS** Disodium Laureth Sulfosuccinate
**CAPB** Cocamidopropyl Betaine
**SLG** PROTELAN AGL 95

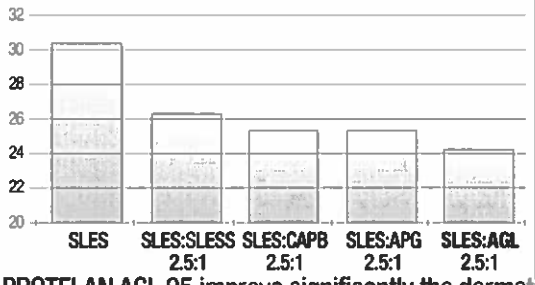



**PROTELAN AGL 95** is non irritant to eyes

Figure 5 shows the results of RBC test made on Sodium Laureth Sulfate (SLES) with different co-surfactant combinations (12).

**Figure 5 - RBC** test results with different co-surfactant combinations

**SLESS** Disodium Laureth Sulfosuccinate
**CAPB** Cocamidopropyl Betaine
**APG** Alkyl Polyglucoside
**AGL** PROTELAN AGL 95




**PROTELAN AGL 95** improve significantly the dermatological properties of Sodium Laureth Sulfate

## Skin sensitization

Skin sensitization potential of **PROTELAN AGL 95** was evaluated through an occlusive application of the product by means of Finn chamber (alluminium cell of some 20 microlitres volume) on 20 selected volunteers' volar part of the forearm for 48 hours (13). After the removal of the occlusion, cutaneous reactions induced by the product were evaluated 24 and 48 hours later. The repeated control of the treated

areas allows us to trace the presence and/or the activity in elicitation of cutaneous reactions of common allergens. Controls were made immediately after removal of the patch and 24 and 48 hours later.

The allergic potential is expressed as a percentage of allergic reactions and it is evaluated considering:

- the number of reactions observed
- severity, type and duration of reactions.

**PROTELAN AGL 95** was tested at 5% active matter.

**Table 3** - Skin Sensitization of **PROTELAN AGL 95**
N° total volunteers = 20

| Observed reaction | $T_1$ | $T_2$ | $T_3$ |
|---|---|---|---|
| Observed reaction | 0 | 0 | 0 |
| Doubtful reaction | 0 | 0 | 0 |
| Minimum reaction | 0 | 0 | 0 |
| Strong reaction | 0 | 0 | 0 |
| N° of allergic reactions | $T_1$0 | $T_2$0 | $T_3$0 |

**PROTELAN AGL 95** can be defined as non sensitizing

## Mutagenic power

Mutagenicity of **PROTELAN AGL 95** was tested through the Ames Test.

**PROTELAN AGL 95 can be classified as non mutagenic (14).**

# References


1. La cute come barriera dinamica e funzionalità cosmetica, ed. RCT, 1994
2. Surfactants in Cosmetics, ed. M.M. Rieger, vol 16, 1985
3. H.W. Spier and G. Pascher, Arch. Klin Exp. Dermatol. 199, 411 to 427 (1955)
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12. Internal Test, Zschimmer & Schwarz GmbH, Lahnstein
13. Ipoallergenicità, ISPE, rapporto 206/00/02 del 21.11.2000
14. Ames Test, Biolab, 1999
15. Miti Test, Biolab, 2001
16. Anaerobic Biodegradability Test (DIN EN ISO 11734 (1998)), Fraunhofer UMSICHT, 2006


# Notes

- This brochure was printed in February 2007 and replaces all the previous data
- The only analytical specifications guaranteed are those mentioned on the certificate of analysis supplied with each delivery
- The information set forth herein is furnished free of charge and is based on technical data that Zschimmer & Schwarz believes to be reliable. It is intended for use by person having technical skill and at their own discretion and risk. Since conditions of use are outside our control, we make no warranties, express or implied, and assume no liability in connection with any use of this information. Nothing herein is to be taken as a licence to operate under or a recommendation to infringe any patents

**ZSCHIMMER & SCHWARZ ITALIANA S.p.A.**

Zschimmer & Schwarz Italiana – 13038 – Tricerro (VC) / ITALY

# INFORMAZIONI TOSSICOLOGICHE
# *TOXICOLOGICAL INFORMATION*

***Revisione n°***
***Revision n° 00***

| | | |
|---|---|---|
| **1.** | **Informazioni generali**<br>***General information*** | |
| **1.1** | **Nome commerciale**<br>***Trade name*** | **PROTELAN AGL 95/C** |
| **1.2** | **Produttore/Fornitore**<br>(indirizzo, telefono, fax, contatto)<br>***Manufacturer/Supplier***<br>*(address, phone no., fax no., contact person)* | ZSCHIMMER & SCHWARZ ITALIANA<br>Via A. Ariotto 1/C - 13038 Tricerro (VC)<br>Italy<br>Tel: +39 (0)161 808111<br>Fax: +39 (0)161 801002<br>elisabetta.merlo@zsi.it |
| **1.3** | **Categoria della materiai prima**<br>(es. tensioattivo anionico)<br>***Raw material category***<br>*(e.g. anionic surfactant)* | Anionic surfactant, moisturizing |
| **1.4** | **Nome chimico**<br>***Chemical name*** | I-Glutamic acid, N-coco acyl derivs., monosodium salts |
| **1.5** | **Nome INCI (CTFA)**<br>**Composizione**<br>***INCI (CTFA) name***<br>**Composition** | Sodium Cocoyl Glutamate: 32.6% - 38.0% as dry matter<br>Aqua: to 100% |
| **1.6** | **N° EC (EINECS-/ELINCS)**<br>***EC (EINECS/ELINCS) no.*** | 269-087-2 |
| **1.7** | **N° CAS**<br>***CAS no.*** | 68187-32-6 |

| | | |
|---|---|---|
| 1.8 | **Registrazioni** (es. UE, USA, Giappone)<br>***Registration status*** *(e.g. EU, USA, Japan)* | TSCA (USA), NDSL (Canada), EINECS (Europe), ECL (Korea), PICCS (Philippines), ASIA-PAC (Asia-Pacific) and AICS (Australia). Japanes have recently changed their system, so that publication in the Japanese list of approved ingredients is no longer necessary. Any cosmetic ingredient is now allowed in Japan with no prior approvation. |

| | | |
|---|---|---|
| **2.** | **Informazioni sulla produzione**<br>**Information on production** | |
| **2.1** | **Origine della materia prima**<br>(vegetale, animale, sintetica)<br>**Origin of starting material**<br>(plant, animal, synthetic) | Vegetable, mineral and synthetic origin.<br>Glutamic acid is obtained through fermentation of glucose/molasses or from wheat.<br>Cocoyl chloride is obtained starting from coconut acid that comes from cleavage and distillation of coconut oil from Cocos Nucifera (South East Asia and Philippines).<br>NaOH is mineral and Propylene Glycol is synthetic. |
| **2.2** | **La materia prima deriva da organismi geneticamente modificati (OGM)?**<br>**Is the starting material derived from genetically modified organisms (GMO)?** | No |
| **2.3** | **Informazioni sul processo di produzione**<br>(descrizione generale)<br>**Information on production process**<br>(general description) | Schotten-Baumann reaction |

| | | |
|---|---|---|
| **3.** | **Additivi**<br>***Additives*** | |
| **3.1** | **Conservanti**<br>***Preservatives*** | Not added |
| **3.2** | **Antiossidanti**<br>***Antioxidants*** | Not added |

| | | |
|---|---|---|
| 3.3 | **Solventi**<br>***Solvents*** | 4.0% - 6.0% of Propylene Glycol |
| 3.4 | **Sbiancanti**<br>***Bleaching agents*** | Not added |
| 3.5 | **Altri**<br>***Others*** | Not added |

| | | |
|---|---|---|
| **4.** | **Specifiche microbiologiche**<br>**Microbiological specification** | |
| 4.1 | **Conta microbica totale (ufc/g)**<br>***Total viable count (colony-forming units/g)*** | less than 10 ufc/g |

| | | |
|---|---|---|
| **5.** | **Residui del processo di lavorazione**<br>La presenza di tracce delle sostanze elencate in Annex II della Direttiva 76/768/CEE (Incl. CMR cat. I - III sostanze contrassegnate con *) deve essere dimostrata come presenza tecnicamente inevitabile lavorando in GMP e deve essere conforme all'Articolo 2 della Direttiva 76/768/CEE.<br>***By-products***<br>*The presence of traces of the substances listed in Annex II of Directive 76/768/EEC (incl. cmr cat. I – III substances marked with *) shall be allowed provided that such presence is technically unavoidable in good manufacturing practice and that it conforms with Article 2 of Directive 76/768/EEC.* | |
| 5.1 | **1,4-Diossano**<br>***1,4-Dioxane*** | Not expected |
| 5.2 | **Ossido di etilene**<br>***Ethylene oxide*** | Not expected |
| 5.3 | **Solventi residui**<br>***Residual solvents*** | 4.0% - 6.0% of Propylene Glycol |
| 5.4 | **Monomeri residui**<br>***Residual monomers*** | Not expected |

| | | |
|---|---|---|
| **5.5** | **Ammine**<br>***Amines*** | Not expected |
| **5.6** | **Nitrosammine**<br>***Nitrosamines*** | Not expected |
| **5.7** | **Metalli pesanti**<br>***Heavy metals*** | Arsenic (As) < 2 ppm, Antimony (Sb) < 5 ppm, Lead (Pb) < 1 ppm, Cadmium (Cd) < 2 ppm, Mercury (Hg) < 2 ppm, Nickel (Ni) < 1 ppm, Chromium (Cr) < 2 ppm, Total heavy metals (as Fe) < 10 ppm |
| **5.8** | **Acido monocloroacetico**<br>**Monochloroacetic acid** | Absent |
| **5.9** | **Acido dicloroacetico**<br>***Dichloroacetic acid*** | Absent |
| **5.10** | **Allergeni**<br>**Allergens according to the 7th Amendment to the Cosmetics Directive (ppm)** | Absent |
| **5.11** | **Altri**<br>***Others*** | Sodium glutamate: 5% maximum<br>Coconut acid: 3%<br>Sodium chloride: 4% - 5.5% |

| | | |
|---|---|---|
| **6.** | **Tossicologia**<br>***Toxicology*** | |
| **6.1** | **Informazioni sulla tossicità acuta**<br>***Information on acute toxicity*** | LD50 on mice (male) = 5.5/kg (from literature) |
| **6.2** | **Informazioni sull'irritazione cutanea**<br>***Information on skin irritation*** | 10% a.m. = Not irritant (our Flex Wash Test n° 990354FWT) |

| | | |
|---|---|---|
| **6.3** | **Informazioni sull'irritazione oculare**<br>***Information on irritation of the mucous membrane*** | Not irritant (our test, Red Blood Cell n° cmrbc) |
| **6.4** | **Informazioni sulla sensibilizzazione**<br>***Information on sensitisation potential*** | Occlusive applications, 5% a.m. = The product hasn't any sensitization danger (our test n° 37/99/02) |
| **6.5** | **Informazioni sulla genotossicità**<br>***Information on gene toxicity*** | Ames test = Not mutagenic (from literature) |
| **6.6** | **Informazioni sull'assorbimento percutaneo**<br>***Information on percutaneous permeation*** | Not determined |
| **6.7** | **Altri**<br>***Others*** | Phototoxicity and photosensitization:<br>40 healthy males, 0.1%-0.5%-1%-3%-5% aqueous solutions = No abnormality was found (from literature) |

| | | |
|---|---|---|
| 7.3 | **Altri**<br>***Others*** | / |

| | | |
|---|---|---|
| 8. | **Informazioni aggiuntive**<br>(Per i dettagli sulle specifiche vedere il bollettino tecnico allegato; per i dettagli sull'etichettatura e la classificazione vedere la scheda di sicurezza allegata.)<br><br>***Additional information***<br>*(For details on specification see enclosed instruction sheet; for details on labelling and classification see enclosed safety data sheet.)* | |
| | **Dichiarazione BSE**<br>***BSE statement*** | The product is not from animal origin. Furthermore it doesn't contain any ingredient of animal origin, it is not produced using ingredients of animal origins and it doesn't come into contact with animal origin ingredients at any stage of its production. It is therefore BSE free. |
| | **Dichiarazione test animali**<br>***Non-animal testing declaration*** | ZSCHIMMER & SCHWARZ ITALIANA has never made or commissioned animal tests on this product. |
| | **Glicol eteri**<br>***Glycol ethers*** | Absent |
| | ***Ftalati***<br>***Phtalates*** | Absent |
| | ***Glutine***<br>***Gluten*** | Absent |
| | ***Formaldeide***<br>***Formaldehyde (Formol)*** | Absent |
| | ***VOC***<br>***VOC compounds*** | The product doesn't contain any of the substances that are classified as VOC according to "Ordonnance sur taxe d'incitation sur les composes organiques volatils (OCOV) du 12 novembre 1997". It contains 4% - 6% of Propylene Glycol. |
| | ***Pesticidi***<br>***Pesticides*** | The product doesn't contain any pesticides or pollutant substances (under detection limits). |
| | ***Grado cosmetico***<br>***Cosmetic grade*** | The product is of cosmetic grade and it can be used in cosmetic products |

**ZSCHIMMER & SCHWARZ ITALIANA S.p.A.**

Zschimmer & Schwarz Italiana – 13038 – Tricerro (VC) / ITALY

| | |
|---|---|
| **8.1 Data di scadenza**<br>***Shelf life*** | The product, if well preserved and in its original containers, maintains its appearance and characteristics for at least one year from delivery date. After this time, product can be used but it must be rechecked. |

Data / *Date* 15/07/10

**Queste informazioni si riferiscono solo ai prodotto sopramenzionato** e non possono essere considerate valide per altri prodotti o in altri processi produttivi. Le informazioni sono corrette e complete secondo le nostre attuali conoscenze e sono date in buona fede ma senza garanzia. E' responsabilità dell'utilizzatore l'assicurarsi che le informazioni siano appropriate e complete per lo specifico uso del prodotto.

***This information refers only to the above mentioned product*** *and does not need to be valid if used with other product(s) or in any process. The information is to our best present knowledge correct and complete and is given in good faith but without warranty. It remains the user's own responsibility to make sure that the information is appropriate and complete for his specific use of this product.*

Personal Care Products Council
Committed to Safety,
Quality & Innovation

**Memorandum**

**TO:** F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

**FROM:** Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

**DATE:** September 14, 2012

**SUBJECT:** Information on Acetyl Hydroxyproline

Anonymous. 2011. An in-use safety evaluation to determine the ocular irritation potential and consumer opinion of cosmetic products (under eye treatment containing 2% Acetyl Hydroxyproline).

Anonymous. 2011. Repeated insult patch test of a product containing 2% Acetyl Hydroxyproline.

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

[redacted]

# Final Report

**An In-Use Safety Evaluation to Determine the Ocular Irritation Potential and Consumer Opinion of Cosmetic Products**

**CLIENT:**

**ATTENTION:**

[redacted]

**TEST MATERIAL:**

**2 Step Under Eye and Crow's Feet Treatment:**
- **Step 1- Gel** = 2% n-acetyl hydroxy proline
- **Step 2- Cream**

[redacted] **STUDY NUMBER:** [redacted]

**AUTHORIZED SIGNATURES:**

[redacted]

**REPORT DATE:** **November 2, 2011**

[redacted]

[redacted]

# Good Clinical Practice
# Quality Assurance Audit Statement

**Clinical Study Number:** [redacted]

**Start Date:** September 27, 2011

**Completion Date:** October 26, 2011

The clinical study listed above was conducted in accordance with [redacted] Standard Operating Procedures, which incorporate the principles of Good Clinical Practice defined by applicable guidelines and regulations established by U.S. Regulatory Agencies. The conduct of the study was monitored for compliance, and the associated records, including source documents or raw data, were reviewed for documentation practices and accuracy by a Project Manager/Study Director and/or a Quality Assurance representative. Standard Quality Assurance audit procedures for this final report and study related documents were conducted.

[redacted]

Nov. 2, 2011
Date

**FINAL REPORT**

**An In-Use Safety Evaluation to Determine the Ocular Irritation Potential and Consumer Opinion of Cosmetic Products**

## OBJECTIVES

The objectives of this study were;

1. to evaluate the potential of cosmetic products to induce subjective and/or objective ocular irritation during four weeks of normal use in contact lens wearers and non-contact lens wearers;
2. to obtain the consumer opinion of cosmetic products following a four-week use period.

## INVESTIGATORS

## INVESTIGATIVE SITE

## SPONSOR

## TEST MATERIAL

The following test materials were provided by and were received by on September 21, 2011:

| Client Identification | | Identification |
|---|---|---|
| 2 Step Under Eye and Crow's Feet Treatment | Step 1- Gel | 97511-1 |
| | Step 2- Cream | 97511-2 |

Test materials were labeled with identification and subject numbers. Test material weights appear in Appendix I.

## STUDY DATES

This study was initiated on September 27, 2011 and was completed on October 26, 2011.

## STUDY POPULATION

A total of 33 female subjects, ranging in age from 40 to 60 years and in generally good health, were selected for the study (Subject Demographics – Appendix II). Sixteen subjects (48%) were contact lens wearers and the remaining 17 subjects (52%) were non-contact lens wearers. Subjects who met all of the inclusion criteria and none of the exclusion criteria listed in the study protocol were enrolled for participation.

## TEST METHOD

This study was conducted according to the attached study protocol, [redacted]7511 AC 1.0 (Attachment I).

## TEST RESULTS

### *Completed and Discontinued Subjects*

All 33 subjects completed the study. Subject #3 missed the Week 2 visit.

### *Ophthalmic Examinations*

Increases from baseline in ocular examination scores observed in each eye for each evaluated tissue at the Week 2 and Week 4 ophthalmic examinations appear in Table I. There were no reports of subjective irritation, lacrimation or eyelid irritation and no changes in corneal tissue integrity or contact lenses observed at either examination. Four subjects exhibited trace increases in palpebral conjunctival irritation at the Week 2 and/or Week 4 examinations. One subject exhibited a trace increase in bulbar conjunctival irritation at the Week 4 examination. In the opinion of the Investigator, these findings were not related to use of the test material and were probably caused by external factors such as hair products, mechanical factors, environmental conditions and/or seasonal factors.

### *Questionnaires*

A summary of questionnaire responses appears in Attachment II. Statistical analysis of questionnaire responses appears below.

## TEST RESULTS (Continued)

### *Questionnaires (Continued)*

| Question | Most Favorable Percentage | Least Favorable Percentage | Z-Score | Significant |
|---|---|---|---|---|
| 1: Please rate the following for this product regimen: | | | | |
| 1a. Spreadability | 95.5% | 4.5% | 5.22 | Yes |
| 1b. Ease of absorption/Rub in | 83.3% | 16.7% | 3.83 | Yes |
| 1c. The feel of the products on skin after both products are applied | 92.4% | 7.6% | 4.87 | Yes |
| 2: Please rate the following characteristics | | | | |
| 2a. Moisturizes/hydrates | 93.9% | 6.1% | 5.05 | Yes |
| 2b. Fine lines are less noticeable | 78.8% | 21.2% | 3.31 | Yes |
| 2c. Wrinkles (crow's feet area) are less noticeable | 74.2% | 25.8% | 2.79 | Yes |
| 2d. Improves skin texture/ smoothness | 80.3% | 19.7% | 3.48 | Yes |
| 2e. Appears to plump and fill in lines around my eyes | 66.7% | 33.3% | 1.91 | No |
| 2f. Skin looks younger | 71.2% | 28.8% | 2.44 | Yes |
| 2g. Eye area firmness / elasticity is increased | 75.8% | 24.2% | 2.96 | Yes |
| 2h. Skin looks brighter | 74.2% | 25.8% | 2.79 | Yes |
| 2i. Dark circles are less apparent | 67.9% | 32.1% | 1.89 | No |
| 2j. Is gentle to the skin | 95.5% | 4.5% | 5.22 | Yes |
| 2k. Is not irritating | 90.9% | 9.1% | 4.70 | Yes |
| 2l. Does not sting or burn | 86.4% | 13.6% | 4.18 | Yes |
| 2m. Does not rub off or 'ball' | 77.3% | 22.7% | 3.13 | Yes |
| 2n. Is compatible with makeup/foundation | 82.3% | 17.7% | 3.59 | Yes |
| 2o. Improves overall appearance of skin around eyes | 77.3% | 22.7% | 3.13 | Yes |
| 3. What is the earliest time that you thought this product regimen made your eye area skin look younger? | 74.2% | 25.8% | 2.79 | Yes |
| 4. Compared to the eye area product that you usually use, how would you rate this product? | 84.5% | 15.5% | 3.71 | Yes |
| 5. What is your overall opinion of this product regimen? | 78.8% | 21.2% | 3.31 | Yes |
| 6. Please rate how much you agree or disagree with how easy it was to use this 2 step product regimen? | 100.0% | 0.0% | 5.74 | Yes |
| 7. Please rate the packaging for each product | | | | |
| 7a. Step 1 Gel | 89.4% | 10.6% | 4.53 | Yes |
| 7b. Step 2 Cream | 93.9% | 6.1% | 5.05 | Yes |
| 8. For the Step 1 Gel, did the ball applicator glide or roll easily when applying it during the study? | 100.0% | 0.0% | 5.74 | Yes |

*=Percentage of the study population reporting the two most favorable responses (included half of the population reporting the central response) or the two least favorable responses (included half of the population reporting the central response, where applicable).

### *Daily Diaries*

There were no comments recorded on the Daily Diaries that were related to reactions or symptoms perceived during test material use.

### *Adverse Events*

There were no adverse events reported during the study period.

[redacted]



## CONCLUSION

Based on the test results of the subjective and objective ophthalmic evaluations during the four-week period, it was determined that use of the test materials, 2 Step Under Eye and Crow's Feet Treatment: Step 1-Gel and Step 2-Cream, did not demonstrate a potential for eliciting ophthalmic irritation. In this test population, the test materials were clinically safe for use by contact lens wearers and non-contact lens wearers.

Questionnaires completed by subjects following the four-week use period indicated that a statistically significant portion of the test population reported favorable test material attributes including application qualities, gentleness, and effect on skin, including the following;

- moisturizes
- improves appearance of fine lines/wrinkles
- improves skin texture/smoothness
- skin looks younger and brighter
- increases eye area firmness/elasticity

[redacted]



## Table I

## Ophthalmic Examinations

| Increase from Baseline – Week 2 | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subject Number | Subjective Irritation | | Lacrimation | | Eyelid Irritation (Upper/Lower) | | Palpebral Conjunctival Irritation (Upper/Lower) | | Bulbar Conjunctival Irritation | | Cornea | | Contact Lens | |
| | R | L | R | L | R | L | R | L | R | L | R | L | R | L |
| 1 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 2 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | Missed Visit | | | | | | | | | | | | NA | NA |
| 4 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 5 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 6 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 1/1 | 1/1 | 0 | 0 | 0 | 0 | NA | NA |
| 7 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 8 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 9 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 11 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 1/1 | 1/1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 15 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 17 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 18 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 19 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 20 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 21 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 22 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 24 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 27 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |

The scoring scale for ophthalmic examinations appears in the attached study protocol.
R = Right Eye L = Left Eye NA = Not Applicable; subject is not a contact lens wearer.

[redacted]



**Table I**
**(Continued)**

## Ophthalmic Examinations

| Subject Number | Increase from Baseline – Week 4 | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Subjective Irritation | | Lacrimation | | Eyelid Irritation (Upper/Lower) | | Palpebral Conjunctival Irritation (Upper/Lower) | | Bulbar Conjunctival Irritation | | Cornea | | Contact Lens | |
| | R | L | R | L | R | L | R | L | R | L | R | L | R | L |
| 1 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 2 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 4 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 5 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 6 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 7 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 8 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 1/1 | 1/1 | 0 | 0 | 0 | 0 | NA | NA |
| 9 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 11 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 1/1 | 1/1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 15 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 17 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 18 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 19 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 20 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 21 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 22 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 1 | 1 | 0 | 0 | NA | NA |
| 24 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 27 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 1/1 | 1/1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |

The scoring scale for ophthalmic examinations appears in the attached study protocol.
R = Right Eye L = Left Eye NA = Not Applicable; subject is not a contact lens wearer.

[redacted]



## Appendix I

### Test Material Weights

| Step 1 -Gel (weight in grams) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Subject # | Baseline | | Week 2 | | Week 4 | | Total Amt. Used |
| | Tube 1 | Tube 2 | Tube 1 | Tube 2 | Tube 1 | Tube 2 | |
| 1 | 25.9 | 25.5 | 24.6 | 25.5 | 23.3 | 25.3 | 2.8 |
| 2 | 25.9 | 26.0 | 25.1 | 26.0 | 22.8 | 22.4 | 6.7 |
| 3 | 25.2 | 25.9 | MV | MV | 24.4 | 24.4 | 2.3 |
| 4 | 25.6 | 26.2 | 25.5 | 25.6 | 24.7 | 25.4 | 1.7 |
| 5 | 25.9 | 25.3 | 25.2 | 25.3 | 24.3 | 25.3 | 1.6 |
| 6 | 25.8 | 26.1 | 24.1 | 26.1 | 23.5 | 26.0 | 2.4 |
| 7 | 25.6 | 25.4 | 25.0 | 25.4 | 24.2 | 25.4 | 1.4 |
| 8 | 25.7 | 26.9 | 23.6 | 26.6 | 23.3 | 24.0 | 5.3 |
| 9 | 26.0 | 25.9 | 24.3 | 25.9 | 22.4 | 25.8 | 3.7 |
| 10 | 25.9 | 26.2 | 23.6 | 26.2 | 23.1 | 24.9 | 4.1 |
| 11 | 25.9 | 25.6 | 22.3 | 25.6 | 20.0 | 25.6 | 5.9 |
| 12 | 25.9 | 25.9 | 25.8 | 25.7 | 25.8 | 25.1 | 0.9 |
| 13 | 26.0 | 25.8 | 25.4 | 25.8 | 25.1 | 25.8 | 0.9 |
| 14 | 25.8 | 26.0 | 17.6 | 26.0 | 17.6 | 13.8 | 20.4 |
| 15 | 26.0 | 25.0 | 25.2 | 25.0 | 24.3 | 24.9 | 1.8 |
| 16 | 25.4 | 25.4 | 24.6 | 25.4 | 18.4 | 21.6 | 10.8 |
| 17 | 25.2 | 26.2 | 25.1 | 26.2 | 17.8 | 24.8 | 8.8 |
| 18 | 25.7 | 25.7 | 25.7 | 24.9 | 25.3 | 24.9 | 1.2 |
| 19 | 25.1 | 25.1 | 20.9 | 24.9 | 19.2 | 22.4 | 8.6 |
| 20 | 25.1 | 25.3 | 24.8 | 25.3 | 24.5 | 25.3 | 0.6 |
| 21 | 25.7 | 25.4 | 25.7 | 24.6 | 23.6 | 20.7 | 6.8 |
| 22 | 26.0 | 25.7 | 24.4 | 25.7 | 23.1 | 25.7 | 2.9 |
| 23 | 25.5 | 26.3 | 24.6 | 26.3 | 21.8 | 25.6 | 4.4 |
| 24 | 25.3 | 25.7 | 24.6 | 25.7 | 23.7 | 25.7 | 1.6 |
| 25 | 26.1 | 26.0 | 25.8 | 25.9 | 25.7 | 25.9 | 0.5 |
| 26 | 24.9 | 25.6 | 24.4 | 25.6 | 23.6 | 25.5 | 1.4 |
| 27 | 25.5 | 25.1 | 23.4 | 25.1 | 20.6 | 23.7 | 6.3 |
| 28 | 26.2 | 25.9 | 25.7 | 25.4 | 25.3 | 24.7 | 2.1 |
| 29 | 26.0 | 25.5 | 25.1 | 25.5 | 24.4 | 25.5 | 1.6 |
| 30 | 25.3 | 26.1 | 25.3 | 25.9 | 24.9 | 25.6 | 0.9 |
| 31 | 25.7 | 25.9 | 24.7 | 25.9 | 23.7 | 25.9 | 2 |
| 32 | 25.5 | 25.7 | 24.9 | 25.7 | 24.3 | 25.7 | 1.2 |
| 33 | 26.2 | 26.0 | 26.2 | 25.4 | 25.6 | 21.0 | 5.6 |

**Appendix I**
**(Continued)**

**Test Material Weights**

| Step 2 -Cream (weight in grams) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Subject # | Baseline | | Week 2 | | Week 4 | | Total Amt. Used |
| | Tube 1 | Tube 2 | Tube 1 | Tube 2 | Tube 1 | Tube 2 | |
| 1 | 31.8 | 31.9 | 29.0 | 31.6 | 26.0 | 31.6 | 6.1 |
| 2 | 31.9 | 32.0 | 25.5 | 32.0 | 22.4 | 28.0 | 13.5 |
| 3 | 32.1 | 31.7 | MV | MV | 29.6 | 30.1 | 4.1 |
| 4 | 31.7 | 31.9 | 27.1 | 30.0 | 23.2 | 29.5 | 10.9 |
| 5 | 31.7 | 31.7 | 30.0 | 31.7 | 27.4 | 31.7 | 4.3 |
| 6 | 31.8 | 32.1 | 28.0 | 32.1 | 24.6 | 32.0 | 7.3 |
| 7 | 32.0 | 32.2 | 30.2 | 32.2 | 28.3 | 32.2 | 3.7 |
| 8 | 31.8 | 32.3 | 26.0 | 32.1 | 25.5 | 26.0 | 12.6 |
| 9 | 31.8 | 31.9 | 29.7 | 31.9 | 28.5 | 31.9 | 3.3 |
| 10 | 31.6 | 32.1 | 27.3 | 32.1 | 25.4 | 29.0 | 9.3 |
| 11 | 31.8 | 31.8 | 26.3 | 31.2 | 21.6 | 31.2 | 10.8 |
| 12 | 32.0 | 31.7 | 30.5 | 31.7 | 27.4 | 31.7 | 4.6 |
| 13 | 31.8 | 32.2 | 31.5 | 30.7 | 31.5 | 29.2 | 3.3 |
| 14 | 32.0 | 31.8 | 17.5 | 31.5 | 17.4 | 17.3 | 29.1 |
| 15 | 31.6 | 31.8 | 29.4 | 31.8 | 27.8 | 31.8 | 3.8 |
| 16 | 31.7 | 32.0 | 29.4 | 32.0 | 17.4 | 20.6 | 25.7 |
| 17 | 31.7 | 32.0 | 29.5 | 32.0 | 17.3 | 30.6 | 15.8 |
| 18 | 31.9 | 31.9 | 30.2 | 31.9 | 30.2 | 30.9 | 2.7 |
| 19 | 31.7 | 31.6 | 25.0 | 31.5 | 24.3 | 27.5 | 11.5 |
| 20 | 31.7 | 32.2 | 27.3 | 29.6 | 26.5 | 29.6 | 7.8 |
| 21 | 32.3 | 32.3 | 32.3 | 30.7 | 30.7 | 24.9 | 9 |
| 22 | 31.7 | 31.9 | 25.0 | 31.9 | 17.3 | 29.1 | 17.2 |
| 23 | 29.3 | 31.8 | 27.0 | 31.8 | 17.4 | 31.0 | 12.7 |
| 24 | 32.0 | 31.9 | 29.0 | 31.9 | 25.9 | 31.9 | 6.1 |
| 25 | 31.9 | 31.7 | 30.0 | 31.7 | 27.6 | 31.7 | 4.3 |
| 26 | 31.7 | 32.1 | 28.3 | 32.1 | 24.1 | 32.1 | 7.6 |
| 27 | 32.1 | 31.9 | 28.1 | 31.9 | 21.4 | 31.9 | 10.7 |
| 28 | 31.6 | 32.0 | 29.4 | 31.0 | 28.1 | 28.8 | 6.7 |
| 29 | 31.9 | 31.6 | 26.7 | 31.6 | 22.3 | 30.7 | 10.5 |
| 30 | 32.0 | 31.8 | 27.8 | 31.7 | 24.9 | 30.1 | 8.8 |
| 31 | 31.8 | 31.8 | 28.3 | 31.8 | 24.3 | 31.8 | 7.5 |
| 32 | 32.0 | 32.2 | 28.9 | 32.2 | 26.0 | 32.2 | 6 |
| 33 | 31.8 | 31.7 | 318.0 | 27.4 | 23.7 | 27.1 | 12.7 |

## Appendix II

### Subject Demographics

| Subject Number | Subject Initials | [redacted] ID # | Eye Type | Age | Sex |
|---|---|---|---|---|---|
| 1 | [redacted] | | NCLW | 55 | F |
| 2 | | | DSCL | 58 | F |
| 3 | | | NCLW | 60 | F |
| 4 | | | NCLW | 43 | F |
| 5 | | | NCLW | 58 | F |
| 6 | | | NCLW | 57 | F |
| 7 | | | NCLW | 50 | F |
| 8 | | | NCLW | 58 | F |
| 9 | | | DISP | 46 | F |
| 10 | | | NCLW | 51 | F |
| 11 | | | DSCL | 49 | F |
| 12 | | | DISP | 40 | F |
| 13 | | | DISP | 46 | F |
| 14 | | | NCLW | 51 | F |
| 15 | | | DISP | 41 | F |
| 16 | | | NCLW | 44 | F |
| 17 | | | NCLW | 50 | F |
| 18 | | | NCLW | 42 | F |
| 19 | | | NCLW | 53 | F |
| 20 | | | NCLW | 51 | F |
| 21 | | | NCLW | 49 | F |
| 22 | | | DISP | 45 | F |
| 23 | | | NCLW | 48 | F |
| 24 | | | DISP | 53 | F |
| 25 | | | DISP | 45 | F |
| 26 | | | NCLW | 56 | F |
| 27 | | | DISP | 54 | F |
| 28 | | | DISP | 45 | F |
| 29 | | | DSCL | 58 | F |
| 30 | | | DISP | 54 | F |
| 31 | | | DISP | 44 | F |
| 32 | | | DISP | 51 | F |
| 33 | | | DISP | 49 | F |

**Eye Type:** **DISP** = Disposable Soft Contact Lenses
**DSCL** = Daily Soft Contact Lenses
**NCLW** = Non-contact Lens Wearer

[redacted]

# FINAL REPORT

**CLIENT:** [redacted]

**ATTENTION:**

**TEST:** Repeated Insult Patch Test
Protocol No.: 1.01S

**TEST MATERIAL:** A33 Plumper Gel Step-1, F#268-46, L#231-125
2% n-acetyl hydroxy proline containing product

**EXPERIMENT REFERENCE NUMBER:** [redacted]

Reviewed by: [redacted]

Approved by:

Approved by:



[redacted]

[redacted]

# QUALITY ASSURANCE UNIT STATEMENT

**Trial Number:** [redacted]

[redacted] Quality Assurance Unit (QAU) is responsible for auditing the conduct, content and reporting of all clinical trials that are conducted at [redacted]

This trial has been conducted in accordance with the Declaration of Helsinki, the ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable laws and regulations, [redacted] Standard Operating Procedures, and the approved protocol.

The [redacted] QAU has reviewed all data, records, and documents relating to this trial and also this Final Report. The following QAU representative signature certifies that all data, records, and documents relating to this trial and also this Final Report have been reviewed and are deemed to be acceptable, and that the trial conforms to all of the requirements as indicated above.

All records and documents pertaining to the conduct of this trial shall be retained in the [redacted] archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the [redacted] QAU to obtain custody of trial records once the [redacted] archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, trial-related records shall be destroyed at the end of the [redacted] archive period in a manner that renders them useless.

[redacted]
Quality Assurance Representative

11/2/11
Date

[redacted]

**Objective:** To determine by repetitive epidermal contact the potential of a test material to induce primary or cumulative irritation and/or allergic contact sensitization.

**Participants:** One hundred-fifteen (115) qualified subjects, male and female, ranging in age from 17 to 77 years, were selected for this evaluation. One hundred-nine (109) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

**Inclusion Criteria:**

a. Male and female subjects, age 16[a] and over.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History form and the understanding and signing of an Informed Consent form.
e. Considered reliable and capable of following directions.

**Exclusion Criteria:**

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

**Test Material:** A33 Plumper Gel Step-1, F#268-46, L#231-125

**Study Schedule:**

| Panel # | Initiation Date | Completion Date |
|---|---|---|
| 20110291 | September 07, 2011 | October 20, 2011 |
| 20110306 | September 14, 2011 | October 20, 2011 |

[a]With parental or guardian consent

**Methodology:**

The upper back between the scapulae served as the treatment area. Approximately 0.2 g of the test material, or an amount sufficient to cover the contact surface, was applied to the 1" x 1" absorbent pad portion of a clear adhesive dressing. This was then applied to the appropriate site to form a semi-occlusive patch.

**Induction Phase:**

Applications were conducted three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period.

If any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications were discontinued for the remainder of this test phase, if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of twenty-four hours following each Monday and Wednesday application, and forty-eight hours following each Friday application.

**Challenge Phase:**

Approximately two (2) weeks after the final Induction application, a Challenge application was conducted to a virgin test site adjacent to the original Induction site, following the same procedure described for Induction. The site was scored at the clinic twenty-four and seventy two hours post-application.

**Methodology (continued):**

**Evaluation Criteria (Erythema and additional Dermal Sequelae):**

| | | | | | |
|---|---|---|---|---|---|
| **0** | **=** | **No visible skin reaction** | **E** | **=** | **Edema** |
| **0.5** | **=** | **Barely perceptible** | **D** | **=** | **Dryness** |
| **1** | **=** | **Mild** | **S** | **=** | **Staining** |
| **2** | **=** | **Moderate** | **P** | **=** | **Papules** |
| **3** | **=** | **Marked** | **V** | **=** | **Vesicles** |
| **4** | **=** | **Severe** | **B** | **=** | **Bullae** |
| | | | **U** | **=** | **Ulceration** |
| | | | **Sp** | **=** | **Spreading** |

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

**Results:**

The results of each participant are appended (Table 1).

Observations remained negative throughout the test interval.

Subject demographics are presented in Table 2.

**Summary:**

Under the conditions of this study, test material, A33 Plumper Gel Step-1, F#268-46, L#231-125, did not indicate a potential for dermal irritation or allergic contact sensitization.

Table 1
Panel #20110291

Individual Results

A33 Plumper Gel Step-1, F#268-46, L#231-125

| Subject Number | Induction Phase | | | | | | | | | | Virgin Challenge Site | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 24*hr | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 24*hr | 72 hr |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0[m] | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | ----------DID NOT COMPLETE STUDY---------- | | | | | | | | | | | |
| 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | 0[m] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | ----------DID NOT COMPLETE STUDY---------- | | | | | | | | | | | |
| 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22 | 0 | 0 | 0 | 0[m] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0[m] | 0 | 0 | 0 | 0 | 0 |
| 27 | 0 | 0 | ----------DID NOT COMPLETE STUDY---------- | | | | | | | | | |
| 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

24* = Supervised removal of 1st Induction and Challenge Patch
m = Additional makeup day granted at the discretion of the clinic supervisor
- = Subject not present for supervised removal

Table 1
(continued)
Panel #20110291

Individual Results

A33 Plumper Gel Step-1, F#268-46, L#231-125

| Subject Number | 24*hr | Induction Phase 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Virgin Challenge Site 24*hr | 72 hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0[m] | 0 | 0 | 0 | 0 |
| 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0[m] | 0 | 0 | 0 | 0 | 0 |
| 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 41 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 47 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 49 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 56 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 57 | ----------DID NOT COMPLETE STUDY---------- | | | | | | | | | | | |
| 58 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

24* = Supervised removal of 1st Induction and Challenge Patch
m = Additional makeup day granted at the discretion of the clinic supervisor

Table 1
Panel #20110306

Individual Results

A33 Plumper Gel Step-1, F#268-46, L#231-125

| Subject Number | 24*hr | Induction Phase 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Virgin Challenge Site 24*hr | 72 hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

24* = Supervised removal of 1st Induction and Challenge Patch
- = Subject not present for supervised removal

Table 1
(continued)
Panel #20110306

Individual Results

A33 Plumper Gel Step-1, F#268-46, L#231-125

| Subject Number | 24*hr | Induction Phase 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Virgin Challenge Site 24*hr | 72 hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 36 | 0 | 0 | 0 | ----------DID NOT COMPLETE---------- | | | | | | | | |
| 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 40 | - | 0 | 0 | 0 | 0 | 0 | 0 | ---------DID NOT COMPLETE-------- | | | | |
| 41 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 47 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 49 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 56 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 57 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

24* = Supervised removal of 1st Induction and Challenge Patch
- = Subject not present for supervised removal

Table 2
Panel #20110291

Subject Demographics

| Subject Number | Initials | Age | Sex |
|---|---|---|---|
| 1 | [redacted] | 53 | F |
| 2 | | 47 | F |
| 3 | | 55 | F |
| 4 | | 18 | M |
| 5 | | 63 | F |
| 6 | | 51 | F |
| 7 | | 50 | F |
| 8 | | 39 | F |
| 9 | | 59 | F |
| 10 | | 36 | F |
| 11 | | 48 | F |
| 12 | | 48 | M |
| 13 | | 65 | F |
| 14 | | 45 | F |
| 15 | | 57 | F |
| 16 | | 62 | M |
| 17 | | 45 | F |
| 18 | | 62 | F |
| 19 | | 66 | F |
| 20 | | 37 | M |
| 21 | | 69 | M |
| 22 | | 19 | F |
| 23 | | 37 | F |
| 24 | | 47 | F |
| 25 | | 43 | F |
| 26 | | 57 | F |
| 27 | | 36 | F |
| 28 | | 44 | F |
| 29 | | 67 | F |

Table 2
(continued)
Panel #20110291

Subject Demographics

| Subject Number | Initials | Age | Sex |
|---|---|---|---|
| 30 | [redacted] | 42 | M |
| 31 | | 51 | F |
| 32 | | 29 | F |
| 33 | | 31 | F |
| 34 | | 62 | M |
| 35 | | 69 | F |
| 36 | | 50 | M |
| 37 | | 65 | M |
| 38 | | 55 | F |
| 39 | | 65 | F |
| 40 | | 49 | F |
| 41 | | 52 | F |
| 42 | | 42 | F |
| 43 | | 69 | F |
| 44 | | 60 | F |
| 45 | | 48 | F |
| 46 | | 62 | F |
| 47 | | 48 | F |
| 48 | | 65 | F |
| 49 | | 53 | F |
| 50 | | 25 | F |
| 51 | | 29 | F |
| 52 | | 58 | F |
| 53 | | 51 | F |
| 54 | | 55 | F |
| 55 | | 69 | F |
| 56 | | 34 | F |
| 57 | | 34 | F |
| 58 | | 70 | F |

Table 2
Panel #20110306

Subject Demographics

| Subject Number | Initials | Age | Sex |
|---|---|---|---|
| 1 | [redacted] | 37 | M |
| 2 | | 77 | F |
| 3 | | 27 | F |
| 4 | | 43 | F |
| 5 | | 60 | F |
| 6 | | 40 | F |
| 7 | | 57 | F |
| 8 | | 51 | F |
| 9 | | 54 | F |
| 10 | | 69 | M |
| 11 | | 66 | F |
| 12 | | 76 | F |
| 13 | | 49 | F |
| 14 | | 33 | F |
| 15 | | 17 | M |
| 16 | | 49 | F |
| 17 | | 34 | F |
| 18 | | 64 | F |
| 19 | | 39 | F |
| 20 | | 37 | F |
| 21 | | 31 | F |
| 22 | | 41 | F |
| 23 | | 40 | F |
| 24 | | 53 | F |
| 25 | | 39 | F |
| 26 | | 53 | M |
| 27 | | 75 | M |
| 28 | | 57 | F |
| 29 | | 74 | F |

Table 2
(continued)
Panel #20110306

Subject Demographics

| Subject Number | Initials | Age | Sex |
|---|---|---|---|
| 30 | [redacted] | 43 | F |
| 31 | | 57 | M |
| 32 | | 74 | F |
| 33 | | 74 | F |
| 34 | | 52 | M |
| 35 | | 53 | F |
| 36 | | 43 | F |
| 37 | | 47 | F |
| 38 | | 54 | M |
| 39 | | 56 | F |
| 40 | | 73 | F |
| 41 | | 73 | F |
| 42 | | 40 | F |
| 43 | | 18 | M |
| 44 | | 27 | F |
| 45 | | 46 | F |
| 46 | | 48 | F |
| 47 | | 40 | F |
| 48 | | 31 | F |
| 49 | | 69 | F |
| 50 | | 67 | M |
| 51 | | 66 | F |
| 52 | | 38 | F |
| 53 | | 54 | F |
| 54 | | 61 | F |
| 55 | | 70 | F |
| 56 | | 57 | M |
| 57 | | 54 | F |

Personal Care Products Council
Committed to Safety,
Quality & Innovation

**Memorandum**

**TO:** F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

**FROM:** Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

**DATE:** September 17, 2012

**SUBJECT:** Information on Acetyl Proline

Anonymous. 2001. Ames II assay A-25 (Acetyl Proline).

Anonymous. 2001 Mattek epiderm assay A-25 (Acetyl Proline).

Anonymous. 2006. Acute oral toxicity - up and down procedure A-25 (Acetyl Proline).

Anonymous. 2002. Evaluation of two creams on the treatment of eczema or active atopic dermatitis - 10% Acetyl Proline. (A25 = Acetyl Proline)

Anonymous. 2002. Repeated insult patch test of a cream containing 10% Acetyl Proline.

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

[redacted]

# AMES II ASSAY

**Prepared For:**

[redacted]

A-25 = Acetyl Proline

---

[redacted]

[redacted]

## TABLE OF CONTENTS

[redacted]

[redacted]

**NOTICE**

[redacted] submits this report with the understanding that no portion of it will be used for advertising or promotional use without the express permission and written consent of [redacted] regarding the proposed use. When such permission is obtained, [redacted] will assist in the preparation of reports acceptable to the Sponsor.

**DISCLAIMER**

[redacted] submits this report with the understanding that the data contained herein may be used as an important component of the Sponsor's Product Safety assessment strategy. However, the data contained herein may not provide sufficient evidence by itself of the toxicity potential (or lack thereof) of a product or raw material. These data should be evaluated along with other sources of information about the test material including, but not limited to, physicochemical data and clinical safety studies. Accordingly, the final assessment of any test material's safety and/or hazard potential is the sole responsibility of the Sponsor.

[redacted]

**SUMMARY**

This Ames II Assay was conducted fo[redacted]o determine the potential of the Sponsor's test material to cause frame shift mutations and base pair substitutions. Six different strains of *Salmonella Typhimurium*, each containing a different missense mutation in the histidine operon and therefore unable to manufacture histidine, were exposed to various concentrations of the Sponsor's test material. If the Sponsor's test material caused a reversion of the original mutation, then the bacteria will survive in a histidine-depleted environment. After exposure to the Sponsor's test material, the bacteria were plated into the wells of 384 well plate and cultured in medium without histidine. Wells containing revertant bacteria were quantified using a colorimetric assay. The greater the number of wells containing revertant bacteria, the greater the potential of the Sponsor's test material to cause frame shift mutations and base pair substitutions.

This assay was performed in the presence of an optional S9 fraction. The S9 fraction contains hepatic enzymes that can breakdown the test materials into their respective metabolites, simulating the breakdown of the material if it were absorbed into the body and taken into the liver for metabolism. Thus this test not only provides information on the test material, but also on any by-product formed by metabolism of the test material that could be potentially mutagenic.

The results of the Ames II Assay show that all four of the Sponsor's test materials did not induce a rate of mutation that was significantly different from the negative control (sterile deionized water).

[redacted]

**PURPOSE**

This Ames II Assay was conducted fo[redacted]o determine the potential of the Sponsor's test material to cause frame shift mutations and base pair substitutions.

**GENERAL INFORMATION**

[redacted]

| | |
|---|---|
| Test: | Ames II Assay |
| Test Materials: | A-25 (10% solution) |
| | [redacted] |
| Investigator and Biostatistics: | [redacted] |
| Quality Assurance Manager: | |
| Administrative and Testing Facility: | |
| Source of Tissue: | MatTek Corporation<br>200 Homer Avenue<br>Ashland, Massachusetts 01721 |
| Sponsor: | [redacted] |
| Sponsor Representative: | |
| Experiment Start Date: | June 12, 2001 |
| Experiment TerminationDate: | June 30, 2001 |

[redacted]

**STORAGE, HANDLING, AND DOCUMENTATION OF TEST MATERIALS**

The receipt of test materials to [redacted] was documented in a logbook, which serves as a permanent record of the receipt, storage, return, and disposition of all study materials. All study materials were kept in a locked product storage room accessible to laboratory staff members only.

**TEST MATERIAL DESCRIPTIONS**

Test Material Identification Number (TMIN):
Sponsor Test Material Identification:
Physical Description:
Concentrations Tested:

[redacted]

Test Material Identification Number (TMIN):
Sponsor Test Material Identification:
Physical Description:
Concentrations Tested:

Test Material Identification Number (TMIN): 0062-01L
Sponsor Test Material Identification: A-25 (10% solution)
Physical Description: Colorless, transparent liquid
Concentrations Tested: 0.4%, 0.2%, 0.1%, 0.05%, 0.025% and 0.0125%

Test Material Identification Number (TMIN):
Sponsor Test Material Identification:
Physical Description:
Concentrations Tested:

[redacted]

**PROCEDURES AND METHODS**

Note: All procedures were performed using sterile technique

**Day 1: Overnight Culture Preparation**

Vials containing the TA98 and Mixed Strains bacteria were removed from storage at -70°C and allowed to thaw to room temperature. Concurrently, vials containing ampicillin were removed from storage at -20°C and also allowed to thaw to room temperature. Three 50-ml culture tubes were then prepared by filling each with 10 ml of growth medium and 10 µl of ampicillin.

The overnight cultures were prepared by adding 10 µl of either the TA98 or Mixed Strains bacteria to a 50-ml culture tube. The third culture tube was used as a negative control to ensure that all reagents were free of contamination from outside bacteria. All three tubes were loosely capped and incubated overnight (14-17 hours) at 37± 2°C on a shaker set for 250 RPM.

**Day 2: Verify Culture Viability, Prepare Test Material And Start Ames II Assay**

After the overnight incubation, a 100 µl aliquot was removed from each of the three culture tubes and added to respective cuvettes containing 900 µl of growth medium (+ histidine). The cuvettes were mixed and read using a spectrophotometer set to a wavelength of 600 nm and blanked with growth medium. If the O.D. for the TA98 culture and the Mixed strains culture was greater than 0.2 (indicating sufficient bacterial growth) and the O.D. for the negative control was ~0.00 (indicating no growth), then the cultures were considered viable and used in the assay.

The Sponsor's test materials were prepared in the following 25x stock concentrations: neat, 50%, 25%, 12.5%, 6.25%, and 3.125% (using sterile deionized water as a diluent). When the test materials are diluted in the assay it will yield the final concentrations of: 4%, 2%, 1%, 0.5%, 0.25% and 0.125%. The positive control for the assay was 2-aminoanthracene, 125 µg/ml prepared in DMSO.

[redacted]

The S9 fraction was prepared by mixing the following freshly prepared reagents (proportions reflect the amount needed to test four materials):

0.332 ml 1.00 M KCl
0.320 ml 0.25 M $MgCl_2 \bullet 6H2O$
0.252 ml 0.20 MGlucose-6-Phosphate
1.000 ml 0.04 M NADP
5.000 ml 0.20 MNaH2PO4 buffer (pH 7.4)
1.596 ml Sterile Deionized Water
3.000 ml S9 Mix (Aroclor Induced, from Male Sprague Dawley Rat Liver)

10.0 ml total volume

For the test material exposure part of the Ames II assay, 6.0 ml of exposure medium (+ histidine) was combined with 1.2 ml of the S9 fraction and 0.8 ml of either TA98 or Mixed Strains Bacteria. From this mixture, 240 µl aliquots were transferred to each well of a 24-well plate. Ten (10) microliters of either test material (6 different concentrations), positive control or negative control was then added to each well to give a final volume of 250 µl. Within each 24-well plate the testing will be done in triplicate. The 24-well plates were then placed into a 37±2°C incubator on a shaker set for 250 RPM for 90 minutes.

After the 90-minute incubation, the 24-well plates were removed and 2.8 ml of indicator medium (pH indicator, -histidine) was added to each well for a total volume of approximately 3.0 ml. From each well of the 24-well plate, forty-eight 50µl aliquots were transferred to the wells of a 384-well plate. Once the 384-well plate was full it was placed into a 37±2°C incubator for two days.

**Day 4: Count The Number Of Revertant Colonies**
After two days of incubation the 384-well plates were removed from the incubator and the number of revertant wells were counted.

**CALCULATIONS AND DATA MANAGEMENT**
The test material was considered to be mutagenic if it induced a statistically significant two-fold increase in the number of revertant wells when compared to the negative control. Statistical significance was determined via an ANOVA followed by a Tukey multiple comparison t-test.

**RESULTS**
The numbers of revertant wells produced by each test material are presented in tables 1 through 4.

[redacted]

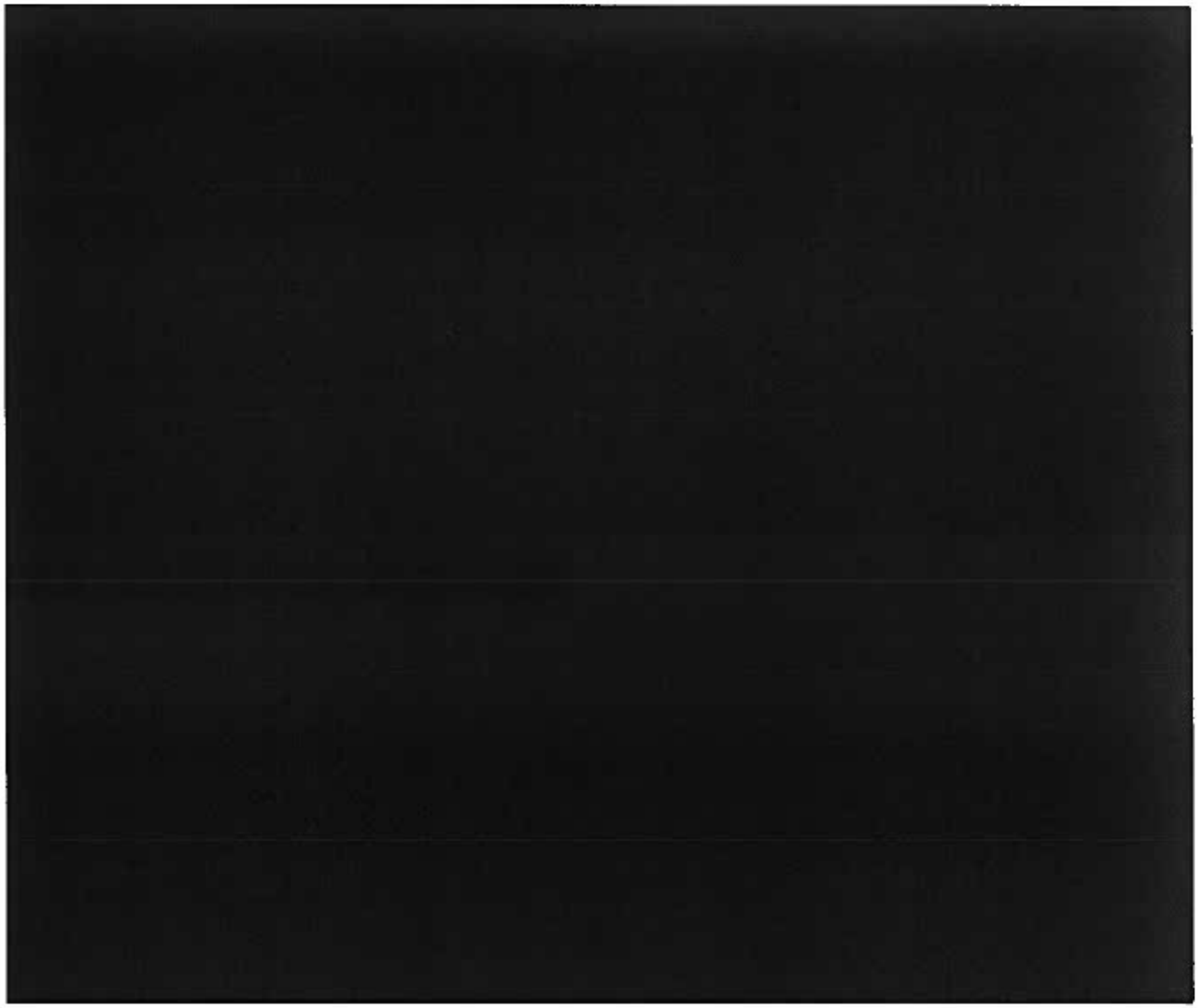
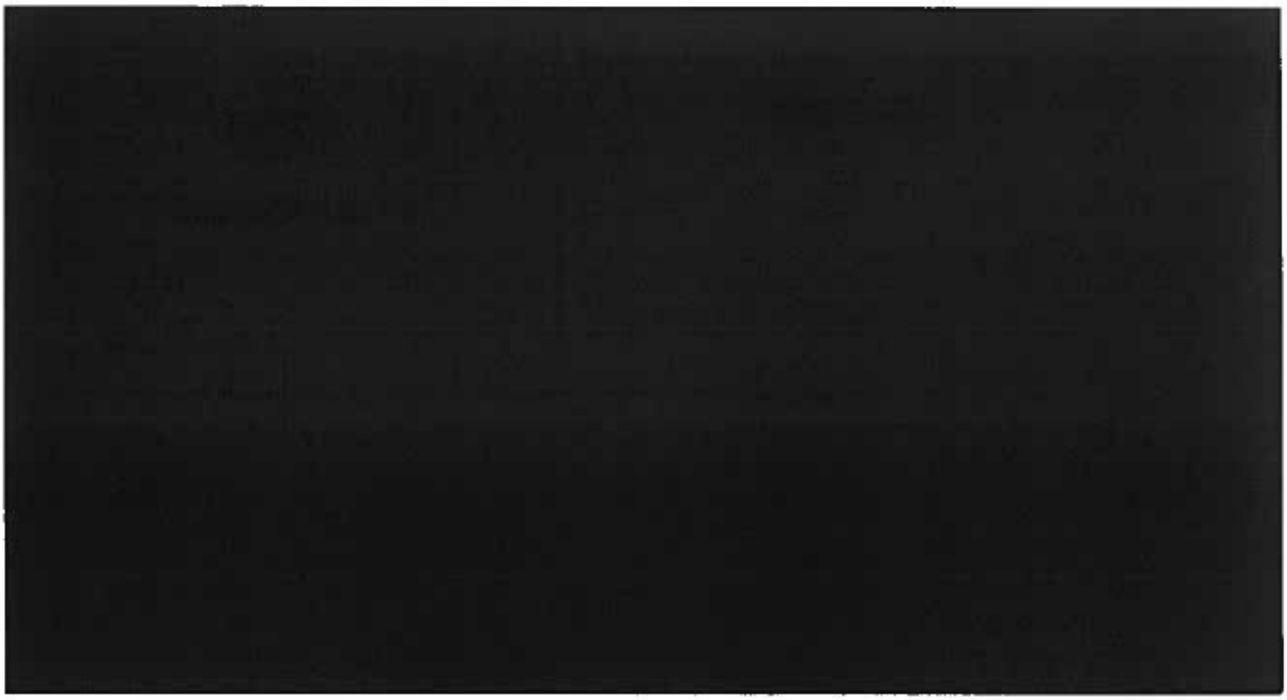


**TABLE 3**
**A-25 Solution**

| Treatment | TA 98 | | Mixed Strains | |
|---|---|---|---|---|
| | Positive Wells (Mean ± S.D.) | Fold Increase over Negative Control | Positive Wells (mean ± S.D.) | Fold Increase over Negative Control |
| Negative Control | 2.3 ± 3.2 | N/A | 0.3 ± 0.6 | N/A |
| 0.125% | 1.7 ± 1.2 | 0.7 | 0.3 ± 0.6 | 1.0 |
| 0.25% | 2.0 ± 1.0 | 0.9 | 1.0 ± 1.0 | 3.0 |
| 0.5% | 1.7 ± 1.5 | 0.7 | 0.3 ± 0.6 | 1.0 |
| 1.0% | 1.0 ± 1.7 | 0.4 | 0.3 ± 0.6 | 1.0 |
| 2.0% | 2.7 ± 2.1 | 1.1 | 1.0 ± 1.0 | 3.0 |
| 4.0% | 0.3 ± 0.6 | 0.1 | 0.0 ± 0.0 | 0.0 |
| Positive Control | 48.0 ± 0.0* | 20.6* | 34.0 ± 1.0* | 102.0* |

* Denotes significantly different from negative control ($p < 0.05$).



Please see Appendix I for complete biostatistics and Appendix II for graphs.

**DISCUSSION AND CONCLUSIONS**

The minimum criteria for classifying a test material as potentially mutagenic is for it to cause a two fold increase in the number of positive wells (wells containing revertant bacteria) when compared to a negative control. Since the negative control (deionized water) is non-mutagenic then any positive wells associated with its exposure represent spontaneous revertants. Thus, any material that produces mutations at twice the rate of spontaneous mutations is considered mutagenic. However, due to the inherent variable nature of mutations, both spontaneous and test material induced, the two fold increase must also be statistically significant.

Test materials [redacted] A-25 at certain concentrations appeared to induce a twofold or greater increase in the number of positive wells when compared to the negative control (primarily in the mixed strain bacteria). However, since these increases were not statistically significant the test materials were not considered to be mutagenic. Also, the various concentrations of the test materials that did induce the higher fold increase in positive wells did not typically follow a dose response relationship. As the concentration of the test material increases, the rate of mutation should also increase if it is truly mutagenic. However this was not observed for test materials [redacted] A-25, where the number of positive wells appeared to slightly fluctuate between doses and did not have a distinct pattern. The only exception was [redacted] which displayed a slight dose response relationship between the testing concentrations of 0.125% and 0.5%. However this relationship did not continue with the higher testing concentrations. This lack of a dose response relationship between the test material concentration and the number of positive wells further supports the observation that these materials should not be considered mutagenic.

[redacted] did not induce a two-fold increase in the number of positive wells at any of the tested concentrations and was not considered mutagenic.

[redacted]

## STATEMENT OF QUALITY ASSURANCE

All data and supporting documentation for this study have been audited by [redacted] Quality Assurance Department and found to be accurate, complete, and in compliance with the requirements of the protocol and [redacted] Standard Operating Procedures. This report has been reviewed and accurately reflects all aspects of the conduct of the study.

All laboratory research studies that are performed by [redacted] are in accordance with federal regulations and Good Laboratory Practice guidelines.

[redacted]

[redacted]

# MATTEK EPIDERM ASSAY

Prepared For:

[redacted]

[redacted]

[redacted]

[redacted]

## TABLE OF CONTENTS

[redacted]

[redacted]

## NOTICE

[redacted] submits this report with the understanding that no portion of it will be used for advertising or promotional use without the express permission and written consent of [redacted] regarding the proposed use. When such permission is obtained, [redacted] will assist in the preparation of reports acceptable to the Sponsor.

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[redacted] submits this report with the understanding that the data contained herein may be used as an important component of the Sponsor's Product Safety assessment strategy. However, the data contained herein may not provide sufficient evidence by itself of the toxicity potential (or lack thereof) of a product or raw material. These data should be evaluated along with other sources of information about the test material including, but not limited to, physicochemical data and clinical safety studies. Accordingly, the final assessment of any test material's safety and/or hazard potential is the sole responsibility of the Sponsor.

[redacted]

**SUMMARY**

This MatTek EpiDerm Assay was conducted for [redacted] to determine the exposure period to the Sponsor's test materials which results in a fifty percent reduction in the uptake of MTT (tetrazolium dye) by the MatTek EpiDerm skin model. The MatTek EpiDerm skin model consists of normal human-derived epidermal keratinocytes, which have been cultured to form a multi-layered, highly differentiated model of the human epidermis. Keratinocytes are cultured on specially prepared permeable cell culture inserts that allow attainment of levels of differentiation on the cutting edge of *in vitro* skin technology. Ultrastructurally, the EpiDerm® skin model closely parallels human skin, thus providing a useful *in vitro* substrate to assess dermal toxicity.

The MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay is a colorimetric analysis of cellular metabolic activity. Reduction of MTT by mitochondria results in the formation of insoluble purple formazin crystals that are extracted from the cells with isopropanol and quantified spectrophotometrically. The intensity of the purple color is directly proportional to the metabolic activity of the cells and inversely proportional to the toxicity of the test material (1,2,3).

Prostaglandins are lipids synthesized in cells from polyunsaturated fatty acids. They belong to various groups (E, F, A, B, D, etc.) on the basis of structure. Prostaglandins, in particular $PGE_2$, are involved in the inflammatory response. *In vivo*, the concentration of $PGE_2$ in inflamed tissues correlates with inflammation related effects, such as increased vascular permeability, dermal ischemia, and granulocyte infiltration (4-8).

IL-1$\alpha$ is a cytokine that is produced by keratinocytes in response to injury. It is identified as a mediator of skin inflammation in response to skin irritation (4-8).

LDH is a glycolytic enzyme commonly found in the cytosolic compartment of all human cells. Normally the enzyme remains within the cell and is only released when the permeability of the cell membrane is altered (i.e. due to membrane damage). The amount of LDH released is proportional to the change in permeability of the cell membrane. Thus assaying cell culture medium for LDH content after exposing the tissue to test material provides a sensitive index for how much cell damage the test material can induce.

Prior to the start of the assay, compatibility of the test materials with MTT was determined. The tissues were dosed at intervals with 100 µl of each test material. The tissues were incubated for one, four, and 24 hours. After each exposure period to the test materials, the tissues were exposed to MTT for cytotoxicty evaluation.

The results of both the MTT assay and the IL-1$\alpha$ assay suggest that test material A-25 was a very mild irritant. None of the four test materials induced a $PGE_2$ response or a LDH response that was different from the negative control suggesting that exposure to these materials does not produce significant inflammation or cell membrane damage.

[redacted]

**PURPOSE**
This MatTek EpiDerm Assay was conducted for [redacted] to determine the exposure period to the Sponsor's test materials which results in a fifty percent reduction in the uptake of MTT (tetrazolium dye) by the MatTek EpiDerm skin model.

**GENERAL INFORMATION**

| | |
|---|---|
| Study Number: | [redacted] |
| Test: | MatTek EpiDerm Assay |
| Test Materials: | A-25 (cream) -8% [redacted] [redacted] |
| Investigator and Biostatistics: | [redacted] |
| Quality Assurance Coordinator: | |
| Administrative and Testing Facility: | |
| Source of Tissue: | MatTek Corporation<br>200 Homer Avenue<br>Ashland, Massachusetts 01721 |
| Sponsor: | [redacted] |
| Sponsor Representatives: | |
| Experiment Start Date: | June 12, 2001 |
| Experiment Termination Date: | June 30, 2001 |

[redacted]

**STORAGE, HANDLING, AND DOCUMENTATION OF TEST MATERIALS**

The receipt of test materials to [redacted] was documented in a logbook, which serves as a permanent record of the receipt, storage, return, and disposition of all study materials. All study materials were kept in a locked product storage room accessible to laboratory staff members only.

**TEST MATERIAL DESCRIPTIONS**

Test Material Identification Number (TMIN): 0056-01L
Sponsor Test Material Identification: A-25 8°/2
Physical Description: White, opaque cream
Concentration Tested: Neat

Test Material Identification Number (TMIN):
Sponsor Test Material Identification:
Physical Description:
Concentration Tested:

Test Material Identification Number (TMIN):
Sponsor Test Material Identification:
Physical Description:
Concentration Tested:

Test Material Identification Number (TMIN):
Sponsor Test Material Identification:
Physical Description:
Concentration Tested:

[redacted]

**PROCEDURES AND METHODS**

**MTT Compatibility Test**

Materials are considered MTT compatible if they do not spontaneously convert MTT to formazin crystals. This was tested by mixing an aliquot of each test material with an equal volume of 1 mg/ml MTT solution in a glass test tube. The test tubes were capped and incubated in the dark at room temperature for approximately three hours. At the end of incubation, the test tubes were read visually for evidence of a color change. Evidence of a color change to purple indicates that the test material may spontaneously reduce MTT, resulting in a false reaction. All four test materials were found to be MTT incompatible. This situation was accounted for by dosing incompatible test materials onto blank meshes, rinsing in the same manner as the tissues, and exposing to MTT for the longest time point. Any MTT conversion on a blank mesh after it has been rinsed was attributed to the conversion of MTT by the test material itself or the mesh. This value, if higher than undosed meshes, was subtracted from the MTT optical density values of tissues treated with the test material in question.

**MTT (Cytotoxicity) Assay**

Prior to dosing with the test materials, the tissue inserts were placed into individual wells of 6-well plate containing 0.9 ml of pre-warmed assay medium per well. The plates were incubated for approximately one hour at 37±2°C and 5±1% $CO_2$ for tissue pre-equilibration. After pre-equilibration, the assay medium was aspirated and replaced with 0.9 ml of fresh, pre-warmed assay medium.

After pre-equilibration, tissues were dosed with 100 µl of each test material. Test materials were tested neat. One percent (1%) Triton X-100 served as the positive control, and de-ionized water served as the negative control. Tissues were incubated for one, four, and 24 hours at 37±2°C and 5±1% $CO_2$. After each exposure period, the spent medium from the wells was collected for LDH, IL-1α and $PGE_2$ analysis, and the tissues were rinsed with Phosphate Buffered Saline (PBS). The excess PBS was removed by shaking and blotting. The tissues were transferred to individual wells of a 24-well plate containing 300 µl of 1mg/ml MTT solution per well. The plates were incubated for approximately three hours at 37±2°C and 5±1% $CO_2$

[redacted]

**PROCEDURES AND METHODS (Continued)**

**MTT (Cytotoxicity) Assay (Continued)**

After exposure to MTT, the tissues were gently rinsed with PBS to remove any residual MTT solution, and the excess PBS was removed by shaking and blotting. The tissues were transferred to individual wells of a 24-well plate containing 2 ml isopropanol per well. The plates were incubated on a rotating plate at 200 rpm at room temperature for at least two hours. After extraction, the extract from each well was vigorously pipetted against each tissue substrate to further extract the formazin precipitate. Two hundred microliters (200 µl) of extract from each well was transferred to individual wells of a 96-well plate, and the absorbance was determined spectrophotometrically at 570 nm against an isopropanol blank.

**Prostaglandin $E_2$ Enzyme Immunoassay**

The spent medium collected during the MTT assay was diluted 1:20 in EIA phosphate buffer. Buffers were prepared and the reagents were reconstituted according to manufacturer directions. Plates used in the assay were rinsed once with Wash Buffer, which was removed from each well by inverting the plate and shaking. One hundred (100) microliters of the EIA Buffer were added to Non-Specific Binding (NSB) wells, and 50 µl of the buffer were added to Maximum Binding ($B_0$) wells.

All the standard solutions were aliquoted into wells S1 through S8. Fifty (50) microliters of each sample were added to the wells, and 50 µl of $PGE_2$ Tracer was added to each well except the Total Activity (TA) and the Blank (B) wells. Fifty (50) microliters of $PGE_2$ antibody were added to each well except the TA, the NSB and the Blank wells. The plate was covered with the plastic film and incubated for approximately 18 hours at 4° ± 2° C. After incubation, the plate was rinsed five times with Wash Buffer. Two hundred (200) microliters of Ellman's Reagent were added to each well, and 5 µl of tracer were added to the TA wells. The plates were covered with plastic film and foil and allowed to develop at room temperature in the dark on a rotating plate for approximately two hours. After development, the plates were read spectrophotometrically at 410 nm.

**IL-1α Assay**

The spent media from all the treatment groups was collected for use in the IL-1α assay. All materials required for the assay were allowed to warm to room temperature prior to use. The standard vial form the kit was removed and reconstituted with 5 ml of assay medium to yield a stock solution of 250 pg/ml. The 250pg/ml IL-1α stock solution was serially diluted in 500 µl of assay medium to generate a series of standards.

Two hundred (200) microliters of each standard solution and sample supernatants were added to the wells of the microtiter plate coated with IL-1α antibody. The plate was tapped gently to mix the samples for 15 seconds, and covered and incubated for approximately two hours at room temperature (15-30°C). During the incubation time, wash buffer was prepared by diluting wash buffer concentrate in deionized water and mixing well. After incubation, each well was drained by aspiration and washed three times with wash buffer. After the final wash, remaining wash buffer was removed by inverting the plate and blotting it against a clean paper towel. Two hundred (200) microliters of the IL-1α conjugate was added to each well, and the plate was covered and incubated for approximately one hour at room temperature.

Prior to the end of the IL-1α conjugate incubation, color reagents A and B were mixed together in equal volumes to make the substrate solution. After incubation, each well was drained by aspiration and washed three times with wash buffer. Two hundred (200) microliters of the substrate solution was added to each well, and the plate was incubated for 20 minutes at room temperature. At the end of the incubation, 50 µl of stop solution were added to each well, and the plate was mixed by gently tapping to ensure mixing until the color changed from blue to yellow. The plate was read spectrophotometrically at a dual wavelength of 450 nm and 540 nm.

**Lactate Dehydrogenase Assay**

NADH was dissolved into pyruvate substrate (from kit) to achieve a concentration of 1.0 mg/ml NADH. One (1.0) ml of the 1.0 mg/ml NADH in pyruvate substrate solution will be transferred into a scintillation vial. The vial was then placed in a 37±2°C water bath for 5 minutes. A 0.10-ml aliquot of spent media was added to

[redacted]

the vial and the vial was then gently mixed and placed back in water for 30 minutes. After 30 minutes, the vials were removed from the water and 1.0 ml of color reagent added. The vials were mixed by swirling and incubated at room temperature for approximately 20 minutes. After 20 minutes, 10.0 ml of 0.40 N NaOH were added to each vial. The vials were capped and mixed by inversion. Two hundred (200) microliter aliquots were then transferred to a 96 well plate and read at 490 nm against the zero standard.

**CALCULATIONS AND DATA MANAGEMENT**

**MTT Assay**

The mean O.D. value and standard deviation for all MTT replicate samples were calculated. The percent of viability was calculated by using the following equation:

$$\frac{\text{Mean O.D. of Test Material}}{\text{Mean O.D. of Negative Control}} \times 100 = \% \text{ Viability}$$

The mean percent viability value for each test material was plotted on the y-axis versus the exposure times for each test material on the x-axis. The $ET_{50}$ value, the exposure time that reduces MTT dye incorporation by 50% when compared to negative controls, was calculated from a regression analysis of the dose-response curve. These values were used to compare the cytotoxicity/irritation potential of different test materials.

The following groupings were used in assigning expected *in vivo* irritancy responses based on the $ET_{50}$ results obtained using the EPI-200.

| $ET_{50}$ (hours) | Expected *in vivo* irritancy | Example |
|---|---|---|
| <1 | severe, probable corrosive | Concentrated Nitric Acid |
| 1-4 | moderate | 1% SDS |
| 4-12 | moderate to mild | 1% Triton X-100 |
| 12-24 | very mild | Baby Shampoo |
| >24 | non-irritating | 10% Tween 20 |

**Prostaglandin Assay**

The logarithm of each $PGE_2$ standard was calculated. Using regression analysis, these logarithms were plotted against their corresponding absorbance values to produce the standard curve. The logarithms of the concentrations of unknown $PGE_2$ in each spent medium sample were calculated from the standard curve. The antilogarithms of these values were taken to calculate the amount of $PGE_2$ in each spent medium sample. Mean corrected $PGE_2$ synthesis values were then calculated for each spent medium sample.

**IL-1 α Assay**

For the IL-1α assay, the IL-1 α standard concentrations were plotted against their corresponding absorbance values to produce a standard curve. Using regression analysis, the concentrations of unknown IL-1 α in each spent medium sample were calculated from the standard curve. The mean IL-1 α release for tissues treated with each sample was then calculated.

**LDH Assay**

For the LDH assay, the LDH standard concentrations were plotted against their corresponding absorbance values to produce a standard curve. Using regression analysis, the concentrations of unknown LDH in each spent medium sample were calculated from the standard curve. The mean LDH release for tissues treated with each sample was then calculated.

**MAINTENANCE OF RECORDS**

All original records (including the study protocol, data sheets, and any other records or forms used in this study) and a copy of the final report will be retained on file in the [redacted] archives for two years from the date of study completion. When the archive time has expired, the study files will either be sent to the Sponsor or destroyed. Any of these options may be carried out upon the Sponsor's request and notification.

[redacted]

**RESULTS**

The level of cytotoxicity was determined in tissues after one, four, and 24 hours of exposure to the test materials using MTT as a marker of cell viability. Table 1 presents the mean percent viability and the exposure time which reduces MTT dye incorporation by 50% when compared to the negative control ($ET_{50}$) for each test material. All test materials were tested neat. Please note that the scores for each test material and positive control are based on mean negative control values. The scores for the negative control are considered zero, and the control is assumed to be non-irritating.

**TABLE 1**
**RESULTS OF THE EPIDERM (MTT) ASSAY**

| Test Material | Exposure Time (Hours) | Mean Percent Viability | $ET_{50}$ (Hours) | *In vitro* Irritancy Classification |
|---|---|---|---|---|
| A-25 | 1 | 100.62 | 21.7 | Very Mild Irritant |
| | 4 | 93.96 | | |
| | 24 | 47.39 | | |
| [redacted] | | | | |
| 1.0% Triton X-100 (Positive Control) | 1 | 108.22 | 8.8 | Mild Irritant |
| | 4 | 81.46 | | |
| | 24 | 10.12 | | |

The results for the $PGE_2$ assay, Il-1α assay and the LDH assay are presented in tables 2,3 and 4, respectively.

[redacted]

**TABLE 2**
**RESULTS OF THE $PGE_2$ ASSAY**

| Test Material | Exposure Time (Hours) | $PGE_2$ (pg/mL) |
|---|---|---|
| A-25 | 1 | 1.22 |
| | 4 | 1.51 |
| | 24 | 35.01 |
| [redacted] | | |
| 1.0% Triton X-100 (Positive Control) | 1 | 4.12 |
| | 4 | 19.53 |
| | 24 | 393.65 |
| Deionized Water (Negative Control) | 1 | 3.19 |
| | 4 | 8.35 |
| | 24 | 36.57 |

**TABLE 3**
**RESULTS OF THE IL-1α ASSAY**

| Test Material | Exposure Time (Hours) | IL-1α (pg/mL) |
|---|---|---|
| A-25 | 1 | 8.65 |
| | 4 | 18.81 |
| | 24 | 86.86 |
| [redacted] | | |
| 1.0% Triton X-100 (Positive Control) | 1 | 7.94 |
| | 4 | 44.55 |
| | 24 | 270.76 |
| Deionized Water (Negative Control) | 1 | 0.52 |
| | 4 | 5.96 |
| | 24 | 17.82 |

[redacted]

**TABLE 4**
**RESULTS OF THE LDH ASSAY**

| Test Material | Exposure Time (Hours) | LDH (B-B Units/mL*) |
|---|---|---|
| A-25 | 1 | 0 |
| | 4 | 107.26 |
| | 24 | 57.62 |
| [redacted] | | |
| 1.0% Triton X-100 (Positive Control) | 1 | 193.00 |
| | 4 | 368.99 |
| | 24 | 1677.62 |
| Deionized Water (Negative Control) | 1 | 152.38 |
| | 4 | 224.58 |
| | 24 | 356.42 |

*One B-B Unit is defined as the amount of LDH needed to reduce 4.8 x $10^{-4}$ µmol of pyruvate per minute.

Please see Appendix I for complete biostatistics and Appendix II for graphs.

**DISCUSSION AND CONCLUSIONS**

Based on the results of the MTT assay only test material A-25 was classified as a very mild irritant, with an $ET_{50}$ value of 21.7 hours. The balance of the test materials [redacted] were classified as non-irritants and did not induced any change in tissue viability over the 24-hour exposure period.

The results of the MTT assay closely parallel the results of the IL-1α assay. Test material A-25 induced a slight increase in IL-1α release at 1 and 4 hours, and a more dramatic increase at 24 hours (when compared to the negative control). Again, this is an indicator of the irritant potential of this test material, although the amount of IL-1α release induced by A-25 was substantially less than that of the positive control. The balance of the test materials [redacted] did not produce an IL-1α response that was different from the negative control.

None of the four test materials evoked a $PGE_2$ response or a LDH response that was different from the negative control. Even test material A-25 failed to evoke a significant change in these indicators of cellular inflammation and membrane damage (respectively) suggesting that's it effect is limited to a potential mild irritant.

[redacted]

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[redacted]

## STATEMENT OF QUALITY ASSURANCE

All data and supporting documentation for this study have been audited by [redacted] Quality Assurance Department and found to be accurate, complete, and in compliance with the requirements of the protocol and [redacted] Standard Operating Procedures. This report has been reviewed and accurately reflects all aspects of the conduct of the study.

All laboratory research studies that are performed by [redacted] are in accordance with federal regulations and Good Laboratory Practice guidelines.

[redacted]

[redacted]

**VOLUME I**

| | | |
|---|---|---|
| **Study Title** | : | Acute Oral Toxicity - Up and Down Procedure (UDP) |
| **Test Article** | : | A25-N-acetyl proline, Lot/batch #2129150601 |
| **Author** | : | [redacted] |
| **Study Completed On** | : | July 31, 2006 |
| **Performing Laboratory** | : | [redacted] |
| [redacted] **Project #** | : | |
| **Protocol #** | : | 1010-01 |
| **Sponsor** | : | [redacted] |
| **Citation** | : | |

[redacted]

## GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

This study was conducted in accordance with the Good Laboratory Practices Regulations of the EPA 40 CFR 160 and 792, FDA 21 CFR 58, and as specified in The Testing of Chemicals, published by the Organization for Economic Cooperation & Development (OECD), 1997.

STUDY DIRECTOR [redacted]

[redacted]

[redacted]

**PROJECT NUMBER :** [redacted]
**TEST ARTICLE :** A25-N-acetyl proline, Lot/batch #2129150601
**SPONSOR :** [redacted]
**TITLE :** Acute Oral Toxicity - Up and Down Procedure (UDP)
**PROTOCOL # :** [redacted]

## ABSTRACT

**Objective:** To determine the potential for toxicity of the test article when administered orally. This study is designed to comply with the standards set forth in EPA Health Effects Test Guidelines, OPPTS 870.1100 December 2002, and in OECD Guidelines for the Testing of Chemicals, Guideline 425 adopted December 17, 2001.

**Method Synopsis:** Initially, a single female Wistar rat was dosed orally with A25-N-acetyl proline, Lot/batch #2129150601 at a dose level of 2000 mg/kg. Since the animal survived, four additional females were dosed at 2000 mg/kg. The rats were observed 1/2, 1, 2 and 4 hours postdose and once daily for 14 days for toxicity and pharmacological effects. All animals were observed twice daily for mortality. Body weights were recorded immediately pretest, weekly and at termination. All animals were examined for gross pathology. The potential for toxicity was based on the mortality response noted.

**Summary:**

All animals survived the 2000 mg/kg oral dose.

There were no abnormal physical signs noted during the observation period.

Body weight changes were normal.

Necropsy results were normal.

**Conclusion:** The $LD_{50}$ of A25-N-acetyl proline, Lot/batch #2129150601 is greater than 2000 mg/kg.

[redacted]

[redacted]

## OBJECTIVE

To determine the potential for toxicity of the test article when administered orally. This study is designed to comply with the standards set forth in EPA Health Effects Test Guidelines, OPPTS 870.1100 December 2002, and in OECD Guidelines for the Testing of Chemicals, Guideline 425 adopted December 17, 2001.

## TEST ARTICLE

| | | |
|---|---|---|
| Identity | : | A25-N-acetyl proline, Lot/batch #2129150601 |
| Test Article Characterization | : | Strength: 100%<br>Purity: 99.9%<br>Composition: N-acetyl proline (solid)<br>Stability: 2 years as solid<br>Uniformity: Uniform |
| Stability | : | The test article is stable for 2 years as solid. |
| Supplied by | : | [redacted] |
| Date Received | : | 04/05/06 |
| Storage | : | Room temperature and humidity |
| Description | : | White powder |
| Specific Gravity | : | Not Applicable |
| Sample Preparation | : | 2.5 g of test article was mixed with distilled water to a total volume of 5 ml and dosed from a stir plate. |

## TEST DATES

| | | | |
|---|---|---|---|
| Study Initiation | (date protocol signed) | : | 05/08/06 |
| Experimental Start Date | (1st exposure to test substance) | : | 05/12/06 |
| Experimental Term Date | (last date data collected) | : | 05/31/06 |
| Draft Report Sent | (if applicable) | : | 06/19/06 |
| Final Report Signed | (study completion) | : | 07/31/06 |

[redacted]

[redacted]

## EXPERIMENTAL DESIGN

**Test Animals**

Animals were received from Ace Animals, Boyertown, PA on 05/02/06, 05/04/06 & 05/09/06. Following an equilibration period of at least five days, five healthy, non-pregnant and nulliparous female Wistar albino rats were selected for dosing without conscious bias.

The animals were born the weeks of 03/07/06 & 03/14/06. The pretest body weight range was 181 - 197 grams. The weight variation of each animal used did not exceed ± 20% of the mean initial weight of all previously dosed animals.

The animals were identified by cage notation and indelible body marks, and housed in suspended wire mesh cages; 1/cage. Bedding was placed beneath the cages and changed at least three times/week. Fresh PMI Rat Chow (Diet #5012) was freely available except for 16-20 hours prior to dosing. Water was freely available at all times. The animal room, reserved exclusively for rats on acute tests, was temperature controlled, had a 12 hour light/dark cycle, and was kept clean and vermin free.

**Dosing**

The test article was mixed with distilled water to make dosing by gavage possible. The dose was based on the dry weight of the test article. Initially, a single female Wistar rat was dosed orally by syringe and dosing needle at a dose level of 2000 mg/kg. Since the animal survived, four additional females were dosed at 2000 mg/kg.

**Type and Frequency of Observations**

In Vivo - Animals were observed 1/2, 1, 2 and 4 hours postdose and once daily for 14 days for toxicity and pharmacological effects. All animals were observed twice daily for mortality. Body weights were recorded immediately pretest, weekly and at termination.

Post Mortem – All animals were humanely sacrificed using $CO_2$ following study termination and examined for gross pathology.

**Analysis of Data**

An estimate of the $LD_{50}$ was made based on the mortality occurring during the study.

**Retention of Data**

Upon signing the final report, all raw data, supporting documentation and reports are submitted to the Archivist by the Study Director. The raw data is filed at [redacted] by project number. The final report is filed at [redacted] by sponsor name and [redacted] number.

The test article will be returned to the sponsor following submission of the report.

**Amendment to the Protocol**

There were no amendments to the protocol.

[redacted]

[redacted]

## RESULTS & DISCUSSION

1. **Mortality**
   All animals survived the 2000 mg/kg oral dose.

2. **Body Weights (Table 1)**
   Body weight changes were normal.

3. **Systemic Observations (Table 2)**
   There were no abnormal physical signs noted during the observation period.

4. **Necropsy Findings (Table 3)**
   Necropsy results were normal.

[redacted]

[redacted]

## CONCLUSION

The $LD_{50}$ of A25-N-acetyl proline, Lot/batch #2129150601 is greater than 2000 mg/kg.

## FINAL REPORT

Approved by: [redacted]

[redacted]

[redacted]

**Table 1: Dose Volume and Body Weights in grams**

**Dose: 2000 mg/kg**

| An. # | Sex | Dose Volume in cc | Body Weights in grams Day 0 | Day 7 | Day 14 |
|---|---|---|---|---|---|
| 1 | F | 0.72 | 181 | 222 | 254 |
| 2 | F | 0.79 | 197 | 247 | 259 |
| 3 | F | 0.76 | 190 | 234 | 255 |
| 4 | F | 0.77 | 193 | 233 | 249 |
| 5 | F | 0.77 | 192 | 237 | 262 |

[redacted]

[redacted]

**Table 2: Systemic Observations**

Test animals were observed for mortality, toxicity and pharmacological effects at time intervals determined by the protocol. The animal, while being gently held, was examined for any abnormal signs and observations were recorded at this time. Also, the animal was observed as it moved around within the cage. The general health of the animal was recorded using the following code, which details the most commonly observed signs of poor health. Additional signs, when observed, are footnoted and described.

| | |
|---|---|
| A = NORMAL | |
| B = LETHARGY | Spontaneous movement is less than or slower than normal. For rabbits, lack of response to poking or failure to hold head alert may be construed as lethargy. |
| C = FLACCID MUSCLE TONE | When picked up, animal hangs loosely. |
| D = DIARRHEA | Brownish stain anogenitally or loose stool in waste pan. |
| E = ATAXIA | Lack of coordination, wobbles, may fall, trouble walking |
| F = PILOERECTION | Hair Stands up |
| G = PROSTRATE | Lying down, stretched out, will respond sluggishly to stimuli |
| H = COMA | No spontaneous movement or response to stimuli; breathing & pulse present |
| I = CONVULSION | Violent involuntary muscle contraction of whole body |
| J = CHROMODACRYORRHEA | Red discharge from eyes. |
| K = NEGATIVE RIGHTING REFLEX | Cannot turn right-side up |
| M = DYSPNEA | Difficult, labored breathing |
| N = TACHYPNEA | Rapid short breaths |
| O = TREMORS | Involuntary trembling or quivering |
| P = SPASM | Localized involuntary muscle contraction |
| Q = SAGGING EYELIDS | One or both eyelids partially closed |
| R = AGA WET | Anogenital area wet |
| S = CHROMORHINORRHEA | Colored discharge from nose, use for red only |
| T = AGA SOILED | Anogenital area soiled |
| V = BLOATED ABDOMEN | |
| W = APPEARS EMACIATED | Apparent thinness. |
| X = FEW FECES | Less than normal fecal output |
| Y = YELLOW NASAL DISCHARGE | Yellow fluid in nasal area |
| Z = DEAD | |

[redacted]

[redacted]

**Table 2 (cont'd): Systemic Observations**

| DOSE | 2000 mg/kg | | | | |
|---|---|---|---|---|---|
| Animal # /Sex | 1/F | 2/F | 3/F | 4/F | 5/F |
| Time Periods | | | | | |
| Hour ½ | A | A | A | A | A |
| Hour 1 | A | A | A | A | A |
| Hour 2 | A | A | A | A | A |
| Hour 4 | A | A | A | A | A |
| Day 1 | A | A | A | A | A |
| Day 2 | A | A | A | A | A |
| Day 3 | A | A | A | A | A |
| Day 4 | A | A | A | A | A |
| Day 5 | A | A | A | A | A |
| Day 6 | A | A | A | A | A |
| Day 7 | A | A | A | A | A |
| Day 8 | A | A | A | A | A |
| Day 9 | A | A | A | A | A |
| Day 10 | A | A | A | A | A |
| Day 11 | A | A | A | A | A |
| Day 12 | A | A | A | A | A |
| Day 13 | A | A | A | A | A |
| Day 14 | A | A | A | A | A |

A = Normal

[redacted]

[redacted]

**Table 3: Necropsy Observations**

| DOSE | 2000 mg/kg | | | | |
|---|---|---|---|---|---|
| Animal number/Sex | 1/F | 2/F | 3/F | 4/F | 5/F |
| Observations | S | S | S | S | S |
| Normal | X | X | X | X | X |

**CODES:** S = sacrifice
X = observed

Note: The external surfaces and orifices were examined. The skin was slit along the central midline from pelvis to chin. The internal organs were examined in situ including the thoracic, abdominal and pelvic cavities and their viscera and the cervical tissues and organs. All findings were recorded.

[redacted]

[redacted]

## QUALITY ASSURANCE EVALUATION

The Quality Assurance Unit has inspected an in-life phase of this study, audited the raw data and the report and determined that the methods and results contained herein accurately reflect the raw data. No changes/modifications from the approved protocol or Standard Operating Procedures were made without proper authorization and documentation. A summary of the compliance inspections is presented below.

| Date of Inspection | Phase | Performed By | Date Findings Reported to Mgmt. | Sty. Dir. |
|---|---|---|---|---|
| 05/12/06 | Dosing administration | [redacted] | 07/31/06 | 07/31/06 |
| 06/05/06 | Raw data audit | | 07/31/06 | 07/31/06 |
| 06/15/06 | Draft report audit | | 07/31/06 | 07/31/06 |
| 07/28/06 | Final report audit | | 07/31/06 | 07/31/06 |

[redacted]

[redacted]

[redacted]

# EVALUATION OF TWO CREAMS ON THE TREATMENT OF ECZEMA OR ACTIVE ATOPIC DERMATITIS

10% n-acetyl proline

**Prepared for:**

[redacted]

[redacted]

[redacted]

[redacted]

**TABLE OF CONTENTS**

[redacted]

**PURPOSE**

This double-blind, randomized controlled usage study was conducted for [redacted] to evaluate the effectiveness of two creams on the treatment of eczema or active atopic dermatitis.

**GENERAL INFORMATION**

| | |
|---|---|
| Study Number: | [redacted] |
| Test: | Evaluation of Two Creams on the Treatment of Eczema or Active Atopic Dermatitis |
| Test Materials: | A: Formula A25 10% n-acetyl proline |
| Investigator: | [redacted] |
| Co-Investigator: | |
| Study Physician: | |
| Site Manager: | |
| Biostatistics Manager: | |
| Quality Assurance Manager: | |
| Testing Facility: | |
| Administrative Facility: | |
| Sponsor: | |
| Sponsor Representative: | |
| Experiment Start Date: | March 12, 2002 |
| Experiment End Date: | April 5, 2002 |

[redacted]

**SUMMARY**

This double-blind, randomized controlled usage study was conducted fo[redacted]o evaluate the effectiveness of two creams on the treatment of eczema or active atopic dermatitis. A total of twenty-seven subjects with eczema and/or active atopic dermatitis completed this study using the following test materials:

- 15 subjects – Product A: Formula A25
- 12 subjects – [redacted]

Subjects qualified for study participation by having a selected target lesion with at least moderate overall lesion severity. During the course of the study, subjects applied the test material to the assigned target lesion and surrounding area twice per day for 14 days (*Product Application Phase* –Day 0 (Baseline), Day 4 (Visit 2), Day 7 (Visit 3), Day 14 (Visit 4). After Day 14, subjects discontinued use of the test material for 4 days (*Regression Phase* –Day 18(Visit 5).

The following clinical grading of the target lesion was performed on Day 0 (Baseline), Day 4, Day 7, Day 14, and Day 18:

- Erythema
- Scaling
- Edema
- Itching
- Lesion Soreness/Discomfort
- Overall Lesion Severity:
- Tactile Texture:

Subjects completed a self-assessment questionnaire regarding skin condition and product benefit at Day 0, Day 4, Day 7, Day 14 and Day 18.

Both test products (Product A: Formula A25 and [redacted] performed well in alleviating the objective and subjective symptoms of target lesions. However, [redacted] had a slight advantage in effectiveness as measured by tactile texture during the treatment phase, and overall lesion severity during the regression phase. Nevertheless, caution is advised in the interpretation of the final results, since the range of lesion severity for both groups was large, and, consequently, high variability is anticipated for lesion responsiveness. This variability is expected to decrease the sensitivity of the test to distinguish treatment differences.

[redacted]

**STORAGE, HANDLING, AND DOCUMENTATION OF TEST MATERIALS**

The receipt of test materials by [redacted] was documented in a log book, which serves as a permanent record of the receipt, storage, return, and disposition of all study materials. All study materials were kept in a locked product storage room accessible to clinical staff members only. At the conclusion of the study, the remaining test materials were disposed of according to all applicable regulations.

**TEST MATERIAL DESCRIPTIONS**

| | |
|---|---|
| Test material identification number (TMIN): | 0065-02CC |
| Sponsor test material identification: | A: Formula A25 |
| Physical description: | White, opaque cream |
| Test material identification number (TMIN): | [redacted] |
| Sponsor test material identification: | |
| Physical description: | |

**INFORMED CONSENT**

Written informed consent conforming to 21 CFR 50.25 was obtained from each subject prior to enrollment in the study. An original signed copy for each subject participating in the study will be retained in the study file. Each subject received a copy of the agreement. Please see Appendix V for a sample of the IRB approved informed consent form.

**INSTITUTIONAL REVIEW BOARD**

The protocol and informed consent agreement for this study were reviewed and approved by IntegReview Institutional Review Board (IRB) on February 19, 2002. Please see Appendix IV for a copy of the IRB approval letter.

**RECORD OF SPONSOR MONITORING VISITS**

There were no monitoring visits conducted by Sponsor representatives during the course of the study.

**ATTRITION**

Twenty-seven subjects completed this study. Thirty-eight subjects enrolled in the study and eleven subjects discontinued study participation due to the following reasons:

- Voluntarily discontinued participation: 001, 006, 018, 032, 036
- Disqualified at grading: 017, 023, 026
- Adverse event: 008, 037
- Investigator discretion (medication usage): 014

Please see Appendix VI for a copy of the Attrition Form, which documents the dates of and reasons for attrition of the eleven subjects.

[redacted]

**ADVERSE EVENTS**

During the course of the study, two subjects reported adverse events. Brief descriptions of the initial adverse event, steps taken to resolve the adverse event, and the relation of the adverse event to the test material are presented below. Please see Appendix VII for complete adverse event forms.

Subject Number: 008
Test Material: C [redacted]
Brief Description: The subject reported a spreading rash on 03/27/02 (after eight days of product usage). The Study Investigator examined the subject in the clinic and observed moderately severe inflammation, with edema and multiple vesicles (occasionally weeping) on the legs, arms, and torso (including areas where test material was not applied).
Resolution: A Board Certified Dermatologist examined the subject and made the following observations: erythematous, edematous plaques on extremities and trunk; intense erythema on lower extremities; pattern highly suggestive of contact dermatitis to something other than the study test material. The subject was discontinued from study participation and prescribed Triamcinolone Cream Gel to apply to the affected areas. Numerous attempts were made by the clinic to contact the subject to determine adverse event resolution. On 05/02/02 the subject revisited the clinic to return the test products and confirmed that the rash had cleared approximately three days following the visit to the Dermatologist.

Relation to Test Material: Not Related
Date Test Material Use Began: 03/19/02
Date of Reaction Onset: 03/25/02
Date of Reaction Cessation: 03/30/02

Subject Number: 037
Test Material: A Formula A25
Brief Description: On 03/22/02, the subject was examined in the clinic and observed to have moderately severe contact dermatitis with erythema, edema, and some weeping.
Resolution: On 03/25/02, a Board Certified Dermatologist examined the subject and made the following observations: erythema and weeping, scaly plaques on neck, chest, and back; consistent with acute chronic dermatitis reaction. The subject was discontinued from study participation and prescribed Clobetasol Cream to apply to the affected areas. On 03/27/02, the subject reported that she has only mild redness remaining and rash has mostly subsided. All symptoms resolved by 04/16/02.
Relation to Test Material: Definitely Related
Date Test Material Use Began: 03/19/02
Date of Reaction Onset: 03/21/02
Date of Reaction Cessation: 04/16/02

[redacted]

**SUBJECT DEMOGRAPHICS**

Twenty-seven subjects completed this study. Table 1 presents each subject's ethnicity, gender, date of birth, and product assignment (randomization). Ethnicity information was obtained from each subject's health and eligibility questionnaire.

**TABLE 1**
**SUBJECT DEMOGRAPHICS**

| Subject Number | Ethnicity | Gender | Date of Birth | Product Assignment |
|---|---|---|---|---|
| 002 | Caucasian | Female | 06/25/51 | [redacted] |
| 003 | Caucasian | Male | 10/16/55 | A: Formula A25 |
| 004 | Hispanic | Male | 10/18/55 | [redacted] |
| 005 | Native American | Male | 01/06/63 | A: Formula A25 |
| 007 | Caucasian | Female | 09/02/43 | A: Formula A25 |
| 009 | Caucasian | Male | 09/14/36 | A: Formula A25 |
| 010 | Caucasian | Female | 03/02/50 | [redacted] |
| 011 | Caucasian | Female | 01/15/65 | A: Formula A25 |
| 012 | Caucasian | Female | 02/23/78 | [redacted] |
| 013 | Caucasian | Female | 05/15/66 | A: Formula A25 |
| 015 | Caucasian | Male | 11/06/64 | A: Formula A25 |
| 016 | Caucasian | Male | 04/28/59 | [redacted] |
| 019 | Caucasian | Male | 04/22/77 | A: Formula A25 |
| 020 | Caucasian | Female | 04/16/67 | [redacted] |
| 021 | Caucasian | Female | 08/26/48 | A: Formula A25 |
| 022 | Caucasian | Female | 06/05/67 | [redacted] |
| 024 | Caucasian | Female | 09/17/57 | [redacted] |
| 025 | Caucasian | Male | 05/18/79 | A: Formula A25 |
| 027 | Caucasian | Female | 12/20/61 | A: Formula A25 |
| 028 | Caucasian/African American | Female | 11/01/85 | [redacted] |
| 029 | Caucasian | Female | 01/29/77 | A: Formula A25 |
| 030 | Caucasian/American Indian | Female | 12/18/54 | [redacted] |
| 031 | Caucasian | Male | 09/11/61 | A: Formula A25 |
| 033 | Caucasian | Female | 04/19/57 | A: Formula A25 |
| 034 | Caucasian | Male | 05/23/63 | [redacted] |
| 035 | Caucasian | Female | 12/08/70 | A: Formula A25 |
| 038 | Caucasian | Male | 11/13/49 | [redacted] |

[redacted]

**PROTOCOL AMENDMENTS**

| | |
|---|---|
| Affected Sections of Protocol: | **TITLE, OBJECTIVE, TEST PRODUCTS** |
| Original Wording: | Four Creams (A, B, C, D) |
| Revised Wording: | Two Creams (A and C) |
| Reason for Change: | Creams B and D were removed from testing in this study due to Sponsor request for enrollment numbers. |
| Affected Section of Protocol: | **TEST PRODUCTS** |
| Added Wording: | A safety summary letter detailing the expected safety of the products and their ingredients will be provided to the Institutional Review Board (IRB). This safety summary letter will be attached to the protocol as Appendix I. If any changes are planned regarding the products, the IRB will immediately be notified prior to the change, so that appropriate review/approval may be obtained prior to use. |
| Reason for Change: | Institutional Review Board request |

**PROTOCOL DEVIATIONS**

| | |
|---|---|
| Affected Section of Protocol: | **STUDY DESIGN** |
| Protocol Specification: | A total of 60 subjects (15 per product) will be expected to complete the study. |
| Deviation: | Twenty-seven subjects completed the study. Fifteen subjects completed using product A: Formula A25 and twelve subjects completed using product [redacted] |
| Impact of Deviation: | The impact of this deviation on the outcome of the study is unknown. |
| Affected Section of Protocol: | **PROCEDURES**, *Product Application Phase* |
| Protocol Specification: | Assessments will be made at baseline (Day 0), and Days 4, 7, and 14. Self-assessment questionnaires will also be administered at these visits. |
| Deviation: | Subjects 005 and 035 did not attend the Day 4 visit. |
| Impact of Deviation: | The grading data and self-assessment questionnaire data for these subjects are unavailable for statistical analysis. |
| Affected Section of Protocol: | **PROCEDURES**, *Product Application Phase* |
| Protocol Specification: | Subjects will be instructed to generously apply their product twice daily (once in the morning and once in the evening approximately 12 hours apart) to the assigned test site. |
| Deviation: | The following subjects either missed application(s) on the listed dates or performed evening application before the specified time point: |

<u>Missed Applications:</u>

- 003: 03/26/02 PM, 03/30/02 PM, 03/31/02 PM
- 009: 03/23/02 PM
- 013: 03/25/02 AM
- 019: 03/22/02 AM, 03/25/02 AM, 03/031/02 AM, 04/01/02 AM
- 035: 03/22/02 AM

<u>Early Product Applications:</u>

- 012: 03/24/02 (3 hours early)
- 035: 03/19/02 (4 hours early), 03/20/02 (5 hours early), 03/21/02 (3 hours early)

| | |
|---|---|
| Impact of Deviation: | This deviation is not expected to have an impact on the outcome of the study. |

[redacted]

**PROCEDURES AND METHODS**

At the Screening Visit, potential subjects were screened by [redacted] Board Certified Dermatologist) for the presence of eczema and/or active atopic dermatitis. Subjects who qualified according to the initial examination completed a health and eligibility questionnaire, and signed an informed consent agreement and confidentiality agreement. Subjects were instructed to discontinue use of any topical products to their bodies other than Cetaphil® cleanser provided by the clinic.

Qualified subjects returned to the clinic for Day 0 after participating in a two day washout period. An expert clinical grader examined subjects for the presence of at least one target lesion of eczema or active atopic dermatitis. A minimum score of 2 for overall lesion severity, according to the scale listed below, was required for continued study eligibility.

The target lesion was assessed for the following parameters using the indicated grading scales (nota that half-point increments were assigned):

- Erythema, Scaling, Edema, Itching, Lesion Soreness/Discomfort and Overall Lesion Severity:
  0 = None
  1 = Mild
  2 = Moderate
  3 = Severe
  4 = Very severe

- Tactile Texture:
  0 = Very smooth
  1 = Mostly smooth/slightly rough
  2 = Moderately rough
  3 = Totally rough and bumpy texture
  4 = Extremely rough

Subjects completed a baseline self-assessment questionnaire regarding skin condition.

Subjects were assigned to use Product A: Formula A25 or [redacted] according to a predetermined randomization (see Appendix VIII for a copy of the Randomization Form). Subjects were instructed to apply the test material to the assigned target lesion and surrounding area twice per day (each morning and evening approximately 12 hours apart), with at least one application performed after cleansing. Subjects were instructed to continue using the provided Cetaphil® cleanser. Subjects were also instructed to apply the test material to the target area at least 8 hours prior to study visits.

*Product Application Phase*

Subjects returned to the clinic on Day 4, Day 7, and Day 14 for assessments. At each of these visits, target lesions were graded for erythema, scaling, edema, itching, lesion soreness/discomfort, overall lesion severity, and tactile texture as described for baseline. Subjects also completed a self-assessment questionnaire at each visit.

*Regression Phase*

At the conclusion of the Day 14 visit, subjects discontinued use of the assigned material but continued using the provided Cetaphil® cleanser. Subjects returned to the clinic on Day 18 and target lesions were graded for erythema, scaling, edema, itching, lesion soreness/discomfort, overall lesion severity, and tactile texture as described for baseline.

[redacted]

**BIOSTATISTICS AND DATA MANAGEMENT**

*Product Application Phase*

Mean scores for clinical grading at Day 4, Day 7, and Day 14 were statistically compared to mean Day 0 scores using a paired t-test at the $p \leq 0.05$ significance level.

*Regression Phase*

Mean scores for clinical grading at Day 18 were statistically compared to mean scores at Day 0 and mean scores at Day 14 scores using a paired t-test at the $p \leq 0.05$ significance level.

Mean percent change from baseline and incidence of improvement were calculated for all attributes at all time points. Comparisons were made between the two test materials using ANOVA with pairwise comparisons (Fisher's LSD) to determine if significant differences exist. Please see Appendix I for complete statistical calculations.

Self-assessment questionnaires were tabulated and are presented in Appendix II.

**MAINTENANCE OF RECORDS**

All original records (including the study protocol, observation records, medical histories, informed consent agreements, attrition forms, and any other records or forms used in this study) and a copy of the final report will be retained on file in the [redacted] archives for two years from the date of completion of the final report. When the archive time has expired, the study files will either be sent to the Sponsor or destroyed, according to the Sponsor's request and notification.

[redacted]

**RESULTS**

At Day 0 (Baseline) and at Day 4, Day 7, Day 14, and Day 18, target lesions were graded for erythema, scaling, edema, itching, lesion soreness/discomfort, overall lesion severity, and tactile texture using the grading scales on page 9.

Table 2 presents the results of the target lesion clinical grading for the product application phase and regression phase. For the product application phase, mean scores at Day 4, Day 7, and Day 14 are compared to mean Day 0 (Baseline) scores. For the regression phase, mean scores at Day 18 are compared to mean scores at Day 0 and mean scores at Day 14.

Mean scores are presented for each grading time point and mean percent changes from baseline are listed in parentheses. Mean percent changes are calculated by averaging each subject's individual percent change from baseline. Please note that mean percent changes are calculated using the scale length (4) as the denominator. In order to avoid a denominator of 0, the scale length rather than the baseline score is used since many subjects exhibited a baseline score of 0 for the irritation parameters. Percent change for those subjects can not be calculated and so they would be excluded from the average percent change calculations. By using the scale length for the denominator, the average percent change will be zero only when the average change is zero.

Significant changes from Day 0/Day 14 are marked with an open arrow. A significant decrease represents an improvement.

**TABLE 2**
**MEAN SCORES FOR CLINICAL GRADING OF TARGET LESIONS**

| | **Product A: Formula A25** (n=15) *Product Application Phase* | | | |
|---|---|---|---|---|
| | Day 0 | Day 4 | Day 7 | Day 14 |
| Erythema | 2.33 | 1.64 ⇩ (-18.8%) | 1.33 ⇩ (-25.0%) | 0.97 ⇩ (-34.2%) |
| Scaling | 1.93 | 1.25 ⇩ (-16.1%) | 0.83 ⇩ (-27.5%) | 0.47 ⇩ (-36.7%) |
| Edema | 1.23 | 0.64 ⇩ (-15.2%) | 0.40 ⇩ (-20.8%) | 0.30 ⇩ (-23.3%) |
| Tactile Texture | 1.87 | 1.46 ⇩ (-8.9%) | 1.10 ⇩ (-19.2%) | 0.70 ⇩ (-29.2%) |
| Itching | 2.20 | 1.11 ⇩ (-26.8%) | 1.13 ⇩ (-26.7%) | 0.83 ⇩ (-34.2%) |
| Lesion Discomfort | 2.10 | 1.21 ⇩ (-21.4%) | 1.17 ⇩ (-23.3%) | 0.80 ⇩ (-32.5%) |
| Overall Lesion Severity | 2.37 | 1.68 ⇩ (-17.9%) | 1.30 ⇩ (-26.7%) | 0.97 ⇩ (-35.0%) |

⇩ Indicates a statistically significant (p≤0.05) decrease compared to Day 0

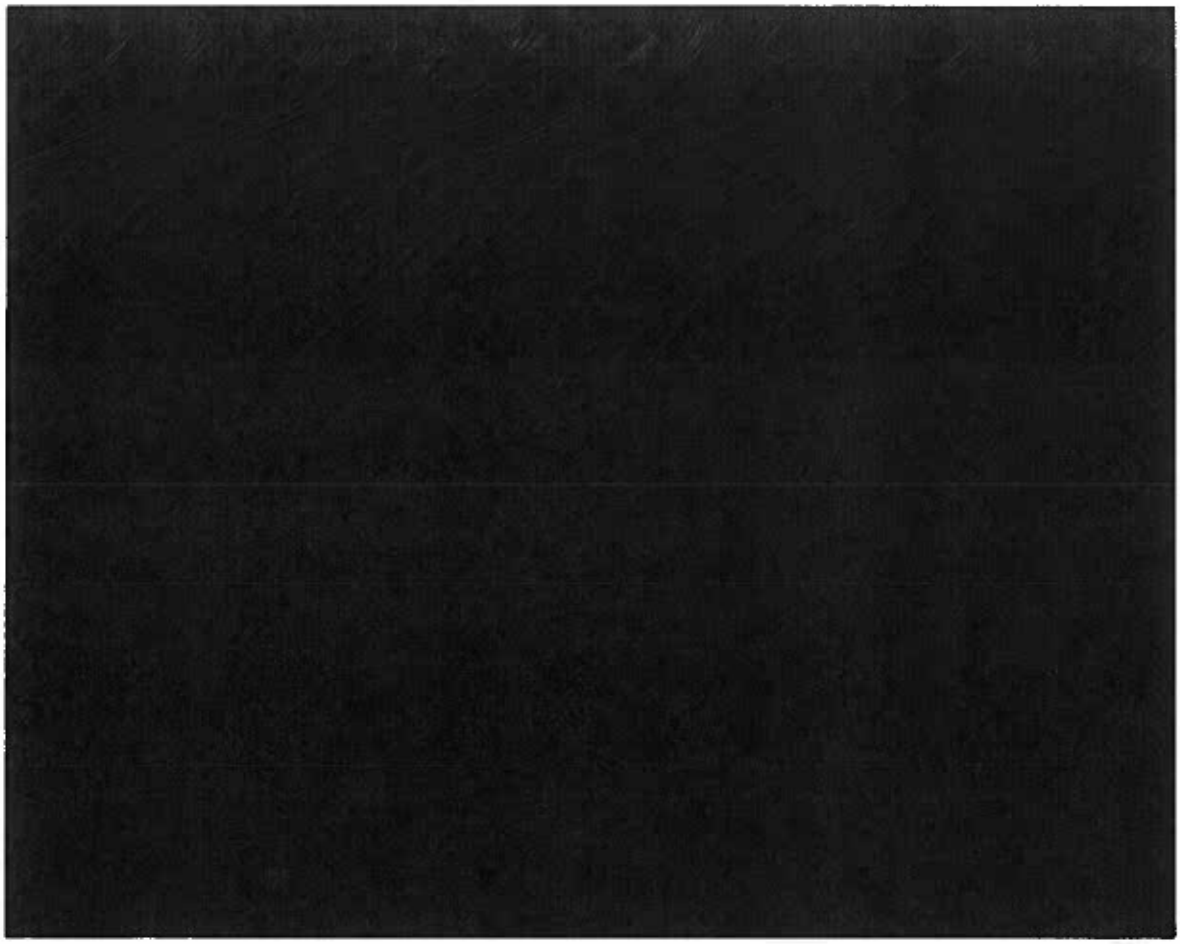
| | **Product A: Formula A25** (n=15) *Regression Phase* | | | | |
|---|---|---|---|---|---|
| | Day 0 | Day 14 | Day 18 | | |
| | | | Mean | Compared to Day 0 | Compared to Day 14 |
| Erythema | 2.33 | 0.97 | 1.33 | ⇩ (-25.0%) | (9.2%) |
| Scaling | 1.93 | 0.47 | 0.97 | ⇩ (-24.2%) | ⇧ (12.5%) |
| Edema | 1.23 | 0.30 | 0.50 | ⇩ (-18.3%) | (5.0%) |
| Tactile Texture | 1.87 | 0.70 | 1.10 | ⇩ (-19.2%) | ⇧ (10.0%) |
| Itching | 2.20 | 0.83 | 1.30 | ⇩ (-22.5%) | ⇧ (11.7%) |
| Lesion Discomfort | 2.10 | 0.80 | 0.93 | ⇩ (-29.2%) | (3.3%) |
| Overall Lesion Severity | 2.37 | 0.97 | 1.43 | ⇩ (-23.3%) | ⇧ (11.7%) |

⇩ Indicates a statistically significant (p≤0.05) decrease compared to Day 0
⇧ Indicates a statistically significant (p≤0.05) increase compared to Day 14

[redacted]

RESULTS (Continued)

TABLE 2 (Continued)
MEAN SCORES FOR CLINICAL GRADING OF TARGET LESIONS



**Results of Test Material Comparisons**
Comparisons, based on average change compared to baseline, were made among the test materials to determine statistically significant differences for the target lesion clinical grading parameters. The following statistically significant ($p \leq 0.05$) differences were found:

There was a greater significant decrease (improvement) for [redacted] for tactile texture at Day 4 and Day 7 compared to Day 0.

[redacted]

**DISCUSSION AND CONCLUSIONS**

This controlled usage study was conducted to evaluate the effectiveness of two creams on the treatment of eczema and active atopic dermatitis. A total of twenty-seven subjects with eczema and/or active atopic dermatitis completed this study using the following test materials:

- 15 subjects – Product A: Formula A25
- 12 subjects – [redacted]

Subjects applied the test material to a selected target lesion for fourteen days (assessments made at Day 0, Day 4, Day 7, and Day 14) and then subjects discontinued test material usage for a four-day regression phase (assessments made at Day 18)

*Product Application Phase*

Both test materials showed a statistically significant decrease (improvement) in all clinically graded parameters (erythema, scaling, edema, tactile texture, itching, lesion discomfort, and overall lesion severity) at Day 4, Day 7, and Day 14, when compared to mean Day 0 (Baseline) scores.

Comparisons between the two test materials for grading parameters showed that [redacted] had a significantly greater improvement in tactile texture at Day 4 and Day 7 compared to Product A: Formula A25.

*Regression Phase*

The mean scores for Day 18 (4 days of regression) were compared to the mean scores for Day 14 to determine the degree of target lesion worsening after treatment was discontinued. Both test materials showed a statistically significant worsening in target lesion scaling, tactile texture, and itching. However, test product A: Formula A25 also showed a statistically significant worsening in overall lesion severity.

*Overall Conclusions*

Both test products (Product A: Formula A25 and [redacted] performed well in alleviating the objective and subjective symptoms of target lesions. However, [redacted] had a slight advantage in effectiveness as measured by tactile texture during the treatment phase, and overall lesion severity during the regression phase. Nevertheless, caution is advised in the interpretation of the final results, since the range of lesion severity for both groups was large, and, consequently, high variability is anticipated for lesion responsiveness. This variability is expected to decrease the sensitivity of the test to distinguish treatment differences.

[redacted]

## STATEMENT OF QUALITY ASSURANCE

All data and supporting documentation for this study have been audited by the [redacted] Quality Assurance Department and found to be accurate, complete, and in compliance with the requirements of the protocol and [redacted] Standard Operating Procedures. This report has been reviewed and accurately reflects all aspects of the conduct of the study.

All clinical research studies that are performed by [redacted] are in accordance with federal regulations and Good Clinical Practice guidelines.

[redacted]

[redacted]

# FINAL REPORT

**CLIENT:**

[redacted]

**ATTENTION:**

**TEST:** Repeated Insult Patch Test
Protocol No.: 1.01

**TEST MATERIAL:** 10% A25 cream, F#61-67, L#64-103

**EXPERIMENT REFERENCE NUMBER:**

[redacted]



[redacted]

[redacted]

# QUALITY ASSURANCE UNIT STATEMENT

**Study No.:** [redacted]

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of clinical laboratory studies. These studies have been performed with adherence to ICH Guideline E6 for Good Clinical Practice and requirements provided for in 21 CFR parts 50 and 56 and in accordance to standard operating procedures and applicable protocols. The QAU maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. The findings of these inspections have been reported to management and the Study Director. All materials and data pertinent to this study will be stored in the Archive Facility at [redacted] unless specified otherwise, in writing by the Sponsor.

**Date(s) of inspection:**

| | |
|---|---|
| December 19, 2001 | January 30, 2002 |
| December 26, 2001 | February 11, 2002 |
| December 28, 2001 | February 12, 2002 |
| January 28, 2002 | |

**Senior personnel involved:**

[redacted]

- Quality Assurance Supervisor
- Quality Assurance Associate
- Quality Assurance Associate

[redacted]

The representative signature of the Quality Assurance Unit signifies that this study has been performed in accordance with standard operating procedures and study protocol as well as government regulations regarding such procedures and protocols.

[redacted]

**Objective:** To determine by repetitive epidermal contact the potential of a test material to induce primary or cumulative irritation and/or allergic contact sensitization.

**Participants:** One hundred thirteen (113) qualified subjects, male and female, ranging in age from 18 to 79 years, were selected for this evaluation. One hundred seven (107) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

**Inclusion Criteria:**

a. Male and female subjects, age 16[a] and over.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History form and the understanding and signing of an Informed Consent form.
e. Considered reliable and capable of following directions.

**Exclusion Criteria:**

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

**Test Material:** 10% A25 cream, F#61-67, L#64-103

**Study Schedule:**

| Panel # | Initiation Date | Proposed Completion Date | Actual Completion Date |
|---|---|---|---|
| 20010761 | December 19, 2001 | January 24, 2002 | January 24, 2002 |
| 20010774 | December 26, 2001 | January 31, 2002 | February 7, 2002 |

[a]With parental or guardian consent

**Methodology:**

The upper back between the scapulae served as the treatment area. Approximately 0.2 g of the test material, or an amount sufficient to cover the contact surface, was applied to the 1" x 1" absorbent pad portion of a clear adhesive dressing*. This was then applied to the appropriate treatment site to form a semi-occluded patch.

**Induction Phase:**

Patches were applied three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of patch application. Following supervised removal and scoring of the first Induction patch, participants were instructed to remove all subsequent Induction patches at home, twenty-four hours after application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period.

With the exception of the first supervised Induction Patch reading, if any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications are discontinued for the remainder of this test phase, if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of twenty-four hours following each Tuesday and Thursday removal, and forty-eight hours following each Saturday removal.

**Challenge Phase:**

Approximately two (2) weeks after the final Induction patch application, a Challenge patch was applied to a virgin test site adjacent to the original Induction patch site, following the same procedure described for Induction. The patch was removed and the site scored at the clinic twenty-four and seventy-two hours post-application.

*Manufactured by TruMed Technologies, Inc., Burnsville, MN

**Evaluation Key:**

0 = No visible skin reaction
+ = Barely perceptible or spotty erythema
1 = Mild erythema covering most of the test site
2 = Moderate erythema, possible presence of mild edema
3 = Marked erythema, possible edema
4 = Severe erythema, possible edema, vesiculation, bullae and/or ulceration

**Results:**

The results of each participant are appended (Table 1).

Observations remained within normal limits throughout the test interval.

It was noted that Subject #16, Panel 20010774, exhibited a barely perceptible (+) response twenty-four hours post-challenge application. However, this site was negative at the final observation. It is the Laboratory's opinion that this weak, transitory response can be considered clinically insignificant.

**Summary:**

Under the conditions of this study, test material, 10% A25 cream, F#61-67, L#64-103, did not indicate a clinically significant potential for dermal irritation or allergic contact sensitization.

Table 1
Panel #20010761

Individual Results

10% A25 cream, F#61-67, L#64-103

| Subject Number | 24*hr | Induction Phase 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Virgin Challenge Site 24*hr | 72 hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | + | +[D] | +[D] | 0[D] | 0[D] | +[D] | 0 | 0 |
| 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | DNC | | | |
| 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 0 | 0 | 0 | 0 | 0 | 0[m] | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 | 0 | 0[m] | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 | 0 | DID NOT COMPLETE STUDY | | | | | | | | | | |
| 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

24* = Supervised removal of 1st Induction and Challenge Patch
m = Additional makeup day granted at the discretion of the clinic supervisor
DNC = Did not complete study
D = Dryness

Table 1
(continued)
Panel #20010761

Individual Results

10% A25 cream, F#61-67, L#64-103

| Subject Number | 24*hr | Induction Phase 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Virgin Challenge Site 24*hr | 72 hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 34 | 0 | ----------DID NOT COMPLETE STUDY---------- | | | | | | | | | | |
| 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 41 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 47 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 49 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ---DNC-- | |
| 53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 56 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

24* = Supervised removal of 1st Induction and Challenge Patch
DNC = Did not complete study

Table 1
(continued)
Panel # 20010774

Individual Results

10% A25 cream, F#61-67, L#64-103

| Subject Number | 24*hr | Induction Phase 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Virgin Challenge Site 24*hr | 72 hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 0 | 0 | + | 0 | 1[P] | + | 0 | 0 | 0 | 0 | + | 0 |
| 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ---DNC--- | |
| 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

24* = Supervised removal of 1st Induction and Challenge Patch
DNC = Did not complete study
P = Papules

Table 1
(continued)
Panel # 20010774

Individual Results

10% A25 cream, F#61-67, L#64-103

| Subject Number | 24*hr | Induction Phase 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Virgin Challenge Site 24*hr | 72 hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 41 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 47 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 49 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50 | ----------DID NOT COMPLETE STUDY---------- | | | | | | | | | | | |
| 51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 56 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 57 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 |

24* = Supervised removal of 1st Induction and Challenge Patch

Table 2
Panel #20010761

Subject Data

| Subject Number | Initials | Age | Sex |
|---|---|---|---|
| 1 | [redacted] | 79 | F |
| 2 | | 77 | F |
| 3 | | 72 | F |
| 4 | | 78 | F |
| 5 | | 34 | F |
| 6 | | 48 | F |
| 7 | | 69 | M |
| 8 | | 47 | F |
| 9 | | 66 | F |
| 10 | | 72 | F |
| 11 | | 64 | F |
| 12 | | 65 | F |
| 13 | | 33 | F |
| 14 | | 48 | F |
| 15 | | 68 | F |
| 16 | | 53 | F |
| 17 | | 49 | M |
| 18 | | 18 | M |
| 19 | | 27 | F |
| 20 | | 66 | F |
| 21 | | 42 | F |
| 22 | | 56 | M |
| 23 | | 24 | F |
| 24 | | 24 | F |
| 25 | | 31 | F |
| 26 | | 32 | F |
| 27 | | 77 | F |
| 28 | | 42 | F |

Table 2
(continued)
Panel #20010761

Subject Data

| Subject Number | Initials | Age | Sex |
|---|---|---|---|
| 29 | [redacted] | 54 | F |
| 30 | | 27 | F |
| 31 | | 29 | F |
| 32 | | 30 | M |
| 33 | | 70 | M |
| 34 | | 36 | F |
| 35 | | 45 | M |
| 36 | | 41 | M |
| 37 | | 43 | F |
| 38 | | 26 | F |
| 39 | | 54 | M |
| 40 | | 71 | F |
| 41 | | 39 | M |
| 42 | | 41 | F |
| 43 | | 40 | F |
| 44 | | 38 | F |
| 45 | | 42 | F |
| 46 | | 39 | F |
| 47 | | 34 | M |
| 48 | | 71 | M |
| 49 | | 44 | M |
| 50 | | 44 | F |
| 51 | | 60 | F |
| 52 | | 23 | F |
| 53 | | 20 | M |
| 54 | | 46 | F |
| 55 | | 42 | M |
| 56 | | 40 | M |

Table 2
(continued)
Panel # 20010774

Subject Data

| Subject Number | Initials | Age | Sex |
|---|---|---|---|
| 1 | [redacted] | 72 | F |
| 2 | | 73 | F |
| 3 | | 72 | F |
| 4 | | 77 | F |
| 5 | | 76 | M |
| 6 | | 40 | F |
| 7 | | 52 | F |
| 8 | | 19 | F |
| 9 | | 54 | M |
| 10 | | 40 | F |
| 11 | | 43 | F |
| 12 | | 43 | F |
| 13 | | 36 | F |
| 14 | | 56 | F |
| 15 | | 68 | F |
| 16 | | 26 | M |
| 17 | | 62 | M |
| 18 | | 54 | F |
| 19 | | 72 | F |
| 20 | | 71 | M |
| 21 | | 37 | F |
| 22 | | 33 | F |
| 23 | | 38 | F |
| 24 | | 32 | F |
| 25 | | 47 | M |
| 26 | | 54 | F |
| 27 | | 37 | F |
| 28 | | 58 | F |
| 29 | | 47 | F |

Table 2
(continued)
Panel # 20010774

Subject Data

| Subject Number | Initials | Age | Sex |
|---|---|---|---|
| 30 | [redacted] | 54 | F |
| 31 | | 70 | M |
| 32 | | 62 | F |
| 33 | | 19 | F |
| 34 | | 74 | M |
| 35 | | 21 | M |
| 36 | | 21 | F |
| 37 | | 65 | M |
| 38 | | 61 | F |
| 39 | | 40 | M |
| 40 | | 63 | F |
| 41 | | 44 | M |
| 42 | | 64 | M |
| 43 | | 59 | F |
| 44 | | 47 | F |
| 45 | | 74 | M |
| 46 | | 73 | F |
| 47 | | 77 | F |
| 48 | | 53 | M |
| 49 | | 54 | F |
| 50 | | 29 | F |
| 51 | | 77 | M |
| 52 | | 37 | F |
| 53 | | 39 | F |
| 54 | | 40 | M |
| 55 | | 44 | F |
| 56 | | 38 | F |
| 57 | | 54 | F |

Personal Care Products Council
Committed to Safety,
Quality & Innovation

**Memorandum**

**TO:** F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

**FROM:** Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

**DATE:** September 17, 2012

**SUBJECT:** Information on Acetyl Tyrosinamide

Anonymous. 2001. Bacterial reverse mutation assay: Princubation method with a confirmatory assay (Acetyl Tyrosinamide).

Anonymous. 2011. Chromosomal aberrations in cultured human peripheral blood lymphocytes (Acetyl Tyrosinamide).

Anonymous. 2011. Topical application ocular irritation screening assay using the Epiocular™ human cell construct (Actyl Tyrosinamide 1.25%).

Anonymous. 2011. An evaluation of the contact-sensitization potential of a topical coded product in human skin by means of the maximization assay (Acetyl Tyrosinamide 1%).

Anonymous. 2011. Neutral red uptake phototoxicity assay in BALB/c3T3 mouse fibroblasts (Acetyl Tyrosinamide).

Anonymous. 2011. An assessment of the photosensitization potential of there topical coded test products using a human photocontact allergenicity test (Acetyl Tyrosinamide 1%).

Anonymous. 2011. 48 Hour patch test of a gel containing 2% Acetyl Tyrosinamide.

Anonymous. 2011. 48 Hour patch tests of products containing 1.5-2% Acetyl Tyrosinamide.

Anonymous. 2011. Repeated insult patch test of a product containing 2% Acetyl Tyrosinamide.

Anonymous. 2011. An in-use safety evaluation to determine the ocular irritation potential and consumer opinion of cosmetic products (product containing 2% Acetyl Tyrosinamide).

Anonymous. 2011. Evaluation of the in vitro human trunk percutaneous absorption of N-Acetyl-L-Tyrosinamide using the Franz finite dose model.

# Final Report

| | |
|---|---|
| Study Title | Bacterial Reverse Mutation Assay:<br>Preincubation Method with a Confirmatory Assay |
| Test Article | N-acetyl-L-tyrosinamide |
| Author | [redacted] |
| Sponsor | [redacted] |
| Testing Facility | [redacted] |
| [redacted] | 8236018 |
| [redacted] | 1001306 |
| Genetic Toxicology Protocol Modifier | 422OECD, Edition 3 |
| Report Issued | 28 February 2011 |
| Page Number | 1 of 66 |

[redacted]

## COMPLIANCE STATEMENT

**Bacterial Reverse Mutation Assay:**
**Preincubation Method with a Confirmatory Assay**

I, the undersigned, hereby declare that the work was performed under my direction and that the findings provide a true and accurate record of the results obtained.

The study was conducted in accordance with the agreed protocol, unless otherwise stated, and the study objectives were achieved.

Except as noted below, all aspects of this study were in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58; the Organisation for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice, ENV/MC/CHEM(98)17; and any applicable amendments.

Exceptions:

1) Documentation of the stability of the test article was not provided by the Sponsor.

   Study Director Impact Statement: The impact of not having documentation of the stability of the test article cannot be fully evaluated at this time without additional information.

2) Documentation that the test article was characterized under GLP or GMP conditions is not available.

   Study Director Impact Statement: The impact of not having documentation that the test article was characterized under GLP or GMP conditions cannot be fully evaluated at this time without additional information.

3) The stability, homogeneity, and/or concentration of the dosing preparations were not analyzed.

   Study Director Impact Statement: The impact of not having verification of the stability, homogeneity, and/or concentration of the dosing formulations cannot be fully evaluated at this time without additional information.

[redacted]

28 FEB 2011
Date

[redacted]

## QUALITY ASSURANCE STATEMENT

### Bacterial Reverse Mutation Assay:
### Preincubation Method with a Confirmatory Assay

This report has been reviewed by the [redacted] and accurately reflects the raw data. The following study specific inspections were conducted and findings reported to the study director (SD) and associated management.

| Inspection Dates | | Phase | Date Reported to SD and SD Management |
|---|---|---|---|
| From | To | | |
| 08 Oct 2010 | 08 Oct 2010 | Protocol Review - [redacted] | 08 Oct 2010 |
| 08 Oct 2010 | 08 Oct 2010 | Protocol Amendment Review [redacted] | 08 Oct 2010 |
| 08 Oct 2010 | 08 Oct 2010 | Scoring of Plates | 08 Oct 2010 |
| 11 Nov 2010 | 11 Nov 2010 | Draft Report and Data Review | 11 Nov 2010 |
| 04 Jan 2011 | 04 Jan 2011 | Protocol Amendment Review - [redacted] | 04 Jan 2011 |
| 05 Jan 2011 | 05 Jan 2011 | Protocol Amendment Review - [redacted] | 05 Jan 2011 |
| 21 Jan 2011 | 21 Jan 2011 | Protocol Amendment Review - [redacted] | 21 Jan 2011 |
| 24 Feb 2011 | 25 Feb 2011 | Final Report Review | 25 Feb 2011 |

[redacted]

28 Feb 2011
Date

[redacted]

## KEY PERSONNEL

### Bacterial Reverse Mutation Assay:
### Preincubation Method with a Confirmatory Assay

[redacted]

## CONTENTS

## ABSTRACT

The objective of this study was to evaluate the test article, N-acetyl-L-tyrosinamide, and/or its metabolites for their ability to induce reverse mutations at the histidine locus in several strains of *Salmonella typhimurium* (*Salmonella*; TA98, TA100, TA1535, and TA1537), and at the tryptophan locus of *Escherichia coli* (*E. coli*) strain WP2*uvr*A in the presence or absence of an exogenous mammalian metabolic activation system (S9).

N-acetyl-L-tyrosinamide was evaluated in the dose range-finding assay in tester strains TA100 and WP2*uvr*A. Ten doses of test article, from 6.67 to 5000 µg/plate, were evaluated with and without S9 (one plate per dose). Normal growth was observed in both tester strains, and the test article was found to be freely soluble, at all doses evaluated with and without S9.

Based upon the results of the dose range-finding assay, N-acetyl-L-tyrosinamide was evaluated in the initial mutagenicity assay, in all five tester strains, at doses of 313, 625, 1250, 2500, and 5000 µg/plate with and without S9. All doses of the test article, as well as the concurrent positive and vehicle controls, were evaluated in triplicate plates. Normal growth again was observed in all five tester strains at all doses evaluated with and without S9. In addition, the test article again was found to be freely soluble in the aqueous top agar at all doses evaluated with and without S9. Revertant frequencies for all doses of N-acetyl-L-tyrosinamide, in all tester strains with and without S9, approximated or were less than those observed in the concurrent vehicle control cultures.

N-acetyl-L-tyrosinamide was re-evaluated in the confirmatory mutagenicity assay under identical conditions, and similar results were observed. Normal growth again was observed in all five tester strains at all doses evaluated with and without S9. In addition, the test article again was found to be freely soluble in the aqueous top agar at all doses evaluated with and without S9. Revertant frequencies for all doses of N-acetyl-L-tyrosinamide, in all tester strains with and without S9, again approximated or were less than control values. All positive and vehicle control values were within acceptable ranges, and all criteria for a valid study were met.

These results indicate N-acetyl-L-tyrosinamide was negative in the Bacterial Reverse Mutation Assay: Preincubation Method with a Confirmatory Assay under the conditions, and according to the criteria, of the test protocol.

[redacted]

## STUDY CONDUCT

### Objective

The objective of this study was to evaluate the test article, N-acetyl-L-tyrosinamide, and/or its metabolites for their ability to induce reverse mutations at the histidine locus in several strains of *Salmonella* (TA98, TA100, TA1535, and TA1537), and at the tryptophan locus of *E. coli* strain WP2*uvr*A, in the presence or absence of an exogenous mammalian metabolic activation system (S9). This assay design is based on OECD Guideline 471 (OECD, 1997) and ICH Guidelines S2A and S2B (ICH, 1995, 1997).

### Study Timetable

| | |
|---|---|
| Study Initiation Date | 17 September 2010 |
| Experimental Start Date | 22 September 2010 |
| Experimental Completion Date | 25 October 2010 |
| Study Completion Date | 28 February 2011 |

### Protocol Adherence

This study was conducted in accordance with the protocol and amendments (Appendix 1).

### Major Computer Systems

| System[a] | *Application Function* |
|---|---|
| EMCS | Monitors and documents facility storage conditions (e.g., constant room temperatures and humidity levels) |
| EMCSDR | Transfers data from EMCS for reporting purposes |
| REES environmental monitoring system | Monitors and documents facility storage conditions (e.g., refrigerators, freezers, and incubator levels) |
| ASM | Collects and processes bacterial reverse mutation assay data |
| MTTS | Test article accessioning and dispensing |

a All version numbers of the applications are maintained by [redacted] Definitions for the acronyms can be found in the Glossary

### Record Retention

The raw data, documentation, specimens, the protocol, and final report for this study will be stored in the [redacted] for at least 1 year after report finalization. The [redacted] staff will contact the sponsor after at least 1 year following report finalization to determine disposition of the archived materials (except for raw data on durable media, study correspondence, the protocol, and final report which will be kept by [redacted]). The sponsor will then provide instructions to retain the materials at [redacted], authorize the transport of the materials to their site (or that of their designee), or request destruction on their behalf if regulatory retention requirements have been satisfied.

## TEST AND CONTROL ARTICLES

### Test Article

The test article was supplied by the Sponsor as a white crystalline powder on 15 September 2010 with the following information/characteristics.

| Test Article | Lot No. | Storage | Purity | Expiration Date |
|---|---|---|---|---|
| N-acetyl-L-tyrosinamide LHY3-100 | | Room temperature | 99.6% | NP |

NP = Not provided

### Vehicle Control

The vehicle control article was dimethylsulfoxide (DMSO), supplied by Sigma Aldrich, with the following information/characteristics.

| Control Article | CAS No. | Lot No. | Purity | Expiration Date |
|---|---|---|---|---|
| DMSO 67-68-5 | | 76396EM | 99.92% | 28 Jul 2013<br>09 Sep 2013 |
| | | 17496KM | 99.99% | 04 Oct 2013 |

Information on synthesis methods, stability, purity, composition, or other characteristics that define the test article and control article components is on file with the Sponsor or the respective manufacturer. The Certificate of Analysis for the test article is attached (Appendix 2).

### Positive Controls

The positive controls were supplied by Sigma-Aldrich with the exception of ICR-191 which was supplied by Molecular Toxicology, Inc. and evaluated in the appropriate tester strain and activation conditions as indicated below (Table I). All positive control articles were dissolved in dimethyl sulfoxide (DMSO) with the exception of sodium azide which was dissolved in water.

**Table I**
**Positive Control Articles**

| Tester Strain(s) | S9 | Positive Control | Dose (µg/plate) | CAS No. | Lot No. |
|---|---|---|---|---|---|
| TA98 | – | 2-nitrofluorene 1.0 | | 607-57-8 | 01508BE |
| TA100, TA1535 | – | sodium azide | 2.0 | 26628-22-8 | MKBD3457 |
| TA1537 | – | ICR-191 2.0 | | 17070-45-0 | 6243ICR |
| WP2*uvr*A | – | 4-nitroquinoline-N-oxide | 0.4 56-57-5 | | 039K1332 |
| TA98 + | | benzo[a]pyrene | 2.5 | 50-32-8 | 087K0733 |
| TA100, TA1535, TA1537 | + | 2-aminoanthracene | 2.5 | 613-13-8 | 12317CE |
| WP2*uvr*A + | | 2-aminoanthracene | 25.0 | 613-13-8 | 12317CE |

**Sterility Controls**
The most concentrated test article dilution and the S9 mix (50 and 500 µL, respectively; the same volumes used in the assay) were checked for sterility by plating on selective agar.

## METABOLIC ACTIVATION SYSTEM (S9)

**Liver Homogenate**
Liver homogenate (S9) was purchased from Molecular Toxicology, Inc. (Lot Nos. 2502 and 2582, containing 36.0 and 34.9 mg/mL protein, respectively). The homogenate was prepared from male Sprague-Dawley rats that had been injected (intraperitoneally) with Aroclor™ 1254 (200 mg/mL in corn oil) at 500 mg/kg, 5 days before sacrifice (Ames, *et al.*, 1975).

**S9 Mix**
S9 mix was prepared on the day of use, maintained on ice, and contained the components indicated below (Table II; the mix and metabolic activation system are referred to as S9 interchangeably).

**Table II**
**S9 Mix Components**

| Component Amount | |
|---|---|
| $H_2O$ 0.70 | mL |
| 1M $NaH_2PO_4/Na_2HPO_4$, pH 7.4 | 0.10 mL |
| 0.25M Glucose-6-phosphate | 0.02 mL |
| 0.10M NADP | 0.04 mL |
| 0.825M KCl/0.2M $MgCl_2$ 0.04 | mL |
| Liver Homogenate | 0.10 mL |
| 1.00 | mL |

## TEST SYSTEM

**Test System Rationale**
The bacterial reverse mutation assay has been shown to be a sensitive, rapid, and accurate indicator of the mutagenic activity of many materials including a wide range of chemical classes. By using several different tester strains, both base pair substitution and frameshift mutations can be detected. *Salmonella* and *E. coli* strains used in this assay are histidine and tryptophan auxotrophs, respectively, by virtue of conditionally lethal mutations in the appropriate operons. When these histidine (*his*$^-$) or tryptophan (*trp*$^-$) dependent cells are exposed to the test article and grown under selective conditions (minimal media with a trace amount of histidine or tryptophan), only those cells which revert to histidine (*his*$^+$) or tryptophan (*trp*$^+$) independence are able to form colonies. Trace amounts of histidine or tryptophan added to the media allow all the plated bacteria to undergo a few cell divisions, which is essential for mutagenesis to be fully expressed.

*his*$^+$ or *trp*$^+$ revertants are readily discernable as colonies against the limited background growth of *his*$^-$ or *trp*$^-$ cells.

**Tester Strains**

The tester strains used were the *Salmonella* histidine auxotrophs TA98, TA100, TA1535, and TA1537 (Ames *et al.* 1975) and the *E. coli* tryptophan auxotroph WP2*uvr*A (Green and Muriel, 1976). Specific genotypes of the strains are shown below (Table III).

**Table III**
**Tester Strain Genotypes**

| Tester Strain | *his/trp* Mutation | Additional Mutations | | Plasmid |
|---|---|---|---|---|
| | | Repair L | PS | |
| TA98 | *his*D3052 | *uvr*B | *rfa* pKM101 | |
| TA100 | *his*G46 | *uvr*B | *rfa* pKM101 | |
| TA1535 | *his*G46 | *uvr*B | *rfa* | – |
| TA1537 | *his*C3076 | *uvr*B | *rfa* | – |
| WP2*uvr*A | *trp* | *uvr*A | – | – |

In addition to a mutation in either the histidine or tryptophan operons, the tester strains contain additional mutations that enhance their sensitivity to some mutagenic compounds. Mutation of the *uvr*A gene (*E. coli)* or the *uvr*B gene (*Salmonella*) results in a deficient DNA excision repair system that greatly enhances the sensitivity of these strains to some mutagens. Since the *uvr*B deletion extends through the *bio* gene, *Salmonella* tester strains containing this deletion also require the vitamin biotin for growth.

*Salmonella* tester strains also contain the *rfa* wall mutation, which results in the loss of one of the enzymes responsible for the synthesis of part of the lipopolysaccharide (LPS) barrier that forms the surface of the bacterial cell wall. The resulting cell wall deficiency increases permeability to certain classes of chemicals, such as those containing large ring systems (i.e., benzo[a]pyrene), that otherwise would be excluded by a normal cell wall.

Tester strains TA98 and TA100 also contain the pKM101 plasmid, which further increases the sensitivity of these strains to some mutagens. The suggested mechanism for this increased sensitivity is modification of an existing bacterial DNA repair polymerase complex involved with the mismatch-repair process.

Tester strains TA98 and TA1537 are reverted from histidine dependence (auxotrophy) to histidine independence (prototrophy) by frameshift mutagens. In contrast, tester strains TA100, TA1535, and WP2*uvr*A are reverted from auxotrophy to prototrophy by base substitution mutagens.

***Source of Tester Strains***
*Salmonella* tester strains were received from Dr. Bruce Ames, Department of Biochemistry, University of California (Berkeley, CA). *E. coli* tester strain WP2*uvr*A was received from The National Collection of Industrial Bacteria, Torrey Research Station, Scotland (United Kingdom).

**Preparation of Overnight Cultures**
***Inoculation***
Overnight cultures were inoculated into flasks containing culturing broth and the flasks were placed in a shaker/incubator programmed to begin operation (shaking, 125 ± 25 rpm; incubation, 37 ± 2°C) so that overnight cultures were in late log phase when optical density (OD) monitoring began.

***Harvest***
To ensure cultures are harvested at the appropriate phase, the growth of the culture was monitored using a spectrophotometer. An aliquot of each culture was diluted 1:4 and its OD determined at 650 nm ($OD_{650(1:4)}$). Cultures were harvested once a predetermined OD was reached which ensured that cultures had reached late exponential or early stationary phase (representative of cultures with $\geq 10^9$ cells/mL) and had not overgrown. Overgrown (stationary) cultures may exhibit decreased sensitivity to some mutagens. Once the target OD was reached ($OD_{650(1:4)}$ = 0.4 to 0.6), the cultures were removed from incubation and held at >0 to 10°C until used in the assay.

**Tester Strain Characterization**
All tester strains were checked, at a minimum, for the following phenotypic characteristics.

***Histidine/Tryptophan Auxotrophy (his⁻/trp⁻ Mutation)***
The presence of the *his*⁻ or *trp*⁻ mutations was confirmed by demonstrating growth on minimal bottom agar plates supplemented with histidine or tryptophan.

***rfa Cell Wall Mutation***
The presence of the *rfa* cell wall mutation was confirmed by demonstrating sensitivity of the strains to crystal violet.

***pKM101 Plasmid***
The presence of the pKM101 plasmid was confirmed by demonstrating resistance of the strains to ampicillin.

***uvrA and uvrB Mutation***
The presence of the *uvr*A and *uvr*B mutations was confirmed by demonstrating sensitivity of the strains to ultraviolet light.

**Tester Strain Media**

***Culturing Broth***

The broth used to grow overnight cultures of the tester strains was Vogel-Bonner salt solution (Vogel and Bonner, 1956) supplemented with 2.5% (w/v) Oxoid Nutrient Broth No. 2 (dry powder).

***Minimal Bottom Agar Plates***

Bottom agar (25 mL per 15 x 100 mm petri dish) was Vogel-Bonner minimal medium E (Vogel and Bonner, 1956), supplemented with 1.5% (w/v) agar and 0.2% (w/v) glucose.

***Top Agar for Selection of Revertants***

Top (overlay) agar contained 0.7% (w/v) agar and 0.5% (w/v) NaCl, supplemented with 0.045mM histidine and 0.045mM biotin, or 0.045mM tryptophan, for *Salmonella*, or *E. coli*, respectively. Two mL of supplemented top agar was used for cultures treated in the presence and absence of S9.

## EXPERIMENTAL DESIGN

**Dose Range-finding Assay**

The growth inhibitory effect (cytotoxicity) of the test article to the test system was determined in order to allow the selection of appropriate doses to be tested in the mutagenicity assay.

***Design***

The dose range-finding assay was performed using tester strains TA100 and WP2*uvr*A. The test article was evaluated at doses of 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, and 5000 µg/plate with and without S9 (one plate per dose).

***Rationale***

Cytotoxicity of the test article observed in tester strain TA100 is generally representative of that observed in the other *Salmonella* tester strains. Because of TA100's comparatively high spontaneous revertant frequency (revertant colonies/plate), gradations of cytotoxicity can be readily discerned from routine experimental variation. *E. coli* tester strain WP2*uvr*A does not have the *rfa* wall mutation possessed by the *Salmonella* strains; therefore a different range of cytotoxicity may be observed. Also, cytotoxicity of a test article in the presence of S9 may vary greatly from that observed in its absence, requiring that different dose ranges be evaluated in the mutagenicity assay with and without S9.

***Evaluation of the Dose Range-finding Assay***

Cytotoxicity is detectable as a decrease in revertant frequency and/or a thinning or disappearance of the bacterial background lawn. However, thinning of the background lawn not accompanied by a reduction in revertant frequency may not necessarily be evaluated as an indication of cytotoxicity.

**Mutagenicity Assay**

***Design***

Based upon the results of the dose range-finding assay, the test article was evaluated in the initial mutagenicity assay at doses of 313, 625, 1250, 2500, and 5000 µg/plate with and without S9. An independent confirmatory assay subsequently was performed under identical conditions.

***Frequency and Route of Administration***

Tester strains were exposed to the test article via the preincubation modification of the Ames Test originally described by Yahagi *et al.* (1975) and Maron and Ames (1983). This methodology has been shown to detect a wide range of classes of chemical mutagens. In the preincubation method, the tester strain, test article, and S9 mix (or phosphate buffer, where appropriate), are preincubated prior to the addition of molten agar. The preincubation reaction mixture then is combined in molten top agar, which is then overlaid onto a minimal bottom agar plate. Following incubation, revertant colonies are counted.

## PROCEDURES

The experimental materials, methods and procedures were based on those described by Ames *et al.* (1975) and Green and Muriel (1976).

**Plating Procedures**

These procedures were used in both the dose range-finding assay and the mutagenicity assays. Each plate was labeled with a code that identified the test article, test phase, tester strain, activation condition, and dose level. Dilutions of the test article were prepared immediately prior to their use.

Treatments were performed by adding 500 µL 0.1M phosphate buffer or 500 µL S9 mix, as appropriate, to 13 x 100 mm glass culture tubes (pre-heated to 37 ± 2°C) and then adding 100 µL tester strain and 50 µL test or control article. The mixture was vortexed and allowed to incubate for 20 ± 2 minutes at 37 ± 2°C. Two mL of molten top agar was added to each tube, and the mixture was vortexed and overlaid onto the surface of bottom agar dishes. After the overlay solidified, the plates were inverted and incubated for 52 ± 4 hours at 37 ± 2°C.

**Scoring the Plates**

Plates which were not evaluated immediately following the incubation period were held at >0 to 10°C until such time that colony counting and bacterial background lawn evaluation could take place.

***Bacterial Background Lawn Evaluation***
The condition of the bacterial background lawn was evaluated macroscopically and microscopically (using a dissecting microscope) for indications of cytotoxicity and test article precipitate. Evidence of cytotoxicity was scored relative to the vehicle control plate and was recorded along with the revertant counts for all plates at that dose level. Lawns were scored as normal (N), reduced (R), obscured by precipitate (O), macroscopic precipitate present (P), absent (A), or enhanced (E); contaminated plates (C) were also noted as applicable.

***Counting Revertant Colonies***
Revertant colonies were counted by automated colony counter and/or by hand.

## DATA

**Data Presentation**
The average revertants/plate and the standard deviation were calculated. Results of these calculations are presented in tabular form in the Data Tables section of this report. Historical control data are included for comparison (Appendix 3).

**Assay Acceptance Criteria**
Before assay data were evaluated, the criteria for a valid assay had to be met. The following criteria were used to determine a valid assay:

***Tester Strain Integrity***
*Histidine/Tryptophan Auxotrophy (his⁻/trp⁻ Mutation)*
*Salmonella* tester strains must exhibit the presence of the *his*⁻ mutation, as demonstrated by the ability to grow on minimal bottom agar plates supplemented with histidine/biotin (but not tryptophan/biotin). Tester strain WP2*uvr*A must exhibit the presence of the *trp*⁻ mutation, as demonstrated by the ability to grow on bottom agar plates supplemented with tryptophan/biotin (but not histidine/biotin).

*rfa Cell Wall Mutation*
*Salmonella* tester strains must exhibit the presence of the *rfa* mutation, as demonstrated by sensitivity to crystal violet. Tester strain WP2*uvr*A must exhibit resistance to crystal violet.

*pKM101 Plasmid*
Tester strains TA98 and TA100 must exhibit the presence of the pKM101 plasmid by demonstrating resistance to ampicillin. In contrast, tester strains TA1535, TA1537, and WP2*uvr*A must be sensitive to ampicillin.

*uvrA and uvrB Mutation*
All tester strains must exhibit the presence of the *uvr*A or *uvr*B mutations, as demonstrated by their sensitivity to ultraviolet light.

*Characteristic Number of Spontaneous Revertants*
The average revertants/plate of the vehicle control cultures must be within the following acceptance limits which are based upon historical data and published reports.

| | |
|---|---|
| TA98 8 | - 60 |
| TA100 60 | - 240 |
| TA1535 4 | - 45 |
| TA1537 2 | - 25 |
| WP2*uvr*A 5 | - 40 |

*Tester Strain Culture Density*
Each culture must reach a target $OD_{650(1:4)}$ = 0.4 to 0.6, which has been demonstrated to be representative of cultures in the late exponential or early stationary phase with $\geq 10^9$ cells/mL.

*Positive Control Values*
The positive controls must induce a ≥3-fold increase in revertants/plate as compared to the concurrent vehicle controls. A 3-fold increase in the absence of S9 indicates that the tester strain can identify a mutagen, while a 3-fold increase in the presence of S9 will be considered to have demonstrated the integrity of the S9 mix as well as the ability of the tester strain to detect a mutagen.

*Number of Dose Levels*
A minimum of three non-toxic doses is required to evaluate assay data.

*Maximum Dose Level*
The highest dose evaluated was the limit dose of 5000 µg/plate.

**Assay Evaluation Criteria**
Once the criteria for a valid assay had been met, responses observed in the assay were evaluated.

*Criteria for a Positive Response*
A test article is considered to have produced a positive response if it induces a dose-dependent increase in revertant frequency that is ≥2.0-fold vehicle control values for tester strains TA98, TA100, and WP2*uvr*A, or ≥3.0-fold vehicle control values for tester strains TA1535 and TA1537. In addition, any response should be reproducible.

*Criteria for a Negative Response*
A test article is considered to have produced a negative response if no dose-dependent, ≥2.0-fold or ≥3.0-fold increases are observed in tester strains TA98, TA100, and WP2*uvr*A, or TA1535 and TA1537, respectively.

[redacted]

***Criteria for an Equivocal Response***

Even after repeated trials, a test article may produce results that are neither clearly positive nor clearly negative (e.g., responses that do not meet the dose-dependency or fold increase requirements but are reproducible). In those rare instances, the test article may be considered to have produced an equivocal response.

Other criteria also may be used in reaching a conclusion about the study results (e.g., comparison to historical control values, biological significance, etc.). In such cases, the Study Director will use sound scientific judgment and clearly report and describe any such considerations.

## RESULTS AND DISCUSSION

### Test Article Handling

In solubility testing, the test article was immiscible in deionized water. In DMSO, the test article was observed to form a transparent, colorless solution at ~100 mg/mL. Based on these results, DMSO was selected as the vehicle for this study. At 100 mg/mL, which was the most concentrated dose formulation prepared for treatment the test article was observed to form a transparent, non-viscous, colorless solution. The test article remained freely soluble at all succeeding lower dilutions prepared.

### Dose Range-finding Assay

N-acetyl-L-tyrosinamide was evaluated in the dose range-finding assay in tester strains TA100 and WP2*uvr*A. Ten doses of test article, from 6.67 to 5000 µg/plate, were evaluated with and without S9 (one plate per dose; Trial 8236018-A1, Table 1 and Table 2). Normal growth was observed in both tester strains, and the test article was found to be freely soluble, at all doses evaluated with and without S9.

### Mutagenicity Assay

Based upon the results of the dose range-finding assay, N-acetyl-L-tyrosinamide was evaluated in the initial mutagenicity assay, in all five tester strains, at doses of 313, 625, 1250, 2500, and 5000 µg/plate with and without S9, (Trial 8236018-B1, Table 3 and Table 4). All doses of the test article, as well as the concurrent positive and vehicle controls, were evaluated in triplicate plates. Normal growth again was observed in all five tester strains at all doses evaluated with and without S9. In addition, the test article again was found to be freely soluble in the aqueous top agar at all doses evaluated with and without S9. Revertant frequencies for all doses of N-acetyl-L-tyrosinamide, in all tester strains with and without S9, approximated or were less than those observed in the concurrent vehicle control cultures.

N-acetyl-L-tyrosinamide was re-evaluated in the confirmatory mutagenicity assay under identical conditions, and similar results were observed (Trial 8236018-C1, Table 5 and Table 6). Normal growth again was observed in all five tester strains at all doses evaluated with and without S9. In addition, the test article again was found to be freely soluble in the aqueous top agar at all doses evaluated with and without S9. Revertant frequencies for all doses of N-acetyl-L-tyrosinamide, in all tester strains with and without S9, again approximated or were less than control values. All positive and vehicle control values were within acceptable ranges, and all criteria for a valid study were met.

## CONCLUSION

These results indicate N-acetyl-L-tyrosinamide was negative in the Bacterial Reverse Mutation Assay: Preincubation Method with a Confirmatory Assay under the conditions, and according to the criteria, of the test protocol.

[redacted]

## DATA TABLES

## Table 1
## Dose Range-finding Assay with S9

Study No.: 8236018
Trial No.: 8236018-A1 Date Plated: 9/28/2010
Plating Method: Pre-incubation assay

| Strain C | ompound | Dose level per plate (µg/plate) | Ratio treated/ vehicle | Individual revertant colony counts |
|---|---|---|---|---|
| **TA100** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *0.9* | 126 N |
| | | 3330 | *0.9* | 127 N |
| | | 1000 | *1.1* | 147 N |
| | | 667 | *1.0* | 139 N |
| | | 333 | *0.9* | 121 N |
| | | 100 | *1.0* | 133 N |
| | | 66.7 | *1.1* | 146 N |
| | | 33.3 | *0.9* | 131 N |
| | | 10.0 | *0.9* | 131 N |
| | | 6.67 | *1.0* | 141 N |
| **D** | **imethyl Sulfoxide** | | | 138 M N |
| **WP2*uvrA*** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *0.9* | 21 N |
| | | 3330 | *0.6* | 14 M N |
| | | 1000 | *0.6* | 14 M N |
| | | 667 | *0.7* | 16 N |
| | | 333 | *0.8* | 20 N |
| | | 100 | *0.8* | 19 N |
| | | 66.7 | *0.7* | 16 N |
| | | 33.3 | *0.9* | 21 N |
| | | 10.0 | *0.7* | 17 N |
| | | 6.67 | *0.7* | 16 N |
| **D** | **imethyl Sulfoxide** | | | 24 M N |

Key to Plate Postfix Codes

| | |
|---|---|
| N | Normal background bacterial lawn |
| M | Plate counted manually |

## Table 2
## Dose Range-finding Assay without S9

Study No.: 8236018
Trial No.: 8236018-A1 Date Plated: 9/28/2010
Plating Method: Pre-incubation assay

| Strain C | ompound | Dose level per plate (µg/plate) | Ratio treated/ vehicle | Individual revertant colony counts |
|---|---|---|---|---|
| **TA100** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *1.0* | 91 N |
| | | 3330 | *1.3* | 114 N |
| | | 1000 | *0.8* | 77 M N |
| | | 667 | *1.0* | 88 M N |
| | | 333 | *1.0* | 91 N |
| | | 100 | *1.0* | 93 M N |
| | | 66.7 | *1.0* | 94 N |
| | | 33.3 | *1.1* | 101 N |
| | | 10.0 | *1.3* | 116 N |
| | | 6.67 | *1.0* | 89 N |
| **D** | **imethyl Sulfoxide** | | | 91 N |
| **WP2*uvrA*** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *0.9* | 16 M N |
| | | 3330 | *0.9* | 15 M N |
| | | 1000 | *0.8* | 14 N |
| | | 667 | *1.0* | 17 N |
| | | 333 | *0.6* | 11 M N |
| | | 100 | *0.8* | 14 M N |
| | | 66.7 | *0.7* | 12 N |
| | | 33.3 | *0.6* | 10 N |
| | | 10.0 | *0.9* | 15 M N |
| | | 6.67 | *0.9* | 15 M N |
| **D** | **imethyl Sulfoxide** | | | 17 N |

Key to Plate Postfix Codes

| | |
|---|---|
| N | Normal background bacterial lawn |
| M | Plate counted manually |

## Table 3
## Initial Mutagenicity Assay Results with S9

Study No.: 8236018
Trial No.: 8236018-B1 Date Plated: 10/5/2010
Plating Method: Pre-incubation assay

| Strain C | ompound | Dose level (µg/plate) | Mean revertants per plate | SD | Ratio treated/ vehicle | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA98** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *15.7 4.* | *6* | *0.9* | 13 N, 21 M N, 13 N |
| | | 2500 | *17.0 7.* | *0* | *1.0* | 14 M N, 25 N, 12 M N |
| | | 1250 | *21.3 2.* | *5* | *1.3* | 21 N, 19 M N, 24 N |
| | | 625 | *20.0 2.* | *0* | *1.2* | 22 N, 18 N, 20 M N |
| | | 313 | *19.3 5.* | *5* | *1.2* | 23 M N, 13 M N, 22 N |
| | **Dimethyl Sulfoxide** | | *16.7 0.* | *6* | | 17 M N, 16 M N, 17 N |
| **TA100** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *115.3 8.* | *1* | *0.8* | 108 N, 124 N, 114 M N |
| | | 2500 | *110.7 6.* | *8* | *0.8* | 113 N, 116 M N, 103 N |
| | | 1250 | *106.3 3.* | *5* | *0.8* | 103 M N, 110 N, 106 N |
| | | 625 | *120.0 15* | *.6* | *0.9* | 130 N, 102 M N, 128 N |
| | | 313 | *104.0 3.* | *0* | *0.7* | 107 M N, 104 N, 101 M N |
| | **Dimethyl Sulfoxide** | | *140.3 20* | *.0* | | 163 M N, 133 M N, 125 M N |
| **TA1535** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *9.0 1.* | *7* | *0.8* | 10 N, 7 N, 10 N |
| | | 2500 | *16.0 2.* | *0* | *1.4* | 14 N, 16 N, 18 M N |
| | | 1250 | *11.7 1.* | *5* | *1.0* | 12 N, 13 N, 10 N |
| | | 625 | *11.7 2.* | *5* | *1.0* | 9 N, 14 M N, 12 N |
| | | 313 | *11.0 4.* | *4* | *1.0* | 16 N, 8 M N, 9 M N |
| | **Dimethyl Sulfoxide** | | *11.3 4.* | *0* | | 12 N, 15 M N, 7 M N |
| **TA1537** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *9.7 1.* | *2* | *1.2* | 9 M N, 11 M N, 9 N |
| | | 2500 | *7.7 2.* | *5* | *0.9* | 10 M N, 5 N, 8 M N |
| | | 1250 | *9.7 2.* | *1* | *1.2* | 12 N, 9 N, 8 N |
| | | 625 | *9.7 3.* | *2* | *1.2* | 11 M N, 12 M N, 6 M N |
| | | 313 | *10.0 3.* | *6* | *1.2* | 14 N, 9 N, 7 N |
| | **Dimethyl Sulfoxide** | | *8.3 0.* | *6* | | 8 M N, 9 M N, 8 M N |

Key to Plate Postfix Codes

| | |
|---|---|
| N | Normal background bacterial lawn |
| M | Plate counted manually |

## Table 3 (cont.)
## Initial Mutagenicity Assay Results with S9

Study No.: 8236018
Trial No.: 8236018-B1 Date Plated: 10/5/2010
Plating Method: Pre-incubation assay

| Strain | Compound | Dose level (µg/plate) | Mean revertants per plate | SD | Ratio treated/ vehicle | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **WP2*uvr*A** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *15.7 3.* | *1* | *1.0* | 19 M N, 13 N, 15 N |
| | | 2500 | *19.0 2.* | *0* | *1.2* | 21 M N, 17 N, 19 M N |
| | | 1250 | *16.7 1.* | *5* | *1.0* | 17 N, 15 M N, 18 M N |
| | | 625 | *17.0 2.* | *0* | *1.1* | 19 M N, 15 M N, 17 M N |
| | | 313 | *16.7 4.* | *7* | *1.0* | 22 M N, 15 N, 13 M N |
| | **Dimethyl Sulfoxide** | | *16.0 2.* | *6* | | 14 M N, 15 M N, 19 M N |
| **TA98 BP** | | 2.5 | *399.7 12* | *.7* | *24.0* | 408 N, 406 N, 385 N |
| **TA100 2A** | **A** | 2.5 | *1589.0 19* | *6.1* | *11.3* | 1813 N, 1448 N, 1506 N |
| **TA1535 2** | **AA** | 2.5 | *149.7 14* | *.0* | *13.2* | 151 N, 135 N, 163 N |
| **TA1537 2** | **AA** | 2.5 | *133.0 8.* | *9* | *16.0* | 140 N, 123 M N, 136 N |
| **WP2*uvr*A 2A** | **A** | 25.0 | *411.3 52* | *.0* | *25.7* | 403 N, 364 N, 467 N |

| Key to Positive Controls | | Key to Plate Postfix Codes | |
|---|---|---|---|
| BP | Benzo{a}pyrene | N | Normal background bacterial lawn |
| 2AA | 2-aminoanthracene | M | Plate counted manually |

## Table 4
## Initial Mutagenicity Assay Results without S9

Study No.: 8236018
Trial No.: 8236018-B1 Date Plated: 10/5/2010
Plating Method: Pre-incubation assay

| Strain | Compound | Dose level (µg/plate) | Mean revertants per plate | SD | Ratio treated/ vehicle | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA98** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *15.0 1.* | *0* | *1.0* | 15 N, 14 N, 16 N |
| | | 2500 | *12.3 2.* | *5* | *0.8* | 10 N, 15 N, 12 N |
| | | 1250 | *13.3 3.* | *1* | *0.9* | 16 N, 14 N, 10 M N |
| | | 625 | *12.7 3.* | *1* | *0.9* | 12 M N, 16 M N, 10 M N |
| | | 313 | *13.0 2.* | *0* | *0.9* | 13 N, 11 M N, 15 M N |
| **D** | **imethyl Sulfoxide** | | *14.7 2.* | *9* | | 13 N, 13 N, 18 N |
| **TA100** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *96.7 8.* | *3* | *1.0* | 90 N, 106 N, 94 N |
| | | 2500 | *95.7 12* | *.7* | *1.0* | 81 N, 103 N, 103 N |
| | | 1250 | *104.0 8.* | *0* | *1.1* | 96 N, 112 N, 104 N |
| | | 625 | *94.3 11* | *.5* | *1.0* | 106 N, 94 N, 83 N |
| | | 313 | *101.7 4.* | *0* | *1.1* | 106 N, 101 N, 98 N |
| **D** | **imethyl Sulfoxide** | | *96.3 11* | *.6* | | 84 N, 107 N, 98 N |
| **TA1535** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *10.3 3.* | *5* | *0.9* | 10 M N, 14 N, 7 N |
| | | 2500 | *10.3 4.* | *5* | *0.9* | 6 N, 10 N, 15 M N |
| | | 1250 | *11.7 3.* | *5* | *1.0* | 15 N, 8 N, 12 N |
| | | 625 | *8.7 1.* | *5* | *0.7* | 9 N, 7 M N, 10 N |
| | | 313 | *8.7 1.* | *2* | *0.7* | 8 N, 10 M N, 8 N |
| **D** | **imethyl Sulfoxide** | | *12.0 2.* | *0* | | 14 N, 10 N, 12 N |
| **TA1537** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *5.3 2.* | *5* | *0.8* | 5 M N, 8 M N, 3 M N |
| | | 2500 | *4.3 2.* | *3* | *0.7* | 7 N, 3 N, 3 N |
| | | 1250 | *3.3 1.* | *2* | *0.5* | 4 M N, 4 M N, 2 M N |
| | | 625 | *6.0 1.* | *0* | *0.9* | 7 M N, 6 N, 5 M N |
| | | 313 | *5.7 0.* | *6* | *0.9* | 6 N, 6 M N, 5 N |
| **D** | **imethyl Sulfoxide** | | *6.3 0.* | *6* | | 6 N M, 7 N M, 6 M N |

Key to Plate Postfix Codes

| | |
|---|---|
| N | Normal background bacterial lawn |
| M | Plate counted manually |

## Table 4 (cont.)
## Initial Mutagenicity Assay Results without S9

Study No.: 8236018
Trial No.: 8236018-B1 Date Plated: 10/5/2010
Plating Method: Pre-incubation assay

| Strain | Compound | Dose level (µg/plate) | Mean revertants per plate | SD | Ratio treated/ vehicle | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **WP2*uvr*A** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *15.3 2.* | *1* | *0.9* | 16 N, 17 M N, 13 M N |
| | | 2500 | *15.3 4.* | *6* | *0.9* | 18 M N, 10 M N, 18 M N |
| | | 1250 | *13.3 1.* | *5* | *0.8* | 13 M N, 15 M N, 12 M N |
| | | 625 | *17.3 3.* | *1* | *1.1* | 18 M N, 20 M N, 14 M N |
| | | 313 | *12.7 2.* | *3* | *0.8* | 14 M N, 14 M N, 10 M N |
| | **Dimethyl Sulfoxide** | | *16.3 3.* | *2* | | 20 M N, 14 N, 15 M N |
| **TA98** | **2 NF** | 1.0 | *382.3 17* | *.2* | *26.1* | 363 N, 396 N, 388 N |
| **TA100** | **SA** | 2.0 | *1268.3 16* | *.1* | *13.2* | 1250 N, 1280 N, 1275 N |
| **TA1535** | **SA** | 2.0 | *883.0 16* | *.8* | *73.6* | 870 N, 902 N, 877 N |
| **TA1537** | **IC R** | 2.0 | *720.7 25* | *0.6* | *113.8* | 1004 N, 528 N, 630 N |
| **WP2*uvr*A** | **4NQO** | 0.4 | *819.0 15* | *6.7* | *50.1* | 689 N, 775 N, 993 N |

| Key to Positive Controls | | Key to Plate Postfix Codes | |
|---|---|---|---|
| 2NF | 2-nitrofluorene | N | Normal background bacterial lawn |
| SA | sodium azide | M | Plate counted manually |
| ICR | ICR-191 | | |
| 4NQO | 4-nitroquinoline-N-oxide | | |

## Table 5
## Confirmatory Mutagenicity Assay Results with S9

Study No.: 8236018
Trial No.: 8236018-C1 Date Plated: 10/15/2010
Plating Method: Pre-incubation assay

| Strain C | ompound | Dose level (µg/plate) | Mean revertants per plate | SD | Ratio treated/ vehicle | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA98** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *20.3 3.* | *1* | *1.0* | 21 N, 23 N, 17 M N |
| | | 2500 | *22.0 3.* | *6* | *1.1* | 26 N, 21 N, 19 N |
| | | 1250 | *24.7 2.* | *5* | *1.2* | 25 N, 27 N, 22 N |
| | | 625 | *25.3 6.* | *1* | *1.3* | 32 N, 24 N, 20 N |
| | | 313 | *21.0 2.* | *0* | *1.1* | 19 N, 23 N, 21 N |
| | **Dimethyl Sulfoxide** | | *20.0 1.* | *0* | | 20 N, 21 N, 19 M N |
| **TA100** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *126.0 8.* | *7* | *1.0* | 121 N, 136 N, 121 N |
| | | 2500 | *128.3 8.* | *1* | *1.0* | 127 N, 137 N, 121 N |
| | | 1250 | *128.0 3.* | *6* | *1.0* | 129 N, 124 N, 131 N |
| | | 625 | *134.0 2.* | *6* | *1.1* | 132 N, 137 N, 133 N |
| | | 313 | *130.0 5.* | *3* | *1.1* | 124 N, 134 N, 132 N |
| | **Dimethyl Sulfoxide** | | *123.7 9.* | *7* | | 113 M N, 132 M N, 126 M N |
| **TA1535** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *11.7 3.* | *5* | *0.8* | 15 N, 8 M N, 12 M N |
| | | 2500 | *13.0 2.* | *6* | *0.9* | 16 N, 11 N, 12 N |
| | | 1250 | *9.0 1.* | *7* | *0.6* | 10 N, 10 N, 7 M N |
| | | 625 | *13.7 2.* | *1* | *1.0* | 16 N, 13 M N, 12 M N |
| | | 313 | *12.0 1.* | *0* | *0.8* | 12 N, 11 N, 13 N |
| | **Dimethyl Sulfoxide** | | *14.3 3.* | *8* | | 10 N, 17 M N, 16 N |
| **TA1537** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *9.3 1.* | *5* | *0.9* | 8 M N, 9 N, 11 M N |
| | | 2500 | *10.0 1.* | *7* | *1.0* | 8 N, 11 N, 11 N |
| | | 1250 | *11.7 3.* | *5* | *1.2* | 15 M N, 12 M N, 8 N |
| | | 625 | *10.0 1.* | *0* | *1.0* | 11 N, 9 N, 10 N |
| | | 313 | *11.0 4.* | *4* | *1.1* | 8 M N, 9 M N, 16 N M |
| | **Dimethyl Sulfoxide** | | *10.0 2.* | *6* | | 11 M N, 7 M N, 12 M N |

Key to Plate Postfix Codes

| | |
|---|---|
| N | Normal background bacterial lawn |
| M | Plate counted manually |

## Table 5 (cont.)
## Confirmatory Mutagenicity Assay Results with S9

Study No.: 8236018
Trial No.: 8236018-C1 Date Plated: 10/15/2010
Plating Method: Pre-incubation assay

| Strain | Compound | Dose level (µg/plate) | Mean revertants per plate | SD | Ratio treated/ vehicle | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **WP2*uvr*A** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *18.3 2.* | *5* | *0.8* | 18 N, 21 N, 16 N |
| | | 2500 | *21.0 3.* | *0* | *1.0* | 24 M N, 21 N, 18 M N |
| | | 1250 | *24.0 1.* | *0* | *1.1* | 23 M N, 24 N, 25 M N |
| | | 625 | *22.3 5.* | *5* | *1.0* | 28 N, 17 N, 22 N |
| | | 313 | *18.7 2.* | *5* | *0.9* | 16 N, 21 N, 19 N |
| | **Dimethyl Sulfoxide** | | *21.7 6.* | *0* | | 21 M N, 28 M N, 16 N |
| **TA98** | **BP** | 2.5 | *492.7 38* | *.2* | *24.6* | 536 N, 464 N, 478 N |
| **TA100** | **2A A** | 2.5 | *1957.0 9.* | *2* | *15.8* | 1947 N, 1965 N, 1959 N |
| **TA1535** | **2 AA** | 2.5 | *216.3 21* | *.5* | *15.1* | 218 N, 194 N, 237 N |
| **TA1537** | **2 AA** | 2.5 | *146.3 6.* | *1* | *14.6* | 145 N, 141 N, 153 N |
| **WP2*uvr*A** | **2A A** | 25.0 | *588.7 42* | *.6* | *27.2* | 548 N, 585 N, 633 N |

| Key to Positive Controls | | Key to Plate Postfix Codes | |
|---|---|---|---|
| BP | Benzo{a}pyrene | N | Normal background bacterial lawn |
| 2AA | 2-aminoanthracene | M | Plate counted manually |

## Table 6
## Confirmatory Mutagenicity Assay Results without S9

Study No.: 8236018
Trial No.: 8236018-C1 Date Plated: 10/15/2010
Plating Method: Pre-incubation assay

| Strain | Compound | Dose level (µg/plate) | Mean revertants per plate | SD | Ratio treated/ vehicle | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| TA98 | N-ACETYL-L-TYROSINAMIDE | 5000 | 12.7 3. | 1 | 0.8 | 16 N, 10 N, 12 N |
| | | 2500 | 11.3 3. | 8 | 0.7 | 14 M N, 7 M N, 13 N |
| | | 1250 | 10.3 3. | 1 | 0.6 | 7 N, 13 N, 11 N |
| | | 625 | 12.0 1. | 0 | 0.8 | 13 N, 11 M N, 12 M N |
| | | 313 | 13.3 3. | 2 | 0.8 | 11 M N, 17 M N, 12 N |
| D | imethyl Sulfoxide | | 16.0 2. | 6 | | 15 M N, 14 M N, 19 M N |
| TA100 | N-ACETYL-L-TYROSINAMIDE | 5000 | 106.0 3. | 0 | 1.0 | 106 N, 103 M N, 109 N |
| | | 2500 | 109.0 12 | .2 | 1.0 | 95 N, 117 N, 115 N |
| | | 1250 | 100.3 7. | 6 | 0.9 | 95 N, 109 N, 97 N |
| | | 625 | 88.3 5. | 5 | 0.8 | 88 M N, 83 N, 94 N |
| | | 313 | 97.0 9. | 8 | 0.9 | 89 M N, 94 M N, 108 N |
| D | imethyl Sulfoxide | | 106.3 3. | 8 | | 109 N, 108 N, 102 N |
| TA1535 | N-ACETYL-L-TYROSINAMIDE | 5000 | 8.7 6. | 0 | 0.7 | 8 N, 15 M N, 3 N |
| | | 2500 | 8.7 4. | 2 | 0.7 | 4 N, 12 N, 10 M N |
| | | 1250 | 12.7 2. | 3 | 1.0 | 14 M N, 14 M N, 10 N |
| | | 625 | 12.3 1. | 2 | 0.9 | 11 M N, 13 M N, 13 M N |
| | | 313 | 8.7 1. | 5 | 0.7 | 10 N, 7 N, 9 M N |
| D | imethyl Sulfoxide | | 13.0 2. | 0 | | 15 N, 13 M N, 11 N |
| TA1537 | N-ACETYL-L-TYROSINAMIDE | 5000 | 4.3 0. | 6 | 0.8 | 4 M N, 5 N, 4 N |
| | | 2500 | 6.0 1. | 7 | 1.1 | 8 N, 5 M N, 5 N |
| | | 1250 | 8.0 4. | 0 | 1.4 | 4 N, 12 N, 8 M N |
| | | 625 | 6.7 2. | 1 | 1.2 | 9 N, 5 N, 6 N |
| | | 313 | 4.7 1. | 2 | 0.8 | 4 M N, 4 M N, 6 M N |
| D | imethyl Sulfoxide | | 5.7 0. | 6 | | 6 N, 6 N, 5 N |

Key to Plate Postfix Codes

| | |
|---|---|
| N | Normal background bacterial lawn |
| M | Plate counted manually |

## Table 6 (cont.)
## Confirmatory Mutagenicity Assay Results without S9

Study No.: 8236018
Trial No.: 8236018-C1
Plating Method: Pre-incubation assay

Date Plated: 10/15/2010

| Strain | Compound | Dose level (µg/plate) | Mean revertants per plate | SD | Ratio treated/ vehicle | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **WP2*uvr*A** | **N-ACETYL-L-TYROSINAMIDE** | 5000 | *19.3 2.* | *3* | *1.0* | 18 N, 22 N, 18 M N |
| | | 2500 | *13.7 2.* | *1* | *0.7* | 13 N, 12 M N, 16 N |
| | | 1250 | *17.7 0.* | *6* | *0.9* | 18 N, 17 N, 18 M N |
| | | 625 | *14.3 4.* | *7* | *0.8* | 16 N, 9 M N, 18 M N |
| | | 313 | *14.7 4.* | *2* | *0.8* | 10 N, 18 N, 16 M N |
| | **Dimethyl Sulfoxide** | | *19.0 1.* | *0* | | 18 N, 19 N, 20 N |
| **TA98** | **2NF** | 1.0 | *505.7 33* | *.5* | *31.6* | 473 N, 504 N, 540 N |
| **TA100** | **SA** | 2.0 | *1317.0 48* | *.1* | *12.4* | 1358 N, 1264 N, 1329 N |
| **TA1535** | **SA** | 2.0 | *990.3 36* | *.5* | *76.2* | 992 N, 1026 N, 953 N |
| **TA1537** | **ICR** | 2.0 | *834.3 46* | *.4* | *147.2* | 824 N, 794 N, 885 N |
| **WP2*uvr*A** | **4NQO** | 0.4 | *710.3 54* | *.6* | *37.4* | 661 N, 701 N, 769 N |

| Key to Positive Controls | |
|---|---|
| 2NF | 2-nitrofluorene |
| SA | sodium azide |
| ICR | ICR-191 |
| 4NQO | 4-nitroquinoline-N-oxide |

| Key to Plate Postfix Codes | |
|---|---|
| N | Normal background bacterial lawn |
| M | Plate counted manually |

[redacted]

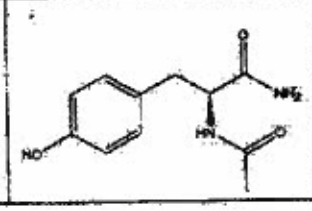
# APPENDICES

[redacted]

**Appendix 2**
**Certificate of Analysis**

[redacted]

# Certificate of Analysis

| Product Name | N-Acetyl-L-tyrosinamide | | |
|---|---|---|---|
| Structure |  | Catalog Number | |
| | | CAS Number | |
| | | Molecular Formula | C11H14N2O3 |
| | | Molecular Weight | 222.24 |

**Lot#: LHY3-100**

| Items | Analysis Results |
|---|---|
| Physical Appearance | White solid |
| Assay | 99.6% (HPLC) |
| pH value (24°C) | 6.0* |
| $[\alpha]^{18}_{D}$ | +55.3° (c=0.8, H2O) |
| TLC | One spot |
| $^{1}$H NMR | Conforms to structure |

*For pH measurement, 1g of sample was dissolved in 10mL of mixture of DMSO and H2O (7:3) to prepare the solution.

[redacted]

# Final Report

| | |
|---|---|
| Study Title | Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes |
| Test Article | N-acetyl-L-tyrosinamide |
| Author | [redacted] |
| Sponsor | [redacted] |
| Test Facility | [redacted] |
| [redacted] Study Number | 8239860 |
| [redacted] Client Code | 1001306 |
| Genetic Toxicology Protocol Modifier | 449OECD, Edition 5 |
| Report Issued | 14 March 2011 |
| Page Number | 1 of 57 |

[redacted]

## COMPLIANCE STATEMENT

**Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes**

I, the undersigned, hereby declare that the work was performed under my direction and that the findings provide a true and accurate record of the results obtained.

The study was conducted in accordance with the agreed protocol, unless otherwise stated, and the study objectives were achieved.

Except as noted below, all aspects of this study were in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58; the Organisation for Economic Co-operation and Development Principles of Good Laboratory Practice, ENV/MC/CHEM(98)17; and any applicable amendments.

Exceptions:

1) Documentation of the stability, purity, and/or characterization of the test article was not provided by the Sponsor;

   Study Director Impact Statement: The impact of not having documentation of the stability, purity and/or characterization of the test article cannot be fully evaluated at this time without additional information.

2) The stability, homogeneity, and/or concentration of the dosing preparations were not analyzed.

   Study Director Impact Statement: The impact of not having verification of the stability, homogeneity, and/or concentration of the dosing formulations cannot be fully evaluated at this time without additional information.

[redacted]

14 March 2011
Date

[redacted]

## QUALITY ASSURANCE STATEMENT

### Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes

This report has been reviewed by the Quality Assurance Unit of [redacted] Inc. and accurately reflects the raw data. The following study specific inspections were conducted and findings reported to the study director (SD) and associated management.

| Inspection Dates | | | Date Reported to SD and SD |
|---|---|---|---|
| From | To | Phase | Management |
| 01 Dec 2010 | 01 Dec 2010 | Protocol Review - [redacted] | 01 Dec 2010 |
| 01 Dec 2010 | 01 Dec 2010 | Test Article Administration | 01 Dec 2010 |
| 07 Feb 2011 | 07 Feb 2011 | Protocol Amendment Review - [redacted] | 07 Feb 2011 |
| 08 Feb 2011 | 09 Feb 2011 | Draft Report and Data Review | 10 Feb 2011 |
| 17 Feb 2011 | 17 Feb 2011 | Protocol Amendment Review - [redacted] | 17 Feb 2011 |
| 07 Mar 2011 | 07 Mar 2011 | Final Report Review | 07 Mar 2011 |

[redacted]

14 MAR 2011
Date

[redacted]

## KEY PERSONNEL

### Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes

| | |
|---|---|
| Sponsor's Authorized Representative | [redacted] |
| Study Director | [redacted] |
| Study Toxicologist | [redacted] |
| Laboratory Supervisor | [redacted] |

## CONTENTS

## ABSTRACT

The objective of this *in vitro* assay was to evaluate the ability of N-acetyl-L-tyrosinamide to cause structural chromosomal aberrations in cultured human peripheral blood lymphocytes without and with an exogenous metabolic activation system.

Dimethylsulfoxide (DMSO) was the vehicle for the study. The highest concentration tested in the assay was 2230 µg/mL, which was approximately 10 mM of N-acetyl-L-tyrosinamide (molecular weight 222.24). The stock formulations and their dilutions were dosed using a dosing volume of 1% (10 µL/mL) and the vehicle control cultures were treated with 10 µL/mL of DMSO.

In the initial chromosomal aberrations assay, the treatment period was for 3 hours without and with metabolic activation and the cultures were harvested ~22 hours from the initiation of treatment. Concentrations of 397, 529, 706, 941, 1254, 1673, and 2230 µg/mL were tested without and with metabolic activation. Cultures treated with concentrations of 1254, 1673, and 2230 µg/mL with and without metabolic activation were analyzed for chromosomal aberrations. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed.

In the confirmatory chromosomal aberrations assay, the treatment period was for ~22 hours without metabolic activation and 3 hours with metabolic activation and the cultures were harvested ~22 hours from the initiation of treatment. Concentrations of 150, 300, 600, 900, 1200, 1600, 2000, and 2230 µg/mL were tested without metabolic activation and 900, 1200, 1600, 2000, and 2230 µg/mL were tested with metabolic activation. Cultures treated with concentrations of 1600, 2000, and 2230 µg/mL without and with metabolic activation were analyzed for chromosomal aberrations. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed.

The vehicle control cultures were in the historical control range for cells with chromosomal aberrations and the positive control cultures had significant increase in cells with chromosomal aberrations as compared with the vehicle control cultures. The high doses selected for analysis was 2230 µg/mL, which was approximately 10 mM of N-acetyl-L-tyrosinamide (molecular weight 222.24).

The test article, N-acetyl-L-tyrosinamide, was considered negative for inducing chromosomal aberrations in cultured human lymphocytes without and with metabolic activation.

[redacted]

## STUDY CONDUCT

### Objective

The objective of this *in vitro* assay was to evaluate the ability of N-acetyl-L-tyrosinamide to cause structural chromosomal aberrations in cultured human lymphocytes without and with an exogenous metabolic activation system. The assay design was based on OECD Guideline 473, updated and adopted 21 July 1997.

### Study Timetable

| | |
|---|---|
| Study Initiation Date | 24 November 2010 |
| Experimental Start Date | 29 November 2010 |
| Experimental Completion Date | 17 January 2011 |
| Study Completion Date | 14 March 2011 |

### Protocol Adherence

This study was conducted in accordance with the protocol and amendment (Appendix 1).

### Major Computer Systems

| System[a] | Application Function |
|---|---|
| EMCS | Monitors and documents facility storage conditions (e.g., constant room temperatures and humidity levels) |
| EMCSDR | Transfers data from EMCS for reporting purposes |
| REES environmental monitoring system | Monitors and documents facility storage conditions (e.g., refrigerators, freezers, and incubator levels) |
| Program Trend | Statistical analysis |
| MTTS | Test article accessioning and dispensing |

a All version numbers of the applications are maintained by [redacted] Definitions for the acronyms can be found in the Glossary.

### Record Retention

The raw data, documentation, specimens, the protocol, and final report for this study will be stored in the [redacted] for at least 1 year after report finalization. The [redacted] staff will contact the sponsor after at least 1 year following report finalization to determine disposition of the archived materials (except for raw data on durable media, study correspondence, the protocol, and final report which will be kept by [redacted]). The sponsor will then provide instructions to retain the materials at [redacted] authorize the transport of the materials to their site (or that of their designee), or request destruction on their behalf if regulatory retention requirements have been satisfied.

## TEST AND CONTROL ARTICLES

The test article was supplied by the Sponsor as a white, crystalline powder on 19 November 2010 and identified as follows.

| Test article | Lot No. | Storage | Purity | Expiration Date |
|---|---|---|---|---|
| N-acetyl-L-tyrosinamide LHY3-100 | | Room Temperature | NP | NP |

NP = Not provided

In the assays without metabolic activation, vehicle controls were cultures containing DMSO, the vehicle for the test article, at 10.0 µL/mL, the highest concentration used in test cultures. In the assays with metabolic activation, vehicle controls were the same as described in the assays without metabolic activation but with the S9 and energy producing system (S9 activation mix) included. The vehicle control article was supplied as follows.

| Control Article | CAS No. | Supplier | Lot No. | Purity | Expiration Date |
|---|---|---|---|---|---|
| Dimethylsulfoxide (DMSO) | 67-68-5 | Sigma Aldrich | 17496KM | >99.99% | 17 Nov 2013<br>08 Dec 2013 |

The positive control agents which were used in the assays were mitomycin C (MMC) for the assays without metabolic activation and cyclophosphamide (CP) in the assays with metabolic activation. Mitomycin C is a clastogen that does not require metabolic activation. Cyclophosphamide does not act directly but must be converted to active intermediates by microsomal enzymes. In the chromosomal aberrations assays, three concentrations of MMC (0.750, 1.00, and 1.50 µg/mL, for the 3-hour treatment; 0.200, 0.300, and 0.400 µg/mL, for the ~22-hour treatment) and CP (20.0, 25.0, and 40.0 µg/mL) were used to induce chromosomal aberrations. Both MMC and CP were dissolved in sterile, deionized water. The positive control articles were supplied as follows.

| Control Article | CAS No. | Supplier | Lot No. |
|---|---|---|---|
| Mitomycin C (MMC) | 50-07-7 | Sigma-Aldrich | 010M0665 |
| Cyclophosphamide (CP) | 6055-19-2 | Sigma-Aldrich | 068K1131 |

Information on synthesis methods, stability, purity, composition, or other characteristics that define the test article and control article is on file with the Sponsor or the respective manufacturer.

## METABOLIC ACTIVATION SYSTEM

The *in vitro* metabolic activation system (Maron and Ames, 1983) consisted of a rat liver post-mitochondrial fraction (S9) and an energy-producing system (NADP plus isocitric acid). Various hepatic P450 isoenzyme levels are increased by treatment of the rats with Aroclor™ 1254 (single concentration of 500 mg/kg) which were sacrificed 5 days later (Molecular Toxicology, Inc., Lot No. 2502). The S9 fraction, prepared in potassium chloride, was retained frozen at ≤-60°C until use.

### S9 Activation Mix

Aliquots of S9 were thawed immediately before use and added to the other components to form the activation system described as follows:

**S9 Activation Mix**

| Component | Amount |
|---|---|
| NADP (sodium salt) | 1.5 mg/mL (1.8 mM) |
| Isocitric acid | 2.7 mg/mL (10.5 mM) |
| Homogenate (S9 fraction) | 15.0 µL/mL* (1.5%) |

* This concentration of rat S9, obtained from Molecular Toxicology Inc., Boone, NC, has consistently caused CP to be highly clastogenic for many different lots.

## TEST SYSTEM

### Test System Rationale

Aberrations are a consequence of failure or mistakes in repair processes such that breaks either do not rejoin or rejoin in abnormal configurations (Evans, 1962, 1976). Descriptions of the types of aberrations are provided in this report. Structural aberrations may be of two types, chromosome or chromatid:

***Chromosome aberration***

Structural chromosome damage expressed as breakage, or breakage followed by reunion, of both sister chromatids at an identical site.

***Chromatid aberration***

Structural chromosome damage expressed as breakage of single chromatids or breakage followed by reunion between chromatids. This is the most common type of structural aberration.

Most known chemical clastogens (chromosome-breaking agents) require a period of DNA synthesis to convert initial DNA damage into chromosome alterations that become visible at mitosis. The lymphocytes in blood do not usually divide, but they were stimulated to divide in cultures by exposure to phytohemagglutinin (PHA-M). At predetermined intervals after exposure to the test article, the lymphocytes were treated with a metaphase-arresting article, Colcemid®, then harvested and stained, and

metaphase cells were analyzed microscopically for the presence of chromosomal aberrations.

Many mutagenic chemicals do not act directly on DNA but do so after being converted to active intermediates by enzymes found in liver. Human lymphocytes have only a limited capacity to metabolize some test articles, so an exogenous metabolic activation system (rat liver S9 homogenate) was included with a series of treatments to enhance the degree of conversion and the ability of the assay to detect clastogenic, metabolic intermediates.

Numerical aberrations (a change in the number of chromosomes from the normal number of 46 for human lymphocytes) were not determined. However, the occurrence of polyploidy or endoreduplication, which were scored, might indicate that the test article had the potential to induce numerical aberrations.

## EXPERIMENTAL DESIGN

### Test System

Human venous blood from healthy, adult donors (nonsmokers without a history of radiotherapy, chemotherapy, or drug usage, and lacking current viral infections) was drawn into sterile, heparinized "vacutainers". Whole blood cultures were initiated in 15 mL centrifuge tubes by adding ~0.6 mL of fresh heparinized blood into a sufficient volume of culture medium so that the final volume was 10 mL in the assay without metabolic activation after the addition of the test article in its chosen vehicle or was 10 mL in the assay with metabolic activation after the addition of the test article in its chosen vehicle and the S9 activation mix. In the chromosomal aberration assays, duplicate cultures were used at each test article concentration, for vehicle controls, and for the positive controls. In the aberration assays, ~22-hour harvests were conducted. This harvest time corresponds to 1.5 times a cell cycle time of ~15 hours after the lymphocytes are induced to divide by the addition of phytohemagglutinin M (PHA-M) (Galloway *et al.*, 1994).

### Media and Cell Culture Conditions

Cultures in 15 mL tubes were incubated at 37°C ± 2°C in a humidified atmosphere of 5% ± 1.5% $CO_2$ in air. The medium was RPMI 1640 supplemented with HEPES buffer (25 mM), approximately 20% heat-inactivated fetal bovine serum (FBS), penicillin (100 units/mL), streptomycin (100 µg/mL), L-glutamine (2 mM) and 2% PHA-M.

## PROCEDURES

### Initial Chromosomal Aberrations Assay Without and With Metabolic Activation

For the assay without metabolic activation, 2 days after culture initiation, cells were incubated at 37 ± 2°C with the test article at predetermined concentrations, vehicle control and positive controls for 3 hours. For the assay with metabolic activation, 2 days after culture initiation, cultures were incubated at 37 ± 2°C for 3 hours in the presence of

the test article at predetermined concentrations, vehicle control and positive controls, and the S9 activation mix. The cultures were then washed with phosphate-buffered saline, refed with complete RPMI 1640 medium and incubated for the rest of the culture period up to the time of harvest with 0.1 µg/mL Colcemid® present during the last 2 ± 0.5 hours of incubation. The cultures were then harvested (~22 hours after initiation of treatment).

***Harvest Procedure***
The cultures were centrifuged, the supernatant discarded, and the cells were swollen with 75 mM KCl hypotonic solution. The cultures were then fixed with absolute methanol: glacial acetic acid (3:1, v/v) fixative.

***Slide Preparation***
Slides were prepared from the harvested cultures on glass slides and air-dried. The slides were stained with ~5% Giemsa solution, air-dried, and mounted permanently for the analysis of mitotic index and chromosomal aberrations.

**Confirmatory Chromosomal Aberrations Assay Without and With Metabolic Activation**
For the assay without metabolic activation, 2 days after culture initiation, cells were incubated at 37 ± 2°C with the test article at predetermined concentrations, vehicle and positive controls for ~22 hours with 0.1 µg/mL Colcemid® added for the last 2 ± 0.5 hours of incubation. The cultures were then harvested. For the assay with metabolic activation, 2 days after culture initiation, cultures were incubated at 37 ± 2°C for 3 hours in the presence of the test article at predetermined concentrations, vehicle and positive controls, and the S9 activation mix. The cultures were then washed with phosphate-buffered saline. The cells were refed with complete RPMI 1640 medium and incubated for the rest of the culture period up to the time of harvest with 0.1 µg/mL Colcemid® present during the last 2 ± 0.5 hours of incubation. The cultures were then harvested (~22 hours after initiation of treatment).

***Harvest Procedure and Slide Preparation***
The cultures were harvested and slides were prepared as described for the initial aberrations assay.

## DATA

**Data Evaluation**
Mitotic index was evaluated from the vehicle controls and a range of concentrations by analyzing the number of mitotic cells in 1000 cells, if possible, and the ratio expressed as a percentage of mitotic cells.

When analysis for aberrations was performed, 100 cells, if possible, from each replicate culture from three concentrations of the test article, vehicle controls, and one dose of the positive control cultures were analyzed for the different types of chromosomal aberrations (Evans, 1962, 1976). At least 25 cells were analyzed from those cultures that

had greater than 25% of cells with one or more aberrations. Cells were selected for good morphology and only cells with the number of centromeres equal to the modal number 46 ± 2 (range 44-48) were analyzed. Percent polyploidy and endoreduplication were also analyzed from the dose levels selected for analysis and controls by evaluating at least 100 metaphases, if possible, and tabulated. For control of bias, the slides will be identified by an abbreviated code which is unknown to the scorer. Cells with aberrations were recorded on the data sheets by the microscope stage location.

**Data Presentation**
Data were summarized in tables showing mitotic index and percent cells with polyploidy and endoreduplication. For structural aberrations, the aberrant cells were presented in two categories: the number and percentages of aberrant cells excluding gaps (-g), and the number and percentages of aberrant cells including gaps (+g). Historical control data are presented in Appendix 2.

**Assay Acceptance Criteria**
An assay was considered acceptable for evaluation of test results only if all of the following criteria were satisfied. The metabolic activation and nonactivation sections of the aberrations assay were independent units and would be repeated independently, as needed, to satisfy the acceptance criteria.

***Acceptable Controls***
The vehicle control cultures must contain less than ~5% cells with aberrations. The positive control result must be significantly higher ($p \leq 0.01$) than the vehicle controls.

***Acceptable High Dose***
If the aberration results are negative and there is no significant reduction (~ ≥50%) in mitotic index, the assay must include the highest applicable dose (a target dose of 10 mM or 5 mg/mL, whichever is lower) or a dose exceeding the solubility limit in culture medium.

***Acceptable Number of Doses***
The assay must include at least three analyzable concentrations.

**Assay Evaluation Criteria**
After completion of microscopic analysis for aberrations, data were decoded. The following factors are taken into account in evaluation of the test article data:

- The number and percentages of aberrant cells excluding gaps (-g).
- The number and percentages of aberrant cells including gaps (+g).
- Evidence of a dose-response relationship.

The experimental unit is the cell, and therefore the percentage of cells with structural aberrations was the basis for evaluation. Statistical analysis employed a Cochran-Armitage test for linear trend and Fisher's Exact Test (Thakur *et al.*, 1985) to

compare the percentage of cells with aberrations in treated cells to the results obtained for the vehicle controls.

Statistical analysis was also performed for cells exhibiting polyploidy and/or endoreduplication in order to detect significant ($p \leq 0.01$) increases in these events as indicators of possible induction of numerical aberrations.

***Evaluation of a Positive Response***
A test article was considered positive for inducing chromosomal aberrations if a significant increase (the difference was considered significant when $p \leq 0.01$) in the number of cells with chromosomal aberrations is observed at one or more concentrations. The linear trend test evaluated the dose responsiveness. A dose-response should be observed if a significant increase was seen at one or more concentrations.

***Evaluation of a Negative Response***
A test article was considered negative for inducing chromosomal aberrations if no significant increase was observed in the number of cells with chromosomal aberrations at any of the concentrations.

***Equivocal Evaluation***
Although most assays give clearly positive or negative results, in rare cases the data set would preclude making a definitive judgment about the activity of the test article. Results might remain equivocal or questionable regardless of the number of times the assay is repeated.

Although the evaluation criteria provided here is normally sufficient, the Study Director might use additional considerations to obtain a final evaluation of the test article based upon the Study Director's scientific judgment.

## RESULTS AND DISCUSSION

### Test Article Handling

***Solubility and Dose Determination***
The highest concentration tested in the assay, 2230 µg/mL, was approximately 10 mM of N-acetyl-L-tyrosinamide (molecular weight 222.24). The stock formulation and its dilutions were dosed using a dosing volume of 1% (10 µL/mL) and the vehicle control cultures were treated with 10 µL/mL of DMSO. The high formulation of 223 mg/mL of the test article in the vehicle, DMSO, was a colorless, transparent solution.

### Initial Chromosomal Aberrations Assay

The initial chromosomal aberrations assay was conducted testing concentrations of 397, 529, 706, 941, 1254, 1673, and 2230 µg/mL without and with metabolic activation with a 3-hour treatment and all cultures were harvested ~22 hours from the initiation of treatment.

A summary of the treatment times for the assay is as follows.

**Summary of Treatment Schedule in Hours (approximate)**

| S9Activation Mix | Test Article Added | Exposure Completed | Colcemid® Added | Harvest Started |
|---|---|---|---|---|
| Without 0 | | 3 | 20 | 22 |
| With 0 | | 3 | 20 | 22 |

In the assay without metabolic activation, mitotic index data are presented in Table 1. Chromosomal aberrations were analyzed from the cultures treated with 1254, 1673, and 2230 µg/mL (Table 2). The high dose selected for analysis, 2230 µg/mL, at a 10 mM concentration, is the high dose recommended for this assay by the OECD Testing Guidelines. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed.

In the assay with metabolic activation, mitotic index data are presented in Table 3. Chromosomal aberrations were analyzed from the cultures treated with 1254, 1673, and 2230 µg/mL (Table 4). The high dose selected for analysis, 2230 µg/mL, at a 10 mM concentration, is the high dose recommended for this assay by the OECD Testing Guidelines. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed.

**Confirmatory Chromosomal Aberrations Assay**

Based on the results of the initial assay, the confirmatory chromosomal aberrations assay was conducted testing concentrations of 150, 300, 600, 900, 1200, 1600, 2000, and 2230 µg/mL for ~22 hours without metabolic activation and 900, 1200, 1600, 2000, and 2230 µg/mL for 3 hours with metabolic activation. All cultures were harvested ~22 hours from the initiation of treatment.

A summary of the treatment times for the assay is as follows.

**Summary of Treatment Schedule in Hours (approximate)**

| S9Activation Mix | Test Article Added | Exposure Completed | Colcemid® Added | Harvest Started |
|---|---|---|---|---|
| Without 0 | | 22 | 20 | 22 |
| With 0 | | 3 | 20 | 22 |

In the assay without metabolic activation, mitotic index data are presented in Table 5. Chromosomal aberrations were analyzed from the cultures treated with 1600, 2000, and 2230 µg/mL (Table 6). The high dose selected for analysis, 2230 µg/mL, at a 10 mM concentration, is the high dose recommended for this assay by the OECD Testing

Guidelines. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed.

In the assay with metabolic activation, mitotic index data are presented in Table 7. Chromosomal aberrations were analyzed from the cultures treated with 1600, 2000, and 2230 µg/mL (Table 8). The high dose selected for analysis, 2230 µg/mL, at a 10 mM concentration, is the high dose recommended for this assay by the OECD Testing Guidelines. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed.

The vehicle control cultures were in the historical control range for cells with chromosomal aberrations and the positive control cultures had significant increase in cells with chromosomal aberrations as compared with the vehicle control cultures. The high doses selected for analysis in the assay, 2230 µg/mL, was approximately 10 mM of N-acetyl-L-tyrosinamide (molecular weight 222.24), as recommended for this assay by the OECD Testing Guidelines.

Under conditions without metabolic activation, the sensitivity of the cell cultures for induction of chromosomal aberrations is shown by the increased frequency of aberrations in the cells exposed to mitomycin C, the positive control agent. The test article, N-acetyl-L-tyrosinamide, was considered negative for inducing chromosomal aberrations, polyploidy, or endoreduplication under conditions without metabolic activation.

Under conditions with metabolic activation, the successful activation of the metabolic system is illustrated by the increased incidence of cells with chromosomal aberrations in the cultures induced with cyclophosphamide, the positive control agent. The test article, N-acetyl-L-tyrosinamide, was considered negative for inducing chromosomal aberrations, polyploidy, or endoreduplication under conditions with metabolic activation.

## CONCLUSION

The test article, N-acetyl-L-tyrosinamide, was considered negative for inducing chromosomal aberrations in cultured human lymphocytes without and with metabolic activation.

# DATA TABLES

## Table 1: Assessment of Toxicity for Chromosomal Aberrations Assay - Without Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Study No.: 8239860 Trial No.: B1 Date: 12/01/10
Test Article: N-acetyl-L-tyrosinamide

| Treatment | | | % Mitotic Index A Culture | % Mitotic Index % B Culture | Average Mitotic Index | % Mitotic Index Reduction |
|---|---|---|---|---|---|---|
| Vehicle Control | DMSO | 10.0 µL/mL | 7.4 | 6.9 | 7.2 | 0 |
| Test Article | | 397 µg/mL | --[a] | --[a] | -- | -- |
| | | 529 µg/mL | --[a] | [a] | -- | -- |
| | | 706 µg/mL | --[a] | [a] | -- | -- |
| | | 941 µg/mL | --[a] | [a] | -- | -- |
| | | 1254 µg/mL | 8.4 | 8.2 | 8.3 | 0 |
| | | 1673 µg/mL | 7.5 | 8.3 | 7.9 | 0 |
| | | 2230 µg/mL | 7.8 | 8.2 | 8.0 | 0 |

[a] Not analyzed since non-toxic dose levels were achieved.
DMSO = dimethylsulfoxide

## Table 2: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Study No.: 8239860 Trial No.: B1 Date: 12/01/10 Test Article: N-acetyl-L-tyrosinamide

| | | | | # Cells Scored for Aberrations | % Mitotic Index Reduction[a] | # Cells Scored for pp and er | # of pp Cells | # of er Cells | Judge-Ment (+/-)[b] | Numbers and Percentages of Cells Showing Structural Chromosome Aberrations | | | | | | | Judge-ment (+/-)[d] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | gaps | simple breaks | chte | chre | mab | Totals[c] -g | Totals[c] +g | |
| Controls | | | | | | | | | | | | | | | | | |
| Vehicle: | DMSO | 10.0 µL/mL | A | 100 | | 100 0 | | 0 | | 5 | | | | | 0 | 5 | |
| | | | B | 100 | | 100 0 | | 0 | | 3 | 1 | | | | 1 | 4 | |
| | | | Total | 200 | | 200 | | | | 8 | 1 | | | | 1 | 9 | |
| | | | Average | % | 0 | | 0.0 | 0.0 | | 4.0 | 0.5 | | | | 0.5 | 4.5 | |
| Positive: | MMC | 1.00 µg/mL | A | 50 | | 100 0 | | 0 | | 8 18 | | 9 | | | 24 | 28 | |
| | | | B | 50 | | 100 0 | | 0 | | 4 13 | | 10 | | 1 20 | | 23 | |
| | | | Total | 100 | | 200 | | | | 12 31 19 | | | | 1 | 44 51 | | |
| | | | Average | % | – | | 0.0 | 0.0 | - | 12.0 31. | 0 19. | 0 | | 1.0 | 44.0 51. | 0 | + |
| Test Article | | 1254 µg/mL | A | 100 | | 100 0 | | 0 | | 3 | | | | | 0 | 3 | |
| | | | B | 100 | | 100 0 | | 0 | | 4 | | | | | 0 | 4 | |
| | | | Total | 200 | | 200 | | | | 7 | | | | | 0 | 7 | |
| | | | Average | % | 0 | | 0.0 | 0.0 | - | 3.5 | | | | | 0.0 | 3.5 | - |
| | | 1673 µg/m L | A | 100 | | 100 0 | | 0 | | 3 | | | | | 0 | 3 | |
| | | | B | 100 | | 100 0 | | 0 | | 3 | | | | | 0 | 3 | |
| | | | Total | 200 | | 200 | | | | 6 | | | | | 0 | 6 | |
| | | | Average | % | 0 | | 0.0 | 0.0 | - | 3.0 | | | | | 0.0 | 3.0 | - |
| | | 2230 µg/m L | A | 100 | | 100 0 | | 0 | | 4 | | | | | 0 | 4 | |
| | | | B | 100 | | 100 0 | | 0 | | 4 | 1 | | | | 1 | 5 | |
| | | | Total | 200 | | 200 | | | | 8 | 1 | | | | 1 | 9 | |
| | | | Average | % | 0 | | 0.0 | 0.0 | - | 4.0 | 0.5 | | | | 0.5 | 4.5 | - |

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduclion as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. DMSO = dimethylsulfoxide MMC = Mitomycin C

**Table 3: Assessment of Toxicity for Chromosomal Aberrations Assay - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest**

Study No.: 8239860 Trial No.: B1 Date: 12/01/10
Test Article: N-acetyl-L-tyrosinamide

| Treatment | | | % Mitotic Index A Culture | % Mitotic Index % B Culture | Average Mitotic Index | % Mitotic Index Reduction |
|---|---|---|---|---|---|---|
| Vehicle Control | DMSO | 10.0 µL/mL | 8.8 | 9.3 | 9.1 | 0 |
| Test Article | | 397 µg/mL | --[a] | --[a] | -- -- | |
| | | 529 µg/mL | --[a] | --[a] | -- -- | |
| | | 706 µg/mL | --[a] | --[a] | -- -- | |
| | | 941 µg/mL | --[a] | --[a] | -- -- | |
| | | 1254 µg/mL | 9.8 | 9.3 | 9.6 | 0 |
| | | 1673 µg/mL | 8.9 | 9.4 | 9.2 | 0 |
| | | 2230 µg/mL | 8.4 | 9.5 | 9.0 | 1 |

[a] Not analyzed since non-toxic dose levels were achieved.
DMSO = dimethylsulfoxide

## Table 4: Chromosomal Aberrations in Human Lymphocytes - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Study No.: 8239860 Trial No.: B1 Date: 12/01/10 Test Article: N-acetyl-L-tyrosinamide

| | | | | # Cells Scored for Aberrations | % Mitotic Index Reduction[a] | # Cells Scored for pp and er | # of pp Cells | # of er Cells | Judge-Ment (+/-)[b] | Numbers and Percentages of Cells Showing Structural Chromosome Aberrations | | | | | | | Judge-ment (+/-)[d] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | gaps | simple breaks | chte | chre | mab | Totals[c] -g | Totals[c] +g | |
| Controls | | | | | | | | | | | | | | | | | |
| Vehicle: | DMSO | 10.0 µL/mL | A | 100 | | 100 0 | | 0 | | | | | | | 0 | 0 | |
| | | | B | 100 | | 100 0 | | 0 | | | | | | | 0 | 0 | |
| | | | Total | 200 | | 200 | | | | | | | | | 0 | 0 | |
| | | | Average | % | 0 | | 0.0 | 0.0 | | | | | | | 0.0 | 0.0 | |
| Positive: | CP | 25.0 µg/mL | A | 75 | | 100 0 | | 0 | | 2 15 | | 4 | | | 18 | 20 | |
| | | | B | 50 | | 100 0 | | 0 | | 4 16 | | 2 | | | 17 | 19 | |
| | | | Total | 125 | | 200 | | | | 6 | 31 | 6 | | | 35 | 39 | |
| | | | Average | % | -- | | 0.0 | 0.0 | - | 4.8 | 24.8 | 4.8 | | | 28.0 | 31.2 | + |
| Test Article | | 1254 µg/mL | A | 100 | | 100 0 | | 0 | | | | | | | 0 | 0 | |
| | | | B | 100 | | 100 0 | | 0 | | 1 | | | | | 0 | 1 | |
| | | | Total | 200 | | 200 | | | | 1 | | | | | 0 | 1 | |
| | | | Average | % | 0 | | 0.0 | 0.0 | - | 0.5 | | | | | 0.0 | 0.5 | - |
| | | 1673 µg/m L | A | 100 | | 100 0 | | 0 | | 2 | | | | | 0 | 2 | |
| | | | B | 100 | | 100 0 | | 0 | | | | | | | 0 | 0 | |
| | | | Total | 200 | | 200 | | | | 2 | | | | | 0 | 2 | |
| | | | Average | % | 0 | | 0.0 | 0.0 | - | 1.0 | | | | | 0.0 | 1.0 | - |
| | | 2230 µg/m L | A | 100 | | 100 0 | | 0 | | 1 | 1 | | | | 1 | 2 | |
| | | | B | 100 | | 100 0 | | 0 | | | 1 | | | | 1 | 1 | |
| | | | Total | 200 | | 200 | | | | 1 | 2 | | | | 2 | 3 | |
| | | | Average | % | 1 | | 0.0 | 0.0 | - | 0.5 | 1.0 | | | | 1.0 | 1.5 | - |

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. DMSO = dimethylsulfoxide CP = Cyclophosphamide

[redacted]

**Table 5: Assessment of Toxicity for Chromosomal Aberrations Assay - Without Metabolic Activation - ~22-Hour Treatment, ~22-Hour Harvest**

Study No.: 8239860 Trial No.: C1 Date: 12/29/10
Test Article: N-acetyl-L-tyrosinamide

| Treatment | | | % Mitotic Index A Culture | % Mitotic Index % B Culture | Average Mitotic Index | % Mitotic Index Reduction |
|---|---|---|---|---|---|---|
| Vehicle Control | DMSO | 10.0 µL/mL | 8.7 | 9.2 | 9.0 | 0 |
| Test Article | | 150 µg/mL | --[a] | --[a] | -- -- | |
| | | 300 µg/mL | --[a] | --[a] | -- -- | |
| | | 600 µg/mL | --[a] | --[a] | -- -- | |
| | | 900 µg/mL | --[a] | --[a] | -- -- | |
| | | 1200 µg/mL | --[a] | --[a] | -- -- | |
| | | 1600 µg/mL | 9.1 | 8.4 | 8.8 | 2 |
| | | 2000 µg/mL | 8.8 | 9.3 | 9.1 | 0 |
| | | 2230 µg/mL | 8.3 | 7.8 | 8.1 | 10 |

[a] Not analyzed since non-toxic dose levels were achieved.
DMSO = dimethylsulfoxide

## Table 6: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation - ~22-Hour Treatment, ~22-Hour Harvest

Study No.: 8239860 Trial No.: C1 Date: 12/29/10 Test Article: N-acetyl-L-tyrosinamide

| | | | | | # Cells Scored for Aberrations | % Mitotic Index Reduction[a] | # Cells Scored for pp and er | # of pp Cells | # of er Cells | Judge-Ment (+/-)[b] | Numbers and Percentages of Cells Showing Structural Chromosome Aberrations | | | | | | | Judge-ment (+/-)[d] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | simple | | | | | Totals[c] | |
| | | | | | | | | | | | gaps | breaks | chte | chre | mab | -g | +g | |
| Controls | | | | | | | | | | | | | | | | | | |
| Vehicle: | DMSO | 10.0 | µL/mL | A | 100 | | 100 0 | | 0 | | 1 | | 1 | | | 1 | 2 | |
| | | | | B | 100 | | 100 0 | | 0 | | 1 | 1 | | | | 1 | 1 | |
| | | | | Total | 200 | | 200 | | | | 2 1 1 | | | | | 2 3 | | |
| | | | | Average | % | 0 | | 0.0 0. | 0 | | 1.0 0. | 5 0. | 5 0. | 5 | | 1.0 1. | 5 | |
| Positive: | MMC | 0.300 | µg/mL | A | 25 | | 100 0 | | 0 | | 1 8 1 | | | | | 9 9 | | |
| | | | | B | 25 | | 100 0 | | 0 | | 3 11 | | 1 | | | 12 | 13 | |
| | | | | Total | 50 | | 200 | | | | 4 | 19 | 2 | | | 21 | 22 | |
| | | | | Average | % | – | | 0.0 | 0.0 | - | 8.0 | 38.0 | 4.0 | | | 42.0 | 44.0 | + |
| Test Article | | 1600 | µg/mL | A | 100 | | 100 0 | | 0 | | 4 | | | | | 0 | 4 | |
| | | | | B | 100 | | 100 0 | | 0 | | 1 | | | | | 0 | 1 | |
| | | | | Total | 200 | | 200 | | | | 5 | | | | | 0 | 5 | |
| | | | | Average | % | 2 | | 0.0 | 0.0 | - | 2.5 | | | | | 0.0 | 2.5 | - |
| | | 2000 | µg/mL | A | 100 | | 100 0 | | 0 | | 1 | 1 | | | | 1 | 2 | |
| | | | | B | 100 | | 100 1 | | 0 | | 2 | | | | | 0 | 2 | |
| | | | | Total | 200 | | 200 | | | | 3 | 1 | | | | 1 | 4 | |
| | | | | Average | % | 0 | | 0.5 0. | 0 | - | 1.5 0. | 5 | | | | 0.5 2. | 0 - | |
| | | 2230 | µg/mL | A | 100 | | 100 0 | | 0 | | 1 | | | | | 0 | 1 | |
| | | | | B | 100 | | 100 0 | | 0 | | 1 | 3 | | | | 3 | 4 | |
| | | | | Total | 200 | | 200 | | | | 2 | 3 | | | | 3 | 5 | |
| | | | | Average | % | 10 | | 0.0 0. | 0 | - | 1.0 1. | 5 | | | | 1.5 2. | 5 - | |

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. DMSO = dimethylsulfoxide MMC = Mitomycin C

## Table 7: Assessment of Toxicity for Chromosomal Aberrations Assay - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Study No.: 8239860 Trial No.: C1 Date: 12/29/10
Test Article: N-acetyl-L-tyrosinamide

| Treatment | | | | % Mitotic Index A Culture | % Mitotic Index % B Culture | Average Mitotic Index | % Mitotic Index Reduction |
|---|---|---|---|---|---|---|---|
| Vehicle Control | DMSO | 10.0 | µL/mL | 10.3 | 9.7 | 10.0 | 0 |
| Test Article | | 900 | µg/mL | --[a] | --[a] | -- -- | |
| | | 1200 | µg/mL | --[a] | --[a] | -- -- | |
| | | 1600 | µg/mL | 10.2 | 11.3 | 10.8 | 0 |
| | | 2000 | µg/mL | 9.8 | 9.1 | 9.5 | 5 |
| | | 2230 | µg/mL | 9.1 | 9.3 | 9.2 | 8 |

[a] Not analyzed since non-toxic dose levels were achieved.
DMSO = dimethylsulfoxide

## Table 8: Chromosomal Aberrations in Human Lymphocytes - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Study No.: 8239860 Trial No.: C1 Date: 12/29/10 Test Article: N-acetyl-L-tyrosinamide

| | | | | # Cells Scored for Aberrations | % Mitotic Index Reduction[a] | # Cells Scored for pp and er | # of pp Cells | # of er Cells | Judge-Ment (+/-)[b] | Numbers and Percentages of Cells Showing Structural Chromosome Aberrations | | | | | | | Judge-ment (+/-)[d] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | simple | | | | Totals[c] | | |
| | | | | | | | | | | gaps | breaks | chte | chre | mab | -g | +g | |
| Controls | | | | | | | | | | | | | | | | | |
| Vehicle: | DMSO | 10.0 µL/mL | A | 100 | | 100 0 | | 0 | | 1 | 1 | | | | 1 | 1 | |
| | | | B | 100 | | 100 0 | | 0 | | 2 | 1 | | | | 1 | 3 | |
| | | | Total | 200 | | 200 | | | | 3 | 2 | | | | 2 | 4 | |
| | | | Average | % | 0 | | 0.0 | 0.0 | | 1.5 | 1.0 | | | | 1.0 | 2.0 | |
| Positive: | CP | 25.0 µg/mL | A | 25 | | 100 0 | | 0 | | 3 7 1 | | | | | 8 | 11 | |
| | | | B | 25 | | 100 0 | | 0 | | 3 8 1 | | | | | 9 | 11 | |
| | | | Total | 50 | | 200 | | | | 6 | 15 | 2 | | | 17 | 22 | |
| | | | Average | % | – | | 0.0 | 0.0 | - | 12.0 | 30.0 | 4.0 | | | 34.0 | 44.0 | + |
| Test Article | | 1600 µg/mL | A | 100 | | 100 0 | | 0 | | 2 | | | | | 0 | 2 | |
| | | | B | 100 | | 100 0 | | 0 | | 2 | | | | | 0 | 2 | |
| | | | Total | 200 | | 200 | | | | 4 | | | | | 0 | 4 | |
| | | | Average | % | 0 | | 0.0 | 0.0 | - | 2.0 | | | | | 0.0 | 2.0 | - |
| | | 2000 µg/m L | A | 100 | | 100 0 | | 0 | | 2 | | | | | 0 | 2 | |
| | | | B | 100 | | 100 1 | | 0 | | 1 | 1 | | | | 1 | 2 | |
| | | | Total | 200 | | 200 | | | | 3 | 1 | | | | 1 | 4 | |
| | | | Average | % | 5 | | 0.5 | 0.0 | - | 1.5 | 0.5 | | | | 0.5 | 2.0 | - |
| | | 2230 µg/m L | A | 100 | | 100 0 | | 0 | | 2 | | | | | 0 | 2 | |
| | | | B | 100 | | 100 0 | | 0 | | | 1 | | | | 1 | 1 | |
| | | | Total | 200 | | 200 | | | | 2 | 1 | | | | 1 | 3 | |
| | | | Average | % | 8 | | 0.0 | 0.0 | - | 1.0 | 0.5 | | | | 0.5 | 1.5 | - |

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. DMSO = dimethylsulfoxide CP = Cyclophosphamide

[redacted]

# APPENDICES

FINAL REPORT

Study Title

# TOPICAL APPLICATION OCULAR IRRITATION SCREENING ASSAY USING THE EPIOCULAR™ HUMAN CELL CONSTRUCT

Test Articles

[redacted]

1018047-007 n-acetyl tyrosinamide (1.25%)

[redacted]

Authors

[redacted]

Study Completion Date

25 April 2011

Performing Laboratory

[redacted]

Study Number

[redacted]

Laboratory Project Number

[redacted]

## TABLE OF CONTENTS

## SIGNATURE PAGE

## TOPICAL APPLICATION OCULAR IRRITATION SCREENING ASSAY USING THE EPIOCULAR™ HUMAN CELL CONSTRUCT

| | |
|---|---|
| Initiation Date: | 27 March 2011 |
| Completion Date: | 25 April 2011 |
| Sponsor: | [redacted] |
| Sponsor's Representative: | [redacted] |
| Testing Facility: | [redacted] |
| Archive Location: | |
| Study Director: | [redacted] |
| Laboratory Manager: | |
| Laboratory Supervisor: | |

## TEST ARTICLE RECEIPT

| [redacted]Test Article Number | Sponsor's Designation | Physical Description | Receipt Date | Storage Conditions* |
|---|---|---|---|---|
| [redacted] | | | | |
| 11AC59 | 1018047-007 | clear colorless non-viscous liquid | 22 March 2011 | room temperature |
| [redacted] | | | | |

* - Protected from exposure to light

# TOPICAL APPLICATION OCULAR IRRITATION SCREENING ASSAY USING THE EPIOCULAR™ HUMAN CELL CONSTRUCT

## INTRODUCTION

The EpiOcular™ Human Cell Construct was used to assess the potential ocular irritation of the test materials. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) conversion assay, which measures the NAD(P)H-dependent microsomal enzyme reduction of MTT (and to a lesser extent, the succinate dehydrogenase reduction of MTT) to a blue formazan precipitate, was used to assess cellular metabolism after exposure to each test article for various exposure times[1]. The duration of exposure resulting in a 50% decrease in MTT conversion in test article-treated EpiOcular™ human cell constructs, relative to control cultures, was determined ($ET_{50}$).

The purpose of this study was to evaluate the potential toxicity of the test articles, supplied by [redacted] as measured by the conversion of MTT by EpiOcular™ human cell constructs after exposure to a test article for various exposure times. The laboratory phase of the study was conducted from 28 March 2011 to 31 March 2011 at the [redacted] The test articles were tested in a screening assay to determine the duration of exposure that resulted in an $ET_{50}$ endpoint.

---

[1] Berridge, M.V., Tan, A.S., McCoy, K.D., Wang, R. (1996) The Biochemical and Cellular Basis of Cell Proliferation Assays That Use Tetrazolium Salts. **Biochemica** 4:14-19.

## MATERIALS AND METHODS

Receipt of the EpiOcular™ Human Cell Construct Model

Upon receipt of the EpiOcular™ Human Cell Construct Kit (MatTek Corporation), the solutions were stored as indicated by the manufacturer. The EpiOcular™ human cell constructs were stored at 2-8°C until used. On the day of dosing, EpiOcular™ Assay Medium was warmed to approximately 37°C. Nine-tenths mL of Assay Medium were aliquoted into the appropriate wells of 6-well plates. The six-well plates were labeled to indicate test article and exposure time. The constructs were inspected for air bubbles between the agarose gel and Millicell® insert prior to opening the sealed package. Cultures with air bubbles covering greater than 50% of the Millicell® area were not used. The 24-well shipping containers were removed from the plastic bag and their surfaces were disinfected with 70% ethanol. The EpiOcular™ human cell constructs were transferred aseptically into the 6-well plates. The constructs were then incubated at 37±1°C in a humidified atmosphere of 5±1% $CO_2$ in air (standard culture conditions) for at least one hour. The medium was then aspirated and 0.9 mL of fresh Assay Medium were added to each assay well below the EpiOcular™ human cell construct. The plates were returned to the incubator until treatment was initiated.

Test Article Preparation

As instructed by the Sponsor, each test article was administered to the test system without dilution.

Assessment of Direct Test Article Reduction of MTT

Each test article was added to a 1.0 mg/mL MTT (Sigma) solution in warm Dulbecco's Modified Eagle's Medium (DMEM) containing 2 mM L-glutamine (MTT Addition Medium) to assess its ability to directly reduce MTT. Approximately 100 µL of each test article were added to 1 mL of the MTT solution and the mixtures were incubated in the dark at standard culture conditions for approximately one hour. A negative control, 100 µL of sterile, deionized water (Quality Biological), was tested concurrently. If the MTT solution color turned blue/purple, the test article was presumed to have reduced the MTT.

The test articles, [redacted] 1018047-007, [redacted] were not observed to directly reduce MTT in the absence of viable cells.

pH Determination

The pH of each test article was measured using pH paper (EMD Chemicals Inc.). Initially, each test article was added to pH paper with a 0-14 pH range in 1.0 pH unit increments to approximate a narrow pH range. Next, each test article was added to pH paper with a narrower range of 0-6 or 5-10 pH units with 0.5 pH unit increments, to obtain a more accurate pH value. The pH values obtained from the narrower range pH paper are presented in Table 1.

MTT Assay

The EpiOcular™ cultures were treated in duplicate with the test articles, [redacted] and 1018047-007, at four exposure times of 4, 8, 16, and 24 hours. The EpiOcular™ cultures were treated in duplicate with the test articles, [redacted] at five exposure times of 0.17, 1, 4, 8, and 24 hours. One hundred microliters of each test article were applied to each EpiOcular™ human cell construct. Duplicate cultures of the negative control (exposure time control), 100 µL of sterile, deionized water (Quality Biological), were exposed for 0.25, 4, 8, and 24 hours. Duplicate cultures of the positive control, 100 µL of 0.3% Triton®-X-100 (Fisher), were exposed for 15 and 45 minutes. The exposed cultures were then incubated for the appropriate amount of time at standard culture conditions.

After the appropriate exposure time, the EpiOcular™ cultures were extensively rinsed with Calcium and Magnesium-Free Dulbecco's Phosphate Buffered Saline ($Ca^{++}Mg^{++}$Free-DPBS) and the wash medium was decanted. After rinsing, the tissue was transferred to 5 mL of Assay Medium for a 10 to 20 minute soak at room temperature to remove any test article absorbed into the tissue. A 1.0 mg/mL solution of MTT in warm MTT Addition Medium was prepared no more than 2 hours before use. Three-tenths mL of MTT solution were added to designated wells in a prelabeled 24-well plate. The EpiOcular™ constructs were transferred to the appropriate wells after rinsing with $Ca^{++}Mg^{++}$Free-DPBS. The trays were incubated for approximately three hours at standard culture conditions.

After the incubation period with MTT solution, the EpiOcular™ cultures were blotted on absorbent paper, cleared of excess liquid, and transferred to a prelabeled 24-well plate containing 2.0 mL of isopropanol in each designated well. The plates were sealed with parafilm and stored in the refrigerator (2-8°C) until the last exposure time was harvested. The plates were then shaken for at least two hours at room temperature.

At the end of the extraction period, the liquid within the Millicell® inserts was decanted into the well from which the Millicell® insert was taken. The extract solution was mixed and 200 µL were transferred to the appropriate wells of a 96-well plate. Two hundred µL of isopropanol were added to the two wells designated as the blanks. The absorbance at 550 nm ($OD_{550}$) of each well was measured with a Molecular Devices Vmax plate reader.

Presentation of Data

The raw absorbance values were captured. The mean $OD_{550}$ value of the blank wells was calculated. The corrected mean $OD_{550}$ values of the negative controls were determined by subtracting the mean $OD_{550}$ value of the blank wells from their mean $OD_{550}$ values. The corrected $OD_{550}$ values of the individual test article exposure times and the positive control exposure times were determined by subtracting the mean $OD_{550}$ value of the blank wells from their $OD_{550}$ values. All calculations were performed using an Excel spreadsheet. The following percent of control calculations were made:

$$\% \text{ of Control} = \frac{\text{corrected } OD_{550} \text{ of Test Article or Positive Control Exposure Time}}{\text{appropriate corrected mean } OD_{550} \text{ Negative Control}} \times 100$$

Exposure time response curves were plotted with the % of Control on the ordinate and the test article or positive control exposure time on the abscissa. The $ET_{50}$ value was interpolated from each plot. To determine the $ET_{50}$, two consecutive points were selected, where one exposure time resulted in a relative survival greater than 50%, and one exposure time resulted in less than 50% survival. Two select points were used to determine the slope and the y-intercept for the equation $y=m(x) + b$. Finally, to determine the $ET_{50}$, the equation was solved for $y=50$. When all of the exposure time points showed greater than 50% survival, the $ET_{50}$ value was presented as greater than the longest test article exposure time.

Criteria for a Valid Test

The assay results were accepted when: 1) The $ET_{50}$ value of the positive control fell within two standard deviations of the historical mean (updated every three months) and 2) The corrected mean $OD_{550}$ value for the minimum negative control exposure time was within 20% of the corrected mean $OD_{550}$ value for the maximum negative control exposure time (up to 4 hours).

## RESULTS AND DISCUSSION

MTT Assay

The EpiOcular™ cultures were treated in duplicate with the test articles [redacted] and 1018047-007, at four exposure times of 4, 8, 16, and 24 hours. The EpiOcular™ cultures were treated in duplicate with the test articles, [redacted] at five exposure times of 0.17, 1, 4, 8, and 24 hours. The negative control was exposed in duplicate for 0.25, 4, 8, and 24 hours. Table 1 summarizes the $ET_{50}$ results of the Topical Application Ocular Irritation Screening Assay Using the EpiOcular™ Human Cell Construct for the test articles and the positive control, 0.3% Triton®-X-100. The exposure time response curves are included in Appendix B. Since the positive control fell within two standard deviations of the historical mean (15.7 – 39.0 minutes), and the corrected mean $OD_{550}$ value for the minimum negative control exposure time (1.236) was within 20% of the corrected mean $OD_{550}$ value for the maximum negative control exposure time (up to 4 hours) (1.338), the assay results were accepted.

The test articles, [redacted] 1018047-007, [redacted] were not observed to directly reduce MTT in the absence of viable cells.

**Table 1**

| Assay Date | [redacted] | Sponsor's Designation | Conc. | $ET_{50}$ (hours) | pH |
|---|---|---|---|---|---|
| 30 March 2011 | [redacted] | | | | |
| | 11AC59 | 1018047-007 | Neat | > 24 | 5.0 |
| | [redacted] | | | | |
| | Positive Control | 0.3% Triton®-X-100 | NA | 30.0 minutes | NA |

NA - Not Applicable

[redacted]

# FINAL REPORT dated June 1, 2011

[redacted] #7264

# Sample: Clear Liquid coded 1018047-011

[redacted]

n-acetyl tyrosinamide (1%)

[redacted]

**Title:** An Evaluation of the Contact-Sensitization Potential of a Topical Coded Product in Human Skin by means of the Maximization Assay

**Sponsor:** [redacted]

**Principal Investigator:** [redacted]

**Testing Facility:** [redacted]

[redacted]

# FINAL REPORT

**STUDY TITLE**:

An assessment of the contact-sensitizing potential of a coded topically-applied test agent using a Human Maximization Assay.

[redacted]

**GUIDELINES FOR THE CONDUCT OF THE STUDY**:

All procedures were conducted in compliance with the regulations of the Food and Drug Administration (FDA) (21 CFR 50, 56, 312) ICH-GCP Consolidated Guidelines, May 9, 1997 Federal Register) and in accordance [redacted]

**STUDY OBJECTIVE**:

The objective of this study was to assess the skin sensitizing potential of any preparation designed for topical use by means of the Maximization Test (see references #1 and #2).

**DESIGN RATIONALE**:

A repeat insult patch test wherein the test product was applied under an occlusive dressing to an SLS (sodium lauryl sulfate) pre-treated site on the upper outer arm or back repeatedly to the same designated area for five 48-hour induction periods followed 7-10 days later by a single challenge to a naïve skin site on the opposite outer arm or the opposite side of the back.

[redacted]

**SPONSOR STUDY**:

[redacted] Submission Form dated April 12, 2011

[redacted]

**INFORMED CONSENT**:

Prior to acceptance into the study, each subject was informed by the Investigator or his designee of the nature and purpose of the study, possible side-effects and any other relevant information. The study procedures and possible risks and discomfort were explained to each panelist during the interview using popular understandable language and terms, and the panelists were encouraged to ask questions regarding the study. Each interviewed panelist who qualified was then asked to read and sign the consent form prior to enrollment. Copies of all consent forms are on file [redacted]

**CONDUCTION DATES**:

This study was conducted between April 18, 2011 and May 20, 2011.

**TEST MATERIAL**:

The test product, supplied by the sponsor, was a Clear Liquid and coded 1018047-011. One jar of the Clear Liquid was supplied for testing purposes. The test product was tested as supplied viz. neat.

**TEST PRODUCT ACCOUNTABILITY**:

The test sample was received in good condition by our Quality Assurance Department. The test material was checked for (1) amount (2) product number or code (3) material container etc. The material was individually listed on a special sheet (drug/test product log form) signed by the receiver, the laboratory supervisor and the investigator (physician). The test sample was stored under ambient conditions in an inaccessible location under the supervision of the investigator.

**DISPOSITION OF REMAINING CLINICAL SUPPLIES**:

All remaining test material(s) will be disposed of in accordance with applicable governmental regulations following completion of the study and submission of the final written report to the Sponsor.

**PANEL COMPOSITION**:

Healthy, adult volunteers over the age of 18 years were recruited for this study. Panelists had no blemishes, excess hair or other marks on their upper outer arms or back that would obscure grading of the test site. Both male and female panelists were eligible. None of the subjects had a medical or dermatological illness and none were sensitive to sunscreens or to topical preparations and/or cosmetics. A completed subject was a subject who satisfied the admission criteria and who completed the scheduled study procedures.

**Inclusion Criteria**:

1. Healthy adult male and female volunteers between the ages of 18 and 65 years.
2. All subjects who were willing to follow the study requirements and voluntarily gave their informed consent.

**Exclusion Criteria**:

1. Subjects with any significant internal diseases e.g., cardiac, pulmonary, renal, hepatic, etc.
2. History of allergy or hypersensitivity to cosmetics, toiletries or other dermatological products
3. History of recurrent dermatological diseases, e.g., psoriasis, atopic eczema, chronic urticaria
4. Pregnancy or mothers who are breastfeeding or planning a pregnancy
5. Scars, moles or other blemishes over the upper arm(s) or back which can interfere with the study
6. Subjects receiving systemic or topical drugs or medications which can interfere with delayed immunologic responses e.g., corticosteroids, non-steroidal anti-inflammatories, retinoids, immunosuppressants
7. Other conditions considered by the investigator as sound reasons for disqualification from enrollment into the study

**SUBJECT ASSIGNMENT**:

Volunteer subjects were screened and selected as described above and assigned a study number. The initials of each subject accepted into the study were recorded sequentially as they were enrolled.

**RECORDING OF DATA**:

The case report forms (CRF's) for this study were provided by the Investigator. All case report forms were completed in actual time, during each subject's visit. Copies of the CRF's will be retained by the investigator along with the original signed informed consent forms.

**HANDLING OF STUDY DOCUMENTS**:

All study related documents, case report forms (CRF's), original informed subject consent forms and any data generated were kept under secure lock in the technician's office for the duration of the study.

**STUDY PROCEDURES**:

**Method and Procedures**[1,2]

Patches were applied to the upper outer arm or back of each subject. The entire test was composed of three distinct phases: (1) an Induction phase and (2) a Rest Phase and (3) a Challenge phase.

(1) **Induction Phase**:

Approximately 0.05ml of aqueous SLS (0.25%) was applied to a designated site under a 15mm disc of Webril cotton cloth and the patch was fastened to the skin with occlusive tape for a period of 24 hours. After 24 hours, the SLS patch was removed and 0.05ml of the test material was applied to the same site before the site was again covered with occlusive tape (induction patch). Prior to application, the test product (Clear Liquid) was allowed to air-dry for approximately 30 minutes before the site was again covered with occlusive tape (induction patch). The induction patch was left in place for 48 hours (or for 72 hours when placed over a weekend) following which it was removed and the site again examined for irritation. If no irritation was present, a 0.25% aqueous SLS patch was again reapplied to the same site for 24 hours, followed by reapplication of a fresh induction patch with the test material to the same site. This sequence viz. 24 hour SLS pre-treatment followed by 48 hours of test material application was continued for a total of 5 induction exposures.

If irritation developed at any time-point during the induction phase as previously outlined, the 24-hour SLS pre-treatment patch was eliminated and only the test material was reapplied to the same site after a 24-hour rest period during which no patch was applied.

The aim during this phase of the study was to maintain at least a minimal degree of irritation in order to enhance penetration through the corneum barrier.

(2) **Rest Period**:

No exposure to the test material was made during this rest period, which lasted for 7-10 days after the last induction patch.

(3) **Challenge Phase**:

After a 7-10 day rest period, the subjects were challenged with a single application of the test material to a new skin site on the opposite upper outer arm or opposite side of the back in order to determine if sensitization had developed.

Pre-treatment with SLS was performed prior to challenge. Approximately 0.05ml of a 5.0% aqueous solution was applied to a fresh skin site under a 15mm disc of Webril cotton and covered with occlusive tape. The SLS patch was left in place for one hour. It was then removed and 0.05ml of the test material was applied to the same site, as outlined above. The challenge patch was then covered by occlusive tape and left in place for 48 hours. After that period, the patch was removed and the site graded, and again 24 hours later for any reactions.

**SCORING SCALE**:

0 = not sensitized

1 = mild sensitization (viz. erythema and a little edema)

2 = moderate sensitization (erythema with infiltration, raised, spreading beyond the borders of the patch, with or without vesiculation)

3 = strong sensitization (large vesiculo-bullous reaction).

Based on these findings the number of subjects with positive responses were tabulated for the test material. The test system shown below was used to classify the allergenic potential of the test substance.

| SENSITIZATION RATES: | GRADES: | CLASSIFICATION: |
|---|---|---|
| 0 - 2/25 | 1 | Weak |
| 3 - 7/25 | 2 | Mild |
| 8 - 13/25 | 3 | Moderate |
| 14 - 20/25 | 4 | Strong |
| 21 - 25/25 | 5 | Extreme |

**ADVERSE EXPERIENCES**:

No adverse experiences or unanticipated reactions were encountered or reported by any of the panelists.

**RESULTS**:

A total of twenty-six (26) healthy, adult, male and female volunteers who satisfied the inclusion criteria were enrolled into this study. There were 19 females and 7 males. Their ages ranged from 18 to 65 years. All 26 volunteers completed this investigation, as outlined in the standard protocol. The demographic data are shown in Table 1. No adverse or unexpected reactions were seen in any of the panelists during the induction phase.

The results of the challenge are shown in the enclosed table (Table 2). No instances of contact allergy were recorded at either 48 or 72 hours after the application of the challenge patches.

**CONCLUSION**:

Under the conditions of this test, the test sample labeled Clear Liquid and coded 1018047-011 does not possess a detectable contact-sensitizing potential and hence is not likely to cause contact sensitivity reactions under normal use conditions.

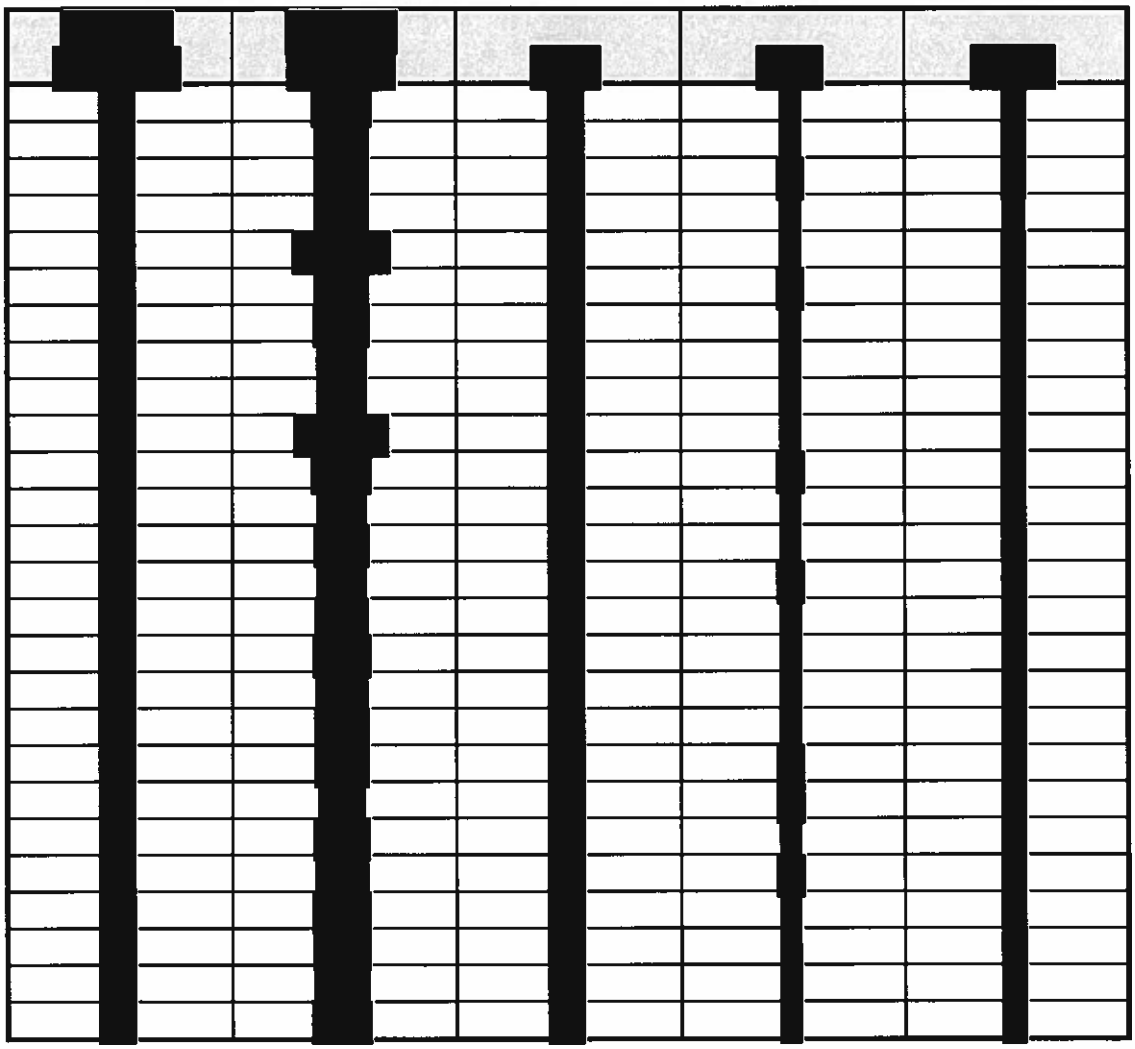
**References**:

(1) Kligman, A.M.: The Maximization Test. J.I.D., Vol. 47, No. 5, pp. 393-409, 1966.

(2) Kligman, A.M. and Epstein W.: Updating the Maximization Test for Identifying Contact Allergens. Contact Dermatitis. Vol. 1, 231-239, 1975.

**TABLE 1**

**DEMOGRAPHIC DATA**



**C = Caucasian**
**B = Black**

**TABLE 2**

**MAXIMIZATION TESTING RESULTS**

**Sample: Clear Liquid coded 1018047-011**

| Subject Number: | 48-Hour Grading | 72-Hour Grading |
|---|---|---|
| 01 | 0 | 0 |
| 02 | 0 | 0 |
| 03 | 0 | 0 |
| 04 | 0 | 0 |
| 05 | 0 | 0 |
| 06 | 0 | 0 |
| 07 | 0 | 0 |
| 08 | 0 | 0 |
| 09 | 0 | 0 |
| 10 | 0 | 0 |
| 11 | 0 | 0 |
| 12 | 0 | 0 |
| 13 | 0 | 0 |
| 14 | 0 | 0 |
| 15 | 0 | 0 |
| 16 | 0 | 0 |
| 17 | 0 | 0 |
| 18 | 0 | 0 |
| 19 | 0 | 0 |
| 20 | 0 | 0 |
| 21 | 0 | 0 |
| 22 | 0 | 0 |
| 23 | 0 | 0 |
| 24 | 0 | 0 |
| 25 | 0 | 0 |
| 26 | 0 | 0 |

**Challenge Readings:**

**48-Hour Reading – May 18, 2011**
**72-Hour Reading – May 19, 2011**

FINAL REPORT

Study Title

**NEUTRAL RED UPTAKE PHOTOTOXICITY ASSAY IN BALB/c 3T3 MOUSE FIBROBLASTS**

Test Article

A33 - n-acetyl tyrosinamide

Authors

[redacted]

Study Completion Date

26 April 2011

Performing Laboratory

[redacted]

Study Number

11AC53.140053

Laboratory Project Number

6222

## TABLE OF CONTENTS

## SIGNATURE PAGE

## NEUTRAL RED UPTAKE PHOTOTOXICITY ASSAY IN BALB/c 3T3 MOUSE FIBROBLASTS

| | |
|---|---|
| Initiation Date: | 22 March 2011 |
| Completion Date: | 26 April 2011 |
| Sponsor: | [redacted] |
| Sponsor's Representative: | [redacted] |
| Testing Facility: | [redacted] |
| Archive Location: | [redacted] |
| Study Director: | [redacted] |
| Laboratory Manager: | [redacted] |
| Laboratory Supervisor: | [redacted] |

## TEST ARTICLE RECEIPT

| [redacted] | Sponsor's Designation | Physical Description | Receipt Date | Storage Conditions* |
|---|---|---|---|---|
| 11AC53 | A33 | white powder | 22 March 2011 | room temperature |

* - Protected from exposure to light

# NEUTRAL RED UPTAKE PHOTOTOXICITY ASSAY IN BALB/c 3T3 MOUSE FIBROBLASTS

## INTRODUCTION

The Neutral Red Uptake Phototoxicity Assay in Balb/c 3T3 Mouse Fibroblasts was used to assess the phototoxicity and cytotoxicity potential of the test article to Balb/c 3T3 mouse fibroblasts (3T3) (American Type Culture Collection, Manassas, VA). The assay is an adaptation of the procedures described in the OECD guideline "*In Vitro* 3T3 NRU Phototoxicity Test" (TG 432)[1]. The bioassay quantitatively determines the cytotoxic potential of a test article by comparing the uptake of neutral red dye (3-amino-7-dimethylamino-2-methylphenazine hydrochloride) in Balb/c 3T3 cultures exposed to serial dilutions of the test article to the dye uptake in control (media or solvent treated) cultures (Borenfreund and Puerner, 1985)[2]. The concentration of test article causing a reduction in neutral red dye uptake of 50% ($IC_{50}$) is determined and used as a measure of cytotoxic potential. The phototoxic potential of the test article is determined by comparing the difference in $IC_{50}$ values of treated cultures in the presence and absence of UVA irradiation (+UVA and –UVA) and shifts in the concentration-response curves. The phototoxic potential is evaluated through the use of two different methods of analysis: Photo-Irritancy Factor (PIF) and Mean Photo Effect (MPE). The PIF is determined by comparing the $IC_{50}$ without UVA exposure to the $IC_{50}$ with UVA exposure and by definition is only useful when $IC_{50}$ values can be determined both with and without UVA exposure. The MPE is determined by comparing the two concentration-response curves (–UVA and +UVA) over the range of active doses.

The purpose of this study was to evaluate the phototoxicity and cytotoxicity potential of the test article supplied by [redacted] as measured by the test article-induced inhibition of neutral red uptake in Balb/c 3T3 cultures, in the presence and absence of UVA exposure. The laboratory phase of this study was conducted from 22 March 2011 to 30 March 2011 at [redacted] After a dose range finding assay, the test article was tested in at least two definitive assays (eight dose levels).

[1]Anonymous, Test Guideline (432) for the *In Vitro* 3T3 NRU Phototoxicity Test, Organization of Economic Cooperation and Development.

[2]Borenfreund, E. and Puerner, J.A. (1985) Toxicity determined in vitro by morphological alterations and neutral red absorption. Toxicology Lett. 24, 119-124.

## MATERIALS AND METHODS

### Reagents

The Trypsin/EDTA Solution and Dimethyl Sulfoxide (DMSO) were stored at -20 ± 5°C. The Dulbecco's Modified Eagle's Medium (DMEM) was stored refrigerated (2-8°C). The Hanks' Balanced Salt Solution (HBSS), $Ca^{++}$ and $Mg^{++}$-Free Hanks' Balanced Salt Solution (CMF-HBSS), and Neutral Red Solvent (ethanol and acetic acid) were stored at room temperature. The Neutral Red 100X Stock Solution (3.3 mg/mL 3-amino-7-dimethylamino-2-methylphenazine hydrochloride) was stored at 2-8°C, protected from light.

The master and working banks of cryopreserved Balb/c 3T3 mouse fibroblasts were stored in a liquid nitrogen freezer. A vial of the cells from the working bank was thawed, diluted in Growth Medium (DMEM supplemented with 4 mM L-glutamine, and 10% Newborn Calf Serum), and seeded into a culture flask. The cultures were incubated at 37 ± 1°C in a humidified atmosphere of 5 ± 1% $CO_2$ in air (standard culture conditions) until the cells attached to the flask (4 to 24 hours), at which time the Growth Medium was removed and replaced with fresh Growth Medium. Unless otherwise indicated, the Balb/c 3T3 cultures were incubated at standard culture conditions. The cells were incubated until they were 50 to 80% confluent.

### Cell Culture Procedures

When cultures were between 50 to 80% confluent, the medium was removed and the cultures were rinsed twice with 7 to 10 mL of CMF-HBSS. Approximately two mL of Trypsin/EDTA solution were added to each 75 $cm^2$ cell monolayer for 15 to 30 seconds. The trypsin was removed and the flasks were incubated at room temperature for 1 to 4 minutes. When more than 50% of the cells became dislodged, the flask was rapped sharply against the palm of the hand. When most of the cells became detached from the surface, the cells were suspended in approximately 8 mL of Growth Medium per 75 $cm^2$ flask. An appropriate volume of the trypsinized cells in Growth Medium was added to new flasks for passaging. Balb/c 3T3 fibroblasts were routinely passaged every 2-4 days (average doubling time, 20 to 24 hr). Since UV sensitivity of the cells increases with aging, cells were used at passage numbers < 100. Stock cells were cultured in the absence of antibiotics and new stock cultures were initiated from the frozen working bank at least every three months.

### Subculturing Balb/c 3T3 into 96-well Bioassay Plates

The Balb/c 3T3 mouse fibroblasts were subcultured into 96-well plates when the flasks were 50 to 80% confluent. The cultures were trypsinized, as described above, and suspended in approximately 5 to 10 mL of Growth Medium per two 75 $cm^2$ flasks. The concentration of cells in the suspension was determined using a Coulter counter, and an appropriate dilution in Growth Medium was made to obtain a cell density of approximately $1.0 \times 10^5$ cells/mL. The cells were subcultured into the designated wells of the 96-well bioassay plates (100 µL cell suspension per well). One hundred µL of Growth Medium were added to all the remaining wells to maintain humidity. All plates were labeled with a unique number (1, 2, 3, etc.), with the cell type, seeding date, and cell density on the day of seeding. In addition, a grid was drawn on the plate lid consistent with the standard plate map to identify dose concentration and solvent control wells. The plates were then incubated at standard culture conditions for approximately twenty-four

hours (or until the cells formed at least a half confluent monolayer) before test article treatment. Prior to dosing, each plate was labeled with the test article or positive control identification, and the appropriate solvent control.

Calibration of Solar Simulator

A Dermalight SOL 3 solar simulator, equipped with a UVA H1 filter (320 - 400 nm), was adjusted to the appropriate height immediately before each use. Measurement of energy through the lid of a 96-well microtiter plate was carried out using a calibrated UV radiometer UVA sensor. Simulator height was adjusted to deliver $1.7 \pm 0.1$ mW/cm$^2$ of UVA energy (the resulting dose was approximately 1 J/cm$^2$ per 10 min.).

Solubility Determination

The solubility test was performed during the preliminary assay to determine the most appropriate solvent for preparing the test article dosing solutions. The test article, A33, was found to be soluble in Hanks' Balanced Salt Solution (HBSS) at 2000 µg/mL and formed a clear colorless non-viscous solution. Accordingly, HBSS was selected as the solvent to dissolve and dilute the test article for the dose range finding trial.

pH Determination

The pH of the highest prepared test article dosing solution in HBSS was measured using pH paper. The prepared dilution was added to pH paper (EMD Chemicals, Inc.) with 5-10 pH range with 0.5 pH unit increments. Since the pH of the highest prepared test article dosing solution in all three assays was within the acceptable range of (6-8 pH), no pH adjustment was required. The pH value obtained from the narrower pH paper is presented in Table 1.

Dose Range Finding Assay

A dose range finding assay was performed to establish appropriate testing concentrations for the test article in the definitive assays. Eight doses of the test article ranging from 1000 µg/mL to 0.291 µg/mL (~ 1/2 log intervals) were tested. The test article, A33, was soluble in HBSS at 2000 µg/mL and was serially diluted in HBSS to prepare the 2X dosing dilutions. The positive control, chlorpromazine, was first diluted in DMSO to a 100X stock dilution series, and subsequently diluted in pre-warmed HBSS to a 2X dosing dilution series. The positive control was tested concurrently using twelve doses ranging from 100 µg/mL to 0.156 µg/mL. The solvent control for the test article was HBSS. The solvent control for the positive control (HBSS containing 1% DMSO) was prepared by diluting 200 µL of DMSO into 10 mL of pre-warmed HBSS to prepare the 2X solvent control dosing dilution. The final concentration of DMSO to be tested was 1%.

Prior to treatment, the Growth Medium was removed from the bioassay plates and rinsed once with 125 µL of pre-warmed HBSS. The HBSS was decanted, and each well received 50 µL of sterile pre-warmed HBSS to prevent cell desiccation while the test article doses were being prepared. Each dose of test article and positive control was tested by treating twelve wells (6 wells on each of two plates) of Balb/c 3T3 mouse fibroblasts with 50 µL of each 2X dilution. Those wells designated as solvent controls for the test article received 50 µL of pre-warmed HBSS. Those wells designated as solvent controls for the positive control received 50 µL of pre-

warmed HBSS containing 2% DMSO. The final concentration of DMSO was 1%. To guard against evaporation, 100 µL of pre-warmed HBSS were added to the remaining wells, which were not treated with the controls or test articles. The treated cultures were then incubated at standard culture conditions for approximately I hour.

Following the 1 hour incubation, the plates designated for the photo exposure were exposed (with the lid on) to 1.7 ± 0.1 mW/cm$^2$ UVA light for 50 ± 2 minutes (+UVA) at room temperature resulting in an irradiation dose of 5 J/cm$^2$. Duplicate plates designated for the cytotoxicity assay (-UVA) were kept in the dark at room temperature for 50 ± 2 minutes. After the 50-minute exposure period, the test article dilutions were decanted from the plates. The cells were washed once with 125 µL of pre-warmed HBSS. The HBSS was gently decanted, the wells were gently blotted dry, and 100 µL of Assay Medium (Growth Medium supplemented with penicillin/streptomycin sulfate) were added to all wells. The cells were incubated at standard culture conditions for 24 ± 1 hours.

After approximately 24 hours of post-treatment incubation, visual observations of the cultures were performed and recorded for each treatment group. Visual observations were recorded to confirm the results of the neutral red uptake end point. Since the visual observations of the treated cultures in the dose range finding and definitive assays supported the neutral red uptake results, no further discussion of the visual observations are presented.

A 1:100 dilution of the Neutral Red 100X Stock Solution was made in warm Assay Medium, and the dilution was filtered through a 0.45 µm filter to remove undissolved crystals (33 µg/mL neutral red). After approximately 24 hours of post-treatment incubation, the Assay Medium was decanted from the wells of the bioassay plates and blotted to remove excess medium. Immediately thereafter, 100 µL of the filtered neutral red solution were added to all test wells. The wells designated as blanks received 100 µL of Assay Medium. The plates were returned to the incubator for approximately 3 hours. After the incubation, the neutral red containing medium was decanted and each well rinsed with 250 µL of pre-warmed HBSS. The plate was decanted and blotted to remove the excess HBSS and 100 µL of Neutral Red Solvent (ethanol and acetic acid) were added to the bioassay wells (including blank wells). The plates were incubated at room temperature, with shaking, for a minimum of 20 minutes.

Finally, after the plates were agitated to evenly distribute the released neutral red, the absorbance at 550 nm ($OD_{550}$) of each well was measured with a Molecular Devices Vmax plate reader using the mean of the blank outer wells as the reference. The relative survival was obtained by comparing the amount of neutral red taken up by test article treated groups to the neutral red taken up by the vehicle treated group on the same plate. An $IC_{50}$ was determined for both the UVA exposed and unexposed plates.

Definitive Assays

Based on the results of the dose range finding assay, eight doses were chosen for the test article for the definitive assays. If possible, these doses were chosen to center on the $IC_{50}$ determined in the dose range finding assay. Since the test article did not induce sufficient cytotoxic effects to achieve an $IC_{50}$ either in the presence or absence of UVA exposure in the dose range finding assay, eight doses up to the maximum concentration of 1000 µg/mL were selected for the definitive trials. The dilution factor was 1.8 (~1/4 log). The dose ranges for the

cells treated in the presence or absence of UVA exposure were overlapped to allow for the Mean Photo Effect analysis.

Both definitive assays were performed simultaneously, yet independently of each other. The assay results are considered independent because: 1) the cell plates were seeded separately using different flasks of 3T3 cells, 2) two separate dilutions of test article and positive control were prepared by two different individuals, and 3) separate batches of neutral red solutions were prepared and added to the plates.

Presentation of Data

The raw data values for each well were imported into the PHOTOTOX (version 2.0) software provided by ZEBET, Berlin, Germany and was used to produce a percent of control survival value for each well at each concentration. The mean Optical Density ($OD_{550}$) of the blank wells for each plate was determined. The corrected $OD_{550}$ for vehicle controls, test article dilutions and positive control dilutions was determined by subtracting the mean $OD_{550}$ of the blank wells from the $OD_{550}$ of each well of the vehicle controls and test article and positive control dilution. The percent of control survival was calculated by dividing the corrected $OD_{550}$ of each test article dilution by the mean corrected $OD_{550}$ of the vehicle control.

$$\text{\% of Control Survival} = \frac{\text{corrected } OD_{550} \text{ of Test Article}}{\text{mean corrected } OD_{550} \text{ of Vehicle Control}} \times 100\%$$

The program then performs a bootstrap resampling of the original data resulting in a set of computer-generated concentration response curves for the concentrations tested. Using the curves, the program computes the $IC_{50}$ for each curve and the average $IC_{50}$ for the test or control article in the presence of UVA (+UVA) and the absence of UVA (–UVA).

The Photo-Irritancy Factor (PIF) was calculated by comparing the $IC_{50}$ –UVA to the $IC_{50}$ +UVA for all possible pairs of concentration response curves generated by the software. Non-rounded numbers were used to perform the calculations. The average PIF is reported.

The program then determines the Mean Photo Effect (MPE) for all possible pairs of concentration response curves. The MPE is defined as a weighted average across a set of individual photo effect (PE) values, where the $PE_C$ at a particular concentration is defined as the product of the response effect ($RE_C$) and the dose effect ($DE_C$), i.e., $PE_C = RE_C \times DE_C$ . (For more details see Peters and Holzhütter, 2002[3]). Non-rounded numbers were used to perform the calculations. The average MPE is reported.

To evaluate whether the corrected mean OD value for the vehicle controls exposed to UVA (+UVA) was >80% of the corrected mean value for the vehicle controls not exposed to UVA (–UVA), the raw data values for each well were imported into an EXCEL spreadsheet. The mean Optical Density ($OD_{550}$) of the blank wells for each plate was determined. The mean corrected $OD_{550}$ for the vehicle controls was determined by subtracting the mean $OD_{550}$ of the blank wells from the mean of the $OD_{550}$ values of the vehicle controls. Finally, the % of the

[3] Peters B and Holzhütter, H.G. (2002) In vitro phototoxicity testing: development and validation of a new concentration response analysis software and biostatistical analysis related to the usage of different prediction models. ATLA 30: 415-432.

corrected mean value for the vehicle controls exposed to UVA (+UVA) was calculated relative to the corrected mean value for the vehicle controls not exposed to UVA (–UVA) by the following equation:

$$\% \text{ of Control (–UVA)} = \frac{\text{corrected } OD_{550} \text{ of Vehicle Control (+UVA)}}{\text{corrected } OD_{550} \text{ of Vehicle Control (–UVA)}} \text{ X } 100\%$$

CRITERIA FOR DETERMINATION OF A VALID TEST

Each definitive assay was accepted (considered valid) when the positive control's Mean Photo Effect (MPE) fell within 2 standard deviations of the historical mean (updated every three months). In addition, the corrected mean OD value for the vehicle or solvent controls exposed to UVA (+UVA) was >80% of the corrected mean value for the vehicle or solvent controls not exposed to UVA (–UVA).

EVALUATION OF TEST RESULTS

Photo-Irritancy Factor

The phototoxicity potential of the test article was determined by comparing the $IC_{50}$ without UVA [$IC_{50}$(–UVA)] with the $IC_{50}$ with UVA [$IC_{50}$(+UVA)] to determine the "factor" difference:

$$\text{factor} = \frac{IC_{50}(\text{–UVA})}{IC_{50}(\text{+UVA})}$$

If both $IC_{50}$ values can be determined, the cut off value of the factor to discriminate between phototoxicants and non-phototoxicants is a factor of 5: A factor > 5 is indicative of phototoxic potential of the test material (European commission, 2000[4]).

OECD guideline 432[5] calls for different limits for the PIF. A PIF value less than two (PIF < 2) predicts "no phototoxicity". A PIF value greater than or equal to two but less than five ($2 \leq$ PIF < 5) predicts "probable phototoxicity" while a PIF value of five or greater (PIF $\geq$ 5) predicts "phototoxicity".

When both $IC_{50}$(–UVA) and $IC_{50}$(+UVA) cannot be calculated because the test article did not show cytotoxicity (50% reduction in viability) up to the highest dose tested, the PIF cannot be used to determine phototoxic potential. The Mean Photo Effect calculation would be used to determine phototoxic potential.

---

[4] European commission (2000) EU Directive 2000/33/EU for the 21st Amendment of Annex V of the EU Directive86/906/EEC for classification and labelling of hazardous chemicals: Test guidelines B-41 "phototoxicity – in vitro 3T3 NRU phototoxicity test". O.J. European Communities June 8, 2000, L136, p.p. 98-107.

[5] Anonymous, Test Guideline (432) for the *In Vitro* 3T3 NRU Phototoxicity Test, Organization of Economic Cooperation and Development.

Mean Photo Effect

This method of analysis is particularly suited to cases where the $IC_{50}$ value can not be calculated for one or both concentration response curves. The MPE measures the effect of UV exposure over a range of concentrations (Holzhütter, 1997 and Peters and Holzhütter, 2002)[6,7].

The mean photo effect, MPE, is calculated at 50 equally spaced grid points within a range of overlapping doses between the +UVA and –UVA response curves. The upper dose for this range is determined so that at least one of the two concentration-response curves shows survival of at least 10%. At each point a photo effect is calculated according to the following formulas,

$$\text{Photo Effect} = \text{Dose Effect}_n \times \text{Response Effect}_d \qquad (\text{ie., } PE = DE_n \times RE_d)$$

$$\text{Dose Effect}_n = \frac{\text{Dose (–UVA) to give survival n / Dose (+UVA) to give survival n} - 1}{\text{Dose (–UVA) to give survival n / Dose (+UVA) to give survival n} + 1}$$

As the ratio of Dose (–UVA) to give survival n / Dose (+UVA) to give survival n increases (indicative of phototoxic potential), then the $\text{Dose Effect}_n$ approaches 1.0.

$$\text{Response Effect}_d = \frac{R(-UVA)_d - R(+UVA)_d}{R_0}$$

where $R_0$ is the Total Survival Range, or 100%, $R(-UVA)_d$ is the survival without UVA at dose d, and $R(+UVA)_d$ is the survival with UVA at dose d.

As the difference between the survival without UVA at dose d and the survival with UVA at dose d [ie., $R(-UVA)_d - R(+UVA)_d$] increases (indicative of phototoxic potential), then the $\text{Response Effect}_d$ approaches 1.0.

A material is considered nonphototoxic if the MPE is $< 0.1$ and phototoxic if the MPE is $\geq 0.1$ (European commission, 2000[8]).

OECD guideline 432[9] calls for different limits for the MPE. An MPE value less than 0.1 ($MPE < 0.1$) predicts "no phototoxicity". An MPE value above or equal to 0.1 but less than 0.15 ($0.1 \leq MPE < 0.15$) predicts "probable phototoxicity" while an MPE value of 0.15 or greater ($MPE \geq 0.15$) predicts "phototoxicity".

---

[6] Holzhütter, H.G. (1997) A general measure of the in vitro phototoxicity derived from pairs of dose response curves and its use for predicting in vivo phototoxicity of chemicals. ATLA 25:445-462.

[7] Peters B and Holzhütter, H.G. (2002) In vitro phototoxicity testing: development and validation of a new concentration response analysis software and biostatistical analysis related to the usage of different prediction models. ATLA 30: 415-432.

[8] European commission (2000) EU Directive 2000/33/EU for the 21st Amendment of Annex V of the EU Directive86/906/EEC for classification and labelling of hazardous chemicals: Test guidelines B-41 "phototoxicity – in vitro 3T3 NRU phototoxicity test". O.J. European Communities June 8, 2000, L136, p.p. 98-107.

[9] Anonymous, Test Guideline (432) for the *In Vitro* 3T3 NRU Phototoxicity Test, Organization of Economic Cooperation and Development.

## RESULTS AND DISCUSSION

Dose Range Finding Assay

A dose range finding assay was performed using eight doses ranging from 1000 µg/mL to 0.291 µg/mL (~1/2 log steps) of the test article supplied by [redacted] Based upon the results of the dose range finding assay, eight doses were selected for the test article for the definitive assays (see Materials and Methods). The positive control, chlorpromazine, was run concurrently (100 µg/mL to 0.156 µg/mL) in the dose range finding assay. The specific doses and the percent survival for the test article and positive control are included in Appendix B. The $IC_{50}$ results for the dose range finding assay are presented in Table 2.

Definitive Assays

Table 1 presents the range of concentrations tested for the test article and the positive control. Table 2 presents the $IC_{50}$ results for the test article and the positive control. Table 3 presents the Mean Photo Effect (MPE) and Photo-Irritation Factors (PIF), calculated where applicable, for the test article and the positive control. Table 4 presents the results for the second criterion for determination of a valid test (the corrected mean OD value for the vehicle or solvent controls exposed to UVA was >80% of the corrected mean value for the vehicle or solvent controls not exposed to UVA). All plates tested in this phototoxicity study met this acceptance criterion. Chlorpromazine, the positive control, gave results indicating phototoxic potential. Since the positive control, chlorpromazine, met the acceptance criteria (MPE between 0.374-0.706), the dose range finding assay, Trial 1, and Trial 2 were considered valid.

The Neutral Red Uptake Phototoxicity Assay in Balb/c 3T3 Mouse Fibroblasts was used to assess the potential phototoxicity and cytotoxicity of the test article. The *in vitro* test system optimizes the detection of potential phototoxic responses by presenting maximum tolerated doses to a cell monoculture in the presence and absence of UVA. According to the prediction model presented in the OECD test guidelines, the test article, A33, is not predicted to have phototoxic potential (i.e., PIF < 2.0 and MPE < 0.100).

**TABLE 1**

**CONCENTRATION OF TEST ARTICLES USED IN DOSING THE DEFINITIVE TRIALS**

| [redacted] | Sponsor Designation | Concentration +UVA[b] (~ ¼ log steps) | Concentration –UVA (~ ¼ log steps) | pH of highest 2X in HBSS |
|---|---|---|---|---|
| 11AC53 | A33 | 1000 to 16.3 µg/mL | 1000 to 16.3 µg/mL | 6.5-7.0 |
| Positive Control | Chlorpromazine | 9.53 to 0.156 µg/mL | 100 to 1.63 µg/mL | 6.5-7.0 |

[b] – See Appendix C for the details of the light spectrum.

## TABLE 2

### $IC_{50}$ SUMMARY

| [redacted] | Sponsor Designation | $IC_{50}$ (µg/mL) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Preliminary 22 March 2011 | | Trial 1 29 March 2011 | | Trial 2 29 March 2011 | |
| | | +UVA | -UVA | +UVA | -UVA | +UVA | -UVA |
| 11AC53 | A33 | > 1000* | > 1000* | > 1000* | > 1000* | > 1000* | > 1000* |
| Positive control | Chlorpromazine | 0.733 | 17.2 | 0.963 | 17.0 | 0.891 | 22.0 |

* –An $IC_{50}$ (µg/mL) value could not be determined for the test article. Therefore, the $IC_{50}$ value is expressed as greater than the highest dose tested.

## TABLE 3

### MEAN PHOTO EFFECT AND PHOTO-IRRITANCY FACTOR SUMMARY

| [redacted] | Sponsor Designation | MPE[1] | | | PIF[2] | | |
|---|---|---|---|---|---|---|---|
| | | Prelim | Trial 1 | Trial 2 | Prelim | Trial 1 | Trial 2 |
| 11AC53 | A33 | 0.005 | 0.023 | -0.033 | ND | ND | ND |
| Positive control | Chlorpromazine | 0.623 | 0.504 | 0.544 | 23.5 | 17.7 | 24.7 |

ND – Not able to be determined because no $IC_{50}$ values were obtained.

[1] – Mean Photo Effect: MPE < 0.1 = predicts no phototoxicity,
0.1 ≤ MPE < 0.150 = predicts a probable phototoxic material
MPE ≥ 0.150 = predicts phototoxicity

[2] – Photo-Irritancy Factor: Photo-Irritancy Factor: PIF < 2.0 = predicts no phototoxicity,
2.0 ≤ PIF < 5.0 = predicts a probable phototoxic material
PIF ≥ 5.0 = predicts phototoxicity

## TABLE 4

### % OF –UVA CONTROL SUMMARY

| [redacted] | Sponsor Designation | % of–UVA Control | | |
|---|---|---|---|---|
| | | Dose Range Finding Trial | Definitive Trial 1 | Definitive Trial 2 |
| 11AC53 | A33 | 91.9% | 95.2% | 87.6% |
| Positive Control | Chlorpromazine | 98.5% | 89.1% | 95.0% |

[redacted] **FINAL REPORT**

**Final Report Date: November 7, 2011**

[redacted]: **#7389**

[redacted]

Title: An Assessment of the Photosensitization Potential of Three Topical Coded Test Products Using a Human Photocontact Allergenicity Assay

Sponsor: [redacted] n-acetyl tyrosinamide (1%)

Sponsor Study: [redacted]

Principal Investigator: [redacted]

# FINAL REPORT

**TITLE**:

An Assessment of the Photosensitization Potential of Three Topical Test Products Using a Human Photocontact Allergenicity Assay.

[redacted]

**GUIDELINES FOR THE CONDUCT OF THE STUDY**:

All procedures were conducted in compliance with the regulations of the Food and Drug Administration (FDA) ([21 CFR 50, 56, 312) ICH-GCP Consolidated Guidelines, May 9, 1997 Federal Register) and in accordance with [redacted]

**OBJECTIVE**:

The objective of this study was to determine the photosensitization (photocontact allergenicity) potential of three topical cosmetic products to determine if these materials have a detectable photocontact allergenic potential when topically applied to human skin (see references #1 and #2).

**DESIGN RATIONALE**:

This was a repeat insult patch test wherein the test materials and ultraviolet radiation (solar simulated radiation) were administered to the same designated test sites over the mid or lower back area repeatedly for a total of six (6) induction exposures over a 3 week period followed by a challenge phase after a rest period of 10 to 14 days. The evaluator was blinded as to the identity of the test products.

**CONDUCTION DATES**:

This study was conducted from September 26, 2011 through October 28, 2011.

[redacted]

[redacted]

**INFORMED CONSENT**:

Prior to acceptance into the study, each subject was informed by the Investigator or his designee of the nature and purpose of the study, possible side-effects and any other relevant information. The study procedures and possible risks and discomfort were explained to each panelist during the interview using popular understandable language and terms, and the panelists were encouraged to ask questions regarding the study. Each interviewed panelist who qualified was then asked to sign a consent form prior to enrollment. A copy of the study schedule of events, visits and dates was then given to the volunteer.

**TEST MATERIALS**:

The test samples used in this study were supplied by the sponsor. The products consisted of separate containers labeled [redacted] Blend coded 1018047-011. One jar of each test product was supplied for testing purposes. The test products coded [redacted] and 1018047-011 (Blend) were allowed to evaporate for ~30 minutes prior to occlusion. The three coded test products were tested as supplied viz., neat.

**TEST DRUG ACCOUNTABILITY**:

The test samples were received in good condition by our Quality Assurance Department. The test materials were checked for (1) amount (2) product number or code (3) material container etc. The materials were individually listed on a special sheet signed by the receiver, the laboratory supervisor and the investigator (physician). The test materials were stored at ambient conditions in an inaccessible location under the supervision of the investigator.

**DISPOSITION OF REMAINING CLINICAL SUPPLIES**:

All remaining test materials will be disposed of in accordance with established procedures following completion of the study and after the final written report has been issued to the Sponsor.

**PANEL COMPOSITION**:

Healthy, Caucasian, adult volunteers with no excess hair or other marks on their back that would obscure grading of the test sites were recruited for this study. These were fair skin individuals with skin types I, II, or III defined as follows (Federal Register 43: 38260, 1978):

Type I - Always burns easily; never tans
Type II - Always burns easily; tans minimally
Type III - Burns moderately; tans gradually

None of the subjects had a medical or dermatological illness and none were sensitive to sunlight or to topical preparations and/or cosmetics.

**Inclusion Criteria**:

1. Healthy adult male and female volunteers (skin types I to III) between the ages of 18 and 65 years.
2. All subjects were willing to follow the study requirements and voluntarily gave their informed consent.

**Exclusion Criteria**:

1. History of sun hypersensitivity and photosensitive dermatoses.
2. History of recurrent dermatological diseases, e.g., psoriasis, atopic eczema, chronic urticaria.
3. Subjects with any significant internal diseases, e.g., cardiac, pulmonary, renal, hepatic, etc.
4. History of allergy or hypersensitivity to cosmetics, toiletries, or other dermatological products.
5. History of allergy or hypersensitivity to sunscreens.
6. History of allergy or hypersensitivity to any type of tape.
7. Scars, moles or other blemishes over the lower back, which could have interfered with the study.
8. Subjects receiving systemic or topical drugs including steroidal or non-steroidal anti-inflammatory drugs, or medications which could have interfered with the development of an inflammatory response, e.g., immunosuppressive agents or retinoids.
9. Subjects receiving potentially photosensitizing medications, e.g., thiazides, tetracyclines, phenothiazines, etc.
10. Pregnancy or mothers who were breastfeeding or planning a pregnancy.
11. Other conditions considered by the Investigator as sound reasons for disqualification from enrollment into the study.

**SUBJECT ASSIGNMENT**:

Volunteer subjects were screened and selected as described above and assigned a study number. The initials of each subject accepted into the study were recorded sequentially as they were enrolled.

**RECORDING OF DATA**:

The case report forms (CRF's) for this study were provided by the Investigator. All case report forms were completed in actual time, during each subject's visit. All scores were recorded on the Case Report Forms. Copies of the CRF's will be retained by the investigator along with the original signed informed consent forms.

**HANDLING OF STUDY DOCUMENTS**

All study related documents, case report forms (CRF's), original informed subject consent forms and any data generated were kept under secure lock in the technician's office for the duration of the study.

**TEST SITE**:

The test site was the mid or lower back. The test sites were inspected prior to test product application to ensure that the skin was normal in appearance and free of irritation or other blemishes.

**METHOD**[1,2]:

Test patches were applied to the lower back of each subject. The entire test was composed of three distinct phases: (1) Pre-testing phase (2) Induction phase and (3) Challenge phase.

(1) **PRE-TESTING PHASE**:

After signing an informed consent form (on Day 1), the Minimal Erythema Dose (MED) of each subject was determined by exposing one side of the midback to a series of exposures (1cm diameter circular areas) in 25% increments from the xenon arc solar simulator, the details of which are listed below. The subject's MED is the shortest exposure time that produces a minimally visible faint erythema 20 to 24 hours later.

(2) **INDUCTION PHASE**:

Approximately 40mgs. of each test material was applied to 2x2cm square skin sites over the lower back and covered with 2x2cm squares of non-woven cotton cloth (Webril, Curity) and covered with occlusive tape (Blenderm, 3M). The patches were left in place for twenty-four (24) hours. At the end of that period, the patches were then removed and

the sites wiped off with dry gauze and exposed to two minimal erythema doses (MED's) from the xenon arc solar simulator. The sites were then left open for a forty-eight (48) hour period, after which the subjects returned to the testing facility and the patches were again reapplied to the same designated test sites under dressings as previously outlined above. Twenty-four (24) hours later, the patches were removed and the sites re-exposed to 2 MED's of solar simulated radiation. This sequence was repeated to the same test sites twice weekly for a total of three weeks (total of 6 exposures).

**(3) CHALLENGE:**

Ten (10) days following the last induction dose, the subjects returned to the testing facility for a single challenge exposure. The test materials were applied as previously specified (40mgs) in **duplicate** to new designated skin sites each measuring 2x2cm on the opposite side of the lower back, under dressings, as previously described, for a period of approximately 24 hours. One set of patches was then removed and any excess test material wiped off with dry gauze. The sites were then irradiated with 1/2 an MED of solar simulated radiation (SSR) plus 4J/cm$^2$ of UVA which was obtained by filtering the beam from the solar simulator to eliminate short (UVB) wavelengths (see Light Source). The duplicate set of patches remained unirradiated and served as control treated sites.

**EVALUATION OF SKIN REACTIONS:**

All test sites were examined for reactions at 48 and 72 hours following exposure of the sites to UV radiation. Each subject reported back to the testing facility at the two time points to have the responses appraised by an evaluator other than the person applying the test products, and who was unaware of the nature of the test substances.

Skin reactions were scored according to the following scale:

0 = Not sensitized
1 = Mild sensitization (viz. erythema and a little edema)
2 = Moderate sensitization (erythema with infiltration, spreading reaction beyond the borders of the patch, with or without vesiculation)
3 = Strong sensitization (large vesicula-bullous reaction)

**LIGHT SOURCE**[3]:

This was a 150-watt compact xenon arc source equipped with UV-reflecting dichroic mirror and a 1mm thick Schott WG-320 filter to produce simulation of the solar spectrum (290nm-400nm). A 1mm thick UG5 filter was added to remove reflected heat and remaining visible radiation. Total irradiance at skin level was measured with a calibrated Eppley Thermopile. The size of the irradiated field was approximately a 1-cm diameter circle. UVA was obtained from this same source by passing the beam through a 1mm Schott WG345 filter (Schott Glass Technologies). This provided a continuous spectrum between 320 and 420nm with a peak between 360-370nm. Total irradiance at skin level was 165.0mW/cm$^2$. The UVA intensity was 67.5mW/cm$^2$.

**ADVERSE EXPERIENCES**:

No adverse experiences or unanticipated reactions of any kind were observed or reported during the study.

**RESULTS**:

A total of 25 healthy, Caucasian volunteers who qualified were enrolled into this study. There were 23 females and 2 males ranging in age from 18 to 62 years. All 25 volunteers completed this investigation, as specified in the protocol. The demography is shown in Table 1.

No side-effects or unexpected reactions of any kind were observed. Following the challenge phase, no reactions suggestive of photocontact allergy were seen in any of the panelists at either 48 or 72 hours post exposure. The results of the challenge are summarized in the enclosed tables (Tables 2 through 7).

**CONCLUSIONS**:

Under the presently described test conditions, the test materials labeled [redacted] Blend (1018047-011) do not possess a detectable photocontact-sensitizing potential in human skin.

## REFERENCES

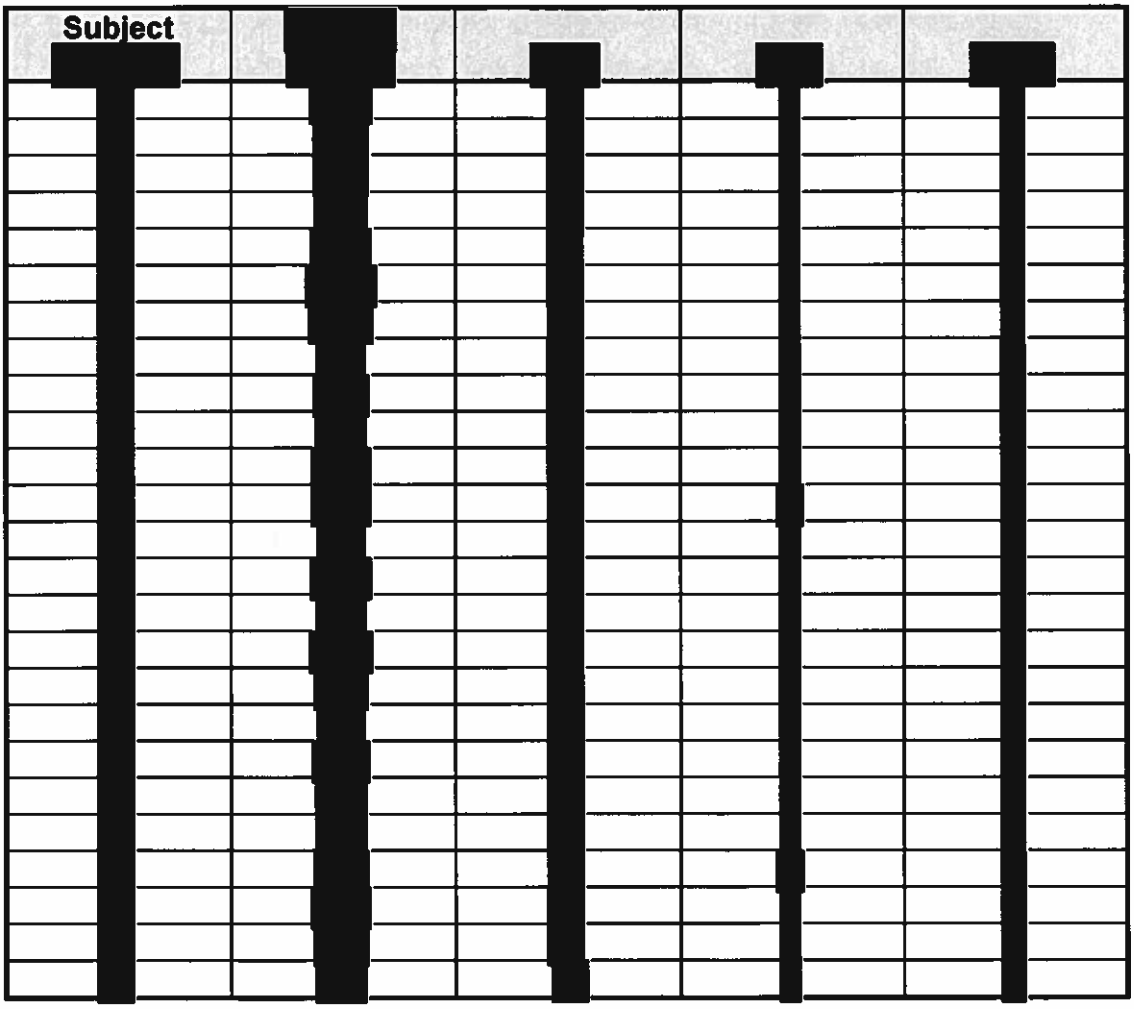
(1) Kaidbey, KH and Kligman AM: Photomaximization test for identifying photoallergic contact sensitizers. Contact Dermatitis, 6: 161-169, 1980.

(2) Kaidbey, KH and Kligman AM: Identification of contact photosensitizers by human assay. In "Current concepts in cutaneous toxicity, edited by V.A. Drill and P. Lazar. Academic Press Inc., pp. 55-68, 1980

(3) Berger DS: Specification and design of solar ultraviolet simulators. J.Invest.Dermtol. 53: 192-199, 1969.

## TABLE 1

## DEMOGRAPHIC DATA




C = Caucasian

## TABLE 6

### RESULTS OF PHOTOMAXIMIZATION TESTING (48 Hour Grading)

**Sample: Blend coded 1018047-011 (tested as supplied)**

| Subject Number: | Unirradiated Control | UV Irradiated |
|---|---|---|
| 001 | 0 | 0 |
| 002 | 0 | 0 |
| 003 | 0 | 0 |
| 004 | 0 | 0 |
| 005 | 0 | 0 |
| 006 | 0 | 0 |
| 007 | 0 | 0 |
| 008 | 0 | 0 |
| 009 | 0 | 0 |
| 010 | 0 | 0 |
| 011 | 0 | 0 |
| 012 | 0 | 0 |
| 013 | 0 | 0 |
| 014 | 0 | 0 |
| 015 | 0 | 0 |
| 016 | 0 | 0 |
| 017 | 0 | 0 |
| 018 | 0 | 0 |
| 019 | 0 | 0 |
| 020 | 0 | 0 |
| 021 | 0 | 0 |
| 022 | 0 | 0 |
| 023 | 0 | 0 |
| 024 | 0 | 0 |
| 025 | 0 | 0 |

**GRADING SCALE**:

0 = Not sensitized
1 = Mild sensitization (viz. erythema and a little edema)
2 = Moderate sensitization (erythema with infiltration, spreading reaction beyond the borders of the patch, with or without vesiculation)
3 = Strong sensitization (large vesiculo-bullous reaction)

## TABLE 7

### RESULTS OF PHOTOMAXIMIZATION TESTING (72 Hour Grading)

**Sample: Blend coded 1018047-011 (tested as supplied)**

| Subject Number: | Unirradiated Control | UV Irradiated |
|---|---|---|
| 001 | 0 | 0 |
| 002 | 0 | 0 |
| 003 | 0 | 0 |
| 004 | 0 | 0 |
| 005 | 0 | 0 |
| 006 | 0 | 0 |
| 007 | 0 | 0 |
| 008 | 0 | 0 |
| 009 | 0 | 0 |
| 010 | 0 | 0 |
| 011 | 0 | 0 |
| 012 | 0 | 0 |
| 013 | 0 | 0 |
| 014 | 0 | 0 |
| 015 | 0 | 0 |
| 016 | 0 | 0 |
| 017 | 0 | 0 |
| 018 | 0 | 0 |
| 019 | 0 | 0 |
| 020 | 0 | 0 |
| 021 | 0 | 0 |
| 022 | 0 | 0 |
| 023 | 0 | 0 |
| 024 | 0 | 0 |
| 025 | 0 | 0 |

**GRADING SCALE**:

0 = Not sensitized
1 = Mild sensitization (viz. erythema and a little edema)
2 = Moderate sensitization (erythema with infiltration, spreading reaction beyond the borders of the patch, with or without vesiculation)
3 = Strong sensitization (large vesiculo-bullous reaction)

[redacted]

# FINAL REPORT

**CLIENT:** [redacted]

**ATTENTION:**

**TEST:** 48 Hour Patch Test
Protocol No.: 1.02

**TEST MATERIAL:** A33 Gel F#239-170
(2% n-acetyl tyrosinamide)

**EXPERIMENT REFERENCE NUMBER:** [redacted]

Reviewed by: [redacted]

Approved by:

Approved by:



[redacted]

[redacted]

# QUALITY ASSURANCE UNIT STATEMENT

**Study Number:** [redacted]

[redacted] Quality Assurance Unit (QAU) is responsible for monitoring the conduct, content and reporting of all clinical laboratory studies that are conducted at [redacted]

This study has been conducted in accordance with ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable regulations, [redacted] Standard Operating Procedures, and the approved Study Protocol.

The [redacted] QAU has reviewed all data, records, and documents relating to this study and also this Final Report. The following QAU representative signature certifies that all data, records, and documents relating to this study and also this Final Report have been reviewed and are deemed to be acceptable, and the study conforms to all of the requirements as indicated above.

All records and documents pertaining to the conduct of this study shall be retained in the [redacted] archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the [redacted] QA Department to obtain custody of study records once the [redacted] archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, study-related records shall be destroyed at the end of the [redacted] archive period in a manner that renders them useless.

[redacted]
Quality Assurance Representative

4/13/11
Date

[redacted]

**Objective:** To determine by epidermal contact the primary irritation potential of a test material.

**Participants:** Fifty-four (54) subjects, male and female, ranging in age from 16 to 79 years, who qualified were selected for this evaluation. Fifty-three (53) subjects completed this study. The remaining subject discontinued her participation for personal reasons unrelated to the use of the test material.

**Inclusion Criteria:**

a. Male and female subjects, age 16[a] and over.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History form and the understanding and signing of an Informed Consent form.
e. Considered reliable and capable of following directions.

**Exclusion Criteria:**

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

**Test Material:** A33 Gel F#239-170

**Study Schedule:**

| Panel # | Initiation Date | Completion Date |
|---|---|---|
| 20110090 | March 29, 2011 | April 1, 2011 |

[a]With parental or guardian consent

**Methodology:**

The upper back between the scapulae served as the treatment area. Approximately 0.2 g of the test material, or an amount sufficient to cover the contact surface, was applied to the 1" x 1" absorbent pad portion of a clear adhesive dressing. When secured to the appropriate treatment site, this dressing formed a semi-occlusive patch.

The test material remained in contact with the skin for a total of forty-eight hours. This site was then evaluated for gross changes. Absence of any visible skin change was assigned a zero value. The test site was re-evaluated at seventy-two hours.

**Evaluation Criteria (Erythema and additional Dermal Sequelae):**

| | | | | | |
|---|---|---|---|---|---|
| **0** | **=** | **No visible skin reaction** | **E** | **=** | **Edema** |
| **0.5** | **=** | **Barely perceptible** | **D** | **=** | **Dryness** |
| **1** | **=** | **Mild** | **S** | **=** | **Staining** |
| **2** | **=** | **Moderate** | **P** | **=** | **Papules** |
| **3** | **=** | **Marked** | **V** | **=** | **Vesicles** |
| **4** | **=** | **Severe** | **B** | **=** | **Bullae** |
| | | | **U** | **=** | **Ulceration** |
| | | | **Sp** | **=** | **Spreading** |

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

**Results:**

The results of each participant are appended (Table 1).

Observations remained negative throughout the test interval.

Subject demographics are presented in Table 2.

**Summary:**

Under the conditions of this study, test material, A33 Gel F#239-170, did not indicate a potential for dermal irritation.

Table 1
Panel #20110090

Individual Results

A33 Gel F#239-170

| Subject Number | Observations 48 Hours | 72 Hours |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 0 | 0 |
| 3 | 0 | 0 |
| 4 | 0 | 0 |
| 5 | 0 | 0 |
| 6 | 0 | 0 |
| 7 | 0 | 0 |
| 8 | 0 | 0 |
| 9 | 0 | 0 |
| 10 | 0 | 0 |
| 11 | 0 | 0 |
| 12 | 0 | 0 |
| 13 | 0 | 0 |
| 14 | 0 | 0 |
| 15 | ---DID NOT COMPLETE STUDY--- | |
| 16 | 0 | 0 |
| 17 | 0 | 0 |
| 18 | 0 | 0 |
| 19 | 0 | 0 |
| 20 | 0 | 0 |
| 21 | 0 | 0 |
| 22 | 0 | 0 |
| 23 | 0 | 0 |
| 24 | 0 | 0 |
| 25 | 0 | 0 |
| 26 | 0 | 0 |
| 27 | 0 | 0 |

Table 1
(continued)
Panel #20110090

Individual Results

A33 Gel F#239-170

| Subject Number | Observations 48 Hours | 72 Hours |
|---|---|---|
| 28 | 0 | 0 |
| 29 | 0 | 0 |
| 30 | 0 | 0 |
| 31 | 0 | 0 |
| 32 | 0 | 0 |
| 33 | 0 | 0 |
| 34 | 0 | 0 |
| 35 | 0 | 0 |
| 36 | 0 | 0 |
| 37 | 0 | 0 |
| 38 | 0 | 0 |
| 39 | 0 | 0 |
| 40 | 0 | 0 |
| 41 | 0 | 0 |
| 42 | 0 | 0 |
| 43 | 0 | 0 |
| 44 | 0 | 0 |
| 45 | 0 | 0 |
| 46 | 0 | 0 |
| 47 | 0 | 0 |
| 48 | 0 | 0 |
| 49 | 0 | 0 |
| 50 | 0 | 0 |
| 51 | 0 | 0 |
| 52 | 0 | 0 |
| 53 | 0 | 0 |
| 54 | 0 | 0 |

Table 2
Panel #20110090

Subject Demographics

| Subject Number | Initials | Age | Sex |
|---|---|---|---|
| 1 | [redacted] | 53 | F |
| 2 | | 50 | F |
| 3 | | 74 | M |
| 4 | | 60 | F |
| 5 | | 38 | F |
| 6 | | 48 | F |
| 7 | | 59 | F |
| 8 | | 55 | F |
| 9 | | 41 | F |
| 10 | | 16 | M |
| 11 | | 76 | M |
| 12 | | 78 | F |
| 13 | | 68 | F |
| 14 | | 29 | F |
| 15 | | 43 | F |
| 16 | | 60 | F |
| 17 | | 67 | F |
| 18 | | 71 | F |
| 19 | | 70 | F |
| 20 | | 22 | F |
| 21 | | 79 | F |
| 22 | | 64 | M |
| 23 | | 56 | F |
| 24 | | 63 | F |
| 25 | | 68 | F |
| 26 | | 49 | F |
| 27 | | 58 | M |

Table 2
(continued)
Panel #20110090

Subject Demographics

| Subject Number | Initials | Age | Sex |
|---|---|---|---|
| 28 | [redacted] | 66 | F |
| 29 | | 49 | F |
| 30 | | 63 | F |
| 31 | | 52 | F |
| 32 | | 79 | F |
| 33 | | 76 | F |
| 34 | | 45 | F |
| 35 | | 45 | M |
| 36 | | 34 | F |
| 37 | | 35 | F |
| 38 | | 69 | F |
| 39 | | 50 | M |
| 40 | | 54 | F |
| 41 | | 58 | F |
| 42 | | 59 | F |
| 43 | | 72 | F |
| 44 | | 57 | F |
| 45 | | 43 | F |
| 46 | | 56 | F |
| 47 | | 50 | F |
| 48 | | 43 | M |
| 49 | | 66 | F |
| 50 | | 66 | F |
| 51 | | 27 | F |
| 52 | | 44 | M |
| 53 | | 53 | F |
| 54 | | 39 | M |

[redacted]

# FINAL REPORT

**CLIENT:** [redacted]

**ATTENTION:**

**TEST:** 48 Hour Patch Test
Protocol No.: 1.02 n-acetyl tyrosinamide

**TEST MATERIALS:**

| | | concentration: |
|---|---|---|
| .01 | [redacted] | |
| .02 | B = Step 2 Skin Plumping Cream Ref: 248-080 | B=1.5% |
| .03 | C = Step 2 Skin Plumping Cream F#245-152 | C=1.25% |
| .04 | D = A33 Gel F#239-170 + [redacted] | D=2% |
| .05 | G = A33 Gel F#239-170 + H = Step 2 Skin Plumping Cream Ref: 248-080 | G=2% |
| .06 | I = A33 Gel F#239-170 + J Step 2 Skin Plumping Cream F#245-152 | H=1.5% |
| | | I=2% |
| | | J=1.25% |

**EXPERIMENT REFERENCE NUMBER:** [redacted]

Reviewed by: [redacted]

Approved by:

Approved by:



[redacted]

[redacted]

# QUALITY ASSURANCE UNIT STATEMENT

**Study Number:** [redacted]

The [redacted] Quality Assurance Unit (QAU) is responsible for monitoring the conduct, content and reporting of all clinical laboratory studies that are conducted at [redacted]

This study has been conducted in accordance with ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable regulations, [redacted] Standard Operating Procedures, and the approved Study Protocol.

The [redacted] QAU has reviewed all data, records, and documents relating to this study and also this Final Report. The following QAU representative signature certifies that all data, records, and documents relating to this study and also this Final Report have been reviewed and are deemed to be acceptable, and the study conforms to all of the requirements as indicated above.

All records and documents pertaining to the conduct of this study shall be retained in the [redacted] archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the [redacted] QA Department to obtain custody of study records once the [redacted] archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, study-related records shall be destroyed at the end of the [redacted] archive period in a manner that renders them useless.

[redacted]

5/10/11
Date

[redacted]

**Objective:** To determine by epidermal contact the primary irritation potential of test materials.

**Participants:** Fifty-three (53) subjects, male and female, ranging in age from 22 to 78 years, who qualified were selected for this evaluation. Fifty-one (51) subjects completed this study. The remaining subjects discontinued their participation for various reasons unrelated to the use of the test material.

**Inclusion Criteria:**

a. Male and female subjects, age 16[a] and over.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History form and the understanding and signing of an Informed Consent form.
e. Considered reliable and capable of following directions.

**Exclusion Criteria:**

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

**Test Materials:**

**.01** [redacted]
**.02** B = Step 2 Skin Plumping Cream Ref: 248-080
**.03** C = Step 2 Skin Plumping Cream F#245-152
**.04** D = A33 Gel F#239-170 + [redacted]
**.05** G = A33 Gel F#239-170 + H = Step 2 Skin Plumping Cream Ref: 248-080
**.06** I = A33 Gel F#239-170 + J Step 2 Skin Plumping Cream F#245-152

**Study Schedule:**

| Panel # | Initiation Date | Completion Date |
|---|---|---|
| 20110106 | April 19, 2011 | April 22, 2011 |

[a]With parental or guardian consent

**Methodology:**

The upper back between the scapulae served as the treatment area. Prior to the initiation of this study, the following test materials were prepared as composites (0.1g:0.1g):

**.04** D = A33 Gel F#239-170 + [redacted]
**.05** G = A33 Gel F#239-170 + H = Step 2 Skin Plumping Cream Ref: 248-080
**.06** I = A33 Gel F#239-170 + J Step 2 Skin Plumping Cream F#245-152

The upper back between the scapulae served as the treatment area. Approximately 0.2g of each of the test materials or an amount sufficient to cover the contact surface, were applied to the 1" x 1" absorbent pad portion of clear adhesive dressings. When secured to the appropriate treatment site, these dressings formed semi-occlusive patches.

The test materials remained in contact with the skin for a total of forty-eight hours. These sites were then evaluated for gross changes. Absence of any visible skin change was assigned a zero value. The test sites were re-evaluated at seventy-two hours.

**Evaluation Criteria (Erythema and additional Dermal Sequelae):**

| | | | | | |
|---|---|---|---|---|---|
| **0** | **=** | **No visible skin reaction** | **E** | **=** | **Edema** |
| **0.5** | **=** | **Barely perceptible** | **D** | **=** | **Dryness** |
| **1** | **=** | **Mild** | **S** | **=** | **Staining** |
| **2** | **=** | **Moderate** | **P** | **=** | **Papules** |
| **3** | **=** | **Marked** | **V** | **=** | **Vesicles** |
| **4** | **=** | **Severe** | **B** | **=** | **Bullae** |
| | | | **U** | **=** | **Ulceration** |
| | | | **Sp** | **=** | **Spreading** |

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

**Results:**

The results of each participant are appended (Table 1).

Observations of the sites treated with the following test materials remained negative throughout the test interval:

**.01** [redacted]
**.02** B = Step 2 Skin Plumping Cream Ref: 248-080
**.04** D = A33 Gel F#239-170 + [redacted]
**.05** G = A33 Gel F#239-170 + H = Step 2 Skin Plumping Cream Ref: 248-080

**.03** C = Step 2 Skin Plumping Cream F#245-152

One Subject (#4) exhibited moderate erythema and edema post-appplication; mild erythema and edema seventy-two hours post-application. Subject #26 exhibited mild erythema and edema forty-eight hours post application; barely perceptible erythema seventy-two hours post-application.

**.06** I = A33 Gel F#239-170 + J Step 2 Skin Plumping Cream F#245-152

Subject #26 exhibited barely perceptible erythema forty-eight hours post-application; negative seventy-two hours post-application.

Subject demographics are presented in Table 2.

**Summary:**

Under the conditions of this study, the following test materials did not indicate a potential for dermal irritation.

**.01** [redacted]
**.02** B = Step 2 Skin Plumping Cream Ref: 248-080
**.03** C = Step 2 Skin Plumping Cream F#245-152
**.04** D = A33 Gel F#239-170 + [redacted]
**.05** G = A33 Gel F#239-170 + H = Step 2 Skin Plumping Cream Ref: 248-080
**.06** I = A33 Gel F#239-170 + J Step 2 Skin Plumping Cream F#245-152

Table 1
Panel #20110106

Individual Results

[redacted]

| Subject Number | Observations 48 Hours | 72 Hours |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 0 | 0 |
| 3 | 0 | 0 |
| 4 | 0 | 0 |
| 5 | 0 | 0 |
| 6 | 0 | 0 |
| 7 | 0 | 0 |
| 8 | 0 | 0 |
| 9 | 0 | 0 |
| 10 | 0 | 0 |
| 11 | 0 | 0 |
| 12 | ---DID NOT COMPLETE STUDY--- | |
| 13 | 0 | 0 |
| 14 | 0 | 0 |
| 15 | 0 | 0 |
| 16 | 0 | 0 |
| 17 | 0 | 0 |
| 18 | 0 | 0 |
| 19 | 0 | 0 |
| 20 | 0 | 0 |
| 21 | 0 | 0 |
| 22 | 0 | 0 |
| 23 | 0 | 0 |
| 24 | 0 | 0 |
| 25 | 0 | 0 |
| 26 | 0 | 0 |
| 27 | 0 | 0 |

Table 1
(continued)
Panel #20110106

Individual Results

[redacted]

| Subject Number | Observations 48 Hours | 72 Hours |
|---|---|---|
| 28 | 0 | 0 |
| 29 | 0 | 0 |
| 30 | 0 | 0 |
| 31 | 0 | 0 |
| 32 | 0 | 0 |
| 33 | 0 | 0 |
| 34 | 0 | 0 |
| 35 | 0 | 0 |
| 36 | 0 | 0 |
| 37 | 0 | 0 |
| 38 | 0 | 0 |
| 39 | 0 | 0 |
| 40 | 0 | 0 |
| 41 | 0 | 0 |
| 42 | 0 | 0 |
| 43 | 0 | 0 |
| 44 | 0 | 0 |
| 45 | 0 | 0 |
| 46 | 0 | 0 |
| 47 | 0 | 0 |
| 48 | 0 | 0 |
| 49 | 0 | 0 |
| 50 | 0 | 0 |
| 51 | 0 | 0 |
| 52 | 0 | 0 |
| 53 | 0 | ---DNC--- |

DNC = Did not complete study

Table 2
Panel #20110106

Individual Results

B = Step 2 Skin Plumping Cream Ref: 248-080

| Subject Number | Observations 48 Hours | 72 Hours |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 0 | 0 |
| 3 | 0 | 0 |
| 4 | 0 | 0 |
| 5 | 0 | 0 |
| 6 | 0 | 0 |
| 7 | 0 | 0 |
| 8 | 0 | 0 |
| 9 | 0 | 0 |
| 10 | 0 | 0 |
| 11 | 0 | 0 |
| 12 | ---DID NOT COMPLETE STUDY--- | |
| 13 | 0 | 0 |
| 14 | 0 | 0 |
| 15 | 0 | 0 |
| 16 | 0 | 0 |
| 17 | 0 | 0 |
| 18 | 0 | 0 |
| 19 | 0 | 0 |
| 20 | 0 | 0 |
| 21 | 0 | 0 |
| 22 | 0 | 0 |
| 23 | 0 | 0 |
| 24 | 0 | 0 |
| 25 | 0 | 0 |
| 26 | 0 | 0 |
| 27 | 0 | 0 |

Table 2
(continued)
Panel #20110106

Individual Results

B = Step 2 Skin Plumping Cream Ref: 248-080

| Subject Number | Observations 48 Hours | 72 Hours |
|---|---|---|
| 28 | 0 | 0 |
| 29 | 0 | 0 |
| 30 | 0 | 0 |
| 31 | 0 | 0 |
| 32 | 0 | 0 |
| 33 | 0 | 0 |
| 34 | 0 | 0 |
| 35 | 0 | 0 |
| 36 | 0 | 0 |
| 37 | 0 | 0 |
| 38 | 0 | 0 |
| 39 | 0 | 0 |
| 40 | 0 | 0 |
| 41 | 0 | 0 |
| 42 | 0 | 0 |
| 43 | 0 | 0 |
| 44 | 0 | 0 |
| 45 | 0 | 0 |
| 46 | 0 | 0 |
| 47 | 0 | 0 |
| 48 | 0 | 0 |
| 49 | 0 | 0 |
| 50 | 0 | 0 |
| 51 | 0 | 0 |
| 52 | 0 | 0 |
| 53 | 0 | ---DNC--- |

DNC = Did not complete study

Table 3
Panel #20110106

Individual Results

C = Step 2 Skin Plumping Cream F#245-152

| Subject Number | Observations 48 Hours | 72 Hours |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 0 | 0 |
| 3 | 0 | 0 |
| 4 | **2**[E2] | **1**[E1] |
| 5 | 0 | 0 |
| 6 | 0 | 0 |
| 7 | 0 | 0 |
| 8 | 0 | 0 |
| 9 | 0 | 0 |
| 10 | 0 | 0 |
| 11 | 0 | 0 |
| 12 | ---DID NOT COMPLETE STUDY--- | |
| 13 | 0 | 0 |
| 14 | 0 | 0 |
| 15 | 0 | 0 |
| 16 | 0 | 0 |
| 17 | 0 | 0 |
| 18 | 0 | 0 |
| 19 | 0 | 0 |
| 20 | 0 | 0 |
| 21 | 0 | 0 |
| 22 | 0 | 0 |
| 23 | 0 | 0 |
| 24 | 0 | 0 |
| 25 | 0 | 0 |
| 26 | **1**[E1] | **0.5** |
| 27 | 0 | 0 |

E = Edema

Table 3
(continued)
Panel #20110106

Individual Results

C = Step 2 Skin Plumping Cream F#245-152

| Subject Number | Observations 48 Hours | 72 Hours |
|---|---|---|
| 28 | 0 | 0 |
| 29 | 0 | 0 |
| 30 | 0 | 0 |
| 31 | 0 | 0 |
| 32 | 0 | 0 |
| 33 | 0 | 0 |
| 34 | 0 | 0 |
| 35 | 0 | 0 |
| 36 | 0 | 0 |
| 37 | 0 | 0 |
| 38 | 0 | 0 |
| 39 | 0 | 0 |
| 40 | 0 | 0 |
| 41 | 0 | 0 |
| 42 | 0 | 0 |
| 43 | 0 | 0 |
| 44 | 0 | 0 |
| 45 | 0 | 0 |
| 46 | 0 | 0 |
| 47 | 0 | 0 |
| 48 | 0 | 0 |
| 49 | 0 | 0 |
| 50 | 0 | 0 |
| 51 | 0 | 0 |
| 52 | 0 | 0 |
| 53 | 0 | ---DNC--- |

DNC = Did not complete study

Table 4
Panel #20110106

Individual Results

D = A33 Gel F#239-170 + [redacted]

| Subject Number | Observations 48 Hours | 72 Hours |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 0 | 0 |
| 3 | 0 | 0 |
| 4 | 0 | 0 |
| 5 | 0 | 0 |
| 6 | 0 | 0 |
| 7 | 0 | 0 |
| 8 | 0 | 0 |
| 9 | 0 | 0 |
| 10 | 0 | 0 |
| 11 | 0 | 0 |
| 12 | ---DID NOT COMPLETE STUDY--- | |
| 13 | 0 | 0 |
| 14 | 0 | 0 |
| 15 | 0 | 0 |
| 16 | 0 | 0 |
| 17 | 0 | 0 |
| 18 | 0 | 0 |
| 19 | 0 | 0 |
| 20 | 0 | 0 |
| 21 | 0 | 0 |
| 22 | 0 | 0 |
| 23 | 0 | 0 |
| 24 | 0 | 0 |
| 25 | 0 | 0 |
| 26 | 0 | 0 |
| 27 | 0 | 0 |

Table 4
(continued)
Panel #20110106

Individual Results

D = A33 Gel F#239-170 + [redacted]

| Subject Number | Observations 48 Hours | 72 Hours |
|---|---|---|
| 28 | 0 | 0 |
| 29 | 0 | 0 |
| 30 | 0 | 0 |
| 31 | 0 | 0 |
| 32 | 0 | 0 |
| 33 | 0 | 0 |
| 34 | 0 | 0 |
| 35 | 0 | 0 |
| 36 | 0 | 0 |
| 37 | 0 | 0 |
| 38 | 0 | 0 |
| 39 | 0 | 0 |
| 40 | 0 | 0 |
| 41 | 0 | 0 |
| 42 | 0 | 0 |
| 43 | 0 | 0 |
| 44 | 0 | 0 |
| 45 | 0 | 0 |
| 46 | 0 | 0 |
| 47 | 0 | 0 |
| 48 | 0 | 0 |
| 49 | 0 | 0 |
| 50 | 0 | 0 |
| 51 | 0 | 0 |
| 52 | 0 | 0 |
| 53 | 0 | ---DNC--- |

DNC = Did not complete study

Table 5
Panel #20110106

Individual Results

G = A33 Gel F#239-170 + H = Step 2 Skin Plumping Cream Ref: 248-080

| Subject Number | Observations 48 Hours | 72 Hours |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 0 | 0 |
| 3 | 0 | 0 |
| 4 | 0 | 0 |
| 5 | 0 | 0 |
| 6 | 0 | 0 |
| 7 | 0 | 0 |
| 8 | 0 | 0 |
| 9 | 0 | 0 |
| 10 | 0 | 0 |
| 11 | 0 | 0 |
| 12 | ---DID NOT COMPLETE STUDY--- | |
| 13 | 0 | 0 |
| 14 | 0 | 0 |
| 15 | 0 | 0 |
| 16 | 0 | 0 |
| 17 | 0 | 0 |
| 18 | 0 | 0 |
| 19 | 0 | 0 |
| 20 | 0 | 0 |
| 21 | 0 | 0 |
| 22 | 0 | 0 |
| 23 | 0 | 0 |
| 24 | 0 | 0 |
| 25 | 0 | 0 |
| 26 | 0 | 0 |
| 27 | 0 | 0 |

Table 5
(continued)
Panel #20110106

Individual Results

G = A33 Gel F#239-170 + H = Step 2 Skin Plumping Cream Ref: 248-080

| Subject Number | Observations 48 Hours | 72 Hours |
|---|---|---|
| 28 | 0 | 0 |
| 29 | 0 | 0 |
| 30 | 0 | 0 |
| 31 | 0 | 0 |
| 32 | 0 | 0 |
| 33 | 0 | 0 |
| 34 | 0 | 0 |
| 35 | 0 | 0 |
| 36 | 0 | 0 |
| 37 | 0 | 0 |
| 38 | 0 | 0 |
| 39 | 0 | 0 |
| 40 | 0 | 0 |
| 41 | 0 | 0 |
| 42 | 0 | 0 |
| 43 | 0 | 0 |
| 44 | 0 | 0 |
| 45 | 0 | 0 |
| 46 | 0 | 0 |
| 47 | 0 | 0 |
| 48 | 0 | 0 |
| 49 | 0 | 0 |
| 50 | 0 | 0 |
| 51 | 0 | 0 |
| 52 | 0 | 0 |
| 53 | 0 | ---DNC--- |

DNC = Did not complete study

Table 6
Panel #20110106

Individual Results

I = A33 Gel F#239-170 + J Step 2 Skin Plumping Cream F#245-152

| Subject Number | Observations 48 Hours | 72 Hours |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 0 | 0 |
| 3 | 0 | 0 |
| 4 | 0 | 0 |
| 5 | 0 | 0 |
| 6 | 0 | 0 |
| 7 | 0 | 0 |
| 8 | 0 | 0 |
| 9 | 0 | 0 |
| 10 | 0 | 0 |
| 11 | 0 | 0 |
| 12 | ---DID NOT COMPLETE STUDY--- | |
| 13 | 0 | 0 |
| 14 | 0 | 0 |
| 15 | 0 | 0 |
| 16 | 0 | 0 |
| 17 | 0 | 0 |
| 18 | 0 | 0 |
| 19 | 0 | 0 |
| 20 | 0 | 0 |
| 21 | 0 | 0 |
| 22 | 0 | 0 |
| 23 | 0 | 0 |
| 24 | 0 | 0 |
| 25 | 0 | 0 |
| 26 | 0.5 | 0 |
| 27 | 0 | 0 |

Table 6
(continued)
Panel #20110106

Individual Results

I = A33 Gel F#239-170 + J Step 2 Skin Plumping Cream F#245-152

| Subject Number | Observations 48 Hours | 72 Hours |
|---|---|---|
| 28 | 0 | 0 |
| 29 | 0 | 0 |
| 30 | 0 | 0 |
| 31 | 0 | 0 |
| 32 | 0 | 0 |
| 33 | 0 | 0 |
| 34 | 0 | 0 |
| 35 | 0 | 0 |
| 36 | 0 | 0 |
| 37 | 0 | 0 |
| 38 | 0 | 0 |
| 39 | 0 | 0 |
| 40 | 0 | 0 |
| 41 | 0 | 0 |
| 42 | 0 | 0 |
| 43 | 0 | 0 |
| 44 | 0 | 0 |
| 45 | 0 | 0 |
| 46 | 0 | 0 |
| 47 | 0 | 0 |
| 48 | 0 | 0 |
| 49 | 0 | 0 |
| 50 | 0 | 0 |
| 51 | 0 | 0 |
| 52 | 0 | 0 |
| 53 | 0 | ---DNC--- |

DNC = Did not complete study

Table 7
Panel #20110106
Subject Demographics

| Subject Number | Initials | Age | Sex |
|---|---|---|---|
| 1 | [redacted] | 53 | F |
| 2 | | 74 | M |
| 3 | | 50 | F |
| 4 | | 62 | F |
| 5 | | 48 | F |
| 6 | | 46 | F |
| 7 | | 57 | M |
| 8 | | 42 | M |
| 9 | | 35 | F |
| 10 | | 56 | F |
| 11 | | 68 | F |
| 12 | | 35 | F |
| 13 | | 43 | F |
| 14 | | 50 | F |
| 15 | | 60 | F |
| 16 | | 65 | F |
| 17 | | 78 | F |
| 18 | | 57 | F |
| 19 | | 64 | M |
| 20 | | 51 | F |
| 21 | | 38 | F |
| 22 | | 68 | M |
| 23 | | 58 | F |
| 24 | | 62 | F |
| 25 | | 42 | M |
| 26 | | 33 | M |
| 27 | | 50 | F |

Table 7
(continued)
Panel #20110106

Subject Demographics

| Subject Number | Initials | Age | Sex |
|---|---|---|---|
| 28 | [redacted] | 22 | M |
| 29 | | 74 | F |
| 30 | | 52 | M |
| 31 | | 42 | F |
| 32 | | 50 | M |
| 33 | | 74 | F |
| 34 | | 55 | F |
| 35 | | 54 | F |
| 36 | | 51 | F |
| 37 | | 44 | F |
| 38 | | 23 | F |
| 39 | | 72 | F |
| 40 | | 50 | M |
| 41 | | 52 | F |
| 42 | | 43 | F |
| 43 | | 47 | F |
| 44 | | 44 | M |
| 45 | | 78 | F |
| 46 | | 53 | F |
| 47 | | 75 | F |
| 48 | | 74 | M |
| 49 | | 58 | F |
| 50 | | 22 | F |
| 51 | | 41 | F |
| 52 | | 25 | F |
| 53 | | 60 | F |

[redacted]

# FINAL REPORT

**CLIENT:** [redacted]

**ATTENTION:**

**TEST:** Repeated Insult Patch Test
Protocol No.: 1.01S

**TEST MATERIAL:** A33 Plumper Gel Step-1, [redacted]
2% n-acetyl tyrosinamide containing product

**EXPERIMENT REFERENCE NUMBER:** [redacted]

Reviewed by: [redacted]

Approved by:

Approved by:



[redacted]

[redacted]

# QUALITY ASSURANCE UNIT STATEMENT

**Trial Number:** [redacted]

[redacted] Quality Assurance Unit (QAU) is responsible for auditing the conduct, content and reporting of all clinical trials that are conducted at [redacted]

This trial has been conducted in accordance with the Declaration of Helsinki, the ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable laws and regulations, [redacted] Standard Operating Procedures, and the approved protocol.

The [redacted] QAU has reviewed all data, records, and documents relating to this trial and also this Final Report. The following QAU representative signature certifies that all data, records, and documents relating to this trial and also this Final Report have been reviewed and are deemed to be acceptable, and that the trial conforms to all of the requirements as indicated above.

All records and documents pertaining to the conduct of this trial shall be retained in the [redacted] archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the [redacted] QAU to obtain custody of trial records once the [redacted] archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, trial-related records shall be destroyed at the end of the [redacted] archive period in a manner that renders them useless.

[redacted]
Quality Assurance Representative

11/2/11
Date

[redacted]

**Objective:** To determine by repetitive epidermal contact the potential of a test material to induce primary or cumulative irritation and/or allergic contact sensitization.

**Participants:** One hundred-fifteen (115) qualified subjects, male and female, ranging in age from 17 to 77 years, were selected for this evaluation. One hundred-nine (109) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

**Inclusion Criteria:**

a. Male and female subjects, age 16[a] and over.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History form and the understanding and signing of an Informed Consent form.
e. Considered reliable and capable of following directions.

**Exclusion Criteria:**

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

**Test Material:** A33 Plumper Gel Step-1, F#268-46, L#231-125

**Study Schedule:**

| Panel # | Initiation Date | Completion Date |
|---|---|---|
| 20110291 | September 07, 2011 | October 20, 2011 |
| 20110306 | September 14, 2011 | October 20, 2011 |

[a]With parental or guardian consent

**Methodology:**

The upper back between the scapulae served as the treatment area. Approximately 0.2 g of the test material, or an amount sufficient to cover the contact surface, was applied to the 1" x 1" absorbent pad portion of a clear adhesive dressing. This was then applied to the appropriate site to form a semi-occlusive patch.

**Induction Phase:**

Applications were conducted three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period.

If any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications were discontinued for the remainder of this test phase, if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of twenty-four hours following each Monday and Wednesday application, and forty-eight hours following each Friday application.

**Challenge Phase:**

Approximately two (2) weeks after the final Induction application, a Challenge application was conducted to a virgin test site adjacent to the original Induction site, following the same procedure described for Induction. The site was scored at the clinic twenty-four and seventy two hours post-application.

**Methodology (continued):**

Evaluation Criteria (Erythema and additional Dermal Sequelae):

| | | | | | |
|---|---|---|---|---|---|
| **0** | = | **No visible skin reaction** | **E** | = | **Edema** |
| **0.5** | = | **Barely perceptible** | **D** | = | **Dryness** |
| **1** | = | **Mild** | **S** | = | **Staining** |
| **2** | = | **Moderate** | **P** | = | **Papules** |
| **3** | = | **Marked** | **V** | = | **Vesicles** |
| **4** | = | **Severe** | **B** | = | **Bullae** |
| | | | **U** | = | **Ulceration** |
| | | | **Sp** | = | **Spreading** |

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

**Results:**

The results of each participant are appended (Table 1).

Observations remained negative throughout the test interval.

Subject demographics are presented in Table 2.

**Summary:**

Under the conditions of this study, test material, A33 Plumper Gel Step-1, F#268-46, L#231-125, did not indicate a potential for dermal irritation or allergic contact sensitization.

Table 1
Panel #20110291

Individual Results

A33 Plumper Gel Step-1, F#268-46, L#231-125

| Subject Number | 24*hr | Induction Phase 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Virgin Challenge Site 24*hr | 72 hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0[m] | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | DID NOT COMPLETE STUDY | | | | | | | | | | | |
| 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | 0[m] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | DID NOT COMPLETE STUDY | | | | | | | | | | | |
| 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22 | 0 | 0 | 0 | 0[m] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0[m] | 0 | 0 | 0 | 0 | 0 |
| 27 | 0 | 0 | DID NOT COMPLETE STUDY | | | | | | | | | |
| 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

24* = Supervised removal of 1st Induction and Challenge Patch
m = Additional makeup day granted at the discretion of the clinic supervisor
- = Subject not present for supervised removal

Table 1
(continued)
Panel #20110291

Individual Results

A33 Plumper Gel Step-1, F#268-46, L#231-125

| Subject Number | 24*hr | Induction Phase 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Virgin Challenge Site 24*hr | 72 hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0[m] | 0 | 0 | 0 | 0 |
| 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0[m] | 0 | 0 | 0 | 0 | 0 |
| 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 41 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 47 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 49 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 56 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 57 | ----------DID NOT COMPLETE STUDY---------- | | | | | | | | | | | |
| 58 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

24* = Supervised removal of 1st Induction and Challenge Patch
m = Additional makeup day granted at the discretion of the clinic supervisor

Table 1
Panel #20110306

Individual Results

A33 Plumper Gel Step-1, F#268-46, L#231-125

| Subject Number | 24*hr | Induction Phase 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Virgin Challenge Site 24*hr | 72 hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

24* = Supervised removal of 1st Induction and Challenge Patch
- = Subject not present for supervised removal

Table 1
(continued)
Panel #20110306

Individual Results

A33 Plumper Gel Step-1, F#268-46, L#231-125

| Subject Number | 24*hr | Induction Phase 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Virgin Challenge Site 24*hr | 72 hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 36 | 0 | 0 | 0 | ----------DID NOT COMPLETE---------- | | | | | | | | |
| 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 40 | - | 0 | 0 | 0 | 0 | 0 | 0 | ---------DID NOT COMPLETE-------- | | | | |
| 41 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 47 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 49 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 56 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 57 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

24* = Supervised removal of 1st Induction and Challenge Patch
- = Subject not present for supervised removal

Table 2
Panel #20110291

Subject Demographics

| Subject Number | Initials | Age | Sex |
|---|---|---|---|
| 1 | [redacted] | 53 | F |
| 2 | | 47 | F |
| 3 | | 55 | F |
| 4 | | 18 | M |
| 5 | | 63 | F |
| 6 | | 51 | F |
| 7 | | 50 | F |
| 8 | | 39 | F |
| 9 | | 59 | F |
| 10 | | 36 | F |
| 11 | | 48 | F |
| 12 | | 48 | M |
| 13 | | 65 | F |
| 14 | | 45 | F |
| 15 | | 57 | F |
| 16 | | 62 | M |
| 17 | | 45 | F |
| 18 | | 62 | F |
| 19 | | 66 | F |
| 20 | | 37 | M |
| 21 | | 69 | M |
| 22 | | 19 | F |
| 23 | | 37 | F |
| 24 | | 47 | F |
| 25 | | 43 | F |
| 26 | | 57 | F |
| 27 | | 36 | F |
| 28 | | 44 | F |
| 29 | | 67 | F |

Table 2
(continued)
Panel #20110291

Subject Demographics

| Subject Number | Initials | Age | Sex |
|---|---|---|---|
| 30 | [redacted] | 42 | M |
| 31 | | 51 | F |
| 32 | | 29 | F |
| 33 | | 31 | F |
| 34 | | 62 | M |
| 35 | | 69 | F |
| 36 | | 50 | M |
| 37 | | 65 | M |
| 38 | | 55 | F |
| 39 | | 65 | F |
| 40 | | 49 | F |
| 41 | | 52 | F |
| 42 | | 42 | F |
| 43 | | 69 | F |
| 44 | | 60 | F |
| 45 | | 48 | F |
| 46 | | 62 | F |
| 47 | | 48 | F |
| 48 | | 65 | F |
| 49 | | 53 | F |
| 50 | | 25 | F |
| 51 | | 29 | F |
| 52 | | 58 | F |
| 53 | | 51 | F |
| 54 | | 55 | F |
| 55 | | 69 | F |
| 56 | | 34 | F |
| 57 | | 34 | F |
| 58 | | 70 | F |

Table 2
Panel #20110306

Subject Demographics

| Subject Number | Initials | Age | Sex |
|---|---|---|---|
| 1 | [redacted] | 37 | M |
| 2 | | 77 | F |
| 3 | | 27 | F |
| 4 | | 43 | F |
| 5 | | 60 | F |
| 6 | | 40 | F |
| 7 | | 57 | F |
| 8 | | 51 | F |
| 9 | | 54 | F |
| 10 | | 69 | M |
| 11 | | 66 | F |
| 12 | | 76 | F |
| 13 | | 49 | F |
| 14 | | 33 | F |
| 15 | | 17 | M |
| 16 | | 49 | F |
| 17 | | 34 | F |
| 18 | | 64 | F |
| 19 | | 39 | F |
| 20 | | 37 | F |
| 21 | | 31 | F |
| 22 | | 41 | F |
| 23 | | 40 | F |
| 24 | | 53 | F |
| 25 | | 39 | F |
| 26 | | 53 | M |
| 27 | | 75 | M |
| 28 | | 57 | F |
| 29 | | 74 | F |

Table 2
(continued)
Panel #20110306

Subject Demographics

| Subject Number | Initials | Age | Sex |
|---|---|---|---|
| 30 | [redacted] | 43 | F |
| 31 | | 57 | M |
| 32 | | 74 | F |
| 33 | | 74 | F |
| 34 | | 52 | M |
| 35 | | 53 | F |
| 36 | | 43 | F |
| 37 | | 47 | F |
| 38 | | 54 | M |
| 39 | | 56 | F |
| 40 | | 73 | F |
| 41 | | 73 | F |
| 42 | | 40 | F |
| 43 | | 18 | M |
| 44 | | 27 | F |
| 45 | | 46 | F |
| 46 | | 48 | F |
| 47 | | 40 | F |
| 48 | | 31 | F |
| 49 | | 69 | F |
| 50 | | 67 | M |
| 51 | | 66 | F |
| 52 | | 38 | F |
| 53 | | 54 | F |
| 54 | | 61 | F |
| 55 | | 70 | F |
| 56 | | 57 | M |
| 57 | | 54 | F |

[redacted]

# Final Report

**An In-Use Safety Evaluation to Determine the Ocular Irritation Potential and Consumer Opinion of Cosmetic Products**

**CLIENT:**

**ATTENTION:**

[redacted]

**TEST MATERIAL:**

**2 Step Under Eye and Crow's Feet Treatment:**
- **Step 1- Gel** = 2% n-acetyl tyrosinamide
- **Step 2- Cream**

[redacted] **STUDY NUMBER:** [redacted]

**AUTHORIZED SIGNATURES:**

[redacted]

**REPORT DATE:** **November 2, 2011**

[redacted]

[redacted]

# Good Clinical Practice
# Quality Assurance Audit Statement

**Clinical Study Number:** [redacted]

**Start Date:** September 27, 2011

**Completion Date:** October 26, 2011

The clinical study listed above was conducted in accordance with [redacted] Standard Operating Procedures, which incorporate the principles of Good Clinical Practice defined by applicable guidelines and regulations established by U.S. Regulatory Agencies. The conduct of the study was monitored for compliance, and the associated records, including source documents or raw data, were reviewed for documentation practices and accuracy by a Project Manager/Study Director and/or a Quality Assurance representative. Standard Quality Assurance audit procedures for this final report and study related documents were conducted.

[redacted]

Nov. 2, 2011
Date

[redacted]

**FINAL REPORT**

**An In-Use Safety Evaluation to Determine the Ocular Irritation Potential and Consumer Opinion of Cosmetic Products**

## OBJECTIVES

The objectives of this study were;

1. to evaluate the potential of cosmetic products to induce subjective and/or objective ocular irritation during four weeks of normal use in contact lens wearers and non-contact lens wearers;
2. to obtain the consumer opinion of cosmetic products following a four-week use period.

## INVESTIGATORS

[redacted]

## INVESTIGATIVE SITE

[redacted]

## SPONSOR

[redacted]

## TEST MATERIAL

The following test materials were provided by [redacted] and were received by [redacted] on September 21, 2011:

| Client Identification | | [redacted] Identification |
|---|---|---|
| 2 Step Under Eye and Crow's Feet Treatment | Step 1- Gel | [redacted]97511-1 |
| | Step 2- Cream | [redacted]97511-2 |

Test materials were labeled with [redacted] identification and subject numbers. Test material weights appear in Appendix I.

## STUDY DATES

This study was initiated on September 27, 2011 and was completed on October 26, 2011.

## STUDY POPULATION

A total of 33 female subjects, ranging in age from 40 to 60 years and in generally good health, were selected for the study (Subject Demographics – Appendix II). Sixteen subjects (48%) were contact lens wearers and the remaining 17 subjects (52%) were non-contact lens wearers. Subjects who met all of the inclusion criteria and none of the exclusion criteria listed in the study protocol were enrolled for participation.

## TEST METHOD

This study was conducted according to the attached study protocol, [redacted]7511 AC 1.0 (Attachment I).

## TEST RESULTS

### *Completed and Discontinued Subjects*

All 33 subjects completed the study. Subject #3 missed the Week 2 visit.

### *Ophthalmic Examinations*

Increases from baseline in ocular examination scores observed in each eye for each evaluated tissue at the Week 2 and Week 4 ophthalmic examinations appear in Table I. There were no reports of subjective irritation, lacrimation or eyelid irritation and no changes in corneal tissue integrity or contact lenses observed at either examination. Four subjects exhibited trace increases in palpebral conjunctival irritation at the Week 2 and/or Week 4 examinations. One subject exhibited a trace increase in bulbar conjunctival irritation at the Week 4 examination. In the opinion of the Investigator, these findings were not related to use of the test material and were probably caused by external factors such as hair products, mechanical factors, environmental conditions and/or seasonal factors.

### *Questionnaires*

A summary of questionnaire responses appears in Attachment II. Statistical analysis of questionnaire responses appears below.

## TEST RESULTS (Continued)

### *Questionnaires (Continued)*

| Question | Most Favorable Percentage | Least Favorable Percentage | Z-Score | Significant |
|---|---|---|---|---|
| 1: Please rate the following for this product regimen: | | | | |
| 1a. Spreadability | 95.5% | 4.5% | 5.22 | Yes |
| 1b. Ease of absorption/Rub in | 83.3% | 16.7% | 3.83 | Yes |
| 1c. The feel of the products on skin after both products are applied | 92.4% | 7.6% | 4.87 | Yes |
| 2: Please rate the following characteristics | | | | |
| 2a. Moisturizes/hydrates | 93.9% | 6.1% | 5.05 | Yes |
| 2b. Fine lines are less noticeable | 78.8% | 21.2% | 3.31 | Yes |
| 2c. Wrinkles (crow's feet area) are less noticeable | 74.2% | 25.8% | 2.79 | Yes |
| 2d. Improves skin texture/ smoothness | 80.3% | 19.7% | 3.48 | Yes |
| 2e. Appears to plump and fill in lines around my eyes | 66.7% | 33.3% | 1.91 | No |
| 2f. Skin looks younger | 71.2% | 28.8% | 2.44 | Yes |
| 2g. Eye area firmness / elasticity is increased | 75.8% | 24.2% | 2.96 | Yes |
| 2h. Skin looks brighter | 74.2% | 25.8% | 2.79 | Yes |
| 2i. Dark circles are less apparent | 67.9% | 32.1% | 1.89 | No |
| 2j. Is gentle to the skin | 95.5% | 4.5% | 5.22 | Yes |
| 2k. Is not irritating | 90.9% | 9.1% | 4.70 | Yes |
| 2l. Does not sting or burn | 86.4% | 13.6% | 4.18 | Yes |
| 2m. Does not rub off or 'ball' | 77.3% | 22.7% | 3.13 | Yes |
| 2n. Is compatible with makeup/foundation | 82.3% | 17.7% | 3.59 | Yes |
| 2o. Improves overall appearance of skin around eyes | 77.3% | 22.7% | 3.13 | Yes |
| 3. What is the earliest time that you thought this product regimen made your eye area skin look younger? | 74.2% | 25.8% | 2.79 | Yes |
| 4. Compared to the eye area product that you usually use, how would you rate this product? | 84.5% | 15.5% | 3.71 | Yes |
| 5. What is your overall opinion of this product regimen? | 78.8% | 21.2% | 3.31 | Yes |
| 6. Please rate how much you agree or disagree with how easy it was to use this 2 step product regimen? | 100.0% | 0.0% | 5.74 | Yes |
| 7. Please rate the packaging for each product | | | | |
| 7a. Step 1 Gel | 89.4% | 10.6% | 4.53 | Yes |
| 7b. Step 2 Cream | 93.9% | 6.1% | 5.05 | Yes |
| 8. For the Step 1 Gel, did the ball applicator glide or roll easily when applying it during the study? | 100.0% | 0.0% | 5.74 | Yes |

*=Percentage of the study population reporting the two most favorable responses (included half of the population reporting the central response) or the two least favorable responses (included half of the population reporting the central response, where applicable).

### *Daily Diaries*

There were no comments recorded on the Daily Diaries that were related to reactions or symptoms perceived during test material use.

### *Adverse Events*

There were no adverse events reported during the study period.

[redacted]



## CONCLUSION

Based on the test results of the subjective and objective ophthalmic evaluations during the four-week period, it was determined that use of the test materials, 2 Step Under Eye and Crow's Feet Treatment: Step 1-Gel and Step 2-Cream, did not demonstrate a potential for eliciting ophthalmic irritation. In this test population, the test materials were clinically safe for use by contact lens wearers and non-contact lens wearers.

Questionnaires completed by subjects following the four-week use period indicated that a statistically significant portion of the test population reported favorable test material attributes including application qualities, gentleness, and effect on skin, including the following;

- moisturizes
- improves appearance of fine lines/wrinkles
- improves skin texture/smoothness
- skin looks younger and brighter
- increases eye area firmness/elasticity

[redacted]



## Table I

## Ophthalmic Examinations

| Subject Number | Increase from Baseline – Week 2 | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Subjective Irritation | | Lacrimation | | Eyelid Irritation (Upper/Lower) | | Palpebral Conjunctival Irritation (Upper/Lower) | | Bulbar Conjunctival Irritation | | Cornea | | Contact Lens | |
| | R | L | R | L | R | L | R | L | R | L | R | L | R | L |
| 1 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 2 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | Missed Visit | | | | | | | | | | | | NA | NA |
| 4 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 5 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 6 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 1/1 | 1/1 | 0 | 0 | 0 | 0 | NA | NA |
| 7 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 8 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 9 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 11 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 1/1 | 1/1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 15 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 17 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 18 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 19 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 20 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 21 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 22 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 24 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 27 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |

The scoring scale for ophthalmic examinations appears in the attached study protocol.
R = Right Eye L = Left Eye NA = Not Applicable; subject is not a contact lens wearer.

[redacted]



**Table I**
**(Continued)**

## Ophthalmic Examinations

| Increase from Baseline – Week 4 | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subject Number | Subjective Irritation | | Lacrimation | | Eyelid Irritation (Upper/Lower) | | Palpebral Conjunctival Irritation (Upper/Lower) | | Bulbar Conjunctival Irritation | | Cornea | | Contact Lens | |
| | R | L | R | L | R | L | R | L | R | L | R | L | R | L |
| 1 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 2 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 4 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 5 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 6 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 7 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 8 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 1/1 | 1/1 | 0 | 0 | 0 | 0 | NA | NA |
| 9 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 11 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 1/1 | 1/1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 15 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 17 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 18 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 19 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 20 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 21 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 22 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 1 | 1 | 0 | 0 | NA | NA |
| 24 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | NA | NA |
| 27 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 1/1 | 1/1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33 | 0 | 0 | 0 | 0 | 0/0 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | 0 | 0 | 0 |

The scoring scale for ophthalmic examinations appears in the attached study protocol.
R = Right Eye L = Left Eye NA = Not Applicable; subject is not a contact lens wearer.

## Appendix I

### Test Material Weights

| Step 1 -Gel (weight in grams) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Subject # | Baseline | | Week 2 | | Week 4 | | Total Amt. Used |
| | Tube 1 | Tube 2 | Tube 1 | Tube 2 | Tube 1 | Tube 2 | |
| 1 | 25.9 | 25.5 | 24.6 | 25.5 | 23.3 | 25.3 | 2.8 |
| 2 | 25.9 | 26.0 | 25.1 | 26.0 | 22.8 | 22.4 | 6.7 |
| 3 | 25.2 | 25.9 | MV | MV | 24.4 | 24.4 | 2.3 |
| 4 | 25.6 | 26.2 | 25.5 | 25.6 | 24.7 | 25.4 | 1.7 |
| 5 | 25.9 | 25.3 | 25.2 | 25.3 | 24.3 | 25.3 | 1.6 |
| 6 | 25.8 | 26.1 | 24.1 | 26.1 | 23.5 | 26.0 | 2.4 |
| 7 | 25.6 | 25.4 | 25.0 | 25.4 | 24.2 | 25.4 | 1.4 |
| 8 | 25.7 | 26.9 | 23.6 | 26.6 | 23.3 | 24.0 | 5.3 |
| 9 | 26.0 | 25.9 | 24.3 | 25.9 | 22.4 | 25.8 | 3.7 |
| 10 | 25.9 | 26.2 | 23.6 | 26.2 | 23.1 | 24.9 | 4.1 |
| 11 | 25.9 | 25.6 | 22.3 | 25.6 | 20.0 | 25.6 | 5.9 |
| 12 | 25.9 | 25.9 | 25.8 | 25.7 | 25.8 | 25.1 | 0.9 |
| 13 | 26.0 | 25.8 | 25.4 | 25.8 | 25.1 | 25.8 | 0.9 |
| 14 | 25.8 | 26.0 | 17.6 | 26.0 | 17.6 | 13.8 | 20.4 |
| 15 | 26.0 | 25.0 | 25.2 | 25.0 | 24.3 | 24.9 | 1.8 |
| 16 | 25.4 | 25.4 | 24.6 | 25.4 | 18.4 | 21.6 | 10.8 |
| 17 | 25.2 | 26.2 | 25.1 | 26.2 | 17.8 | 24.8 | 8.8 |
| 18 | 25.7 | 25.7 | 25.7 | 24.9 | 25.3 | 24.9 | 1.2 |
| 19 | 25.1 | 25.1 | 20.9 | 24.9 | 19.2 | 22.4 | 8.6 |
| 20 | 25.1 | 25.3 | 24.8 | 25.3 | 24.5 | 25.3 | 0.6 |
| 21 | 25.7 | 25.4 | 25.7 | 24.6 | 23.6 | 20.7 | 6.8 |
| 22 | 26.0 | 25.7 | 24.4 | 25.7 | 23.1 | 25.7 | 2.9 |
| 23 | 25.5 | 26.3 | 24.6 | 26.3 | 21.8 | 25.6 | 4.4 |
| 24 | 25.3 | 25.7 | 24.6 | 25.7 | 23.7 | 25.7 | 1.6 |
| 25 | 26.1 | 26.0 | 25.8 | 25.9 | 25.7 | 25.9 | 0.5 |
| 26 | 24.9 | 25.6 | 24.4 | 25.6 | 23.6 | 25.5 | 1.4 |
| 27 | 25.5 | 25.1 | 23.4 | 25.1 | 20.6 | 23.7 | 6.3 |
| 28 | 26.2 | 25.9 | 25.7 | 25.4 | 25.3 | 24.7 | 2.1 |
| 29 | 26.0 | 25.5 | 25.1 | 25.5 | 24.4 | 25.5 | 1.6 |
| 30 | 25.3 | 26.1 | 25.3 | 25.9 | 24.9 | 25.6 | 0.9 |
| 31 | 25.7 | 25.9 | 24.7 | 25.9 | 23.7 | 25.9 | 2 |
| 32 | 25.5 | 25.7 | 24.9 | 25.7 | 24.3 | 25.7 | 1.2 |
| 33 | 26.2 | 26.0 | 26.2 | 25.4 | 25.6 | 21.0 | 5.6 |

**Appendix I**
**(Continued)**

**Test Material Weights**

| Step 2 -Cream (weight in grams) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Subject # | Baseline | | Week 2 | | Week 4 | | Total Amt. Used |
| | Tube 1 | Tube 2 | Tube 1 | Tube 2 | Tube 1 | Tube 2 | |
| 1 | 31.8 | 31.9 | 29.0 | 31.6 | 26.0 | 31.6 | 6.1 |
| 2 | 31.9 | 32.0 | 25.5 | 32.0 | 22.4 | 28.0 | 13.5 |
| 3 | 32.1 | 31.7 | MV | MV | 29.6 | 30.1 | 4.1 |
| 4 | 31.7 | 31.9 | 27.1 | 30.0 | 23.2 | 29.5 | 10.9 |
| 5 | 31.7 | 31.7 | 30.0 | 31.7 | 27.4 | 31.7 | 4.3 |
| 6 | 31.8 | 32.1 | 28.0 | 32.1 | 24.6 | 32.0 | 7.3 |
| 7 | 32.0 | 32.2 | 30.2 | 32.2 | 28.3 | 32.2 | 3.7 |
| 8 | 31.8 | 32.3 | 26.0 | 32.1 | 25.5 | 26.0 | 12.6 |
| 9 | 31.8 | 31.9 | 29.7 | 31.9 | 28.5 | 31.9 | 3.3 |
| 10 | 31.6 | 32.1 | 27.3 | 32.1 | 25.4 | 29.0 | 9.3 |
| 11 | 31.8 | 31.8 | 26.3 | 31.2 | 21.6 | 31.2 | 10.8 |
| 12 | 32.0 | 31.7 | 30.5 | 31.7 | 27.4 | 31.7 | 4.6 |
| 13 | 31.8 | 32.2 | 31.5 | 30.7 | 31.5 | 29.2 | 3.3 |
| 14 | 32.0 | 31.8 | 17.5 | 31.5 | 17.4 | 17.3 | 29.1 |
| 15 | 31.6 | 31.8 | 29.4 | 31.8 | 27.8 | 31.8 | 3.8 |
| 16 | 31.7 | 32.0 | 29.4 | 32.0 | 17.4 | 20.6 | 25.7 |
| 17 | 31.7 | 32.0 | 29.5 | 32.0 | 17.3 | 30.6 | 15.8 |
| 18 | 31.9 | 31.9 | 30.2 | 31.9 | 30.2 | 30.9 | 2.7 |
| 19 | 31.7 | 31.6 | 25.0 | 31.5 | 24.3 | 27.5 | 11.5 |
| 20 | 31.7 | 32.2 | 27.3 | 29.6 | 26.5 | 29.6 | 7.8 |
| 21 | 32.3 | 32.3 | 32.3 | 30.7 | 30.7 | 24.9 | 9 |
| 22 | 31.7 | 31.9 | 25.0 | 31.9 | 17.3 | 29.1 | 17.2 |
| 23 | 29.3 | 31.8 | 27.0 | 31.8 | 17.4 | 31.0 | 12.7 |
| 24 | 32.0 | 31.9 | 29.0 | 31.9 | 25.9 | 31.9 | 6.1 |
| 25 | 31.9 | 31.7 | 30.0 | 31.7 | 27.6 | 31.7 | 4.3 |
| 26 | 31.7 | 32.1 | 28.3 | 32.1 | 24.1 | 32.1 | 7.6 |
| 27 | 32.1 | 31.9 | 28.1 | 31.9 | 21.4 | 31.9 | 10.7 |
| 28 | 31.6 | 32.0 | 29.4 | 31.0 | 28.1 | 28.8 | 6.7 |
| 29 | 31.9 | 31.6 | 26.7 | 31.6 | 22.3 | 30.7 | 10.5 |
| 30 | 32.0 | 31.8 | 27.8 | 31.7 | 24.9 | 30.1 | 8.8 |
| 31 | 31.8 | 31.8 | 28.3 | 31.8 | 24.3 | 31.8 | 7.5 |
| 32 | 32.0 | 32.2 | 28.9 | 32.2 | 26.0 | 32.2 | 6 |
| 33 | 31.8 | 31.7 | 318.0 | 27.4 | 23.7 | 27.1 | 12.7 |

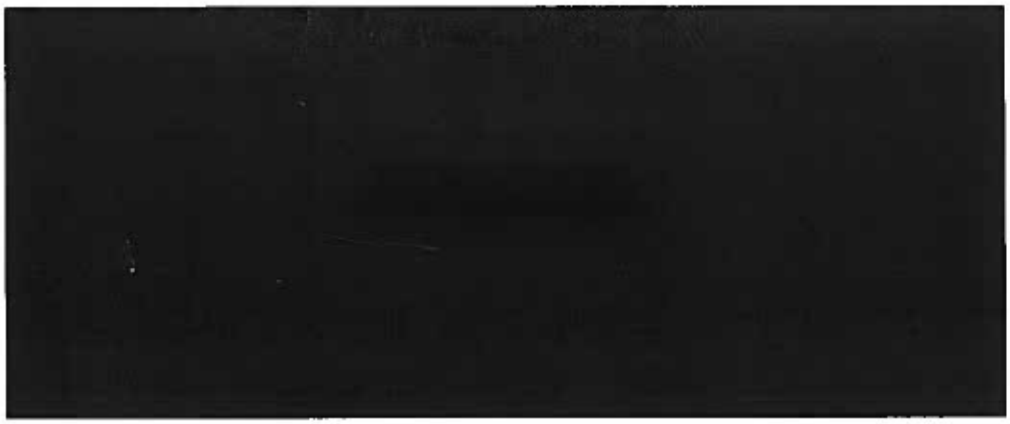
[redacted]

## Appendix II

### Subject Demographics

| Subject Number | Subject Initials | [redacted] ID # | Eye Type | Age | Sex |
|---|---|---|---|---|---|
| 1 | | | NCLW | 55 | F |
| 2 | | | DSCL | 58 | F |
| 3 | | | NCLW | 60 | F |
| 4 | | | NCLW | 43 | F |
| 5 | | | NCLW | 58 | F |
| 6 | | | NCLW | 57 | F |
| 7 | | | NCLW | 50 | F |
| 8 | | | NCLW | 58 | F |
| 9 | | | DISP | 46 | F |
| 10 | | | NCLW | 51 | F |
| 11 | | | DSCL | 49 | F |
| 12 | | | DISP | 40 | F |
| 13 | | | DISP | 46 | F |
| 14 | | | NCLW | 51 | F |
| 15 | | | DISP | 41 | F |
| 16 | | | NCLW | 44 | F |
| 17 | | | NCLW | 50 | F |
| 18 | | | NCLW | 42 | F |
| 19 | | | NCLW | 53 | F |
| 20 | | | NCLW | 51 | F |
| 21 | | | NCLW | 49 | F |
| 22 | | | DISP | 45 | F |
| 23 | | | NCLW | 48 | F |
| 24 | | | DISP | 53 | F |
| 25 | | | DISP | 45 | F |
| 26 | | | NCLW | 56 | F |
| 27 | | | DISP | 54 | F |
| 28 | | | DISP | 45 | F |
| 29 | | | DSCL | 58 | F |
| 30 | | | DISP | 54 | F |
| 31 | | | DISP | 44 | F |
| 32 | | | DISP | 51 | F |
| 33 | | | DISP | 49 | F |

Eye Type: **DISP** = Disposable Soft Contact Lenses
**DSCL** = Daily Soft Contact Lenses
**NCLW** = Non-contact Lens Wearer



[redacted] Protocol Number: R11-0248
Master Project Code: C11-0364

# EVALUATION OF THE *IN VITRO* HUMAN TRUNK PERCUTANEOUS ABSORPTION OF N-ACETYL-L-TYROSINAMIDE USING THE FRANZ FINITE DOSE MODEL

Prepared for:

[redacted]

By

[redacted]

TITLE PAGE

| | |
|---|---|
| **STUDY TITLE:** | Evaluation of the *in vitro* Human Trunk Percutaneous Absorption of N-Acetyl-L-Tyrosinamide using the Franz Finite Dose Model |
| **COMPOUND:** | N-Acetyl-L-Tyrosinamide |
| **SPONSOR:** | [redacted] |
| **PROTOCOL NO.:** | R11-0248/C11-0364 |
| **PHASE OF DEVELOPMENT:** | Pre-Clinical |
| **FIRST CHAMBER DOSE DATE:** | April 20, 2011 |
| **DATE OF LAST SAMPLE COLLECTION:** | April 22, 2011 |
| **PRINCIPAL INVESTIGATOR:** | [redacted] |
| **SPONSOR REPRESENTATIVE:** | [redacted] |
| **INVESTIGATIVE ORGANIZATION:** | [redacted] |
| **PRE-CLINICAL FACILITY:** | [redacted] |
| **ANALYTICAL FACILITY:** | [redacted] |

| | |
|---|---|
| **STUDY DATES:** | Pilot Study:<br>Chamber conduct: April 04 – 07, 2011<br><br>Analytical conduct: April 15 – May 15, 2011<br><br>Pivotal Study:<br>Chamber conduct: April 19 – 22, 2011<br><br>Analytical conduct: April 26 – May 16, 2011 |
| **NUMBER OF DONORS AND REPLICATES PER DONOR:** | Pilot: 1 Donor, 6 Replicates<br><br>Pivotal: 2 Donors, 3 Replicates |

## TABLE OF CONTENTS

## SIGNATURES

The [redacted] - [redacted] Quality Assurance Unit has reviewed the report for [redacted] Protocol Number R11-0248/C11-0364, "Evaluation of the *in vitro* Human Trunk Percutaneous Absorption of N-Acetyl-L-Tyrosinamide using the Franz Finite Dose Model" to assess adherence to the study protocol. While the study contained both Pilot and Pivotal aspects, only the Pivotal portion was reviewed by Quality Assurance. The report was found to be an accurate representation of the Pre-Clinical diffusion cell and analytical work conducted in this study. The summary below lists the Quality Assurance audits conducted for this study.

| QA Activity | Dates Conducted | Date Reported to Investigator |
|---|---|---|
| Data Audit [redacted]-R11-0248-DD-01 | 08/29/11 – 08/31/11 | 08/31/11 |
| Final Report Audit [redacted]-R11-0248-FR-01 | 08/31/11 | 08/31/11 |

[redacted]

10/21/11
Date

[redacted]

[redacted]

## 1. SUMMARY

[redacted] in [redacted], conducted this study for [redacted] The study was designed to evaluate the percutaneous absorption pharmacokinetics of three (3) topically applied formulations containing N-Acetyl-L-Tyrosinamide. Absorption was measured in human, *ex vivo,* trunk skin, *in vitro,* using the finite dose technique and Franz Diffusion Cells.

Three (3) N-Acetyl-L-Tyrosinamide formulations were tested in this study. In the Pivotal study, each formulation was evaluated on three (3) replicate sections from two (2) different *ex vivo* human trunk skin donors. The percutaneous absorption of N-Acetyl-L-Tyrosinamide was determined over a 48 hour dose period. At pre-selected times after dose application; the dermal receptor solution was removed in its entirety, replaced with stock receptor solution, and an aliquot saved for subsequent analysis. In addition, the glass rod used for dosing, the surface wash, stratum corneum, epidermis and dermis were recovered and evaluated for compound content. The samples were analyzed for N-Acetyl-L-Tyrosinamide content using a High Performance Liquid Chromatography (HPLC) method.

The *in vitro* Franz human skin finite dose model has proven to be a valuable tool for the study of percutaneous absorption and the determination of the pharmacokinetics of topically applied drugs. The model uses *ex vivo* human trunk skin mounted in specially designed diffusion cells that allow the skin to be maintained at a temperature and humidity that match typical *in vivo* conditions.[1] A finite dose (for example, 2-10 mg/cm$^2$) of formulation is applied to the outer surface of the skin and drug absorption is measured by monitoring its rate of appearance in the receptor solution bathing the inner surface of the skin. Data defining total absorption, rate of absorption, as well as skin content can be accurately determined in this model. The method has historic precedent for accurately predicting *in vivo* percutaneous absorption kinetics.[2,3]

The objective of this study was to evaluate the percutaneous absorption of N-Acetyl-L-Tyrosinamide from three (3) different formulations into and through human trunk skin using the Franz Finite Dose Model.

[1] Franz, TJ: Percutaneous absorption: on the relevance of *in vitro* data. J Invest Dermatol, 1975, 64:190-195.
[2] Franz TJ: The cadaver skin absorption mode and the drug development process. Pharmacopeial Forum 34(5): 1349-1356, 2008.
[3] Franz TJ: Lehman PA, Raney S: Use of excised human skin to assess the bioequivalence of topical products. Skin Pharmacol Physiol 22:276-286, 2009

### 1.1. TEST ARTICLES

**Table 1: Test Articles Formulations (Provided by Sponsor)**

| Formulation Identity | Batch Number |
|---|---|
| N-Acetyl-L-Tyrosinamide (water in Silicone) | F# 239-166 |
| N-Acetyl-L-Tyrosinamide Gel | F# 239-168 |
| N-Acetyl-L-Tyrosinamide Cream | F# 239-169 |

Test articles were indicated to contain 1.75% N-Acetyl-L-Tyrosinamide by study Sponsor.

## 2. METHODS OF ANALYSIS

### 2.1. Study Skin Preparation

Percutaneous absorption was measured using the *in vitro* Franz human skin finite dose technique. Human, *ex vivo*, trunk skin without obvious signs of skin disease, was used in this study. The skin was dermatomed, cryopreserved, sealed in a water impermeable plastic bag, and stored at ~ -70° C until the day of the experiment. Prior to use it was thawed in ~37°C water, then rinsed in water to remove any adherent blood or other material from the surface.

Human, *ex vivo*, trunk skin from three (3) donors were cut into multiple smaller sections of sufficient size to fit on nominal 2 $cm^2$ static Franz diffusion cells. The dermal receptor compartment was filled to capacity with a receptor solution consisting of normal PBS with 0.008% Gentamicin, and the epidermal cell left open to ambient laboratory environment. All cells were then placed in a diffusion apparatus in which the dermal receptor solution was stirred magnetically at approximately 600 RPM and the skin surface temperature maintained at 32.0° C ± 1.0° C using a temperature controlled water circulation system. Surface temperature was confirmed using a non-contact validated infra-red thermometer.

To assure the integrity of each skin section, its permeability to tritiated water was determined before application of the test products.[4] Following a brief (0.5-1 hour) equilibrium period, $^3H_2O$ (PerkinElmer, Boston, MA, sp. Act. ~ 0.5 µCi/mL) was layered across the top of the skin so that the entire exposed surface was covered (approximately 200 - 500 µL). After 5 minutes the $^3H_2O$ aqueous layer was removed. At 30 minutes the receptor solution was collected and analyzed for radioactive content by liquid scintillation counting. Skin specimens in which absorption of $^3H_2O$ was less than 1.56

[4] Franz TJ, Lehman PA: The use of water permeability as a means of validation for skin integrity in *in vitro* percutaneous absorption studies. Abst. J Invest Dermatol 1990, 94:525.

µL-equ/cm$^2$ were considered acceptable. Donor demographics and the mean integrity test results are presented in Table 2.1.1.

**Table 2.1.1: Donor Demographics and Mean Integrity Test Results**

| Donor ID | Age | Race | Sex | Location | Study Phase | Integrity Test Result* |
|---|---|---|---|---|---|---|
| AR072009 | 51 | Caucasian | Male | Posterior Torso (Trunk) | Pilot | 0.28 ± 0.10 |
| CC010609 | 53 | Hispanic or Latino | Male | Posterior Torso (Trunk) | Pivotal | 0.65 ± 0.31 |
| MA081110 | 44 | Middle Eastern | Male | Posterior Torso (Trunk) | Pivotal | 0.19 ± 0.02 |

*Results are reported as µL-equ $^3H_2O$; Acceptance ≤ 1.56 µl-equ/cm$^2$

## 2.2. Dosing and Sample Collection

### 2.2.1. Pilot Study

An initial pilot study was performed where one (1) formulation (water in silicone, Batch number F# 239-166), containing N-Acetyl-L-Tyrosinamide was evaluated. The purpose of the pilot study was to evaluate different reservoir solutions, different sample extraction solvents, and to generate actual samples to test the analytical method. Static 2 cm$^2$ diffusion cells were filled to capacity with the appropriate receptor solution. Three (3) receptor solutions were analyzed: 1) 1x-PBS with 0.008% Gentamicin, 2) 1x-PBS with 0.1% Oleth-20 (Volpo 20) and 0.008% Gentamicin, and 3) 0.1x-PBS with 0.1% Oleth-20 (Volpo 20) and 0.008% Gentamicin. The formulation was dosed to six (6) 2 cm$^2$ skin sections (chambers) with each receptor solution and surface wash solvent being conducted on pairs of diffusion cells. To each chamber a targeted amount of 10 µL/cm$^2$ dose of the test formulation was applied. Each receptor solution and extraction solvent was evaluated with duplicate skin sections (chambers) for each formulation. Receptor solutions were collected at pre-dose, 6, 12, 24, 32, and 48 hours. Following the last receptor solution sample, the skin surface was washed and the skin was collected for analysis (stratum corneum, epidermis, and dermis as separate samples). Surface wash and extraction solvents evaluated included neat Methanol, 50:50 Methanol/Water, and 80:20 Methanol/Water. Tape strip sample extraction was evaluated in neat Acetonitrile, 50:50 Acetonitrile/Water and neat Water[5]. All samples were processed and analyzed for N-Acetyl-L-Tyrosinamide content. The results of the pilot study are included in APPENDIX III.

[5] All indicates of the use of water throughout the report are to be considered as distilled de-ionized water.

2.2.2. Pivotal Study

The following solutions were utilized in the Pivotal study:

Receptor Solution: 1x-PBS with 0.1% Oleth-20 and 0.008% Gentamicin
Surface wash and tissue extraction: 50:50 Methanol:Water (v/v)
Tape strip samples: 50:50 Acetonitrile:Water (v/v)

The Pivotal study consisted of the evaluation of all three (3) test formulations on skin from two (2) different donors with each formulation being test on three (3) replicate skin sections per donor. One (1) non-dosed control cell was included per donor as a non-dosed blank control. Skin mounting and integrity testing was as previously described. Prior to administration of the topical test formulations to the skin sections, the receptor solution was refreshed with PBS with 0.1% Oleth-20 and 0.008% Gentamicin. The donor compartment chimney of the 2.0 $cm^2$ diffusion cell was removed to allow full access to the epidermal surface of the skin. Prior to dosing, the volume corresponding to a consistent disposition by pipette of a nominal 10 $mg/cm^2$ amount, for each individual formulation, was determined. At the time of dosing, the predetermined 10 mg formulation/$cm^2$/skin-section equivalent volume was drawn up and dispensed by pipette (volumes included in APPENDIX III). A glass rod was used to evenly distribute and rub the formulation into the skin. The glass rod was retained for analysis to correct for the actual applied dose. Five to ten minutes after application the chimney of the diffusion cell was re-installed.

At pre-selected times after dosing (6, 12, 24, 32, and 48 hours) the receptor solution was removed in its entirety, and a 4 mL volume aliquot saved for subsequent analysis. After collection of the last receptor sample, the surface of the skin was washed twice (0.5 mL volume each, refluxed on the skin surface) with 50:50 Methanol:Water to collect un-absorbed formulation from the skin surface.

Following the surface wash, the skin sections were tape-stripped 10 times to remove the stratum corneum. The tape strip samples were collected using 3M Transpore® tape and pooled into a single stratum corneum sample. The tape strips were extracted overnight in 50:50 Acetonitrile:Water. The skin was then removed from the diffusion cell, separated into epidermis and dermis by manual dissection, and each skin sample extracted overnight in 50:50 Methanol:Water. The samples were stored at ~ -20° C (± 10° C) pending analysis for N-Acetyl-L-Tyrosinamide content.

Spare cells for each donor were included, but not dosed, and were used as non-dosed blank control chambers to evaluate for the appearance of substances diffusing out of the skin that might interfere with the analytic method.

### 2.3. Analytical Methods

Quantification of N-Acetyl-L-Tyrosinamide was by High Performance Liquid Chromatography with mass spectroscopy (HPLC/MS). Description of the method used in this study is attached in APPENDIX II.

#### 2.3.1. Receptor solution sample analysis

Receptor solution samples (4 mL) were concentrated using vacuum centrifugation with reconstitution in 50:50 Methanol:Water.

An isocratic solvent system consisting of A: 93%, (0.08% Ammonium acetate in water) and B: 7% (Methanol) was run through a Phenomenex Gemini C18 (50 x 3.0mm, 3.0µ) column at a flow rate of 0.40 mL/min. with an injection volume of 3µL sample, 9µL $H_2O$. Eluting peaks were quantified using a diode-array detector set at 222 nm referenced to 500 nm or using a mass spectroscopy detector monitoring 245.1 m/z with a concentration range of 0.04-1µg/mL (limit of detection was nominally considered at 0.005 µg/mL).

#### 2.3.2. All Mass Balance Sample (Glass Rod, Surface Wash, Tape Strips, Epidermis, Dermis) analysis

An isocratic solvent system consisting of 90%, (0.08% Ammonium acetate in water) versus (10% Methanol) was run through an Phenomenex Gemini C18 (50 x 3.0mm, 3.0µ) column at a flow rate of 0.40 mL/min. Eluting peaks were quantified using a diode-array detector set at 222 nm referenced to 500 nm or using a mass spectroscopy detector monitoring 245.1 m/z (limit of detection is 0.009 µg/mL).

## 3. ABSORPTION

All results and calculations are provided in APPENDIX III.

Rate of percutaneous absorption is presented as the flux of N-Acetyl-L-Tyrosinamide that appears in the receptor solution under the skin. Individual chamber values were calculated and averaged across replicates for a Donor mean ± SD. N-Acetyl-L-Tyrosinamide absorption is outlined in Table 3.1.1.1 and Figure 3.1.1.1 Individual cell calculations are included in APPENDIX III.

## 3.1. Absorption Data Tables

### 3.1.1. N-Acetyl-L-Tyrosinamide

**Table 3.1.1.1: Mean Flux (µg/cm²/hr) Results: Across Donor Summary: N-Acetyl-L-Tyrosinamide**

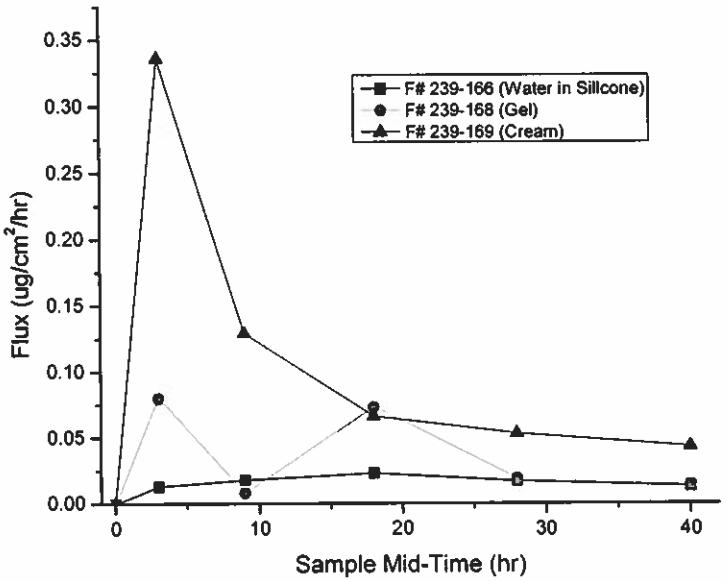
Percutaneous Absorption of N-Acetyl-L-Tyrosinamide through *ex vivo* Human Trunk Skin over a 48 hr Dose Duration. (Mean ± SD, n=2 Donors)

| Time (hr)* | N-Acetyl-L-Tyrosinamide (Water in Silicone) F# 239-166 | N-Acetyl-L-Tyrosinamide Gel F# 239-168 | N-Acetyl-L-Tyrosinamide Cream F# 239-169 |
|---|---|---|---|
| 3.0 | 0.013 ± 0.015 | 0.080 ± 0.112 | 0.336 ± 0.445 |
| 9.0 | 0.018 ± 0.020 | 0.008 ± 0.010 | 0.129 ± 0.160 |
| 18.0 | 0.023 ± 0.029 | 0.073 ± 0.102 | 0.066 ± 0.074 |
| 28.0 | 0.017 ± 0.021 | 0.019 ± 0.026 | 0.053 ± 0.057 |
| 40.0 | 0.013 ± 0.015 | 0.014 ± 0.018 | 0.043 ± 0.046 |

* Time as midpoint between samples.

**Figure 3.1.1.1: Mean Flux (µg/cm²/hr) Results: Across Donor Summary: N-Acetyl-L-Tyrosinamide**

Percutaneous Absorption of N-Acetyl-L-Tyrosinamide through *ex vivo* Human Trunk Skin over a 48 hr Dose Duration. (Mean, n=2 Donors)



## 4. DISTRIBUTION

All results and calculations are provided in APPENDIX III.

Distribution of N-Acetyl-L-Tyrosinamide following a 48 hour dose exposure to *ex vivo* human skin is presented as mass recovered per square centimeter and as percent of applied dose. Percent recovery has been corrected for actual amount applied (less residue on glass rod used to distribute the applied dose) and formulation densities, along with the indicated formulation potencies. Individual chamber values were calculated and averaged across replicates for a Donor mean. N-Acetyl-L-Tyrosinamide distribution is outlined in Table 4.1.1.1 and Figure 4.1.1.1.

### 4.1. Distribution Data Table

#### 4.1.1. N-Acetyl-L-Tyrosinamide

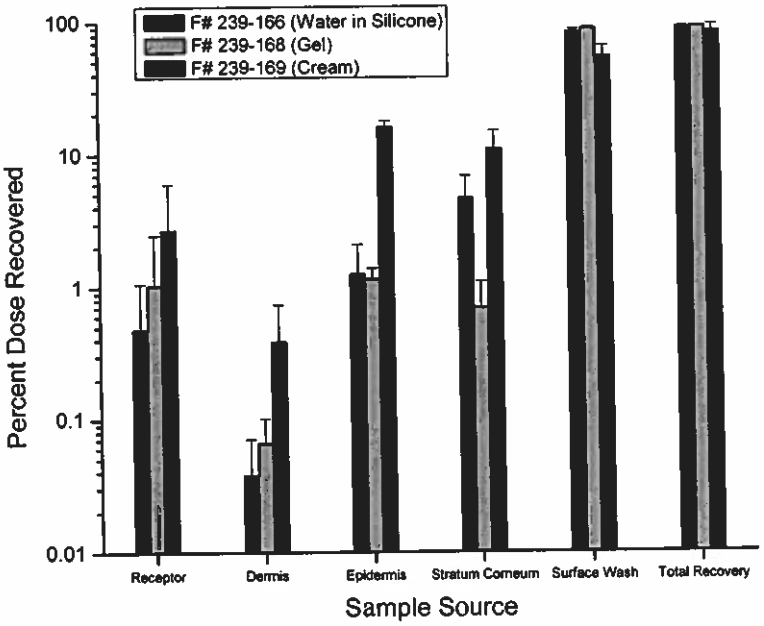
**Table 4.1.1.1: Mass Balance and Distribution Results: Across Skin Donors: N-Acetyl-L-Tyrosinamide**

Distribution of N-Acetyl-L-Tyrosinamide into and through *ex vivo* Human Trunk Skin from a 48 hour Dose Duration Exposure Period
Mean ± SD as Percent of Applied Dose and Total Mass ($\mu g/cm^2$)

| Parameter | N-Acetyl-L-Tyrosinamide (water in Silicone) F# 239-166 | N-Acetyl-L-Tyrosinamide Gel F# 239-168 | N-Acetyl-L-Tyrosinamide Cream F# 239-169 |
|---|---|---|---|
| Receptor ($\mu g/cm^2$) | 0.803 ± 0.973 | 1.771 ± 2.458 | 4.694 ± 5.705 |
| Dermis ($\mu g/cm^2$) | 0.064 ± 0.055 | 0.113 ± 0.059 | 0.668 ± 0.604 |
| Epidermis ($\mu g/cm^2$) | 2.122 ±1.459 | 1.983 ± 0.398 | 27.719 ± 3.366 |
| Stratum corneum ($\mu g/cm^2$) | 7.850 ± 3.791 | 1.202 ± 0.697 | 20.655 ± 4.605 |
| Surface Wash ($\mu g/cm^2$) | 140.100 ± 5.643 | 153.201 ± 3.914 | 94.229 ±19.721 |
| Receptor (%) | 0.479 ± 0.581 | 1.031 ± 1.431 | 2.702 ± 3.281 |
| Dermis (%) | 0.038 ± 0.033 | 0.066 ± 0.035 | 0.386 ± 0.349 |
| Epidermis (%) | 1.252 ± 0.850 | 1.149 ± 0.235 | 15.963 ± 1.924 |
| Stratum corneum (%) | 4.639 ± 2.197 | 0.695 ± 0.405 | 11.909 ± 4.09 |
| Surface Wash (%) | 83.15 ± 4.07 | 88.59 ± 1.97 | 54.336 ± 11.403 |
| Total Recovery (%) | 89.554 ± 1.639 | 91.532 ± 0.140 | 85.296 ± 8.529 |

**Figure 4.1.1.1: Mass Balance and Distribution Results: Across Skin Donors: N-Acetyl-L-Tyrosinamide**

Distribution of N-Acetyl-L-Tyrosinamide into and through *ex vivo* Human Trunk Skin from a 48 hour Dose Duration Exposure Period. Mean ± SD as Percent of Applied Dose




## 5. CONCLUSIONS

The data indicate that N-Acetyl-L-Tyrosinamide does penetrate into and through *ex vivo* human skin using the *in vitro* finite dose model with Franz diffusion cells from all three formulations evaluated.

It is noteworthy to appreciate that with this 2 donor study, one donor demonstrated substantially greater permeability to N-Acetyl-L-Tyrosinamide than the other donor. The individual donor results can be found as within and across donor summary tables in Appendix III.

The greatest extent of absorption was seen from the cream formulation demonstrating a rapid rise to a peak flux at approximately 5 hours after dose application followed by a slow decline in penetration there after. Both the gel and water-in-silicone based formulations demonstrate lower rates of penetration with peak absorption approximately 18 hrs after dose application. From 0.5% (water-in-silicone vehicle) to 2.7% (cream vehicle) of the applied dose penetrated through the skin over the 48 hour dose exposure period, as measured in the receptor fluid.

Dermal content for N-Acetyl-L-Tyrosinamide correlated with the receptor contents with the highest level seen with the cream formulation and the lowest from the water in silicone formulation.

Epidermal and Stratum Corneum contents were the highest with the cream formulation, followed by the water-in-silicone formulation, with the gel showing the lowest levels across the three formulations. It is a likely scenario that the selected surface wash solvent chosen in the pilot study for the water-in-silicone formulation may not have been optimal for surface recovery of the Cream formulation, and could account for the higher stratum corneum and epidermal levels observed from the Cream formulation data. However, the data also indicates that the Cream formulation delivered appreciably more N-Acetyl-L-Tyrosinamide through the skin than the other 2 formulations, which would also be consistent with a higher stratum corneum and epidermal content.

Mass balance (overall accountability of the applied dose) was moderately good and ranged from 84% to 92% of the applied dose across the three formulations tested.

*----/End of Report Text/----*

# 7. APPENDIX II

## Analytical Methods

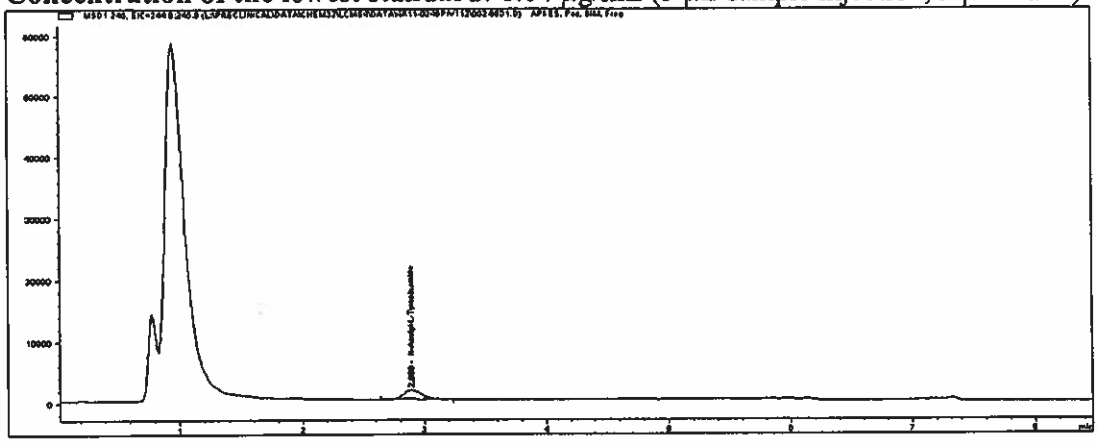
# N-Acetyl-L-Tyrosinamide

| | |
|---|---|
| Method Name: | N-Acetyl-L-Tyrosinamide_Skin_RS_NVAL_a |
| Name of Compound(s): | N-Acetyl-L-Tyrosinamide |
| Validated Method: | ☐ Yes ☒ No ☐ N/A |
| Stock Solution Matrix: | Methanol |
| Internal Standard Matrix: | N/A |
| Standard Curve Matrix (Diluent): | 10x PBS (May be adjusted for non-validated method) |
| Instrumentation: | Agilent 1100 Series LC and LC/MS Systems |
| Detector(s) Used: | MSD |
| Solvent A: | 93 % (0.08% Ammonium acetate in water) |
| Solvent B: | 7 % (Methanol) |
| Gradient or Isocratic: | Isocratic |
| Column Description: | Phenomenex Gemini C18, 3µm, 50 x 3.0 mm |
| Flow-rate (mL/min): | 0.400 |
| Run Time (min): | 8.50 (May be adjusted.) |
| Post Time (min): | Off (May be adjusted.) |
| Column temperature (°C): | 30.0 |
| Injection Volume (µL): | 3 µL sample, 9 µL $H_2O$ (May be adjusted.) |
| IS Concentration (µg/mL): | N/A |
| DAD: | |
| Wavelength1: | 222 nm (4 nm) – 500 nm (50 nm) |
| Limit of Detection (µg/mL): | N/A |
| Curve Type/Weighting: | N/A |
| Concentration Range (µg/mL): | N/A |
| List of All Standards per Curve: | N/A |
| MSD: | |
| Ions Monitored: | 245.1 |
| Compound Used as Internal Std: | N/A |
| Ions Monitored for Internal Std: | N/A |
| Polarity (positive or negative): | Positive |
| Limit of Detection (µg/mL): | 0.005 |
| Curve Type/Weighting: | Quadratic/Linear (Amnt) |
| Concentration Range (µg/mL): | 0.04-1 (May be adjusted for non-validated method) |
| Nitrogen Temp (°C): | 350 |
| Nitrogen Gas Flow (L/min): | 12.0 |
| Nebulizer Pressure (psig): | 35 |
| Fragmentor: | 50 ( May be adjusted.) |
| VCap: | 2500 (May be adjusted.) |

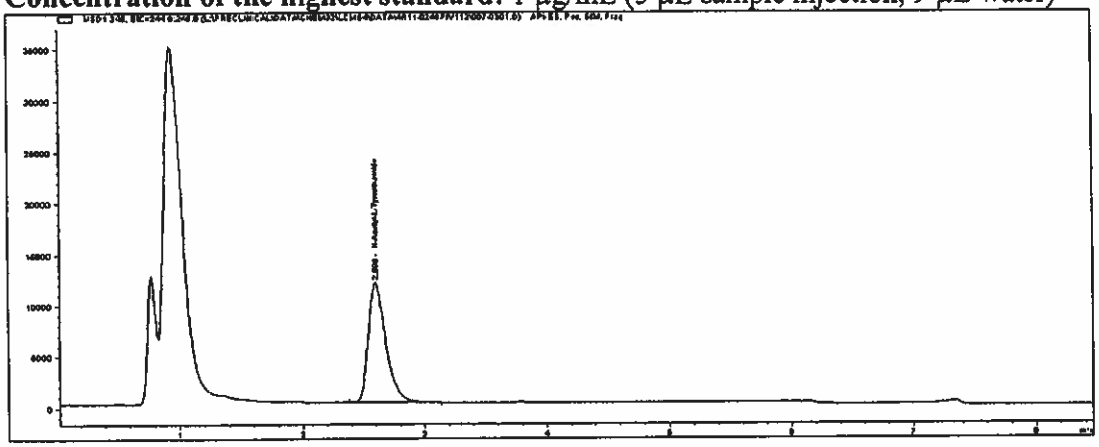
# N-Acetyl-L-Tyrosinamide

**CHROMATOGRAM:**

**Concentration of the lowest standard:** 0.04 µg/mL (3 µL sample injection, 9 µL water)



**Concentration of the highest standard:** 1 µg/mL (3 µL sample injection, 9 µL water)



**Comments/Notes:**

These curve ranges and injection volumes may need to be adjusted based on the formulation.
The curve range and matrices may be adjusted for studies that do not require validation.
Column Switching Valve is recommended at 6.20 min.

Prepared/Revised by : [redacted] Date: 06/15/11

Reviewed by : [redacted] Date: 06/15/11

Approved by : [redacted] Date: 06-20-11

**Initiation Date:** 04/29/11 **Close-out Date:** _____

**Close-out Reason:**

**Is there a method to replace this method?** ☐ **Yes** ☐ **No** ☐ **N/A**

**Explain:** _____

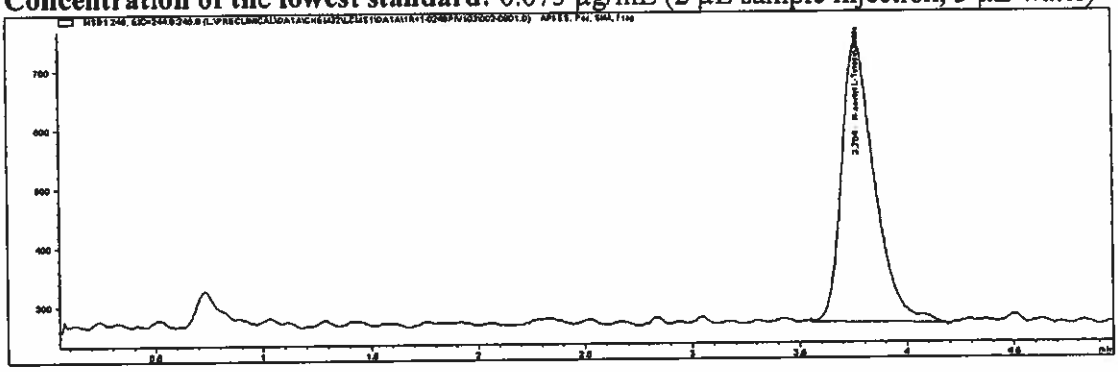
# N-Acetyl-L-Tyrosinamide

| | |
|---|---|
| Method Name: | N-Acetyl-L-Tyrosinamide_Skin_E, D, SW, GR, TS_NVAL_a |
| Name of Compound(s): | N-Acetyl-L-Tyrosinamide |
| Validated Method: | ☐ Yes ☒ No ☐ N/A |
| Stock Solution Matrix: | Methanol |
| Internal Standard Matrix: | N/A |
| Standard Curve Matrix (Diluent): | 50:50 Methanol:Water (May be adjusted for non-validated method) |
| Instrumentation: | Agilent 1100 Series LC and LC/MS Systems |
| Detector(s) Used: | MSD |
| Solvent A: | 90 % (0.08% Ammonium acetate in water) |
| Solvent B: | 10 % (Methanol) |
| Gradient or Isocratic: | Isocratic |
| Column Description: | Phenomenex Gemini C18, 3µm, 50 x 3.0 mm |
| Flow-rate (mL/min): | 0.400 |
| Run Time (min): | 5.00 (May be adjusted.) |
| Post Time (min): | Off (May be adjusted.) |
| Column temperature (°C): | 30.0 |
| Injection Volume (µL): | 2 µL sample, 3 µL $H_2O$ (May be adjusted.) |
| IS Concentration (µg/mL): | N/A |
| DAD: | |
| Wavelength1: | 222 nm (4 nm) – 500 nm (50 nm) |
| Limit of Detection (µg/mL): | N/A |
| Curve Type/Weighting: | N/A |
| Concentration Range (µg/mL): | N/A |
| List of All Standards per Curve: | N/A |
| MSD: | |
| Ions Monitored: | 245.1 |
| Compound Used as Internal Std: | N/A |
| Ions Monitored for Internal Std: | N/A |
| Polarity (positive or negative): | Positive |
| Limit of Detection (µg/mL): | 0.009 |
| Curve Type/Weighting: | Quadratic/Linear (Amnt) |
| Concentration Range (µg/mL): | 0.5-10, 0.075-4 (May be adjusted for non-validated method) |
| Nitrogen Temp (°C): | 350 |
| Nitrogen Gas Flow (L/min): | 12.0 |
| Nebulizer Pressure (psig): | 35 |
| Fragmentor: | 50 ( May be adjusted.) |
| VCap: | 2500 (May be adjusted.) |

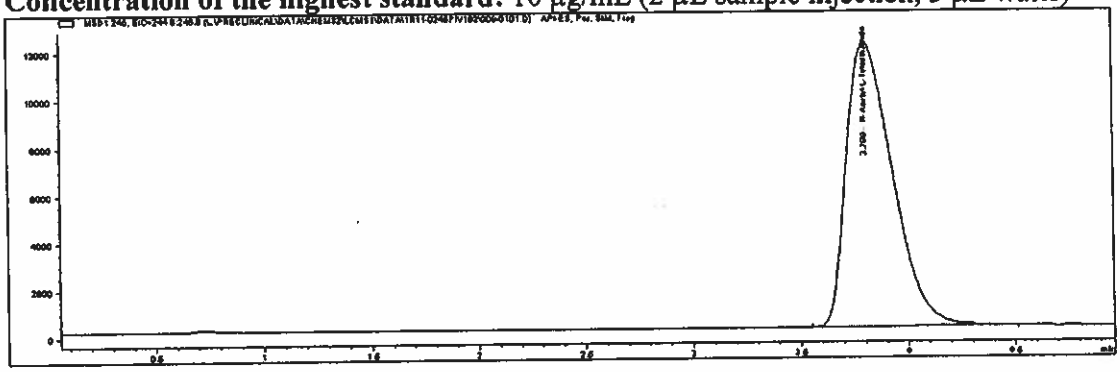
# N-Acetyl-L-Tyrosinamide

**CHROMATOGRAM:**

**Concentration of the lowest standard:** 0.075 µg/mL (2 µL sample injection, 3 µL water)



**Concentration of the highest standard:** 10 µg/mL (2 µL sample injection, 3 µL water)



**Comments/Notes:**
These curve ranges and injection volumes may need to be adjusted based on the formulation. The curve range and matrices may be adjusted for studies that do not require validation.

Prepared/Revised by: [redacted] Date: 06/06/11

Reviewed by: [redacted] Date: 06/15/11

Approved by: [redacted] Date: 06-15-11

**Initiation Date:** _____ **Close-out Date:** _____

**Close-out Reason:**

**Is there a method to replace this method?** ☐ **Yes** ☐ **No** ☐ **N/A**

**Explain:** _____

Personal Care Products Council
Committed to Safety, Quality & Innovation

**Memorandum**

**TO:** F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

**FROM:** Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

**DATE:** October 31, 2012

**SUBJECT:** Concentration of Use by FDA Product Category: Amino Acid Alkyl Amides

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

**Concentration of Use by FDA Product Category***

Acetyl Arginine
Acetyl Cysteine
Acetyl Glutamic Acid
Acetyl Glutamine
Acetyl Histidine
Acetyl Methionine
Acetyl Proline
Acetyl Tyrosine
Capryloyl Collagen Amino Acids
Capryloyl Glycine
Capryloyl Gold of Pleasure Amino Acids
Capryloyl Keratin Amino Acids
Capryloyl Pea Amino Acids
Capryloyl Quinoa Amino Acids
Capryloyl Silk Amino Acids
Cocoyl Glutamic Acid
Dipalmitoyl Cystine
Dipotassium Caprylol Glutamate
Dipotassium Undecylenoyl Glutamate
Disodium Capryloyl Glutamate
Disodium Cocoyl Glutamate
Disodium Hydrogenated Tallow Glutamate
Disodium N-Lauroyl Aspartate
Disodium Lauroyl Glutamate
Disodium Malyl Tyrosinate
Disodium Stearoyl Glutamate
Disodium Undecylenoyl Glutamate
Lauroyl Arginine
Lauroyl Collagen Amino Acids
Lauroyl Glutamic Acid
Lauroyl Lysine
Lauroyl Silk Amino Acids
Magnesium Palmitoyl Glutamate
Myristoyl Glutamic Acid
Myristyl Alaninate
Oleoyl Tyrosine
Palmitoyl Alanine
Palmitoyl Arginine
Palmitoyl Collagen Amino Acids
Palmitoyl Glutamic Acid
Palmitoyl Glycine
Palmitoyl Gold of Pleasure Amino Acids
Palmitoyl Isoleucine
Palmitoyl Keratin Amino Acids
Palmitoyl Millet Amino Acids
Palmitoyl Oat Amino Acids
Palmitoyl Pea Amino Acids
Palmitoyl Quinoa Amino Acids
Palmitoyl Silk Amino Acids
Potassium Capryloyl Glutamate
Potassium Cocoyl Glutamate
Potassium Cocoyl Glycinate
Potassium Lauroyl Collagen Amino Acids
Potassium Lauroyl Glutamate
Potassium Lauroyl Oat Amino Acids
Potassium Lauroyl Pea Amino Acids
Potassium Lauroyl Silk Amino Acids
Potassium Lauroyl Wheat Amino Acids
Potassium Myristoyl Glutamate
Potassium Olivoyl/Lauroyl Wheat Amino Acids
Potassium Stearoyl Glutamate
Potassium Undecylenoyl Glutamate
Propionyl Collagen Amino Acids
Sodium Capryloyl Glutamate
Sodium Cocoyl Alaninate
Sodium Cocoyl Amino Acids
Sodium Cocoyl Apple Amino Acids
Sodium Cocoyl Collagen Amino Acids
Sodium Cocoyl Glutamate
Sodium Cocoyl Glycinate
Sodium Cocoyl/Hydrogenated Tallow Glutamate
Sodium Cocoyl Oat Amino Acids
Sodium Cocoyl/Palmoyl/Sunfloweroyl Glutamate
Sodium Cocoyl Proline
Sodium Cocoyl Threoninate
Sodium Cocoyl Wheat Amino Acids
Sodium Hydrogenated Tallowoyl Glutamate
Sodium Lauroyl Aspartate
Sodium Lauroyl Collagen Amino Acids
Sodium Lauroyl Glutamate
Sodium Lauroyl Millet Amino Acids
Sodium Lauroyl/Myristoyl Aspartate
Sodium Lauroyl Oat Amino Acids
Sodium Lauroyl Silk Amino Acids
Sodium Lauroyl Wheat Amino Acids
Sodium Myristoyl Glutamate
Sodium Olivoyl Glutamate
Sodium Palmitoyl Proline
Sodium Palmoyl Glutamate
Sodium Stearoyl Glutamate

Sodium/TEA-Lauroyl Collagen Amino Acids
Sodium/TEA-Lauroyl Keratin Amino Acids
Sodium/TEA-Undecylenoyl Collagen Amino Acids
Sodium Undecylenoyl Glutamate
Stearoyl Glutamic Acid
Stearoyl Leucine
TEA-Cocoyl Alaninate
TEA-Cocoyl Glutamate
TEA-Cocoyl Glutaminate
TEA-Hydrogenated Tallowoyl Glutamate
TEA-Lauroyl Collagen Amino Acids
TEA-Lauroyl Glutamate
TEA-Lauroyl Keratin Amino Acids
TEA-Lauroyl/Myristoyl Aspartate
Undecylenoyl Collagen Amino Acids
Undecylenoyl Glycine
Undecylenoyl Phenylalanine
Undecylenoyl Wheat Amino Acids
Zinc Lauroyl Aspartate

| **Ingredient** | **FDA Code†** | **Product Category** | **Maximum Concentration of Use** |
|---|---|---|---|
| Acetyl Cysteine | 05G | Tonics, dressings and other hair grooming aids | 0.1% |
| Acetyl Cysteine | 12C | Face and neck creams, lotions and powders<br>not spray | <br>0.0005% |
| Acetyl Cysteine | 12J | Other skin care preparations | 0.03% |
| Acetyl Glutamine | 11E | Shaving cream (aerosol, brushless and lather) | 0.1% |
| Acetyl Glutamine | 12C | Face and neck creams, lotions and powders<br>not spray<br>spray | <br>0.5-1%<br>1% |
| Acetyl Glutamine | 12J | Other skin care preparations | 0.01% |
| Acetyl Methionine | 05G | Tonics, dressings and other hair grooming aids | 0.00001% |
| Acetyl Tyrosine | 03D | Eye lotions | 0.3% |
| Acetyl Tyrosine | 05I | Other hair preparations (noncoloring) | 0.3% |
| Acetyl Tyrosine | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 0.03% |
| Acetyl Tyrosine | 12D | Body and hand creams, lotions and powders<br>not spray | <br>0.08% |
| Acetyl Tyrosine | 12G | Night creams, lotions and powders<br>not spray | <br>0.2% |

| | | | |
|---|---|---|---|
| Capryloyl Glycine | 03C | Eye shadow | 0.4% |
| Capryloyl Glycine | 03G | Other eye makeup preparations | 2% |
| Capryloyl Glycine | 05A | Hair conditioners | 0.4-0.5% |
| Capryloyl Glycine | 05F | Shampoos (noncoloring) | 1-2% |
| Capryloyl Glycine | 07C | Foundations | 1% |
| Capryloyl Glycine | 10B | Deodorants<br>not spray | 0.1% |
| Capryloyl Glycine | 12A | Skin cleansing (cold creams cleansing lotions, liquids and pads) | 0.09-1% |
| Capryloyl Glycine | 12C | Face and neck creams, lotions and powders<br>not spray | 0.2-0.5% |
| Capryloyl Glycine | 12D | Body and hand creams, lotions and powders<br>not spray | 0.5% |
| Capryloyl Glycine | 12F | Moisturizing creams, lotions and powders<br>not spray | 0.09% |
| Capryloyl Glycine | 12H | Paste masks and mud packs | 0.05% |
| Capryloyl Glycine | 12J | Other skin care preparations | 1% |
| Cocoyl Glutamic Acid | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 24% |
| Disodium Capryloyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 0.4% |
| Disodium Cocoyl Glutamate | 03C | Eye shadow | 0.02% |
| Disodium Cocoyl Glutamate | 03G | Other eye makeup preparations | 0.05% |
| Disodium Cocoyl Glutamate | 07B | Face powders | 0.1% |
| Disodium Cocoyl Glutamate | 07C | Foundations<br>spray | 0.3% |
| Disodium Cocoyl Glutamate | 08C | Nail creams and lotions | 0.05% |
| Disodium Cocoyl | 10E | Other personal cleanliness products | 2% |

| | | | |
|---|---|---|---|
| Glutamate | | hand soap | 0.6% |
| Disodium Cocoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions liquids and pads) | 2-3% |
| Disodium Cocoyl Glutamate | 12D | Body and hand creams, lotions and powders not spray | 0.08% |
| Disodium Hydrogenated Tallow Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 2-3% |
| Disodium Hydrogenated Tallow Glutamate | 12D | Body and hand creams, lotions and powders not spray | 0.08% |
| Disodium Stearoyl Glutamate | 03B | Eye liner | 0.1% |
| Disodium Stearoyl Glutamate | 03C | Eye shadow | 0.8-1% |
| Disodium Stearoyl Glutamate | 03D | Eye lotion | 0.05% |
| Disodium Stearoyl Glutamate | 07A | Blushers (all types) | 0.03-2% |
| Disodium Stearoyl Glutamate | 07B | Face powders | 0.2-6% |
| Disodium Stearoyl Glutamate | 07C | Foundations | 0.04-6% |
| Disodium Stearoyl Glutamate | 07E | Lipstick | 0.02% |
| Disodium Stearoyl Glutamate | 07F | Makeup bases | 0.5% |
| Disodium Stearoyl Glutamate | 07H | Makeup fixatives | 0.5% |
| Disodium Stearoyl Glutamate | 07I | Other makeup preparations | 0.2% |
| Disodium Stearoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 0.1-0.3% |
| Disodium Stearoyl Glutamate | 12C | Face and neck creams, lotions and powders | |

| | | | |
|---|---|---|---|
| | | not spray | 0.5-0.9% |
| Disodium Stearoyl Glutamate | 12F | Moisturizing creams, lotions and powders not spray | 0.9% |
| Lauroyl Lysine | 03A | Eye brow pencil | 0.005-4% |
| Lauroyl Lysine | 03B | Eye liner | 0.005-4% |
| Lauroyl Lysine | 03C | Eye shadow | 0.3-10.2% |
| Lauroyl Lysine | 03D | Eye lotion | 0.5-2% |
| Lauroyl Lysine | 03F | Mascara | 0.005-0.5% |
| Lauroyl Lysine | 04C | Powders (dusting and talcum) | 0.5% |
| Lauroyl Lysine | 05A | Hair conditioners | 0.3% |
| Lauroyl Lysine | 05E | Rinses (noncoloring) | 0.001% |
| Lauroyl Lysine | 05F | Shampoos (noncoloring) | 0.001% |
| Lauroyl Lysine | 07A | Blushers (all types) | 0.005-12% |
| Lauroyl Lysine | 07B | Face powders | 0.005-12% |
| Lauroyl Lysine | 07C | Foundations | 0.06-14% |
| Lauroyl Lysine | 07E | Lipstick | 0.4-45% |
| Lauroyl Lysine | 07F | Makeup bases | 0.1% |
| Lauroyl Lysine | 07G | Rouges | 0.9% |
| Lauroyl Lysine | 07H | Makeup fixatives | 0.09% |
| Lauroyl Lysine | 07I | Other makeup preparations | 0.9-3% |
| Lauroyl Lysine | 08E | Nail polish and enamel | 0.001% |
| Lauroyl Lysine | 12C | Face and neck creams, lotions and powders not spray | 0.5-1% |
| Lauroyl Lysine | 12D | Body and hand creams, lotions and powders not spray | 0.1-0.3% |
| Lauroyl Lysine | 12F | Moisturizing creams, lotions and powders not spray | 0.5% |
| Lauroyl Lysine | 12G | Night creams, lotions and powders not spray | 0.5% |

| | | | |
|---|---|---|---|
| Magnesium Palmitoyl Glutamate | 05B | Hair sprays<br>pump spray | 0.2% |
| Magnesium Palmitoyl Glutamate | 05G | Tonics, dressings and other hair grooming aids<br>spray | 0.2% |
| Magnesium Palmitoyl Glutamate | 07C | Foundations | 0.0006-0.09% |
| Magnesium Palmitoyl Glutamate | 08A | Basecoates and undercoats (manicuring preparations) | 0.002% |
| Magnesium Palmitoyl Glutamate | 08G | Other manicuring preparations | 0.001% |
| Magnesium Palmitoyl Glutamate | 12C | Face and neck creams, lotions and powders<br>not spray | 0.1% |
| Magnesium Palmitoyl Glutamate | 12D | Body and hand creams, lotions and powders<br>spray | 0.2% |
| Palmitoyl Glycine | 12C | Face and neck creams, lotions and powders<br>not spray | 1% |
| Potassium Cocoyl Glutamate | 02D | Other bath preparations | 6% |
| Potassium Cocoyl Glutamate | 05F | Shampoos (noncoloring) | 8% |
| Potassium Cocoyl Glutamate | 10A | Bath soaps and detergents | 3% |
| Potassium Cocoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 12% |
| Potassium Cocoyl Glutamate | 12C | Face and neck creams, lotions and powders<br>not spray | 0.03% |
| Potassium Cocoyl Glycinate | 10A | Bath soaps and detergents | 1% |
| Potassium Cocoyl Glycinate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 3-39% |
| Potassium Cocoyl Glycinate | 12D | Body and hand creams, lotions and powders | |

| | | | |
|---|---|---|---|
| | | not spray | 2% |
| Potassium Lauroyl Wheat Amino Acids | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 0.7% |
| Potassium Myristoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 11-22% |
| Sodium Cocoyl Amino Acids | 05A | Hair conditioners | 0.4% |
| Sodium Cocoyl Amino Acids | 05B | Hair sprays<br>pump spray | 0.4% |
| Sodium Cocoyl Amino Acids | 05F | Shampoos (noncoloring) | 0.4% |
| Sodium Cocoyl Amino Acids | 05G | Tonics, dressings and other hair grooming aids | 0.4% |
| Sodium Cocoyl Amino Acids | 05G | Other hair preparations (noncoloring) | 1% |
| Sodium Cocoyl Amino Acids | 10A | Bath soaps and detergents | 2.8% |
| Sodium Cocoyl Apple Amino Acids | 03B | Eye liner | 0.3% |
| Sodium Cocoyl Apple Amino Acids | 05F | Shampoos (noncoloring) | 0.5% |
| Sodium Cocoyl Apple Amino Acids | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 0.6-3% |
| Sodium Cocoyl Collagen Amino Acids | 05A | Hair conditioners | 0.02% |
| Sodium Cocoyl Collagen Amino Acids | 05G | Tonics, dressings and other hair grooming aids | 0.02% |
| Sodium Cocoyl Glutamate | 03B | Eye liner | 0.5% |
| Sodium Cocoyl Glutamate | 03C | Eye shadow | 0.004% |
| Sodium Cocoyl Glutamate | 03D | Eye lotion | 0.6% |

| | | | |
|---|---|---|---|
| Sodium Cocoyl Glutamate | 05F | Shampoos (noncoloring) | 0.2-10% |
| Sodium Cocoyl Glutamate | 05G | Tonics, dressings and other hair grooming aids | 0.5% |
| Sodium Cocoyl Glutamate | 06A | Hair dyes and colors (all types requiring caution statement and patch test) | 3% |
| Sodium Cocoyl Glutamate | 07C | Foundations<br>spray | 0.004-0.005%<br>0.03% |
| Sodium Cocoyl Glutamate | 10A | Bath soaps and detergents | 3% |
| Sodium Cocoyl Glutamate | 10E | Other personal cleanliness products | 0.2% |
| Sodium Cocoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions liquids and pads) | 1-9% |
| Sodium Cocoyl Glutamate | 12C | Face and neck creams, lotions and powders<br>not spray | 0.1-0.6 |
| Sodium Cocoyl Glutamate | 12D | Body and hand creams, lotions and powders<br>not spray | 0.04-3% |
| Sodium Cocoyl Glutamate | 12H | Paste masks and mud packs | 0.01% |
| Sodium Cocoyl Glycinate | 10A | Bath soaps and detergents | 3% |
| Sodium Cocoyl Glycinate | 10E | Other personal cleanliness products<br>hand soap | 0.2% |
| Sodium Cocoyl Glycinate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 5-20% |
| Sodium Hydrogenated Tallowoyl Glutamate | 12C | Face and neck creams, lotions and powder<br>not spray | 0.8% |
| Sodium Lauroyl Aspartate | 05F | Shampoos (noncoloring) | 2% |
| Sodium Lauroyl Aspartate | 07B | Face powders | 0.2% |
| Sodium Lauroyl | 07C | Foundations | 0.04% |

| | | | |
|---|---|---|---|
| Aspartate | | | |
| Sodium Lauroyl Aspartate | 07G | Rouges | 0.005% |
| Sodium Lauroyl Aspartate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 2% |
| Sodium Lauroyl Glutamate | 02A | Bath oils, tablets and salts | 4% |
| Sodium Lauroyl Glutamate | 05F | Shampoos (non-coloring) | 3% |
| Sodium Lauroyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 10-40% |
| Sodium Lauroyl Glutamate | 12C | Face and neck cream, lotions and powders not spray | 0.03-1% |
| Sodium Lauroyl Glutamate | 12D | Body and hand cream, lotions and powders not spray | 4% |
| Sodium Lauroyl Glutamate | 12F | Moisturizing creams, lotions and powders not spray | 0.03-0.1% |
| Sodium Lauroyl Glutamate | 12H | Paste masks and mud packs | 0.003% |
| Sodium Lauroyl Oat Amino Acids | 03E | Eye makeup remover | 5% |
| Sodium Lauroyl Oat Amino Acids | 05F | Shampoos (noncoloring) | 0.04-0.4% |
| Sodium Lauroyl Oat Amino Acids | 05G | Tonics, dressings and other hair grooming aids | 0.4% |
| Sodium Lauroyl Oat Amino Acids | 10A | Bath soaps and detergents | 5% |
| Sodium Lauroyl Oat Amino Acids | 10E | Other personal cleanliness products | 0.3% |
| Sodium Lauroyl Oat Amino Acids | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 0.6-5% |
| Sodium Lauroyl Oat Amino Acids | 12C | Face and neck creams, lotions and pads not spray | 0.8% |
| Sodium Lauroyl Oat | 12D | Body and hand creams, lotions and | |

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| Amino Acids | | powders<br>not spray | 0.6% |
| Sodium Lauroyl Oat Amino Acids | 12F | Moisturizing creams, lotions and powders<br>not spray | 0.5% |
| Sodium Myristoyl Glutamate | 03E | Eye makeup remover | 0.1% |
| Sodium Myristoyl Glutamate | 07C | Foundations | 0.1% |
| Sodium Myristoyl Glutamate | 07F | Makeup bases | 0.4% |
| Sodium Myristoyl Glutamate | 08B | Cuticle softeners | 0.5% |
| Sodium Myristoyl Glutamate | 10A | Bath soaps and detergents | 31% |
| Sodium Myristoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 5-30% |
| Sodium Myristoyl Glutamate | 12J | Other skin care preparations | 5% |
| Sodium Palmoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 2-22% |
| Sodium Stearoyl Glutamate | 03D | Eye lotion | 1% |
| Sodium Stearoyl Glutamate | 05A | Hair conditioners | 0.03-0.2% |
| Sodium Stearoyl Glutamate | 07C | Foundations | 0.4-1.1% |
| Sodium Stearoyl Glutamate | 07E | Lipstick | 1% |
| Sodium Stearoyl Glutamate | 07F | Makeup bases | 0.4% |
| Sodium Stearoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 1.1% |
| Sodium Stearoyl Glutamate | 12C | Face and neck creams, lotions and powders<br>not spray | 0.6-1.1% |

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| Sodium Stearoyl Glutamate | 12D | Body and hand creams, lotions and powders<br>not spray<br>spray | <br>0.8-1.3%<br>0.3% |
| Sodium Stearoyl Glutamate | 12F | Moisturizing creams, lotions and powders<br>not spray | <br>0.8-2% |
| Sodium Stearoyl Glutamate | 12G | Night creams, lotions and powders<br>not spray | <br>1% |
| Sodium Stearoyl Glutamate | 12H | Paste masks and mud packs | 1% |
| TEA-Cocoyl Alaninate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 0.8% |
| TEA-Cocoyl Glutamate | 05F | Shampoos (noncoloring) | 2-10% |
| TEA-Cocoyl Glutamate | 05G | Tonics, dressings and other hair grooming aids | 2% |
| TEA-Cocoyl Glutamate | 10A | Bath soaps and detergents | 2.1% |
| TEA-Cocoyl Glutamate | 11E | Shaving cream (aerosol, brushless and lather) | 10.5% |
| TEA-Cocoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions liquids and pads) | 4-4.4% |
| TEA-Lauroyl Collagen Amino Acids | 05G | Tonics, dressings and other hair grooming aids | 0.4% |
| Undecylenoyl Glycine | 03B | Eye liner | 0.3% |
| Undecylenoyl Phenylalanine | 12D | Body and hand creams, lotions and powders<br>not spray | <br>0.5% |
| Undecylenoyl Phenylalanine | 12J | Other skin care preparations | 0.5-1% |

*Ingredients included in the title of the table, but not found in the table were included in the concentration of use survey, but no uses were reported

†Product category codes used by FDA

Information collected in 2012
Table prepared October 25, 2012

Personal Care Products Council
Committed to Safety, Quality & Innovation

**Memorandum**

**TO:** F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

**FROM:** Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

**DATE:** January 2, 2013

**SUBJECT:** Updated Concentration of Use by FDA Product Category: Amino Acid Alkyl Amines

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

**Concentration of Use by FDA Product Category***

Acetyl Arginine
Acetyl Cysteine
Acetyl Glutamic Acid
Acetyl Glutamine
Acetyl Histidine
Acetyl Methionine
Acetyl Proline
Acetyl Tyrosine
Capryloyl Collagen Amino Acids
Capryloyl Glycine
Capryloyl Gold of Pleasure Amino Acids
Capryloyl Keratin Amino Acids
Capryloyl Pea Amino Acids
Capryloyl Quinoa Amino Acids
Capryloyl Silk Amino Acids
Cocoyl Glutamic Acid
Dipalmitoyl Cystine
Dipotassium Caprylol Glutamate
Dipotassium Undecylenoyl Glutamate
Disodium Capryloyl Glutamate
Disodium Cocoyl Glutamate
Disodium Hydrogenated Tallow Glutamate
Disodium N-Lauroyl Aspartate
Disodium Lauroyl Glutamate
Disodium Malyl Tyrosinate
Disodium Stearoyl Glutamate
Disodium Undecylenoyl Glutamate
Lauroyl Arginine
Lauroyl Collagen Amino Acids
Lauroyl Glutamic Acid
Lauroyl Lysine
Lauroyl Silk Amino Acids
Magnesium Palmitoyl Glutamate
Myristoyl Glutamic Acid
Myristyl Alaninate
Oleoyl Tyrosine
Palmitoyl Alanine
Palmitoyl Arginine
Palmitoyl Collagen Amino Acids
Palmitoyl Glutamic Acid
Palmitoyl Glycine
Palmitoyl Gold of Pleasure Amino Acids
Palmitoyl Isoleucine
Palmitoyl Keratin Amino Acids
Palmitoyl Millet Amino Acids
Palmitoyl Oat Amino Acids
Palmitoyl Pea Amino Acids
Palmitoyl Quinoa Amino Acids
Palmitoyl Silk Amino Acids
Potassium Capryloyl Glutamate
Potassium Cocoyl Glutamate
Potassium Cocoyl Glycinate
Potassium Lauroyl Collagen Amino Acids
Potassium Lauroyl Glutamate
Potassium Lauroyl Oat Amino Acids
Potassium Lauroyl Pea Amino Acids
Potassium Lauroyl Silk Amino Acids
Potassium Lauroyl Wheat Amino Acids
Potassium Myristoyl Glutamate
Potassium Olivoyl/Lauroyl Wheat Amino Acids
Potassium Stearoyl Glutamate
Potassium Undecylenoyl Glutamate
Propionyl Collagen Amino Acids
Sodium Capryloyl Glutamate
Sodium Cocoyl Alaninate
Sodium Cocoyl Amino Acids
Sodium Cocoyl Apple Amino Acids
Sodium Cocoyl Collagen Amino Acids
Sodium Cocoyl Glutamate
Sodium Cocoyl Glycinate
Sodium Cocoyl/Hydrogenated Tallow Glutamate
Sodium Cocoyl Oat Amino Acids
Sodium Cocoyl/Palmoyl/Sunfloweroyl Glutamate
Sodium Cocoyl Proline
Sodium Cocoyl Threoninate
Sodium Cocoyl Wheat Amino Acids
Sodium Hydrogenated Tallowoyl Glutamate
Sodium Lauroyl Aspartate
Sodium Lauroyl Collagen Amino Acids
Sodium Lauroyl Glutamate
Sodium Lauroyl Millet Amino Acids
Sodium Lauroyl/Myristoyl Aspartate
Sodium Lauroyl Oat Amino Acids
Sodium Lauroyl Silk Amino Acids
Sodium Lauroyl Wheat Amino Acids
Sodium Myristoyl Glutamate
Sodium Olivoyl Glutamate
Sodium Palmitoyl Proline
Sodium Palmoyl Glutamate
Sodium Stearoyl Glutamate

Sodium/TEA-Lauroyl Collagen Amino Acids
Sodium/TEA-Lauroyl Keratin Amino Acids
Sodium/TEA-Undecylenoyl Collagen Amino Acids
Sodium Undecylenoyl Glutamate
Stearoyl Glutamic Acid
Stearoyl Leucine
TEA-Cocoyl Alaninate
TEA-Cocoyl Glutamate
TEA-Cocoyl Glutaminate
TEA-Hydrogenated Tallowoyl Glutamate
TEA-Lauroyl Collagen Amino Acids
TEA-Lauroyl Glutamate
TEA-Lauroyl Keratin Amino Acids
TEA-Lauroyl/Myristoyl Aspartate
Undecylenoyl Collagen Amino Acids
Undecylenoyl Glycine
Undecylenoyl Phenylalanine
Undecylenoyl Wheat Amino Acids
Zinc Lauroyl Aspartate

| **Ingredient** | **FDA Code†** | **Product Category** | **Maximum Concentration of Use** |
|---|---|---|---|
| Acetyl Cysteine | 05G | Tonics, dressings and other hair grooming aids | 0.1% |
| Acetyl Cysteine | 12C | Face and neck creams, lotions and powders<br>not spray | <br>0.0005% |
| Acetyl Cysteine | 12J | Other skin care preparations | 0.03% |
| Acetyl Glutamine | 11E | Shaving cream (aerosol, brushless and lather) | 0.1% |
| Acetyl Glutamine | 12C | Face and neck creams, lotions and powders<br>not spray<br>spray | <br>0.5-1%<br>1% |
| Acetyl Glutamine | 12J | Other skin care preparations | 0.01% |
| Acetyl Methionine | 05G | Tonics, dressings and other hair grooming aids | 0.00001% |
| Acetyl Tyrosine | 03D | Eye lotion | 0.3% |
| Acetyl Tyrosine | 05I | Other hair preparations (noncoloring) | 0.3% |
| Acetyl Tyrosine | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 0.03% |
| Acetyl Tyrosine | 12D | Body and hand creams, lotions and powders<br>not spray | <br>0.08% |
| Acetyl Tyrosine | 12G | Night creams, lotions and powders<br>not spray | <br>0.2% |

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| Capryloyl Glycine | 03C | Eye shadow | 0.4-0.5% |
| Capryloyl Glycine | 03G | Other eye makeup preparations | 2% |
| Capryloyl Glycine | 05A | Hair conditioners | 0.4-0.5% |
| Capryloyl Glycine | 05F | Shampoos (noncoloring) | 1-2% |
| Capryloyl Glycine | 07C | Foundations | 1% |
| Capryloyl Glycine | 10B | Deodorants<br>not spray | 0.1% |
| Capryloyl Glycine | 12A | Skin cleansing (cold creams cleansing lotions, liquids and pads) | 0.09-1% |
| Capryloyl Glycine | 12C | Face and neck creams, lotions and powders<br>not spray | 0.2-0.5% |
| Capryloyl Glycine | 12D | Body and hand creams, lotions and powders<br>not spray | 0.5% |
| Capryloyl Glycine | 12F | Moisturizing creams, lotions and powders<br>not spray | 0.09% |
| Capryloyl Glycine | 12H | Paste masks and mud packs | 0.05% |
| Capryloyl Glycine | 12J | Other skin care preparations | 1% |
| Cocoyl Glutamic Acid | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 24% |
| Disodium Capryloyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 0.4% |
| Disodium Cocoyl Glutamate | 03C | Eye shadow | 0.02% |
| Disodium Cocoyl Glutamate | 03G | Other eye makeup preparations | 0.05% |
| Disodium Cocoyl Glutamate | 07B | Face powders | 0.1% |
| Disodium Cocoyl Glutamate | 07C | Foundations<br>spray | 0.3% |
| Disodium Cocoyl Glutamate | 08C | Nail creams and lotions | 0.05% |
| Disodium Cocoyl | 10E | Other personal cleanliness products | 2% |

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| Glutamate | | hand soap | 0.6% |
| Disodium Cocoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions liquids and pads) | 2-3% |
| Disodium Cocoyl Glutamate | 12D | Body and hand creams, lotions and powders not spray | 0.08% |
| Disodium Hydrogenated Tallow Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 1% |
| Disodium Hydrogenated Tallow Glutamate | 12C | Face and neck creams, lotions and powders not spray | 0.1% |
| Disodium Stearoyl Glutamate | 03B | Eye liner | 0.1-0.24% |
| Disodium Stearoyl Glutamate | 03C | Eye shadow | 0.8-1% |
| Disodium Stearoyl Glutamate | 03D | Eye lotion | 0.05% |
| Disodium Stearoyl Glutamate | 03G | Other eye makeup preparations | 0.4% |
| Disodium Stearoyl Glutamate | 07A | Blushers (all types) | 0.03-2% |
| Disodium Stearoyl Glutamate | 07B | Face powders | 0.2-6% |
| Disodium Stearoyl Glutamate | 07C | Foundations | 0.04-6% |
| Disodium Stearoyl Glutamate | 07E | Lipstick | 0.000006-0.02% |
| Disodium Stearoyl Glutamate | 07F | Makeup bases | 0.5% |
| Disodium Stearoyl Glutamate | 07H | Makeup fixatives | 0.5% |
| Disodium Stearoyl Glutamate | 07I | Other makeup preparations | 0.2% |
| Disodium Stearoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 0.1-0.3% |

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| Disodium Stearoyl Glutamate | 12C | Face and neck creams, lotions and powders<br>not spray | 0.5-0.9% |
| Disodium Stearoyl Glutamate | 12F | Moisturizing creams, lotions and powders<br>not spray | 0.9% |
| Lauroyl Lysine | 03A | Eye brow pencil | 0.005-4% |
| Lauroyl Lysine | 03B | Eye liner | 0.005-4% |
| Lauroyl Lysine | 03C | Eye shadow | 0.3-10.2% |
| Lauroyl Lysine | 03D | Eye lotion | 0.5-2% |
| Lauroyl Lysine | 03F | Mascara | 0.005-0.5% |
| Lauroyl Lysine | 04C | Powders (dusting and talcum) | 0.5% |
| Lauroyl Lysine | 04E | Other fragrance preparations<br>not spray | 2% |
| Lauroyl Lysine | 05A | Hair conditioners | 0.3% |
| Lauroyl Lysine | 05E | Rinses (noncoloring) | 0.001% |
| Lauroyl Lysine | 05F | Shampoos (noncoloring) | 0.001% |
| Lauroyl Lysine | 07A | Blushers (all types) | 0.005-12% |
| Lauroyl Lysine | 07B | Face powders | 0.005-12% |
| Lauroyl Lysine | 07C | Foundations | 0.06-14% |
| Lauroyl Lysine | 07E | Lipstick | 0.2-45% |
| Lauroyl Lysine | 07F | Makeup bases | 0.1% |
| Lauroyl Lysine | 07G | Rouges | 0.9% |
| Lauroyl Lysine | 07H | Makeup fixatives | 0.09% |
| Lauroyl Lysine | 07I | Other makeup preparations | 0.9-3% |
| Lauroyl Lysine | 08E | Nail polish and enamel | 0.001% |
| Lauroyl Lysine | 12C | Face and neck creams, lotions and powders<br>not spray | 0.5-1% |
| Lauroyl Lysine | 12D | Body and hand creams, lotions and powders<br>not spray | 0.1-0.3% |

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| Lauroyl Lysine | 12F | Moisturizing creams, lotions and powders<br>not spray | 0.5% |
| Lauroyl Lysine | 12G | Night creams, lotions and powders<br>not spray | 0.5% |
| Magnesium Palmitoyl Glutamate | 05B | Hair sprays<br>pump spray | 0.2% |
| Magnesium Palmitoyl Glutamate | 05G | Tonics, dressings and other hair grooming aids<br>spray | 0.2% |
| Magnesium Palmitoyl Glutamate | 07C | Foundations | 0.0006-0.09% |
| Magnesium Palmitoyl Glutamate | 08A | Basecoates and undercoats (manicuring preparations) | 0.002% |
| Magnesium Palmitoyl Glutamate | 08G | Other manicuring preparations | 0.001% |
| Magnesium Palmitoyl Glutamate | 12C | Face and neck creams, lotions and powders<br>not spray | 0.1% |
| Magnesium Palmitoyl Glutamate | 12D | Body and hand creams, lotions and powders<br>spray | 0.2% |
| Palmitoyl Glycine | 12C | Face and neck creams, lotions and powders<br>not spray | 1% |
| Potassium Cocoyl Glutamate | 02D | Other bath preparations | 6% |
| Potassium Cocoyl Glutamate | 05F | Shampoos (noncoloring) | 8% |
| Potassium Cocoyl Glutamate | 10A | Bath soaps and detergents | 3% |
| Potassium Cocoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 12% |
| Potassium Cocoyl Glutamate | 12C | Face and neck creams, lotions and powders<br>not spray | 0.03% |
| Potassium Cocoyl Glycinate | 10A | Bath soaps and detergents | 1% |

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| Potassium Cocoyl Glycinate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 3-39% |
| Potassium Cocoyl Glycinate | 12D | Body and hand creams, lotions and powders<br>not spray | 2% |
| Potassium Lauroyl Wheat Amino Acids | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 0.7% |
| Potassium Myristoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 11-27% |
| Sodium Cocoyl Amino Acids | 05A | Hair conditioners | 0.4% |
| Sodium Cocoyl Amino Acids | 05B | Hair sprays<br>pump spray | 0.4% |
| Sodium Cocoyl Amino Acids | 05F | Shampoos (noncoloring) | 0.4% |
| Sodium Cocoyl Amino Acids | 05G | Tonics, dressings and other hair grooming aids | 0.4% |
| Sodium Cocoyl Amino Acids | 05I | Other hair preparations (noncoloring) | 1% |
| Sodium Cocoyl Amino Acids | 10A | Bath soaps and detergents | 2.8% |
| Sodium Cocoyl Apple Amino Acids | 03B | Eye liner | 0.3% |
| Sodium Cocoyl Apple Amino Acids | 05F | Shampoos (noncoloring) | 0.5% |
| Sodium Cocoyl Apple Amino Acids | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 0.6-3% |
| Sodium Cocoyl Collagen Amino Acids | 05A | Hair conditioners | 0.02% |
| Sodium Cocoyl Collagen Amino Acids | 05G | Tonics, dressings and other hair grooming aids | 0.02% |
| Sodium Cocoyl Glutamate | 03B | Eye liner | 0.5% |
| Sodium Cocoyl | 03C | Eye shadow | 0.004% |

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| Glutamate | | | |
| Sodium Cocoyl Glutamate | 03D | Eye lotion | 0.6% |
| Sodium Cocoyl Glutamate | 05F | Shampoos (noncoloring) | 0.2-10% |
| Sodium Cocoyl Glutamate | 05G | Tonics, dressings and other hair grooming aids | 0.5% |
| Sodium Cocoyl Glutamate | 06A | Hair dyes and colors (all types requiring caution statement and patch test) | 3% |
| Sodium Cocoyl Glutamate | 07C | Foundations<br>spray | 0.004-0.005%<br>0.03% |
| Sodium Cocoyl Glutamate | 10A | Bath soaps and detergents | 3% |
| Sodium Cocoyl Glutamate | 10E | Other personal cleanliness products | 0.2% |
| Sodium Cocoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions liquids and pads) | 1-9% |
| Sodium Cocoyl Glutamate | 12C | Face and neck creams, lotions and powders<br>not spray | 0.1-0.6 |
| Sodium Cocoyl Glutamate | 12D | Body and hand creams, lotions and powders<br>not spray | 0.04-3% |
| Sodium Cocoyl Glutamate | 12H | Paste masks and mud packs | 0.01% |
| Sodium Cocoyl Glycinate | 10A | Bath soaps and detergents | 3% |
| Sodium Cocoyl Glycinate | 10E | Other personal cleanliness products<br>hand soap | 0.2% |
| Sodium Cocoyl Glycinate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 2-20% |
| Sodium Hydrogenated Tallowoyl Glutamate | 12C | Face and neck creams, lotions and powder<br>not spray | 0.8% |
| Sodium Lauroyl Aspartate | 03B | Eye liner | 0.1% |

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| Sodium Lauroyl Aspartate | 05F | Shampoos (noncoloring) | 2% |
| Sodium Lauroyl Aspartate | 07B | Face powders | 0.2% |
| Sodium Lauroyl Aspartate | 07C | Foundations | 0.04% |
| Sodium Lauroyl Aspartate | 07G | Rouges | 0.005% |
| Sodium Lauroyl Aspartate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 2% |
| Sodium Lauroyl Glutamate | 02A | Bath oils, tablets and salts | 4% |
| Sodium Lauroyl Glutamate | 05F | Shampoos (non-coloring) | 3% |
| Sodium Lauroyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 10-40% |
| Sodium Lauroyl Glutamate | 12C | Face and neck cream, lotions and powders not spray | 0.03-1% |
| Sodium Lauroyl Glutamate | 12D | Body and hand cream, lotions and powders not spray | 4% |
| Sodium Lauroyl Glutamate | 12F | Moisturizing creams, lotions and powders not spray | 0.03-0.1% |
| Sodium Lauroyl Glutamate | 12H | Paste masks and mud packs | 0.003% |
| Sodium Lauroyl Oat Amino Acids | 02B | Bubble baths | 0.9% |
| Sodium Lauroyl Oat Amino Acids | 03E | Eye makeup remover | 5% |
| Sodium Lauroyl Oat Amino Acids | 05F | Shampoos (noncoloring) | 0.04-0.4% |
| Sodium Lauroyl Oat Amino Acids | 05G | Tonics, dressings and other hair grooming aids | 0.4% |
| Sodium Lauroyl Oat Amino Acids | 10A | Bath soaps and detergents | 5% |

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| Sodium Lauroyl Oat Amino Acids | 10E | Other personal cleanliness products | 0.09-4% |
| Sodium Lauroyl Oat Amino Acids | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 0.6-5% |
| Sodium Lauroyl Oat Amino Acids | 12C | Face and neck creams, lotions and pads<br>not spray | 0.8% |
| Sodium Lauroyl Oat Amino Acids | 12D | Body and hand creams, lotions and powders<br>not spray | 0.6% |
| Sodium Lauroyl Oat Amino Acids | 12F | Moisturizing creams, lotions and powders<br>not spray | 0.5% |
| Sodium Myristoyl Glutamate | 03E | Eye makeup remover | 0.1% |
| Sodium Myristoyl Glutamate | 07C | Foundations | 0.1% |
| Sodium Myristoyl Glutamate | 07F | Makeup bases | 0.2% |
| Sodium Myristoyl Glutamate | 08B | Cuticle softeners | 0.5% |
| Sodium Myristoyl Glutamate | 10A | Bath soaps and detergents | 31% |
| Sodium Myristoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 5-30% |
| Sodium Myristoyl Glutamate | 12J | Other skin care preparations | 5% |
| Sodium Palmoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 2-22% |
| Sodium Stearoyl Glutamate | 03D | Eye lotion | 1% |
| Sodium Stearoyl Glutamate | 05A | Hair conditioners | 0.03-0.2% |
| Sodium Stearoyl Glutamate | 07C | Foundations | 0.4-1.1% |
| Sodium Stearoyl Glutamate | 07E | Lipstick | 1% |

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| Sodium Stearoyl Glutamate | 07F | Makeup bases | 0.4% |
| Sodium Stearoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 1.1% |
| Sodium Stearoyl Glutamate | 12C | Face and neck creams, lotions and powders<br>not spray | <br>0.6-1.1% |
| Sodium Stearoyl Glutamate | 12D | Body and hand creams, lotions and powders<br>not spray<br>spray | <br>0.3-1.3%<br>0.3% |
| Sodium Stearoyl Glutamate | 12F | Moisturizing creams, lotions and powders<br>not spray | <br>0.8-2% |
| Sodium Stearoyl Glutamate | 12G | Night creams, lotions and powders<br>not spray | <br>1% |
| Sodium Stearoyl Glutamate | 12H | Paste masks and mud packs | 1% |
| Sodium Stearoyl Glutamate | 13B | Indoor tanning preparations | 0.2% |
| TEA-Cocoyl Alaninate | 12A | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 0.8% |
| TEA-Cocoyl Glutamate | 05F | Shampoos (noncoloring) | 2-10% |
| TEA-Cocoyl Glutamate | 05G | Tonics, dressings and other hair grooming aids | 2% |
| TEA-Cocoyl Glutamate | 10A | Bath soaps and detergents | 2.1% |
| TEA-Cocoyl Glutamate | 10E | Other personal cleanliness products | 3% |
| TEA-Cocoyl Glutamate | 11E | Shaving cream (aerosol, brushless and lather) | 10.5% |
| TEA-Cocoyl Glutamate | 12A | Skin cleansing (cold creams, cleansing lotions liquids and pads) | 3-4.4% |
| TEA-Lauroyl Collagen Amino Acids | 05G | Tonics, dressings and other hair grooming aids | 0.4% |

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| Undecylenoyl Glycine | 03B | Eye liner | 0.3% |
| Undecylenoyl Phenylalanine | 12D | Body and hand creams, lotions and powders<br>not spray | 0.5% |
| Undecylenoyl Phenylalanine | 12J | Other skin care preparations | 0.5-2% |

*Ingredients included in the title of the table, but not found in the table were included in the concentration of use survey, but no uses were reported

†Product category codes used by FDA

Information collected in 2012

Table prepared October 25, 2012

Updated January 2, 2013: Capryloyl Glycine: eye shadow added 0.5% as high concentration; Disodium Hydrogenated Tallow Glutamate: body and hand products deleted; skin cleansing changed to 1%; added face and neck products 0.1%; Disodium Stearoyl Glutamate: eye liner added 0.24% as high concentration; added other eye makeup preparations; lipstick, added 0.000006% as low concentration; Lauroyl Lysine: added other fragrance preparation (not spray); lipstick low end changed to 0.2%; Potassium Myristoyl Glutamate: skin cleansing changed high concentration to 27%; Sodium Cocoyl Glycinate: skin cleansing changed low to 2%; Sodium Lauroyl Aspartate: added eye liner; Sodium Lauroyl Oat Amino Acids: added bubble baths; changed other personal cleanliness products to 0.09-4%; Sodium Myristoyl Glutamate: changed makeup bases to 0.2%; Sodium Stearoyl Glutamate: body products low changed to 0.3%; added indoor tanning products; TEA-Cocoyl Glutamate: added other personal cleanliness products skin cleansing changed 4% to 3%; Undecylenoyl Phenylalanine: other skin care preparations changed high concentration to 2%

Personal Care Products Council
Committed to Safety, Quality & Innovation

**Memorandum**

**TO:** F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

**FROM:** Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

**DATE:** March 20, 2013

**SUBJECT:** Comments on the Scientific Literature Review on Amino Acid Alkyl Amides as Used in Cosmetics

Please note that the Council has no suppliers listed for the following ingredients:

Acetyl Glutamine
Capryloyl Keratin Amino Acids
Dipalmitoyl Cystine
Dipotassium Caprylol Glutamate
Dipotassium Undecylenoyl Glutamate
Disodium N-Lauroyl Aspartate
Disodium Malyl Tyrosinate
Disodium Undecylenoyl Glutamate
Lauroyl Collagen Amino Acids
Myristoyl Glutamic Acid
Palmitoyl Glutamic Acid
Potassium Capryloyl Glutamate
Potassium Lauroyl Collagen Amino Acids
Potassium Lauroyl Oat Amino Acids
Potassium Stearoyl Glutamate
Potassium Undecylenoyl Glutamate
Propionyl Collagen Amino Acids
Sodium Capryloyl Glutamate
Sodium Cocoyl Collagen Amino Acids
Sodium Cocoyl/Hydrogenated Tallow Glutamate
Sodium Cocoyl Oat Amino Acids
Sodium Lauroyl Collagen Amino Acids
Sodium/TEA-Lauroyl Keratin Amino Acids
Sodium/TEA-Undecylenoyl Collagen Amino Acids
Sodium Undeylenoyl Glutamate
Stearoyl Glutamic Acid
Stearoyl Leucine
TEA-Hydrogenated Tallowoyl Glutamate
TEA-Lauroyl Keratin Amino Acids
Undecylenoyl Collagen Amino Acids
Undecylenoyl Wheat Amino Acids

p.1 - In the second paragraph, please correct: "the ingredients in this report will report will rapidly dissociate..."

p.1 - As the other specific ingredients are named, please name the specific dicarboxylic acids (relevant to this report) that were found safe for use in cosmetics.

p.1, 13, Table 2 - The Introduction states that "data are sufficient to determine safety for malic acid." Table 2 says the data were insufficient. The actual conclusion of the 2001 published report is that Malic Acid was found safe for use as a pH adjuster, and the data were insufficient for other uses.

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p.2, 4 - 21CFR172.372 refers to the use of Acetyl Methionine as a food additive permitted for direct addition to food. FDA does not "approve" dietary supplements (see: http://www.fda.gov/Food/DietarySupplements/default.htm).

p.12, Table 2 - Please list the dicarboxylic acid relevant to this report that were found safe as used.

p.14, Table 2 - Rather than having separate entries for Oleic Acid, Olive Acid, Palm Acid, Palmitic Acid, Sunflower Seed Acid and Stearic Acid, these ingredients should be listed under the report in which they were reviewed.

p.37, Table 7 - Please correct the spelling of "Tyroinamide"

p.38, Tables 8 and 10 - In these tables, it would be helpful if the *in vitro* studies were separated from the *in vivo* studies.