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# Safety Assessment of Milk Proteins and Protein Derivatives as Used in Cosmetics

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Status: Draft Report for Panel Review  
Release Date: March 17, 2017  
Panel Meeting Date: April 10-11, 2017

The 2017 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 Lillian J. Gill, DPA. This safety assessment was prepared by Christina L. Burnett, Scientific Analyst/Writer and Bart Heldreth, Ph.D., Chemist CIR.

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Memorandum

To: CIR Expert Panel Members and Liaisons  
From: Christina L. Burnett, Senior Scientific Writer/Analyst  
Date: March 17, 2017  
Subject: Draft Safety Assessment on Milk Proteins and Protein Derivatives

Enclosed is the Draft Report of the Safety Assessment of Milk Proteins and Protein Derivatives as Used in Cosmetics. (It is identified as *mlkpro042017rep* in the pdf document).

In February 2017, CIR issued the Scientific Literature Review (SLR) for these ingredients, which mainly function as skin and hair conditioning agents in personal care products. The majority of the ingredients in this report were originally included in a larger hydrolyzed source proteins safety assessment from 2013. At the time, the Panel determined to divide the ingredients in that larger report into smaller, more manageable safety assessments, with Hydrolyzed Wheat Protein and Hydrolyzed Wheat Gluten being the first ingredients reviewed. The Panel agreed upon the grouping of the 16 ingredients in this current safety assessment in September 2015.

The milk proteins and protein derivatives in this assessment are found in bovine-sourced milk. Bovine milk, milk proteins, and milk protein derivatives are generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (FDA). Because daily exposures from food use would result in much greater systemic exposures than those resulting from use in cosmetic products, this safety assessment focuses on the potential for irritation and sensitization from topical exposure to these milk proteins.

Data requested with the issuance of the SLR included dermal and ocular irritation and sensitization data, molecular weight ranges, composition, impurities, and any additional toxicological data that would help the Panel assess the safe use of these ingredients in cosmetics.

Applicable unpublished data were provided previously by the Personal Care Products Council (Council) for the larger hydrolyzed source proteins safety assessment; the Panel reviewed those data in 2013. More recent data from Industry has also been submitted. Both the refresher data and the new data, along with the most current concentration of use survey, have been incorporated into the report and included in this report's package as a refresher (identified as *mlkpro042017data1* through *mlkpro042017data8*). Comments provided by the Council on the SLR have been considered (*mlkpro042017pcpc*).

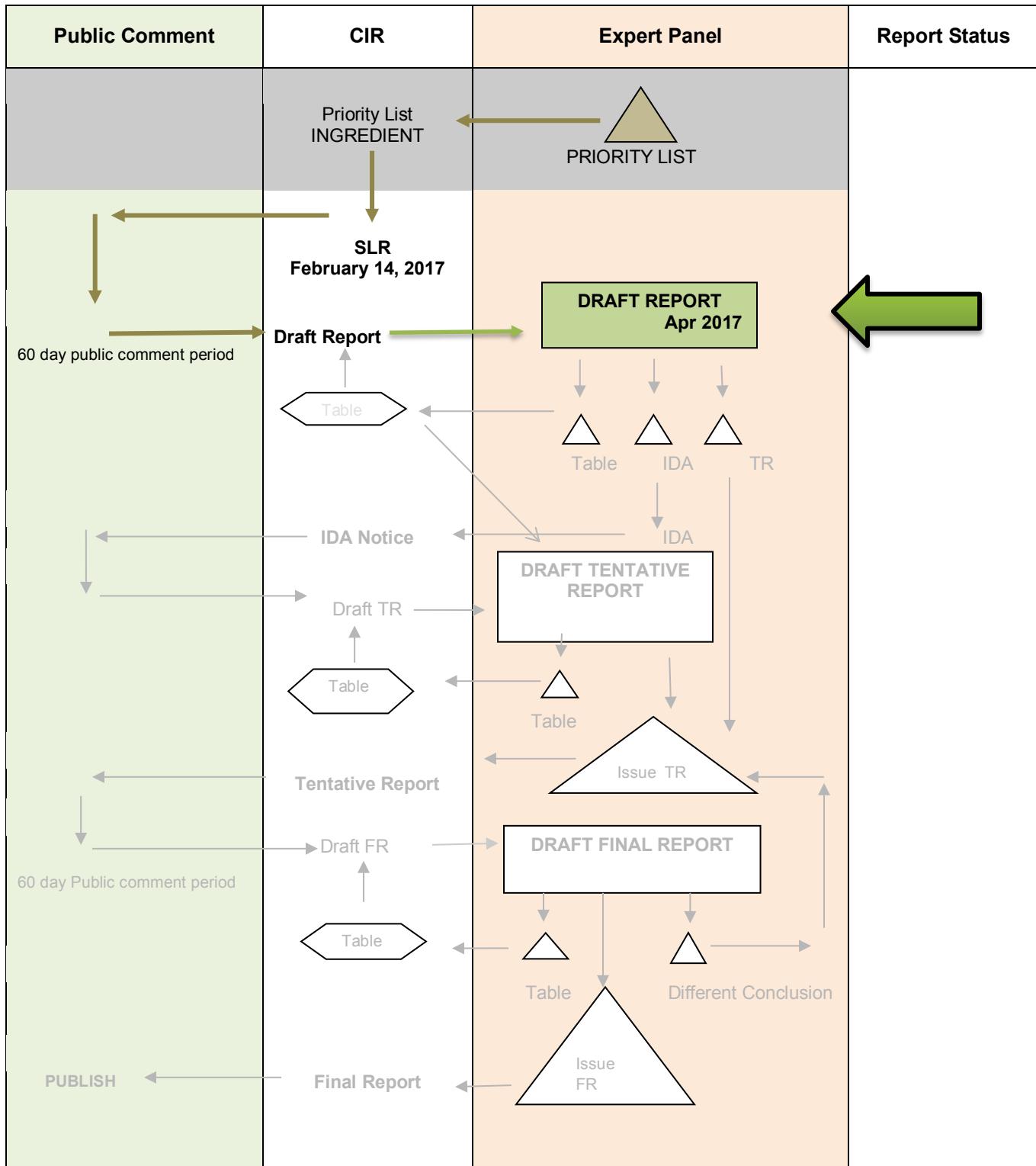
According to 2017 FDA VCRP data, Hydrolyzed Milk Protein is used in 189 formulations; the majority of uses are in leave-on products. Whey Protein has the second greatest number of overall uses reported, with a total of 67; the majority of the uses are in leave-on formulations. The results of the concentration of use survey conducted in 2016 by the Council indicate Sodium Caseinate has the highest reported maximum concentration of use; it is used at up to 96.9% in bath oils, tablets and salts. The highest reported maximum concentration of use in a leave-on for this ingredient is 0.1% in a face and neck skin care product. Casein is used at up to 2% in makeup preparations.

If no further data are needed, the Panel should formulate a Discussion and issue a Tentative Report. However, if additional data are required, the Panel should be prepared to identify those needs and issue an Insufficient Data Announcement.

# SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Milk Proteins and Protein Derivatives

MEETING April 2017



**Milk Proteins and Protein Derivatives**

**February 2017** – Scientific Literature Review announced.

**Milk Proteins and Protein Derivatives Data Profile -April 2017 – Writer, Christina Burnett**

	<b>In-Use</b>	<b>Physical/Chemical Properties</b>	<b>Molecular Weight Range</b>	<b>Method of Manufacturing</b>	<b>Composition/Impurities</b>	<b>Acute Toxicity</b>	<b>Repeated Dose Toxicity</b>	<b>Genotoxicity</b>	<b>Reproductive and Developmental Toxicity</b>	<b>Carcinogenicity</b>	<b>Other Relevant Toxicity Studies</b>	<b>Irritation/Sensitization - Nonhuman</b>	<b>Irritation/Sensitization - Human</b>	<b>Ocular/Mucosal</b>	<b>Phototoxicity</b>	<b>Clinical/Case Studies</b>
Ammonium Caseinate		X			X											
Calcium Caseinate		X			X											
Casein	X	X	X	X	X					X						
Casein Extract																
Hydrolyzed Casein	X		X	X	X		X					X	X			
Hydrolyzed Lactalbumin																
Hydrolyzed Milk Protein	X		X	X		X					X		X	X	X	
Hydrolyzed Whey Protein	X									X						
Hydrolyzed Yogurt Protein	X															
Lactoglobulin	X															
Milk Protein	X															
Milk Protein Extract	X															
Potassium Caseinate		X				X										
Sodium Caseinate	X	X			X											
Sodium Hydrolyzed Casein																
Whey Protein	X				X	X				X						

“X” indicates that data were available in the category for that ingredient.

**Search Strategy for Milk Protein and Protein Derivatives**  
**(Performed by Christina Burnett)**

- SciFinder

- December 2016 - Search for ingredients by “toxicity of milk protein”, 26 hits for close association = 0 relevant to cosmetic use. Most concerned milk allergy from consumption. When the 16 ingredients and available CAS# were entered in substance identifier search, returns were found for Hydrolyzed Milk Protein, Whey Protein, Milk Protein, Potassium Caseinate, Hydrolyzed Casein, Lactoglobulin, Sodium Caseinate, Calcium Caseinate, Ammonium Caseinate, and Casein. References on “adverse events, including toxicity” were only found for Hydrolyzed Casein (20 hits) and were similar to those found in the PubMed search.

Search Terms	TOXLINE Hits (excluding PUBMED)	PUBMED Hits	SCCS/SCCP Opinion	ECHA Hits	NICNAS Assessment
ammonium caseinate OR 9005-42-9	0	4; 0 retrieved	No	No	No
calcium caseinate OR 9005-43-0	5; 0 retrieved	76; 3 retrieved	No	No	No
casein	1981; limited w/ “dermal” = 3; 0 retrieved	30440; limited w/ “toxicity” = 693; limited w/ “toxicity and dermal” = 1; 0 retrieved	No	No	Tier 1 assessment; low concern for human health
casein extract	79; limited w/ “dermal” = 0	800; limited w/ “toxicity” = 20; 0 retrieved	No	No	No
hydrolyzed casein	122; limited w/ “dermal” = 0	739; limited w/ “toxicity” = 14; 0 retrieved	No	No	No
hydrolyzed lactalbumin	3; 0 retrieved	58; 0 retrieved	No	No	No
hydrolyzed milk protein	1; 0 retrieved	823; limited w/ “toxicity” = 7; 0 retrieved	No	No	No
hydrolyzed whey protein	4; 0 retrieved	310; 0 retrieved	No	No	No
hydrolyzed yogurt protein	0	6; 0 retrieved	No	No	No
lactoglobulin	165; limited w/ “dermal” = 0	3990; limited w/ “toxicity” = 41; 1 retrieved	No	No	No
milk protein	2150; limited w/ “dermal” = 3; 0 retrieved	53092; limited w/ “toxicity” = 1028; limited w/ “toxicity and dermal” = 2; both retrieved	No	No	No
milk protein extract	83; limited w/ “dermal” = 0	815; limited w/ “toxicity NOT thistle” = 38; 0 retrieved	No	No	No
potassium caseinate	3; 0 retrieved	19; 1 retrieved	No	No	No

<b>Search Terms</b>	<b>TOXLINE Hits (excluding PUBMED)</b>	<b>PUBMED Hits</b>	<b>SCCS/SCCP Opinion</b>	<b>ECHA Hits</b>	<b>NICNAS Assessment</b>
sodium caseinate	1958; limited w/ “dermal” = 3; 0 retrieved	15020; limited w/“toxicity” = 308; 0 retrieved	No	No	No
sodium hydrolyzed casein	2; 0 retrieved	91; 1 retrieved	No	No	No
whey protein	52; 2 retrieved	15639; limited w/ “toxicity” = 267; 6 retrieved	No	No	No

Total references ordered or downloaded: 16

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## INTRODUCTION

Milk and dairy products, especially bovine (cow) sourced, are considered vital sources of nutrition for billions of people around the world.<sup>1</sup> Milk proteins and protein derivatives form a broad category of materials that are prepared by extraction from bovine milk and partial hydrolysis to yield cosmetic ingredients. The Food and Drug Administration (FDA) defines the term “protein” to mean any  $\alpha$ -amino acid polymer with a specific defined sequence that is greater than 40 amino acids in size.<sup>2</sup> The bovine milk proteins and protein derivatives detailed in this report are described by the *International Cosmetic Ingredient Dictionary and Handbook (Dictionary)* to function mainly as skin and hair conditioning agents in personal care products.<sup>3</sup> This report assesses the safety of the following 16 milk-derived ingredients:

Ammonium Caseinate	Hydrolyzed Yogurt Protein
Calcium Caseinate	Lactoglobulin
Casein	Milk Protein
Casein Extract	Milk Protein Extract
Hydrolyzed Casein	Potassium Caseinate
Hydrolyzed Lactalbumin	Sodium Caseinate
Hydrolyzed Milk Protein	Sodium Hydrolyzed Casein
Hydrolyzed Whey Protein	Whey Protein

The safety of several hydrolyzed proteins as used in cosmetics has been reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel (Panel) in several previous assessments. The Panel concluded that Hydrolyzed Keratin (finalized in 2016), Hydrolyzed Collagen (published in 1985, re-review published in 2006) Hydrolyzed Soy Protein (finalized in 2015), Hydrolyzed Silk (finalized in 2015), Hydrolyzed Rice Protein (published in 2006), and Hydrolyzed Corn Protein (published in 2011) are safe for use in cosmetics.<sup>4-10</sup> Additionally, the Panel concluded that Hydrolyzed Wheat Gluten and Hydrolyzed Wheat Protein are safe for use in cosmetics when formulated to restrict peptides to a weight-average MW of 3500 Da or less.<sup>11</sup> The CIR is concurrently reviewing the safety of plant-derived proteins and peptides in a separate report.

While relevant data on the cosmetic ingredient Hydrolyzed Lactalbumin could not be identified in the published literature, information on the unprocessed protein lactalbumin was discovered and has been incorporated into this report to aid in the review of safety.

## CHEMISTRY

### Definition

The definitions and functions of the milk proteins and protein derivatives are described in Table 1.

Bovine milk proteins are synthesized in the mammary epithelial cells and are only produced by the mammary gland.<sup>12</sup> There are numerous milk proteins, but the most prevalent are caseins (~79% of all milk proteins; the gelatinous material of the curd), and whey; whey is primarily lactalbumin (~4%) and lactoglobulin (~10%).<sup>13</sup> While other proteins exist in milk (e.g., enzymes, antibodies, and growth factors; all together comprising the other ~7%), the ingredients in this report predominantly comprise casein, lactalbumin, and/or lactoglobulin proteins.

Protein hydrolysates can be prepared via acid hydrolysis, enzymatic hydrolysis or other methodologies. The methodology selected and the conditions and duration of the hydrolysis may profoundly affect the size and reactivity of the hydrolysates. Most of the ingredients in this report, even those without “hydrolyzed” in the name, are hydrolyzed to some degree as necessary for extraction or solubilization. Further steps towards solubilization of these macromolecules commonly include reaction with an alkaline substance to produce a protein salt (e.g., Calcium Caseinate). Additionally, the fermentation processes used to create yogurt from milk involve not only the conversion of lactose to lactic acid, but also partial proteolytic hydrolysis (because the lactic acid bacteria need a nitrogen source).<sup>14</sup>

## Physical and Chemical Properties

### **Casein and Caseinate Salts**

Casein is described as an off-white to cream-colored granular or fine powder. It is insoluble in water and alcohol, but can be dissolved by aqueous alkalis to form caseinate salts.<sup>15</sup> Caseinate salts are white to cream-colored granules or powders that are soluble or dispersible in water. The amino acid sequence of  $\beta$ -casein contains 209 residues with an approximate molecular weight (MW) of 23,600 Da.<sup>16</sup>

### **Hydrolyzed Casein**

A supplier has reported that the molecular weight of a Hydrolyzed Casein product is approximately 600 daltons (Da).<sup>17</sup>

### **Hydrolyzed Milk Protein**

A supplier has reported that the molecular weight of Hydrolyzed Milk Protein is ~1000 Da.<sup>18</sup> Another supplier has reported the molecular weight distribution of Hydrolyzed Milk Protein that is described in Figure 1.<sup>19</sup>

### **Hydrolyzed Lactalbumin**

$\alpha$ -Lactalbumin (non-hydrolyzed) is described as a homogenous, free-flowing, semi-hygroscopic, light cream-colored powder.<sup>15</sup> Physical and chemical properties on Hydrolyzed Lactalbumin were not found.

### **Method of Manufacturing**

Methods used to manufacture protein hydrolysates typically yield broad molecular weight distributions of peptides, ranging from 500 to 30,000 Da.<sup>20</sup> However, certain enzymes, such as papain, can routinely produce narrower distributions of 500 to 10,000 Da. For example, if the average molecular weight of an amino acid is 135 Da, then, under the broader distribution figures (i.e., 500 to 30,000 Da), these ingredients are approximately 4 to 220 amino acids in length (and approximately 4 to 74 amino acids in length under the narrower distribution, i.e., 500 to 10,000 da).<sup>21</sup>

### **Casein**

Casein is derived from the coagulum formed by treating skim milk with a food-grade acid (acid casein), enzyme (rennet casein), or other food-grade precipitating agent.<sup>15</sup> After precipitation, Casein is separated from the soluble milk fraction, washed and dried. Casein is a mixture of at least 20 electrophoretically distinct phosphoproteins, with the main fractions being  $\alpha$ -casein,  $\beta$ -casein, and  $\kappa$ -casein.

### **Hydrolyzed Casein**

A supplier reported that a Hydrolyzed Casein product (MW = 600 Da; 30% solution in water) is prepared by acidic, alkaline, and/or enzymatic hydrolysis of bovine milk until the molecular weight reached the target range.<sup>17</sup>

### **Hydrolyzed Lactalbumin**

$\alpha$ -Lactalbumin (non-hydrolyzed) is isolated from either bovine milk or from whey.<sup>15</sup> A method of manufacture for the hydrolysis of lactalbumin (specifically) to Hydrolyzed Lactalbumin was not found.

### **Hydrolyzed Milk Protein**

A supplier reported that Hydrolyzed Milk Protein is produced from milk intended for human consumption.<sup>22</sup> The milk solids are separated and hydrolyzed with a protease for 2 hours. When the target molecular weight is achieved, the enzyme is inactivated by heating the solution to 140°C for 30 minutes. The inactivation step is repeated if gelatin mixed with a sample loses viscosity, indicating the presence of active protease.

Another supplier reported that Hydrolyzed Milk Protein is manufactured by enzymatic hydrolysis for a specific duration and at an elevated temperature (details not provided).<sup>23</sup> The resultant hydrolyzed proteins have molecular weights in the 2000-4000 Da range and all contain di- and tri-peptides.

### **Whey Protein**

Whey is the liquid obtained by separating the coagulum from milk, cream, and/or skim milk (usually in cheese making).<sup>15</sup> Acid-type whey is produced by converting a significant amount of lactose to lactic acid or by direct acidification of milk. Sweet-type whey is derived from a process in which there is insignificant conversion of lactose to lactic acid. Whey protein concentrate is a liquid or dry product that is obtained by the removal of sufficient non-protein constituents from whey so that the finished dry product contains not less than 25.0% protein, while whey protein isolate is a liquid or dry product that is obtained by removing sufficient non-protein constituents from whey so that the finished dry product contains not less than 90% protein. Whey protein concentrate and whey protein isolate are produced by physical separation techniques such as precipitation, filtration, dialysis and/or ion exchange.

## Composition

### **Casein**

Casein is reported to have all the amino acids considered to be essential for human nutrition.<sup>15</sup>

### **Impurities**

The ingredients in this safety assessment are bovine sourced; however, the FDA does not consider milk or processed milk ingredients as risk materials for transmission of infectious agents (i.e. bovine spongiform encephalopathy) in cosmetic products (21 CFR §700.27).

The *Food Chemicals Codex* states that the acceptable lead limit for Casein and caseinate salts is no more than 1 mg/kg.<sup>15</sup> Acid casein should contain not less than 90% protein calculated on a dry basis. The acceptable lead limit in  $\alpha$ -lactalbumin (non-hydrolyzed form of Hydrolyzed Lactalbumin) is no more than 0.5 mg/kg on the dried basis, and the acceptable phosphorus limit is no more than 700 $\mu$ g/g.  $\alpha$ -Lactalbumin may also contain  $\beta$ -lactoglobulin (no more than 6.5% calculated on total protein basis), lactose (no more than 1.0%), lipids (no more than 1.0%). Whey, whey protein concentrate, and whey protein isolate may contain no more than 0.5 mg/kg lead calculated on the dried basis. Whey protein isolate should contain not less than 90% protein calculated on a dry basis.

### **Hydrolyzed Casein**

A supplier reported that a Hydrolyzed Casein product (MW = 600 Da, 30% solution in water) did not contain more than 5 ppm heavy metals and not more than 0.5 ppm arsenic.<sup>17</sup>

### **USE** **Cosmetic**

The safety of the cosmetic ingredients included in this assessment is evaluated based on data received from the U.S. Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by Industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2017 VCRP data, Hydrolyzed Milk Protein is used in 189 formulations; the majority of uses are in leave-on products (Table 2).<sup>24</sup> Whey Protein has the second greatest number of overall uses reported, with a total of 67; the majority of the uses are in leave-on formulations. The results of the concentration of use survey conducted in 2016 by the Council indicate Sodium Caseinate has the highest reported maximum concentration of use; it is used at up to 96.9% in bath oils, tablets and salts.<sup>25</sup> The highest reported maximum concentration of use in a leave-on formulation for this ingredient is 0.1% in a face and neck skin care product. Casein has the highest reported maximum concentration of use in a leave-on product and is used at up to 2% in makeup preparations. Ingredients with no reported uses in the VCRP or by Council are listed in Table 3.

In some cases, reports of use were received from the VCRP, but no concentration of use data were provided. For example, Lactoglobulin is reported to be used in 1 formulation, but no use concentration data were provided. In other cases, no uses were reported to the VCRP, but a maximum use concentration was provided in the industry survey. For example, Casein was not reported in the VCRP database to be in use, but the industry survey indicated that it is used at concentrations up to 2%. It should be presumed that Casein is used in at least one cosmetic formulation for each category it is reported to be used in.

Some of these ingredients may be used in products that can come into contact with mucous membranes and the eyes. For example, Sodium Caseinate is used in bath oils, tablets, and salts at up to 96.9% and Milk Protein is used in eye makeup preparations at up to 0.5%.<sup>25</sup> Additionally, some of these ingredients were reported to be used in spray deodorants, hair sprays, face powders, face and neck sprays, body and hand sprays, and fragrances and could possibly be inhaled. For example, Casein was reported to be used in a spray deodorant at 0.013% and Milk Protein was reported to be used in face powders at 0.0002%. In practice, 95% to 99% of the droplets/ particles released from cosmetic sprays have aerodynamic equivalent diameters >10  $\mu$ m, with propellant sprays yielding a greater fraction of droplets/particles below 10  $\mu$ m compared with pump sprays.<sup>26-29</sup> Therefore, most droplets/ particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.<sup>26,28</sup> There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable.<sup>28</sup> However, the information is not

sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.<sup>30-32</sup>

The milk protein and protein-derived ingredients described in this safety assessment are not restricted from use in any way under the rules governing cosmetic products in the European Union.<sup>33</sup>

#### **Non-Cosmetic**

According to the FDA, bovine milk is considered generally recognized as safe (GRAS) as it is a substance used in food prior to January 1, 1958, through experience based on common use in food (21 CFR§170.30). The FDA has also determined that the use of peptones as direct food substances is GRAS. These GRAS peptones are defined as “the variable mixture of polypeptides, oligopeptides, and amino acids that are produced by partial hydrolysis of casein...or lactalbumin (whey protein) (21 CFR §184.1553). Additionally, Casein is GRAS as substances migrating to food from paper and paperboard products (21CFR §182.90). Sodium Caseinate is GRAS for human and animal consumption (21CFR§182.1748, 21CFR§582.1748). Whey is GRAS for human consumption (21CFR§184.1979). Labeling requirements for milk-related ingredients and hydrolyzed proteins in food that is GRAS for human consumption are defined in 21CFR101.4 and 21CFR102.22.

Calcium Caseinate and Sodium Caseinate are used in over the counter (OTC) weight control drug products, but these active ingredients do not have adequate data available to be generally recognized as safe and effective for these specified uses (21 CFR§ 310.545). These casein salts and whey protein, in mixtures with other substances, are also being investigated for use as drug coatings and topical drug delivery systems, respectively.<sup>34-36</sup>

The FDA requires allergen labeling when one or more of the eight major food allergens, which includes milk, are included in food.<sup>37</sup>

Casein and caseinate salts,  $\alpha$ -lactalbumin, whey, whey protein concentrate, and whey protein isolate are all listed in the *Food Chemicals Codex*, a compendium of internationally recognized standards published by the United States Pharmacopeia (USP) for the purity and identity of food ingredients.<sup>15</sup> Casein and caseinate salts are described as binders, extenders, clarifying agents, emulsifiers, and stabilizers in food.  $\alpha$ -Lactalbumin is described as a nutrient and a source of tryptophan. Whey and whey protein concentrate are described as texturizers and nutrients, with the concentrate also used as an emulsifier, water-binding aid, and gelling agent in foods. Whey protein isolate is considered a source of high-quality protein that may also be used as a gelling agent, water-binding aid, foaming or whipping aid, emulsifier, and an edible coating used as a moisture barrier.

#### **TOXICOKINETICS**

##### ***Hydrolyzed Milk Protein***

While no experimental data were available for the dermal absorption of Hydrolyzed Milk Protein, it was noted that gastro-intestinal absorption allows for substantially greater bioavailability than dermal absorption.<sup>38</sup> In worst-case scenarios of oral exposures greater than 2000 mg/kg, no signs of systemic toxicity were observed and, therefore, it was concluded that no systemic toxicity would occur with cutaneous exposure.

#### **TOXICOLOGICAL STUDIES**

Bovine milk, milk proteins, and milk protein derivatives are GRAS, and daily exposures from food use would result in much greater systemic exposures than those resulting from use in cosmetic products. Consequently, systemic toxicity potential is not addressed further in this report. The safety assessment focuses on the potential for irritation and sensitization from topical exposure to these milk ingredients.

#### **GENOTOXICITY**

##### ***Hydrolyzed Casein***

The mutagenic potential of a Hydrolyzed Casein product (MW = 600 Da, 30% solution in water) was studied in an Ames test using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 and *Escherichia coli* strain WP2uvrA, with and without S9 metabolic activation.<sup>17</sup> Concentrations were tested up to 5000  $\mu$ g/plate. The test material did not induce reverse mutations with or without S9. It was concluded that Hydrolyzed Casein was not mutagenic.

##### ***Hydrolyzed Milk Protein***

The potential of Hydrolyzed Milk Protein to induce gene mutation was studied in *S. typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 with and without S9 metabolic activation.<sup>38</sup> Concentrations were tested up to 5000 µg/plate. The test material did not induce reverse mutations with or without S9. It was concluded that Hydrolyzed Milk Protein was not mutagenic.

## **CARCINOGENICITY**

### **Tumor Suppression**

Several studies have investigated the carcinogenic effects of milk and its related proteins and protein derivatives in the diet.<sup>39-41</sup> Review articles of the role of milk proteins and hydrolyzed proteins on cancer reported that Casein and casein peptides have antimutagenic properties, and that animal models for colon and mammary tumorigenesis (like the study described below) showed that Whey Protein and Hydrolyzed Whey Protein suppressed tumor development.<sup>42-44</sup> The tumor suppression observed in studies with Whey Protein has been attributed to the high content of cystine/cysteine and γ-glutamylcyst(e)ine dipeptides in the milk proteins, which are efficient substrates for synthesizing glutathione, an important cellular antioxidant.

An example of research of the tumor suppression of milk proteins studied the effects of milk proteins on the ability of dimethylbenzanthracene (DMBA) to induce mammary tumors in pregnant Sprague-Dawley rats.<sup>41</sup> The rats (number not reported) were fed diets that included 20% Casein, Hydrolyzed Casein, Whey Protein, or Hydrolyzed Whey Protein starting on gestation day 4. The offspring of these rats were fed the same diet. At 50 days, the female offspring (44-49 rats/group) were dosed by gavage with sesame oil containing 80 mg/kg DMBA and were killed 62 days post-treatment. The rats that were fed Hydrolyzed Whey Protein had an adenocarcinoma incidence of 17% compared to rats fed Casein (34%), Hydrolyzed Casein (33%), and Whey Protein (36%) ( $P < 0.001$ ). The median palpable tumor latency for rats fed Hydrolyzed Whey Protein (61 days,  $P < 0.001$ ) was greater compared to those fed Casein (44 days), Hydrolyzed Casein (42 days), or Whey Protein (45 days). When compared to rats fed Casein and Hydrolyzed Casein, tumor multiplicity was lower in rats fed Hydrolyzed Whey Protein (1.5 vs 3.0,  $P < 0.05$ ). The authors of the study concluded that dietary intake of Whey Protein reduced DMBA-induced mammary tumor.

## **OTHER RELEVANT STUDIES**

### **Type 1 Hypersensitivity**

Bovine or cow's milk protein is a major food allergen that can produce Type 1 (immediate) reactions in sensitized individuals, including up to 8% of children.<sup>45,46</sup> The allergy to bovine milk protein usually occurs in infancy and childhood and is often outgrown by age 5, but approximately 15% to 20% of allergic children remain allergic into adulthood with increased levels of immunoglobulin E (IgE), especially bovine-specific IgE. The IgE-mediated reaction may include cutaneous, respiratory, and gastrointestinal reactions that may on rare occasions result in systemic anaphylaxis.<sup>1,45,46</sup> Non-IgE-mediated reactions may also occur, but these are not as well characterized.<sup>46</sup> While the reactions may be to any of the proteins found in milk, reactions are most commonly linked to α-lactalbumin, β-lactoglobulin, and casein.

## **DERMAL IRRITATION AND SENSITIZATION STUDIES**

### **Irritation and Sensitization**

Dermal irritation and sensitization studies are presented in Table 4.<sup>17,23,38,47</sup> The results of an in vitro assay predicted no potential for irritation to Hydrolyzed Milk Protein (concentration not reported). Hydrolyzed Milk Protein was not irritating to rabbits or humans when tested at up to 25% and 5%, respectively. Hydrolyzed Casein (MW = 600 Da) was not irritating to humans when tested in a 30% solution in water. No irritation or sensitization was observed in a guinea pig maximization study of 5% (v/v) Hydrolyzed Milk Protein in water. Hydrolyzed Casein (MW = 600 Da) was not sensitizing in a human repeated insult patch test (HRIPT) when tested in a 30% solution in water.

### **Phototoxicity**

Phototoxicity studies are presented in Table 5.<sup>38</sup> Hydrolyzed Milk Protein was not a photoirritant or a photosensitizer in human subjects when tested at 5%.

### **OCULAR IRRITATION STUDIES**

In vitro and animal ocular irritation studies are presented in Table 6.<sup>17,23,38,48</sup> No irritation was predicted to Hydrolyzed Milk Protein (concentration not reported) or Hydrolyzed Casein (1.5% active ingredient) in in vitro assays. Hydrolyzed Milk Protein was not irritating to rabbit eyes when tested at up to 25%.

### **CLINICAL STUDIES**

#### ***Hydrolyzed Milk Protein***

A study of sensitization to protein hydrolysates in hair care products was performed in 3 groups of patients.<sup>49</sup> The first group, which comprised 11 hairdressers with hand dermatitis, submitted to scratch and prick tests with 22 trademarked protein hydrolysates, including Hydrolyzed Milk Protein, as well as quaternized hydrolyzed proteins. The second test group comprised 2160 consecutive adults with suspected allergic respiratory disease: they were subjected to skin prick tests with 1 to 3 of the protein hydrolysates (only 1232 patients in this group were tested with Hydrolyzed Milk Protein). The third group of patients comprised 28 adults with atopic dermatitis and was also tested with 1 to 3 protein hydrolysates via a skin prick test.

Out of all 3 groups tested, positive reactions were seen in a total of 12 patients (all female with atopic dermatitis) from exposure to 3 of the 22 protein hydrolysates. All 12 had reactions to hydroxypropyl trimonium hydrolyzed collagen. One of the 12 also had a reaction to hydroxypropyl trimonium hydrolyzed milk protein while 3 others had a reaction to one trademarked version of hydrolyzed collagen. No adverse reactions Hydrolyzed Milk Protein were observed in 1271 patients tested.<sup>49</sup>

### **SUMMARY**

Hydrolyzed Milk Protein is used in 189 formulations; the majority of uses are in leave-on products. Whey Protein has the second greatest number of overall uses reported, with a total of 67; the majority of the uses are in leave-on formulations. Sodium Caseinate has the highest reported maximum concentration of use; it is used at up to 96.9% in bath oils, tablets and salts. The highest reported maximum concentration of use in a leave-on formulation for this ingredient is 0.1% in a face and neck skin care product. Casein has the highest reported maximum concentration of use in a leave-on product and is used at up to 2% in makeup preparations.

Bovine milk, milk proteins, and milk protein derivatives are GRAS, and daily exposures from food use would result in much greater systemic doses than those resulting from use in cosmetic products. The safety assessment focuses on the potential for irritation and sensitization from topical exposure to these milk ingredients.

Hydrolyzed Milk Protein and Hydrolyzed Casein were not mutagenic at concentration up to 5000 µg/plate in Ames assays.

Casein and casein peptides are reported to have antimutagenic properties, and animal models for colon and mammary tumorigenesis have shown that Whey Protein and Hydrolyzed Whey Protein suppressed tumor development. The tumor suppression observed in studies with Whey Protein have been attributed to the high content of cystine/cysteine and  $\gamma$ -glutamylcyst(e)ine dipeptides in the milk proteins, which are efficient substrates for synthesizing glutathione, an important cellular antioxidant.

Bovine milk protein is a major food allergen that can produce Type 1 (immediate) reactions in sensitized individuals, especially children. The IgE-mediated reaction may include cutaneous, respiratory, and gastrointestinal reactions that may on rare occasions result in systemic anaphylaxis. While the reactions may be to any of the proteins found in milk, reactions are most commonly linked to  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, and casein.

No dermal irritation was predicted to Hydrolyzed Milk Protein (concentration not reported) in an in vitro assay. Hydrolyzed Milk Protein was not irritating to rabbits or humans when tested at up to 25% and 5%, respectively. Hydrolyzed Casein (MW = 600 Da) was not irritating to humans when tested in a 30% solution in water.

No dermal sensitization was observed in a guinea pig maximization study of Hydrolyzed Milk Protein at up to 100%. No sensitization was observed in a study of Hydrolyzed Milk Protein in sensitized patients (concentration not reported). Hydrolyzed Casein (MW = 600 Da) was not sensitizing in a human repeated insult patch test when tested in a 30% solution in water.

Hydrolyzed Milk Protein was not a photoirritant or a photosensitizer in human subjects when tested at 5%.

No ocular irritation was predicted to Hydrolyzed Milk Protein (concentration not reported) or Hydrolyzed Casein (1.5% active ingredient) in in vitro assays. Hydrolyzed Milk Protein was not irritating to rabbit eyes when tested at up to 25%.

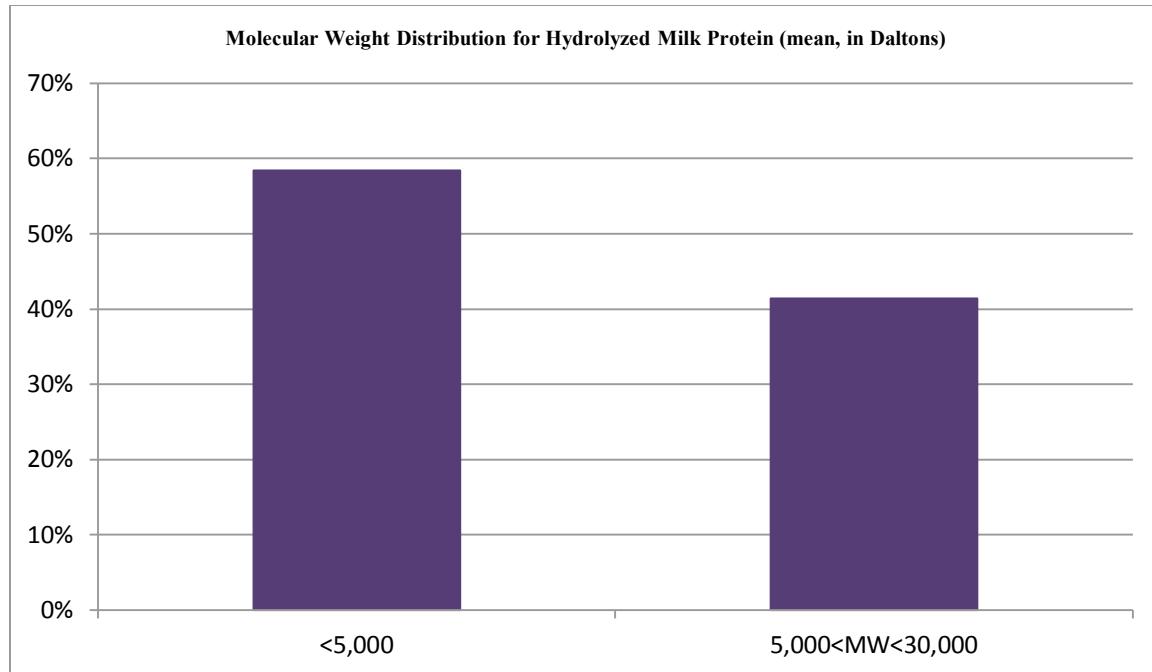
No adverse effects from cosmetic use of milk protein or protein-derived ingredients were discovered in the published literature.

**DISCUSSION**

To be determined...

**CONCLUSION**

To be determined...



**Figure 1.** Molecular weight distribution of hydrolyzed milk protein.<sup>19</sup>

**TABLES****Table 1.** Definitions and functions of the ingredients in this safety assessment.<sup>3</sup>

<b>Ingredient CAS No.</b>	<b>Definition</b>	<b>Function</b>
Casein 9000-71-9	Casein is a mixture of phosphoproteins obtained from cow's milk.	hair conditioning agents; skin-conditioning agents-misc.
Casein Extract	Casein Extract is the extract of Casein.	not reported
Calcium Caseinate 9005-43-0	Calcium Caseinate is the calcium salt of Casein.	binders; bulking agents; hair conditioning agents; skin-conditioning agents-misc.
Ammonium Caseinate 9005-42-9	Ammonium Caseinate is the ammonium salt of Casein.	hair conditioning agents; skin-conditioning agents-misc.
Sodium Caseinate 9005-46-3	Sodium Caseinate is the sodium salt of Casein.	hair conditioning agents; skin-conditioning agents-misc.
Potassium Caseinate 68131-54-4	Potassium Caseinate is the potassium salt of Casein.	hair conditioning agents; skin-conditioning agents-misc.
Hydrolyzed Casein 65072-00-6 73049-73-7	Hydrolyzed Casein is the hydrolysate of Casein derived by acid, enzyme or other method of hydrolysis.	hair conditioning agents; skin-conditioning agents-misc.
Sodium Hydrolyzed Casein	Sodium Hydrolyzed Casein is the sodium salt of Hydrolyzed Casein.	hair conditioning agents; skin-conditioning agents-misc.
Hydrolyzed Lactalbumin 68458-87-7 73049-73-7	Hydrolyzed Lactalbumin is the hydrolysate of milk albumins derived by acid, enzyme, or other method of hydrolysis. [Lactalbumin is a member of the whey protein family]	skin-conditioning agents-misc.
Milk Protein 91053-68-8	Milk Protein is a mixture of proteins obtained from cow's milk.	hair conditioning agents; skin-conditioning agents-misc.
Milk Protein Extract	Milk Protein Extract is the extract of Milk Protein.	not reported
Hydrolyzed Milk Protein 92797-39-2	Hydrolyzed Milk Protein is the hydrolysate of milk protein derived by acid, enzyme or other method of hydrolysis.	hair conditioning agents; skin-conditioning agents-misc.
Whey Protein 84082-51-9	Whey Protein is a polypeptide obtained from the fluid part of Milk after separation from curds.	hair conditioning agents; skin-conditioning agents-misc.
Hydrolyzed Whey Protein	Hydrolyzed Whey Protein is the hydrolysate of Whey Protein derived by acid, enzyme or other method of hydrolysis.	skin-conditioning agents-misc.
Lactoglobulin	Lactoglobulin is a globular protein isolated from milk. [Lactoglobulin is a member of the whey protein family]	hair conditioning agents; skin-conditioning agents-misc.
Hydrolyzed Yogurt Protein	Hydrolyzed Yogurt Protein is the hydrolysate of yogurt protein derived by acid, enzyme or other method of hydrolysis.	hair conditioning agents; skin-conditioning agents-misc.

**Table 2.** Frequency and concentration of use according to duration and type of exposure for milk proteins and protein derivatives.<sup>24,25</sup>

**Table 2.** Frequency and concentration of use according to duration and type of exposure for milk proteins and protein derivatives.<sup>24,25</sup>

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Sodium Caseinate		Whey Protein					
<b>Totals<sup>†</sup></b>	<b>3</b>	<b>0.0005-96.9</b>	<b>67</b>	<b>0.0001-0.5</b>				
<b>Duration of Use</b>								
Leave-On	NR	0.1	62	0.0001-0.5				
Rinse Off	3	2.5	4	0.0075-0.25				
Diluted for (Bath) Use	NR	96.9	1	0.0065				
<b>Exposure Type</b>								
Eye Area	NR	0.001	16	0.05-0.5				
Incidental Ingestion	NR	NR	NR	NR				
Incidental Inhalation-Spray	NR	0.0005; 0.05 <sup>a</sup>	22 <sup>a</sup> ; 13 <sup>b</sup>	0.0001-0.0075; 0.026-0.2 <sup>a</sup>				
Incidental Inhalation-Powder	NR	0.001-0.1 <sup>c</sup>	13 <sup>b</sup>	0.0001-0.5 <sup>c</sup>				
Dermal Contact	3	0.0005-96.9	66	0.0001-0.5				
Deodorant (underarm)	NR	NR	NR	0.0075 <sup>d</sup>				
Hair - Non-Coloring	NR	0.05-2	1	0.0075-0.032				
Hair-Coloring	NR	NR	NR	NR				
Nail	NR	NR	NR	NR				
Mucous Membrane	NR	0.1-96.9	1	0.0065-0.012				
Baby Products	NR	NR	NR	NR				

NR = Not reported. NS = Not surveyed.

<sup>†</sup> Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.<sup>a</sup> It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.<sup>b</sup> Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.<sup>c</sup> It is possible these products may be powders, but it is not specified whether the reported uses are powders.<sup>d</sup> Use reported in an aerosol deodorant.**Table 3.** Ingredients not reported in use.<sup>24,25</sup>

Ammonium Caseinate
Calcium Caseinate
Hydrolyzed Lactalbumin
Potassium Caseinate
Sodium Caseinate
Sodium Hydrolyzed Casein

**Table 4.** Dermal irritation and sensitization studies for Hydrolyzed Milk Protein.

Ingredient	Concentration	Method	Results	Reference
<b><i>In Vitro</i></b>				
Hydrolyzed Milk Protein	In solution (MW = 2000-4000), concentration not reported	MatTek EpiDerm assay	Non-irritating	<sup>23</sup>
<b><i>Animal - Irritation</i></b>				
Hydrolyzed Milk Protein	10% (v/v) aqueous dilution, pH 6.7	Dermal irritation study performed under OECD* Guideline 404 in 6 White New Zealand rabbits; semi-occluded for 24 h	Non-irritating	<sup>38</sup>
Hydrolyzed Milk Protein	25% w/v in water (MW = 1500)	Primary skin irritation study in 6 female New Zealand White rabbits, occluded for 24 h	Primary irritation index = 1.3. Not a primary irritant.	<sup>47</sup>
<b><i>Animal - Sensitization</i></b>				
Hydrolyzed Milk Protein	5% v/v in water	Guinea pig maximization study using male and female Pirbright white guinea pigs (number not reported); induced intracutaneously with 5% of the test material in adjuvant and water and epicutaneously with 100% of the test material; challenged with 100% of the test material	No irritation or sensitization	<sup>38</sup>
<b><i>Human - Irritation</i></b>				
Hydrolyzed Casein (MW = 600 Da)	30% solution in water	24-hpatch test in 20 female subjects using Finn Chambers (occluded)	No irritation	<sup>17</sup>
Hydrolyzed Milk Protein	5% aq. dilution	Phototoxicity study in 10 volunteers with single topical application	No skin irritation	<sup>38</sup>
<b><i>Human - Sensitization</i></b>				
Hydrolyzed Casein (MW = 600 Da)	30% solution in water	HRIPT with 0.2 mL of the test material applied using an occlusive patch on the infrascapular region of 50 subjects	No sensitization	<sup>17</sup>

\* OECD = Organisation for Economic Co-operation and Development

**Table 5.** Phototoxicity/Photosensitization studies in humans for Hydrolyzed Milk Protein.

<b>Ingredient</b>	<b>Concentration</b>	<b>Method</b>	<b>Results</b>	<b>Reference</b>
Hydrolyzed Milk Protein (molecular weight distribution as shown in Figure 1)	5% aq. dilution, v/v	Photoirritation study in 10 subjects; occluded. After 24 h exposure, 1 treated site irradiated with UVA (320-400 nm) for 15 min, other site was control.	Not a photoirritant	<sup>38</sup>
Hydrolyzed Milk Protein (molecular weight distribution as shown in Figure 1)	5% dilution in water, v/v	Photosensitization study in 29 subjects; 3 weeks of 6 induction patches in duplicate. After 24 h exposure, 1 treated site irradiated with UV (260-400 nm) for 15 min, other site was control. After 2 week rest, challenge on virgin irradiated and non-irradiated sites.	Not a photosensitizer	<sup>38</sup>

**Table 6.** Ocular irritation studies for Hydrolyzed Milk Protein.

Ingredient	Concentration	Method	Results	Reference
<i>In Vitro</i>				
Hydrolyzed Casein	1.5% active ingredient (MW = 600 Da)	HET-CAM assay	Non-irritating	<sup>17</sup>
<i>Animal</i>				
Hydrolyzed Milk Protein	10% aq. dilution at pH 6.7	Ocular irritation study performed under OECD guideline 405 using 6 albino White New Zealand rabbits	Not irritating	<sup>38</sup>
Hydrolyzed Milk Protein	25% in distilled water (MW = 1500)	Ocular irritation study in 6 female New Zealand White rabbits; unrinsed eyes	Not irritating	<sup>48</sup>

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**2017 FDA VCRP Raw Data**

03E - Eye Makeup Remover	CASEIN HYDROLYSATE	1
03G - Other Eye Makeup Preparations	CASEIN HYDROLYSATE	1
05A - Hair Conditioner	CASEIN HYDROLYSATE	1
12D - Body and Hand (exc shave)	CASEIN HYDROLYSATE	1
12F - Moisturizing	CASEIN HYDROLYSATE	5
12G - Night	CASEIN HYDROLYSATE	1
12J - Other Skin Care Preps	CASEIN HYDROLYSATE	1
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02A - Bath Oils, Tablets, and Salts	HYDROLYZED MILK PROTEIN	1
02B - Bubble Baths	HYDROLYZED MILK PROTEIN	2
02D - Other Bath Preparations	HYDROLYZED MILK PROTEIN	3
03D - Eye Lotion	HYDROLYZED MILK PROTEIN	1
03E - Eye Makeup Remover	HYDROLYZED MILK PROTEIN	1
03G - Other Eye Makeup Preparations	HYDROLYZED MILK PROTEIN	3
05A - Hair Conditioner	HYDROLYZED MILK PROTEIN	7
05F - Shampoos (non-coloring)	HYDROLYZED MILK PROTEIN	8
05G - Tonics, Dressings, and Other Hair Grooming Aids	HYDROLYZED MILK PROTEIN	4
05I - Other Hair Preparations	HYDROLYZED MILK PROTEIN	2
07E - Lipstick	HYDROLYZED MILK PROTEIN	3
10A - Bath Soaps and Detergents	HYDROLYZED MILK PROTEIN	14
10B - Deodorants (underarm)	HYDROLYZED MILK PROTEIN	2
10E - Other Personal Cleanliness Products	HYDROLYZED MILK PROTEIN	12
12A - Cleansing	HYDROLYZED MILK PROTEIN	5
12C - Face and Neck (exc shave)	HYDROLYZED MILK PROTEIN	6
12D - Body and Hand (exc shave)	HYDROLYZED MILK PROTEIN	21
12F - Moisturizing	HYDROLYZED MILK PROTEIN	87
12G - Night	HYDROLYZED MILK PROTEIN	3
12H - Paste Masks (mud packs)	HYDROLYZED MILK PROTEIN	2
12J - Other Skin Care Preps	HYDROLYZED MILK PROTEIN	2
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10A - Bath Soaps and Detergents	HYDROLYZED YOGURT PROTEIN	4
12F - Moisturizing	HYDROLYZED YOGURT PROTEIN	1
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12C - Face and Neck (exc shave)	LACTOGLOBULIN	1
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02D - Other Bath Preparations	MILK PROTEIN	1
03B - Eyeliner	MILK PROTEIN	1
03D - Eye Lotion	MILK PROTEIN	1
03G - Other Eye Makeup Preparations	MILK PROTEIN	3
05A - Hair Conditioner	MILK PROTEIN	1
05I - Other Hair Preparations	MILK PROTEIN	3
10A - Bath Soaps and Detergents	MILK PROTEIN	3
10E - Other Personal Cleanliness Products	MILK PROTEIN	7
12A - Cleansing	MILK PROTEIN	4
12C - Face and Neck (exc shave)	MILK PROTEIN	4

12F - Moisturizing	MILK PROTEIN	3
12H - Paste Masks (mud packs)	MILK PROTEIN	2
12I - Skin Fresheners	MILK PROTEIN	1
12J - Other Skin Care Preps	MILK PROTEIN	1
12D - Body and Hand (exc shave)	MILK PROTEIN EXTRACT	2
12F - Moisturizing	MILK PROTEIN EXTRACT	1
12J - Other Skin Care Preps	MILK PROTEIN EXTRACT	1
12A - Cleansing	SODIUM CASEINATE	2
12H - Paste Masks (mud packs)	SODIUM CASEINATE	1
02A - Bath Oils, Tablets, and Salts	WHEY PROTEIN	1
03D - Eye Lotion	WHEY PROTEIN	8
03G - Other Eye Makeup Preparations	WHEY PROTEIN	8
05A - Hair Conditioner	WHEY PROTEIN	1
11F - Shaving Soap	WHEY PROTEIN	1
12A - Cleansing	WHEY PROTEIN	1
12C - Face and Neck (exc shave)	WHEY PROTEIN	11
12D - Body and Hand (exc shave)	WHEY PROTEIN	2
12F - Moisturizing	WHEY PROTEIN	14
12G - Night	WHEY PROTEIN	6
12H - Paste Masks (mud packs)	WHEY PROTEIN	1
12J - Other Skin Care Preps	WHEY PROTEIN	11
13C - Other Suntan Preparations	WHEY PROTEIN	2



## **Memorandum**

**TO:** Lillian Gill, D.P.A.  
Director - COSMETIC INGREDIENT REVIEW (CIR)

**FROM:** Beth A. Lange, Ph.D.  
Industry Liaison to the CIR Expert Panel

**DATE:** February 12, 2016

**SUBJECT:** Concentration of Use by FDA Product Category: Milk Proteins

**Concentration of Use by FDA Product Category – Milk Proteins\***

Hydrolyzed Yogurt Protein	Sodium Caseinate	Milk Protein
Casein	Potassium Caseinate	Milk Protein Extract
Casein Extract	Sodium Hydrolyzed Casein	Hydrolyzed Milk Protein
Calcium Caseinate	Hydrolyzed Casein	Whey Protein
Ammonium Caseinate	Hydrolyzed Lactalbumin	Hydrolyzed Whey Protein

Ingredient	Product Category	Maximum Concentration of Use
Hydrolyzed Yogurt Protein	Face and neck products Not spray	0.1%
Hydrolyzed Yogurt Protein	Body and hand products Not spray, not powder	0.02%
Casein	Hair conditioners	0.0075%
Casein	Shampoos (noncoloring)	0.0075%
Casein	Other makeup preparations	2%
Casein	Bath soaps and detergents	0.015%
Casein	Deodorants Not spray Aerosol	0.0076% 0.013%
Sodium Caseinate	Bath oils, tablets and salts	0.1-96.9%
Sodium Caseinate	Eye lotions	0.001%
Sodium Caseinate	Permanent waves	2%
Sodium Caseinate	Shampoos (noncoloring)	0.3%
Sodium Caseinate	Tonics, dressings and other hair grooming aids	0.05%
Sodium Caseinate	Bath soaps and detergents	2.5%
Sodium Caseinate	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.1%
Sodium Caseinate	Face and neck products Not spray	0.001-0.1%
Sodium Caseinate	Body and hand products Not spray Spray	0.01% 0.0005%
Sodium Caseinate	Paste masks and mud packs	0.5%
Hydrolyzed Casein	Mascara	0.003%
Hydrolyzed Casein	Hair sprays Pump spray	0.00072%
Hydrolyzed Casein	Rinses (noncoloring)	0.01%
Hydrolyzed Casein	Shampoos (noncoloring)	0.011%
Hydrolyzed Casein	Tonics, dressings and other hair grooming aids	0.01%
Hydrolyzed Casein	Other hair preparations (noncoloring)	0.01%
Hydrolyzed Casein	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.003%
Hydrolyzed Casein	Face and neck products	

	Not spray	0.015-0.75%
Hydrolyzed Casein	Moisturizing products Not spray	0.015%
Milk Protein	Eye lotions	0.5%
Milk Protein	Mascara	0.01%
Milk Protein	Hair conditioners	0.1%
Milk Protein	Rinses (noncoloring)	0.1%
Milk Protein	Shampoos (noncoloring)	0.05%
Milk Protein	Tonics, dressings and other hair grooming aids	0.01%
Milk Protein	Face powders	0.0002%
Milk Protein	Lipstick	0.01%
Milk Protein	Shaving soap	0.1%
Milk Protein	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.01%
Milk Protein	Face and neck products Not spray	0.01%
Milk Protein	Body and hand products Not spray	0.06%
Milk Protein	Paste masks and mud packs	0.0002%
Milk Protein	Other skin care preparations	0.88%
Milk Protein	Suntan products Not spray	0.05%
Milk Protein Extract	Body and hand products Not spray	0.0015%
Hydrolyzed Milk Protein	Bubble baths	0.01-0.05%
Hydrolyzed Milk Protein	Other bath preparations	0.05%
Hydrolyzed Milk Protein	Eye lotions	0.0075%
Hydrolyzed Milk Protein	Other eye makeup preparations	0.02%
Hydrolyzed Milk Protein	Colognes and toilet waters	0.01%
Hydrolyzed Milk Protein	Hair conditioners	0.001-0.01%
Hydrolyzed Milk Protein	Shampoos (noncoloring)	0.001-0.01%
Hydrolyzed Milk Protein	Tonics, dressings and other hair grooming aids Not spray	0.00001-0.011% 0.001%
Hydrolyzed Milk Protein	Lipstick	0.05%
Hydrolyzed Milk Protein	Bath soaps and detergents	0.00024-0.2%
Hydrolyzed Milk Protein	Deodorant Not spray	0.02%
Hydrolyzed Milk Protein	Other personal cleanliness products	0.02%
Hydrolyzed Milk Protein	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0018-0.2%
Hydrolyzed Milk Protein	Face and neck products Not spray Spray	0.024-0.05 0.0075%
Hydrolyzed Milk Protein	Body and hand products	

	Not spray	0.021-0.2%
Hydrolyzed Milk Protein	Moisturizing products Not spray	0.05%
Hydrolyzed Milk Protein	Night products Not spray	0.02-0.024%
Hydrolyzed Milk Protein	Paste masks and mud packs	0.1%
Hydrolyzed Milk Protein	Skin fresheners	0.02%
Hydrolyzed Milk Protein	Other skin care preparations	0.02%
Whey Protein	Bubble baths	0.0065%
Whey Protein	Eye lotions	0.05-0.5%
Whey Protein	Hair conditioners	0.0075%
Whey Protein	Tonics, dressings and other hair grooming aids	0.032%
Whey Protein	Foundations	0.2%
Whey Protein	Bath soaps and detergents	0.012%
Whey Protein	Deodorants Aerosol	0.0075%
Whey Protein	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.032-0.25%
Whey Protein	Face and neck products Not spray	0.0001-89.1%
Whey Protein	Body and hand products Not spray Spray	0.0013-0.1% 0.0001%
Whey Protein	Moisturizing products Not spray	0.0013%
Whey Protein	Skin fresheners	0.026%
Whey Protein	Other skin care preparations	0.5%
Whey Protein	Suntan products Not spray	0.0099-0.032%
Whey Protein	Indoor tanning preparations	0.2%
Hydrolyzed Whey Protein	Hair conditioners	0.5%
Hydrolyzed Whey Protein	Shampoos (noncoloring)	0.5%
Hydrolyzed Whey Protein	Face and neck products Not spray	0.5%
Hydrolyzed Whey Protein	Body and hand products Not spray	0.5%

\*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.

Information collected in 2015-2016  
Table prepared February 11, 2016



## **Memorandum**

**TO:** Lillian Gill, D.P.A.  
Director - COSMETIC INGREDIENT REVIEW (CIR)

**FROM:** Beth A. Jonas, Ph.D.  
Industry Liaison to the CIR Expert Panel

**DATE:** February 16, 2017

**SUBJECT:** Updated Concentration of Use by FDA Product Category: Milk Proteins

**Concentration of Use by FDA Product Category – Milk Proteins\***

Hydrolyzed Yogurt Protein	Sodium Caseinate	Milk Protein
Casein	Potassium Caseinate	Milk Protein Extract
Casein Extract	Sodium Hydrolyzed Casein	Hydrolyzed Milk Protein
Calcium Caseinate	Hydrolyzed Casein	Whey Protein
Ammonium Caseinate	Hydrolyzed Lactalbumin	Hydrolyzed Whey Protein

Ingredient	Product Category	Maximum Concentration of Use
Hydrolyzed Yogurt Protein	Face and neck products Not spray	0.1%
Hydrolyzed Yogurt Protein	Body and hand products Not spray, not powder	0.02%
Casein	Hair conditioners	0.0075%
Casein	Shampoos (noncoloring)	0.0075%
Casein	Other makeup preparations	2%
Casein	Bath soaps and detergents	0.015%
Casein	Deodorants Not spray Aerosol	0.0076% 0.013%
Sodium Caseinate	Bath oils, tablets and salts	0.1-96.9%
Sodium Caseinate	Eye lotions	0.001%
Sodium Caseinate	Permanent waves	2%
Sodium Caseinate	Shampoos (noncoloring)	0.3%
Sodium Caseinate	Tonics, dressings and other hair grooming aids	0.05%
Sodium Caseinate	Bath soaps and detergents	2.5%
Sodium Caseinate	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.1%
Sodium Caseinate	Face and neck products Not spray	0.001-0.1%
Sodium Caseinate	Body and hand products Not spray Spray	0.01% 0.0005%
Sodium Caseinate	Paste masks and mud packs	0.5%
Hydrolyzed Casein	Mascara	0.003%
Hydrolyzed Casein	Hair sprays Pump spray	0.00072%
Hydrolyzed Casein	Rinses (noncoloring)	0.01%
Hydrolyzed Casein	Shampoos (noncoloring)	0.011%
Hydrolyzed Casein	Tonics, dressings and other hair grooming aids	0.01%
Hydrolyzed Casein	Other hair preparations (noncoloring)	0.01%
Hydrolyzed Casein	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.003%
Hydrolyzed Casein	Face and neck products	

	Not spray	0.015-0.75%
Hydrolyzed Casein	Moisturizing products Not spray	0.015%
Milk Protein	Eye lotions	0.5%
Milk Protein	Mascara	0.01%
Milk Protein	Hair conditioners	0.1%
Milk Protein	Rinses (noncoloring)	0.1%
Milk Protein	Shampoos (noncoloring)	0.05%
Milk Protein	Tonics, dressings and other hair grooming aids	0.01%
Milk Protein	Face powders	0.0002%
Milk Protein	Lipstick	0.01%
Milk Protein	Shaving soap	0.1%
Milk Protein	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.01%
Milk Protein	Face and neck products Not spray	0.01%
Milk Protein	Body and hand products Not spray	0.06%
Milk Protein	Paste masks and mud packs	0.0002%
Milk Protein	Other skin care preparations	0.88%
Milk Protein	Suntan products Not spray	0.05%
Milk Protein Extract	Body and hand products Not spray	0.0015%
Hydrolyzed Milk Protein	Bubble baths	0.01-0.05%
Hydrolyzed Milk Protein	Other bath preparations	0.05%
Hydrolyzed Milk Protein	Eye lotions	0.0075%
Hydrolyzed Milk Protein	Other eye makeup preparations	0.02%
Hydrolyzed Milk Protein	Colognes and toilet waters	0.01%
Hydrolyzed Milk Protein	Hair conditioners	0.001-0.01%
Hydrolyzed Milk Protein	Shampoos (noncoloring)	0.001-0.01%
Hydrolyzed Milk Protein	Tonics, dressings and other hair grooming aids Not spray	0.00001-0.011% 0.001%
Hydrolyzed Milk Protein	Lipstick	0.05%
Hydrolyzed Milk Protein	Bath soaps and detergents	0.00024-0.2%
Hydrolyzed Milk Protein	Deodorant Not spray	0.02%
Hydrolyzed Milk Protein	Other personal cleanliness products	0.02%
Hydrolyzed Milk Protein	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0018-0.2%
Hydrolyzed Milk Protein	Face and neck products Not spray Spray	0.024-0.05 0.0075%
Hydrolyzed Milk Protein	Body and hand products	

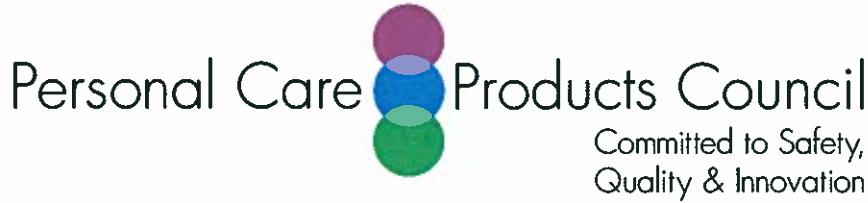
	Not spray	0.021-0.2%
Hydrolyzed Milk Protein	Moisturizing products Not spray	0.05%
Hydrolyzed Milk Protein	Night products Not spray	0.02-0.024%
Hydrolyzed Milk Protein	Paste masks and mud packs	0.1%
Hydrolyzed Milk Protein	Skin fresheners	0.02%
Hydrolyzed Milk Protein	Other skin care preparations	0.02%
Whey Protein	Bubble baths	0.0065%
Whey Protein	Eye lotions	0.05-0.5%
Whey Protein	Hair conditioners	0.0075%
Whey Protein	Tonics, dressings and other hair grooming aids	0.032%
Whey Protein	Foundations	0.2%
Whey Protein	Bath soaps and detergents	0.012%
Whey Protein	Deodorants Aerosol	0.0075%
Whey Protein	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.032-0.25%
Whey Protein	Face and neck products Not spray	0.0001-0.5%
Whey Protein	Body and hand products Not spray Spray	0.0013-0.1% 0.0001%
Whey Protein	Moisturizing products Not spray	0.0013%
Whey Protein	Skin fresheners	0.026%
Whey Protein	Other skin care preparations	0.5%
Whey Protein	Suntan products Not spray	0.0099-0.032%
Whey Protein	Indoor tanning preparations	0.2%
Hydrolyzed Whey Protein	Hair conditioners	0.5%
Hydrolyzed Whey Protein	Shampoos (noncoloring)	0.5%
Hydrolyzed Whey Protein	Face and neck products Not spray	0.5%
Hydrolyzed Whey Protein	Body and hand products Not spray	0.5%

\*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.

Information collected in 2015-2016

Table prepared February 11, 2016

Updated February 16, 2017: Whey Protein 89.1% in face products replaced with a high concentration of 0.5%.



## 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 3, 2012

**SUBJECT:** Information on Hydrolyzed Keratin, Hydrolyzed Silk Protein, Hydrolyzed Elastin, Hydrolyzed Soy Protein, Hydrolyzed Milk Protein and Hydrolyzed Vegetable Protein

### Hydrolyzed Keratin

Consumer Product Testing Co. 2004. Repeated insult patch test: Hydrolyzed Keratin (MW ~ 3,000 Da). Experiment Reference Number: C04-0181.01.

Consumer Product Testing Co. 2004. The Hen's egg test - utilizing the chorioallantoic membrane (HET-CAM): Hydrolyzed Keratin (MW ~ 3,000 Da). Experiment Reference Number: V04-0033-1.

Consumer Product Testing Co. 2004. The MatTek Corporation EpiDerm™ skin model *in vitro* toxicity testing system: Hydrolyzed Keratin (MW ~ 3,000 Da). Experiment Reference Number: V04-0032-1.

Consumer Product Testing Co. 1984. Primary dermal irritation in rabbits; primary ocular irritation in rabbits; acute oral toxicity in rabbits: Hydrolyzed Keratin (MW ~ 500 Da). Experiment Reference No. 84229-1.

Toxicol Laboratories Limited. 1986. Primary skin irritation study: Hydrolyzed Keratin (MW ~ 125,000 Da). Study Ref. No. 63/8601.

Toxicol Laboratories Limited. 1986. Eye irritation study: Hydrolyzed Keratin (MW ~ 125,000 Da). Study Ref. No. 64/8601.

Toxicol Laboratories Limited. 1986. Single dose oral toxicity study in the rat: Hydrolyzed Keratin (MW ~ 125,000 Da). Study Ref. No. 62/8601.

Consumer Product Testing. 1981. Primary dermal irritation in rabbits; primary ocular irritation in rabbits; acute oral toxicity in rats: Hydrolyzed Keratin (MW ~ 600 Da). Experiment Reference No.: 81103-S.

Hydrolyzed Silk

Toxicol Laboratories Limited. 1985. Single dose oral toxicity study in the rat: Hydrolyzed Silk (MW ~ 300 Da). Study Ref. No. 107/8412.

Toxicol Laboratories Limited. 1985. Eye irritation study: Hydrolyzed Silk (MW ~ 300 Da). Study Ref. No. 108/8412.

Toxicol Laboratories Limited. 1985. Primary skin irritation study: Hydrolyzed Silk (MW ~ 300 Da). Study Ref. No. 109/8412.

Consumer Product Testing Co. 1997. Repeated insult patch test: Hydrolyzed Silk (MW ~ 1,000 Da). Experiment Reference Number: C97-0170.

Essex Testing Clinic, Inc. 1985. Repeated insult patch test: Hydrolyzed Silk (MW ~ 1,000 Da). ETC Entry No: 0194a.

Consumer Product Testing. 1985. Primary dermal irritation in rabbits; primary ocular irritation in rabbits; acute oral toxicity in rats: Hydrolyzed Silk (MW ~ 1,000 Da). Experiment Reference No.: 85084-3.

Hydrolyzed Elastin

Consumer Product Testing. 1980. Primary dermal irritation in rabbits; primary ocular irritation in rabbits; acute oral toxicity in rats: Hydrolyzed Elastin (MW ~ 3,000 Da). Experiment Reference No.: 80229-5.

CPTC Inc. 1982. Repeated insult patch test: Hydrolyzed Elastin (MW ~ 3,000 Da). Experiment Reference No.: C-1-82.

Hydrolyzed Soy Protein

Consumer Product Testing. 1984. Primary dermal irritation in rabbits; primary ocular irritation in rabbits; acute oral toxicity in rats: Hydrolyzed Soy Protein (MW ~ 2,000 Da). Experiment Reference No.: 84287-1.

Hydrolyzed Milk Protein

Toxicol Laboratories Limited. 1985. Single dose oral toxicity study in the rat: Hydrolyzed Milk Protein (MW ~ 1,500 Da). Study Ref. No. 110/8412.

Toxicol Laboratories Limited. 1985. Eye irritation study: Hydrolyzed Milk Protein (MW ~ 1,500 Da). Study Ref. No. 111/8412.

Toxicol Laboratories Limited. 1985. Primary skin irritation study: Hydrolyzed Milk Protein (MW ~ 1,500 Da). Study Ref. No. 112/8412.

Hydrolyzed Vegetable Protein

Consumer Product Testing Co. 2005. The hen's egg test - utilizing the chorioallantoic membrane (HET-CAM): Hydrolyzed Vegetable Protein (MW ~ 750 Da). Experiment Reference No.: V05-0133-6.

Consumer Product Testing Co. 2005. The MatTek Corporation EpiDerm™ skin model *in vitro* toxicity testing system: Hydrolyzed Vegetable Protein (MW ~ 750 Da). Experiment Reference No.: V05-0133-3.



EST. 1975

# Consumer Product Testing Co.

## FINAL REPORT

**CLIENT:****ATTENTION:**

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

**TEST MATERIAL:** Hydrolyzed Keratin  
MW N 3,000 Da

**EXPERIMENT**  
**REFERENCE NUMBER:** enzyme hydrolysis  
C04-0181.01

Richard Eisenberg  
Richard R. Eisenberg, M.D.  
Board Certified Dermatologist

Michael Traudt  
Michael Traudt, Ph.D.  
Director, Clinical Evaluations

R.W. Shanahan  
Robert W. Shanahan, Ph.D.  
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Joy Frank, R.N.  
Executive Vice President, Clinical Evaluations

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# Consumer Product Testing Co.

EST. 1975

## QUALITY ASSURANCE UNIT STATEMENT

Study No.: C04-0181.01

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 70 New Dutch Lane, Fairfield, New Jersey, 07004, unless specified otherwise, in writing by the Sponsor.

Date(s) of inspection: May 18, 2004  
May 28, 2004

Senior personnel involved:

A handwritten signature in black ink.

Richard Hettenbach, M.A.  
Senior Director of Regulatory Affairs & Quality Assurance

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.

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**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:** Fifty-eight (58) qualified subjects, male and female, ranging in age from 18 to 79 years, were selected for this evaluation. Fifty-one (51) 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<sup>a</sup> 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:**

<b>Study Schedule:</b>	<u>Panel #</u>	<u>Initiation Date</u>	<u>Proposed Completion Date</u>	<u>Actual Completion Date</u>
	20040177	March 31, 2004	May 6, 2004	May 13, 2004

<sup>a</sup>With parental or guardian consent

C04-0181.01

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**Methodology:**

The upper back between the scapulae served as the treatment area. Approximately 0.2 ml 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

C04-0181.01

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**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 negative throughout the test interval.

**Summary:**

Under the conditions of this study, test material, did not indicate a potential for dermal irritation or allergic contact sensitization.

C04-0181.01

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Table 1  
Panel #20040177

Individual Results

Subject Number	24*hr	Induction Phase									Virgin Challenge Site			
		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	DID NOT COMPLETE STUDY													
5	DID NOT COMPLETE STUDY													
6	0	0	0	0	0	0	0	0	0	0	0	0		
7	DID NOT COMPLETE STUDY													
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	DID NOT COMPLETE STUDY													
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	DID NOT COMPLETE STUDY								
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	DID NOT COMPLETE STUDY						
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 <sup>m</sup>	0	0	0		

24\* = Supervised removal of 1<sup>st</sup> Induction and Challenge Patch

m = Additional makeup day granted at the discretion of the clinic supervisor

C04-0181.01  
Page 7Table 1  
(continued)  
Panel #20040177Individual Results

Subject Number	24*hr	Induction Phase									Virgin Challenge Site	
		1	2	3	4	5	6	7	8	9	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	DID NOT COMPLETE STUDY											
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
58	0	0	0	0	0	0	0	0	0	0	0	0

24\* = Supervised removal of 1<sup>st</sup> Induction and Challenge Patch

C04-0181.01

Page 8

Table 2  
Panel #20040177

Subject Data

Subject Number	Initials	Age	Sex
1	JP	36	M
2	MO	68	F
3	BS	57	F
4	MB	30	F
5	EB	53	F
6	AC	21	F
7	MC	46	F
8	AD	35	F
9	VF	35	M
10	PW	45	F
11	DR	38	F
12	AR	20	M
13	JV	30	F
14	MS	18	M
15	LS	41	F
16	HM	79	M
17	AC	73	M
18	MM	62	F
19	AB	20	M
20	JT	31	F
21	TB	54	M
22	JB	54	F
23	AT	20	M
24	CO	60	F
25	MG	36	F
26	CB	61	F
27	MB	43	F
28	JD	59	M
29	SF	26	F

C04-0181.01  
Page 9Table 2  
(continued)  
Panel #20040177Subject Data

Subject Number	Initials	Age	Sex
30	LH	70	F
31	JC	24	M
32	WF	58	F
33	LF	63	M
34	JN	61	F
35	VM	22	M
36	CA	58	M
37	CB	70	F
38	NO	40	M
39	KR	48	F
40	RB	68	F
41	JW	51	F
42	MH	65	F
43	VY	36	F
44	RO	40	F
45	JS	69	F
46	TD	43	F
47	MM	68	F
48	CG	74	F
49	EG	74	M
50	WT	65	F
51	PT	67	M
52	JL	45	M
53	AG	64	F
54	EH	69	M
55	VF	34	F
56	CS	41	F
57	TD	34	F
58	KK	42	F



## FINAL REPORT

**CLIENT:**

**ATTENTION:**

**TEST:**

The Hen's Egg Test - Utilizing the Chorioallantoic Membrane (HET-CAM)

Hydrolyzed Keratin

MW ~ 3,000 Da

enzymic hydrolysis

**TEST ARTICLE:**

**EXPERIMENT**

**REFERENCE NO.:**

V04-0033-1

Scott Krupa 3-25-04

Scott Krupa  
Quality Assurance Supervisor

3/24/04  
Steven Nitka  
Vice President  
Laboratory Director

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70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234



# Consumer Product Testing Co.

EST. 1975

## QUALITY ASSURANCE UNIT STATEMENT

Study No.: V04-0033-1

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practice principles (including government regulations to the extent applicable) and in accordance with standard operating procedures and applicable standard 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 may have been reported to management and the Study Director.

Date of data inspection: March 22, 2004

**Professional personnel involved:**

Steven Nitka, B.S.	-	Vice President Laboratory Director (Study Director)
Lillian Deniza, B.S.	-	Laboratory Supervisor
Melissa Pandorf, B.S.	-	Technician
Scott Krupa	-	Quality Assurance Supervisor

The representative signature of the Quality Assurance Unit on the front page signifies that this study has been performed in accordance with standard operating procedures, study protocols and the Good Laboratory Practice principles.

V04-0033-1  
Page 3 of 6

**Objective:**

To evaluate the test article for irritancy potential utilizing the HET-CAM test. The test is a modification of that described by Kemper and Luepke.<sup>1</sup>

**Introduction:**

The chick embryo has been used extensively in toxicology. "The chorioallantoic membrane (CAM) of the chick embryo is a complete tissue with organoid elements from all germ cell layers. The chorionic epithelium is ectodermal and the allantoic epithelium is endodermal. The mesoderm located between these epithelia is a complete connective tissue including arteries, capillaries, veins and lymphatic vessels. The CAM responds to injury with a complete inflammatory reaction, comparable to that induced in the rabbit eye test. It is technically easy to study, and is without nerves to sense pain."<sup>2</sup>

**Test Article:**

**Reference Article:** Distilled Water

<sup>1</sup>Kemper, F.H. & Luepke, N.P., (1986). The HET-CAM Test: An Alternative to the Draize Test. *FD Chem. Toxic.* 24, p. 495 - 496.

<sup>2</sup>Leighton, J., Tchao, R., Verdone, J. & Nassauer, J. Macroscopic Assay of Focal Injury in the Chorioallantoic Membrane. In: *Alternative Methods in Toxicology*, Vol. 3, *In Vitro Toxicology* E2, pp. 357 - 369, Alan M. Goldberg, (ed.), Mary Ann Liebert Publishers, Inc., New York, 1985.

V04-0033-1  
Page 4 of 6**Method:**

Fresh, fertile, White Leghorn eggs were obtained from Avian Services in Frenchtown, New Jersey. They were stored at this facility for up to seven (7) days, at 13° C, before being incubated. For incubation the eggs were placed, on their sides, in a Kuhl incubator. The incubator is such that the eggs are automatically rotated once every hour. The temperature was controlled at 99° F ( $\pm 1^{\circ}$ ) with a relative humidity of 60 - 70% for the ten (10) days of incubation. On day eight (8) the eggs were turned so that the acutely angled end faced down.

On day ten (10) each egg was removed from the incubator and placed in a Plexiglas work enclosure. This enclosure had been preheated and humidified so that its environment approached that of the incubator. A cut was made in the larger end of each egg, where the air sack is located. A Dremel® Moto-Flex Tool (model 232-5) equipped with a Dremel® Cut-Off Wheel (No. 409) was used to make each cut. Forceps were then used to remove the shell down to the shell-membrane junction. The inner egg membrane was then hydrated with a warm, physiological saline solution. The saline was removed after a two (2) to five (5) minute exposure. Utilizing pointed forceps, the inner egg membrane was then carefully removed to reveal the CAM.

The test or reference article, at a dosage of three-tenths of one milliliter (0.3 ml) of a liquid or three-tenths of one gram (0.3 g) of a solid was then administered to each of four (4) CAM's. Twenty seconds later, the test or control article was rinsed from each CAM with five (5) milliliters of physiological saline. All CAM's were observed immediately prior to test article administration and at 30 seconds, two (2) and five (5) minutes after exposure to the test article. The reactions of the CAM, the blood vessels, including the capillaries, and the albumin were examined and scored for irritant effects as detailed below:

Effect	Time (min.)	Score		
		0.5	2	5
Hyperemia		5	3	1
Minimal Hemorrhage ("Feathering")		7	5	3
Hemorrhage (Obvious Leakage)		9	7	5
<u>Coagulation and/or Thrombosis</u>		11	9	7

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

V04-0033-1  
Page 5 of 6**Results:**

<b>Reference Article (%)</b>	<b>CAM #</b>	<b>Scores @</b>				<b>Total</b>
		<b>0.5 min.</b>	<b>2 min.</b>	<b>5 min.</b>		
Distilled Water (100%)	1	0	0	0		0
	2	0	0	1		1
	3	0	3	0		3
	4	0	3	0		3
	<b>Average:</b>				<b>1.75</b>	

<b>Test Article (%)</b>	<b>CAM #</b>	<b>Scores @</b>				<b>Total</b>
		<b>0.5 min.</b>	<b>2 min.</b>	<b>5 min.</b>		
	1	0	0	1		1
	2	0	0	1		1
Date 10/31/03 (1%)	3	0	0	1		1
	4	0	0	1		1
	<b>Average:</b>				<b>1.00</b>	

<b>Test Article (%)</b>	<b>CAM #</b>	<b>Scores @</b>				<b>Total</b>
		<b>0.5 min.</b>	<b>2 min.</b>	<b>5 min.</b>		
	1	5	0	0		5
	2	5	0	0		5
Date 10/31/03 (5%)	3	0	3	0		3
	4	0	0	1		1
	<b>Average:</b>				<b>3.50</b>	

<b>Test Article (%)</b>	<b>CAM #</b>	<b>Scores @</b>				<b>Total</b>
		<b>0.5 min.</b>	<b>2 min.</b>	<b>5 min.</b>		
	1	0	3	5	0	8
	2	0	0	1		1
Date 10/31/03 (10%)	3	5	5	0		10
	4	0	3	0		3
	<b>Average:</b>				<b>5.50</b>	

V04-0033-1  
Page 6 of 6

Each article was then classified as indicated in the following:

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

**Discussion:**

Previous studies have shown that the CAM of the hen's egg is more sensitive to liquid irritants than is the rabbit eye. Therefore, the CAM results for the test article at a specific concentration equate to Draize results for the test article at two times that concentration. For example, Johnson's Baby Shampoo, when dosed at 50% in the CAM assay, elicits an average score of approximately 11. This result would correlate to the Draize score of approximately 10 to 15 that would be elicited by Johnson's Baby Shampoo, when dosed at 100%.

**Conclusion:**

Under the conditions of this test, the results indicate that the sponsor-submitted product,

Date 10/31/03, at 2% and at 10% would have practically no irritation potential *in vivo* (The CAM results for the test article at 1% and 5% are equivalent to Draize results for the test article at 2% and at 10%). Under the conditions of this test, the results indicate that the sponsor-submitted product,

Date 10/31/03,

at 20% would have a slight irritation potential *in vivo* (The CAM results for the test article at 10% are equivalent to Draize results for the test article at 20%).



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Under the conditions of this test, the results indicate that the sponsor-submitted product,

Date 10/31/03, at 2% and at 10% would have practically no irritation potential *in vivo* (The CAM results for the test article at 1% and 5% are equivalent to Draize results for the test article at 2% and at 10%). Under the conditions of this test, the results indicate that the sponsor-submitted product,

Date 10/31/03,  
at 20% would have a slight irritation potential *in vivo* (The CAM results for the test article at 10% are equivalent to Draize results for the test article at 20%).



324101

Steven Nitka  
Vice President  
Laboratory Director

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## FINAL REPORT

**CLIENT:**

**ATTENTION:**

**TEST:**

The MatTek Corporation EpiDerm™ Skin Model  
*In Vitro* Toxicity Testing System

Hydrolyzed keratin

MWN 3,000 Da

enzyme hydrolys

**TEST ARTICLE:**

V04-0032-1

**EXPERIMENT  
REFERENCE NO.:**

Scott Krupa 3.8.04

Scott Krupa  
Quality Assurance Supervisor

SN 3/8/04

Steven Nitka  
Vice President  
Laboratory Director

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# Consumer Product Testing Co.

## QUALITY ASSURANCE UNIT STATEMENT

**Study No.: V04-0032-1**

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practice principles (including government regulations to the extent applicable) and in accordance to standard operating procedures and applicable standard 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 may have been reported to management and the Study Director.

**Date of data inspection:** March 5, 2004

**Professional personnel involved:**

Steven Nitka, B.S.	- Vice President Laboratory Director (Study Director)
Lillian Deniza, B.S.	- Laboratory Supervisor
Melissa Pandorf, B.S.	- Technician
Scott Krupa	- Quality Assurance Supervisor

The representative signature of the Quality Assurance Unit on the front page signifies that this study has been performed in accordance with standard operating procedures and applicable study protocols.

V04-0032-1

Page 3 of 5

**Objective:**

To evaluate the test article for irritancy potential utilizing the MatTek Corporation EpiDerm *in vitro* toxicity testing system.

**Introduction:**

"MatTek's patented EpiDerm Skin Model consists of normal, human-derived epidermal keratinocytes (NHEK) which have been cultured to form a multilayered, highly differentiated model of the human epidermis. Keratinocytes are cultured on specially prepared, permeable cell culture inserts . . ." This system . . . closely parallels human skin. EpiDerm consists of highly organized basal, spinous, granular and cornified layers analogous to those found *in vivo*. Epiderm cultured keratinocytes are mitotically and metabolically active."<sup>1</sup>

EpiDerm, when used with the recommended cell metabolism assay, can quickly provide toxicological profiles. The procedure utilizes a water-soluble, yellow, tetrazolium salt (MTT {3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide}), which is reduced by succinate dehydrogenase in the mitochondria of viable cells to a purple, insoluble formazan derivative. Substances which damage this mitochondrial enzyme inhibit the reduction of the tetrazolium salt. The amount of MTT reduced by a culture is therefore proportional to the number of viable cells.

**Test Article:**

**Method:**

After the appropriate tissue preparation, one-hundred microliters of the liquid test article and the negative control article were added to the Millicells containing the EpiDerm samples. The six (6) well plates containing the dosed EpiDerm samples were then incubated at 37°C, five (5)% carbon dioxide and  $\geq$  90% humidity. After the appropriate exposure periods, each insert was individually removed from its plate and rinsed with phosphate buffered saline (PBS) to remove any residual material. Each was then rinsed a second time. Excess liquid was shaken off and each EpiDerm sample was placed into 300 microliters of MTT solution. The EpiDerm samples were then returned to the incubator.

<sup>1</sup> MatTek Corporation, 200 Homer Avenue, Ashland, Massachusetts 01721.

V04-0032-1  
Page 4 of 5**Method (continued):**

After the three (3) hour MTT exposure, each insert was removed and gently rinsed with PBS to remove any residual MTT solution. Excess PBS was shaken from each of the inserts which were then blotted on the bottom on paper towels. The inserts were then each placed into one (1) well of a 24 well extraction plate. Each insert was then immersed in two (2) milliliters of extraction solution overnight. After the exposure, the liquid within each insert was decanted back into the well from which it was taken. The remaining extractant solution was then agitated and a 200 microliter aliquot of each extract was removed for evaluation. A Dynatech MR 4000 Automatic Microplate Reader was used to determine the absorbance of each extract at 570nm. With the absorbance of the negative control (at 20 hours for the 20 hour test article exposures and at 4.5 hours for the 4.5 and 1 hour exposures) defined as 100%, the percent absorbancies of the test articles were determined. The percentages listed below directly correlate with the cell metabolism in the EpiDerm samples.

**Results:**

<u>Test Article (% &amp; Exposure)</u>	<u>Epiderm System</u>	<u>Percent Viability</u>	<u>Percent Inhibition</u>
(100% - 1 hr.)		110	-10
(100% - 4.5 hr.)		107	-7
(100% - 20 hr.)		116	-16

**Discussion:**

For the article, a semi-log scale was used to plot the percent viabilities, on the linear y axis, versus the dosing times, on the log x axis. By interpolation and where possible, the time at which the percent viability would be 50% (ET-50) was estimated.

Croda, Inc.  
V04-0032-1  
Page 5 of 5

**Discussion (continued):**

The test article, 11/06/03, elicited an ET-50 greater than 24 hours. According to MatTek Corporation, as a general guideline, the following groupings can be used in assigning expected *in vivo* irritancy responses based on the ET-50 results obtained using MatTek's EpiDerm:

<u>ET-50 (hrs)</u>	<u>Expected <i>In vivo</i> Irritancy</u>	<u>Example</u>
<0.5	Severe, probably corrosive	Conc. Nitric Acid
0.5-4	Moderate	1% Sodium Dodecyl Sulfate
4-12	Moderate to Mild	1% Triton X-100
12-24	Very Mild	Baby Shampoo
24	Non-irritating	10% Tween 20

**Conclusion:**

Under the conditions of this test, the test article, 11/06/03, has an expected *in vivo* dermal irritancy potential in the non-irritating range.

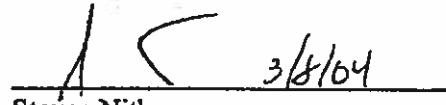
Date



# Consumer Product Testing Co.

Under the conditions of this test, the test article,  
11/06/03, has an expected *in vivo* dermal irritancy potential in the non-irritating range.

Date

  
Steven Nitka  
Vice President  
Laboratory Director

3/8/04

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Single Dose Oral Toxicity Study  
in the Rat

Hydrolyzed Milk Protein

MW N 1,500 Da

Enzyme hydrolysis

Study Ref. No. 110/8412  
January 1985

Toxicol Laboratories Limited,  
Bromyard Road,  
Ledbury,  
Herefordshire.  
HR8 1LG  
England.

Tel : Ledbury (0531) 4121  
Telex : 35644 Toxlab G

SINGLE DOSE ORAL TOXICITY STUDY IN THE RAT

MILK PROTEIN

TOXICOL REF. NO. 110/8412

At the time of this study the Quality Assurance Unit was inspecting one of each critical phase of every type of acute study each month, in accordance with the Good Laboratory Practice regulations as set forth in the following:

- 1) The U.S. Code of Federal Regulations, Title 21, part 58 (1978).
- 2) Notification 313 of the Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare (1982).
- 3) Notification of New Substances Regulations of the U.K. Health and Safety Commission (1982).
- 4) OECD Guidelines for the Testing of Chemicals (1981).
- 5) Swiss I.K.S. Guidelines for G.L.P. in non-clinical laboratory studies (1980).

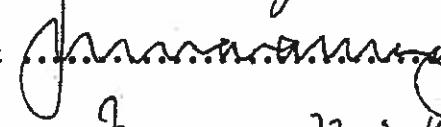
The final report was audited by the Quality Assurance Unit.

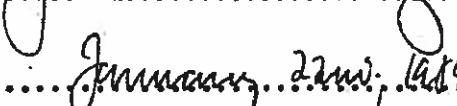
Inspection and audit findings were reported to Management and the Study Director.

All raw data relating to this study will be stored in Toxicol Laboratories' archives for a period of seven years after which it will either be returned to the Sponsor or appropriately destroyed after due consultation.

We, the undersigned, state that this report gives an accurate account of the methods used and the results obtained during the course of the above study and, to the best of our knowledge and belief, the study was conducted in compliance with the Good Laboratory Practice regulations stated above.

Study Director.......... M. Lerego

Quality Assurance Unit .......... S. Trenchard-Morgan

Date of report audit .......... January 22nd, 1985....

C O N T E N T S

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SINGLE DOSE ORAL TOXICITY STUDY IN THE RAT

MILK PROTEIN

**1.0 OBJECTIVE**

To assess the toxicity of the test material following its oral administration to a group of five male and five female rats at a dose level of 10.0g/kg bodyweight.

**2.0 TEST MATERIAL**

The test material was an off white powder supplied in a clear plastic bag labelled Milk Protein and was delivered to the testing facilities on 14th December 1984. The material was coded with the Toxicol Ref. No. 110/8412 for this study and testing commenced on 24th December 1984 using the material suspended in vegetable oil.

**3.0 SUMMARY**

Following overnight fasting a group of five male and five female rats were administered the prepared test material by peroral injection, at a dose level of 10.0g/kg.

No signs of toxicity were observed throughout the fourteen day period of the study.

**4.0 CONCLUSION**

The results of this study demonstrate that the test material, Milk Protein has no toxic effects following oral administration as a single dose at 10.0g/kg bodyweight.

## 5.0 METHODS

### 5.1 Animals and Husbandry

Young adult Sprague Dawley derived rats were supplied by A. Smith, 7 Kydecomb Road, Warlingham, Surrey. After arrival at the testing facilities animals were permitted an acclimatisation period of at least five days.

Animals were housed in single sex groups of five in grid bottomed polypropylene cages. A commercially available pelleted rodent diet (Modified 41B supplied by Pilsbury's Limited of Birmingham) and mains drinking water via polypropylene bottles were provided ad libitum.

The animal room was illuminated by fluorescent light to give a 24 hour cycle of 12 hours light/12 hours dark and the room was air conditioned with the air temperature maintained within the range 18°C - 25°C and relative humidity within the range 50% - 90% during the study.

### 5.2 Dosing

Healthy animals were fasted overnight prior to dosing then five male and five female animals were selected for treatment and weighed, the weight being used to calculate the amount of material to be administered. For dosing, the material was bulked in vegetable oil to give a dose volume of 30ml/kg bodyweight at a dose level of 10.0g/kg. The material was dosed by peroral injection using a metal cannula attached to a syringe of suitable capacity.

After dosing the animals were returned to their cages and permitted access to both food and water.

### 5.3 Observations

All animals were examined frequently after dosing and then daily for fourteen consecutive days. Any signs of toxicity or other effects were noted along with the time of onset and duration.

Animals were weighed at weekly intervals.

Eye Irritation Study

Hydrolyzed Milk Protein

MWN 1,500 Da

Enzyme hydrolysis

Study Ref. No. 111/8412  
January 1985

Toxicol Laboratories Limited,  
Bromyard Road,  
Ledbury,  
Herefordshire.  
HR8 1LG  
England.

Tel : Ledbury (0531) 4121  
Telex : 35644 Toxlab G

EYE IRRITATION STUDY

MILK PROTEIN

STUDY REF. NO. 111/8412

At the time of this study the Quality Assurance Unit was inspecting one of each critical phase of every type of acute study each month, in accordance with the Good Laboratory Practice regulations as set forth in the following :

- 1) The U.S. Code of Federal Regulations, Title 21, part 58 (1978).
- 2) Notification 313 of the Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare (1982).
- 3) Notification of New Substances Regulations of the U.K. Health and Safety Commission (1982).
- 4) OECD Guidelines for the Testing of Chemicals (1981).
- 5) Swiss I.K.S. Guidelines for G.L.P. in non-clinical laboratory studies (1980).

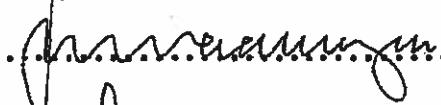
The final report was audited by the Quality Assurance Unit.

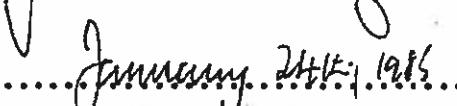
Inspection and audit findings were reported to Management and the Study Director.

All raw data relating to this study will be stored in Toxicol Laboratories' archives for a period of seven years after which it will either be returned to the Sponsor or appropriately destroyed after due consultation.

We, the undersigned, state that this report gives an accurate account of the methods used and the results obtained during the course of the above study and, to the best of our knowledge and belief, the study was conducted in compliance with the Good Laboratory Practice regulations stated above.

Study Director .....  ..... G. Haynes

Quality Assurance Unit .....  ..... S. Trenchard-Morgan

Date of report audit .....  ..... January 24th, 1986

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EYE IRRITATION STUDY

MILK PROTEIN

1.0 OBJECTIVE

To investigate the degree of ocular irritation produced when the test material are introduced into the eye of a group of albino rabbits.

The procedure used was the test for eye irritants described in the U.S. Federal Hazardous Substances Act.

2.0 TEST MATERIAL

The test material was an off white powder supplied in a plastic bag labelled Milk Protein and arrived at the testing facilities on 14th December 1984. The material was coded with the Toxicol Ref. No. 111/8412 for this study and testing commenced on 7th January 1985 using the material at a concentration of 25% in water.

3.0 SUMMARY

The test material was instilled, in 0.1ml aliquots, into the right eye of a group of six albino rabbits, the left eye remaining untreated as a control. The irritation produced was assessed twenty four, forty eight and seventy two hours after dosing.

Slight conjunctival redness was observed in the treated eye of one rabbit of the group twenty four hours after dosing. No other response to treatment was observed and the numerical values given to the irritation observed during the course of the study, when averaged for the group of six rabbits, were :- cornea 0.0, iris 0.0, conjunctivae 0.3, no rabbit exhibiting a positive response to treatment.

4.0 CONCLUSION

The results of this study indicate that the test material, Milk Protein, at a concentration of 25% in water is unlikely to prove irritating to the eye. The material would not be classified as an eye irritant according to the definitions of the U.S. Federal Hazardous Substances Act.

5.1 Methods

The method used was designed to meet the requirements of the test for eye irritants described in the U.S. Federal Hazardous Substances Act. (U.S. Federal Register, 1973, Vol. 38, No. 187, Section 1500 : 42 and revised annually in the U.S. Code of Federal Regulations, Title 16 Part 1500, Subchapter C - Federal Hazardous Substances Act Regulations).

5.2 Animals and Husbandry

Female New Zealand White rabbits were obtained from recognised breeders whose standards are acceptable to the Animal Welfare Department of Toxicol Laboratories. Animals were identified by tattoo in the ear with an individual unique number.

The rabbits were housed in grid bottomed metal cages. A commercially available antibiotic free pelleted rabbit diet (Product Ref. 680, Dalgety-Spillers Limited) and mains drinking water via holding tanks were available ad libitum.

The rabbit holding room was air-conditioned with temperature in the range of 18-21°C and relative humidity in the range of 45-65%. Fluorescent lighting was controlled to give an artificial cycle of 12 hours light/12 hours dark per day.

5.3 Dosing

Six animals were selected for treatment that were healthy and free from any eye defect or abnormality. A 25% (w/v) dispersion of the supplied material in distilled water was prepared for dosing and this prepared material was instilled, in 0.1ml aliquots, into the right eye of each animal by gently pulling away the lower lid from the eyeball to form a cup into which the material was placed. The lids were then held shut for a few seconds and moved gently about to distribute the test material around the surfaces of the eye and the lids.

5.4 Observations

Twenty four, forty eight and seventy two hours following instillation of the test material the animals were examined under a standard light source designed to comply with the requirements of B.S. 950 Part 1 (Artificial Daylight for the Assessment of Colour). The treated eyes were assessed for damage or irritation to the cornea, iris and conjunctivae using the untreated eye as a control. Examination was confined to a macroscopic observation and aids such as a binocular loupe, slit lamp or fluorescein staining were not employed.

The Federal Register method states that an animal shall be considered as exhibiting a positive reaction if the test material at any of the readings produces ulceration of the cornea, or opacity of the cornea (other than slight dulling of the normal lustre), or inflammation of the iris (other than a slight deepening of the fold (rugae) or a slight circumcorneal injection of the blood vessels), or if such material produces in the conjunctivae (excluding the cornea and iris) an obvious swelling with partial eversion of the lids or a diffuse crimson-red with individual vessels not easily discernible.

#### Numerical Scoring System

The scoring system is not part of the Federal Register Test but is utilised to simplify describing reactions observed. The scoring scale and system of weighting followed was that described by Draize, Woodward and Calvery ("Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes". J.H. Draize, G. Woodward and H.O. Calvery, J. Pharmacol. Exp. Ther., 83, 377-390, 1944) and given in more detail by Kay and Calandra ("Interpretation of eye irritation tests", J. H. Kay and J.C. Calandra, J.Soc. Cosmet. Chem., 13, 281-289, 1962).

#### Cornea

##### A. Opacity-degree of density (area most dense taken for reading).

No opacity .....	0
Scattered or diffuse area, details of iris clearly visible....	1*
Easily discernible translucent area, details of iris	
slightly obscured .....	2*
Opalescent areas, no details of iris visible, size of pupil	
barely discernible .....	3*
Opaque, iris invisible .....	4*

##### B. Area of cornea involved

One quarter (or less) but not zero .....	1*
Greater than one quarter, but less than half .....	2*
Greater than half, but less than three quarters .....	3*
Greater than three quarters, up to whole area .....	4*

#### Iris

##### A. Values

Normal .....	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive) .....	1*
No reaction to light, haemorrhage, gross destruction (any or all of these) .....	2*

\*Grades considered positive for irritation in the Federal Register Test.

Conjunctivae

- A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris).

Vessels normal .....	0
Vessels definitely injected above normal.....	1
More diffuse, deeper crimson red, individual vessels not easily discernible .....	2*
Diffuse beefy red .....	3*

- B. Chemosis

No swelling .....	0
Any swelling above normal (includes nictitating membrane).....	1
Obvious swelling with partial eversion of lids .....	2*
Swelling with lids about half closed .....	3*
Swelling with lids about half closed to completely closed ....	4*

- C. Discharge

No discharge .....	0
Any amounts different from normal (does not include small amounts observed in inner canthus of normal animals) .....	1
Discharge with moistening of the lids and hairs just adjacent to lids .....	2
Discharge with moistening of the lids and hairs and considerable area around the eye .....	3

\*Grades considered positive for irritation in the Federal Register Test

The numerical scores obtained on assessment of irritation were weighted following standard procedures to achieve a 'ranking' of the types of reaction to treatment, reflecting the significance given to reaction in different regions of the eye. The scores were weighted in the following manner :-

Conjunctivae

$$(\text{Discharge score} + \text{chemosis score} + \text{redness score}) \times 2$$

Iris

$$\text{Iris score} \times 5$$

Cornea

$$(\text{Opacity score} \times \text{area score}) \times 5$$

TABLE 1 - IRRITATION - SUMMARY

Milk Protein

Toxicol Ref. No. 111/8412

Time	Cornea	Iris	Conjunctivae	Total
24 hours	0	0	2	2
48 hours	0	0	0	0
72 hours	0	0	0	0
Total	0	0	2	2
Average	0.0	0.0	0.3	0.3

Number of animals exhibiting positive response : 0

Federal Register Classification : Negative

Primary Skin Irritation Study

Milk Protein

MW N 1,500 Da

Enzyme hydrolysis

Study Ref. No. 112/8412  
January 1985

Toxicol Laboratories Limited,  
Bromyard Road,  
Ledbury,  
Herefordshire.  
HR8 1LG  
England.

Tel : Ledbury (0531) 4121  
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PRIMARY SKIN IRRITATION STUDY

MILK PROTEIN

Toxicol Ref. No. 112/8412

At the time of this study the Quality Assurance Unit was inspecting one of each critical phase of every type of acute study each month, in accordance with the Good Laboratory Practice regulations as set forth in the following:-

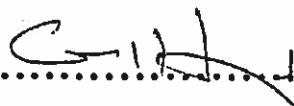
- 1) The U.S. Code of Federal Regulations, Title 21, part 58 (1978).
- 2) Notification 313 of the Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare (1982).
- 3) Notification of New Substances Regulations of the U.K. Health and Safety Commission (1982).
- 4) OECD Guidelines for the Testing of Chemicals (1981).
- 5) Swiss I.K.S. Guidelines for G.L.P. in non-clinical laboratory studies. (1980).

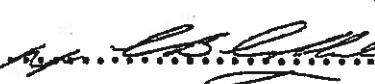
The final report was audited by the Quality Assurance Unit.

Inspection and audit findings were reported to Management and the Study Director.

All raw data relating to this study will be stored in Toxicol Laboratories' archives for a period of seven years after which it will either be returned to the Sponsor or appropriately destroyed after due consultation.

We, the undersigned, state that this report gives an accurate account of the methods used and the results obtained during the course of the above study and, to the best of our knowledge and belief, the study was conducted in compliance with the Good Laboratory Practice regulations stated above.

Study Director .....  ..... G. Haynes

Quality Assurance Unit .....  ..... S. Trenchard-Morgan

Date of report audit ..... 6.2.85 .....

C O N T E N T S

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## PRIMARY SKIN IRRITATION STUDY

### MILK PROTEIN

#### 1.0 OBJECTIVE

To investigate the degree of irritation produced to the abraded and intact skin of six albino rabbits following twenty four hours occluded contact with the test material.

The procedure used meets the requirements of the test for primary irritant substances described in the U.S. Federal Hazardous Substances Act.

#### 2.0 TEST MATERIAL

The test material was an off-white powder supplied in a plastic bag labelled Milk Protein and arrived at the testing facilities on 14th December 1984. The material was coded with the Toxicol Ref. No. 112/8412 for this study and testing commenced on 7th January 1985 using the material diluted to 25% (w/v) in water.

#### 3.0 SUMMARY

The test material was applied in 0.5ml aliquots to the dorsal skin, clipped free of fur, of six albino rabbits, one aliquot on abraded skin and one aliquot on intact skin. Treated areas were maintained under an occlusive dressing for twenty four hours after which the dressings were removed and irritation assessed.

Well defined erythema was apparent at five abraded and three intact treatment sites and very slight erythema was observed at one abraded site and one intact site following twenty four hours contact with the material. Very slight oedema was observed at the abraded skin site of one rabbit at this time. The erythematous reaction described declined during the study and very slight erythema remained at all six abraded sites and four intact treatment sites at the seventy two hour observation. The numerical values given to the irritation observed during the course of the study, when averaged for the group of six rabbits, were 4.7 for erythema and 0.3 for oedema giving a primary irritation score of 1.3.

#### 4.0 CONCLUSION

The results from this study indicate that a 25% (aq) concentration of the test material, Milk Protein, had some irritant effect but may not be classified as a primary irritant to the skin according to the definitions of the U.S. Federal Hazardous Substances Act.

## 5.0 METHOD

### 5.1 Study Requirements

The method used was designed to meet the requirements of the test for primary irritant substances described in the U.S. Federal Hazardous Substances Act. (U. S. Federal Register, 1973, Vol. 38, No. 187, Section 1500 : 41 and revised annually in the U.S. Code of Federal Regulations, Title 16, Part 1500, Subchapter C - Federal Hazardous Substances Act Regulations).

### 5.2 Animals and Husbandry

Female New Zealand White rabbits were obtained from recognised breeders whose standards are acceptable to the Animal Welfare Department of Toxicol Laboratories. Animals were identified by tattoo in the ear with an individual unique number.

The rabbits were housed in grid bottomed metal cages. A commercially available antibiotic free pelleted rabbit diat (Product Ref. 680 Dalgety Spillers Limited) and mains drinking water via holding tanks were available ad libitum.

The rabbit holding room was air-conditioned with temperature in the range of 10-15°C and relative humidity in the range of 65-75%. Fluorescent lighting was controlled to give an artificial cycle of 12 hours light/12 hours dark per day.

### 5.3 Dosing

Six animals were selected for treatment that were healthy and free from any abnormality and placed in restraining stocks. The dorsal surfaces of the trunk were clipped free of hair using an Oster Model A2 clipper with Angra blade. Immediately before dosing the left flank of the animals' back was lightly abraded in a criss-cross pattern using the point of a 25 x 0.6mm sterile disposable hypodermic needle. The abrasions were sufficiently deep to damage the stratum corneum but not to penetrate the dermis. The skin of the right flank remained intact.

A 25% (w/v) dispersion of the supplied material in distilled water was prepared for dosing and this prepared material was placed, in 0.5ml aliquots, onto two 2.5cm square surgical lint pads attached to a length of 5cm wide 'Sleek' plastic adhesive wrapping. The lint squares were then placed in contact with the animal's skin, one lint square in contact with abraded skin and one lint square in contact with intact skin, bilateral to the midline, and secured in position by the attached 'Sleek' adhesive tape. The trunk of the animal was then encircled with a length of 'Elastoplast' elastic adhesive bandage 7.5cm wide. All six animals of the group were treated in this manner and remained in the restraining stocks overnight after which time they were returned to their cages. After a contact period of twenty four hours the occlusive dressings were removed and any excess test material remaining on the skin removed by gentle wiping with cotton wool soaked in warm water.

#### 5.4 Observations

One hour after the end of the dosing period the treated sites were assessed for signs of reaction to treatment. Similar examination was undertaken forty eight hours later (seventy two hours from dosing). Animals were examined under a standard light source designed to comply with the requirements of B.S. 950 Part 1 (Artificial Daylight for the Assessment of Colour). Irritation was described and allocated a numerical value based on the table below :

<u>Erythema and eschar formation</u>	<u>Value</u>
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (best redness) to slight eschar formation (injuries in depth)	4

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (best redness) to slight eschar formation (injuries in depth)	4

<u>Oedema formation</u>	<u>Value</u>
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1mm)	3
Severe oedema (raised more than 1mm and extending beyond the area of exposure)	4

#### 5.5 Calculation and interpretation of results

The values obtained for erythema at both abraded and intact sites at the twenty four and seventy two hour readings were summed and the total divided by six, the number of rabbits, to give an average score for erythema. The values obtained for oedema were treated in a similar manner to give an average score for oedema. A primary irritation score was calculated by adding the total value obtained for erythema to the total value obtained for oedema and dividing by six, the number of rabbits, to give an average of the total score. This average was then divided by four to give a value referred to as the primary irritation score.

The definitions given in the U.S. Federal Register (Vol. 38, No. 187, Section 1500 : 3, (c), (4)) define a 'primary irritant to the skin' as a substance that is not corrosive and that human experience data indicates is a primary irritant and/or means a substance that results in an empirical score of five or more when tested by the method described in Section 1500 : 41. In addition, J. H. Draize in 'Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics (1959)' considers that 'compounds producing combined averages (primary irritation scores) of 2 or less are only mildly irritating whereas those with scores of 2 to 5 are moderate irritants, and those with scores above 6 are considered severe irritants'.

APPENDIX 1 - IRRITATION - INDIVIDUAL SCORES

MILK PROTEIN

Toxicol Ref. No. 112/8412

## 24 hour observation

Rabbit Number	Erythema		Oedema	
	Abraded	Intact	Abraded	Intact
124	2	2	1	0
125	2	2	0	0
126	1	0	0	0
128	2	2	0	0
129	2	1	0	0
130	2	0	0	0

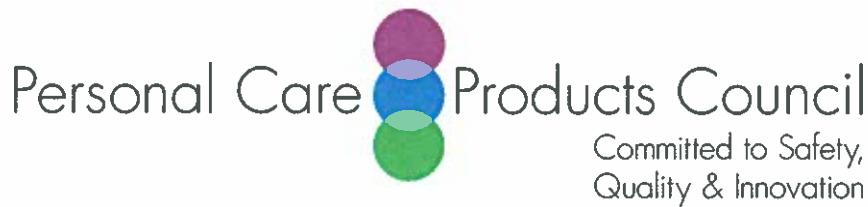
## 72 hour observation

Rabbit Number	Erythema		Oedema	
	Abraded	Intact	Abraded	Intact
124	1	1	1	0
125	1	1	0	0
126	1	0	0	0
128	1	1	0	0
129	1	1	0	0
130	1	0	0	0

Summary of Results

	Erythema	Oedema
Total	28	2
Average	4.7	0.3
Total	30	
Average	30/6 = 5.0	
Primary Irritation Score	5.0/4 = 1.3	

Rabbit Number	124	125	126	128	129	130
Body-weight (Kg)	2.5	2.9	2.9	2.3	3.0	2.7



## 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 2, 2012

**SUBJECT:** Information on Hydrolyzed Milk Protein

A supplier indicated that the mean molecular weight distribution of 3 batches of Hydrolyzed Milk Protein was as follows:

Molecular weight (MW) in Daltons (Da)	Mean of 3 batches (%)
5,000<MW<30,000	41.4 (main peak: 9,430 Da)
MW<5,000	58.4 (main peak: 2,570 Da)

This ingredient was tested in the studies described in the attached summary.

Anonymous. 2000. Safety assessment for human health Hydrolyzed Milk Protein.

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## - Safety Assessment for Human Health - Hydrolyzed Milk Protein

### Summary

### Hydrolyzed Milk Protein

was not toxic or harmful in an acute oral toxicity test in mice. Data from tests in the rabbit do not indicate to be irritating to the skin or eyes under use conditions. Its good skin tolerance was further supported in dermatological studies. Results from a study in guinea pigs as well as with human volunteers gave no evidence for to be a sensitizer. Furthermore, experimental studies in human volunteers in the presence of UV light did not reveal any evidence of phototoxic or photosensitizing potential. Based on the chemical nature of the ingredients, adverse effects after long-term exposure are not to be expected. No potential for gene mutations was detected in the Ames test.

## General

This evaluation refers exclusively to the product in the quality as specified and provided by the COGNIS company. (INCI: Hydrolyzed milk protein), a liquid milk derivative containing hydrolyzed milk protein, amino acids, lactose, and lactic acid as well as mineral salts, is used in skin and hair care products as a nutritive, protective and moisturizing surfactant.

Each cosmetic finished product is an individual and unique combination of ingredients. To comply with his product liability any provider of cosmetic finished products has to safeguard additionally the harmlessness of his product by a toxicological evaluation of the formulation as well.

This toxicological evaluation complies with the "SCC-Notes of guidance for testing of cosmetic ingredients for their safety evaluation"<sup>(1)</sup>. Besides of screening assays, studies dated later than 1984 were conducted in full compliance with the "OECD-principles of Good Laboratory Practice"<sup>(2)</sup>. Classification with respect to the individual endpoints complies with the criteria for the classification and labelling of substances and preparations in the European Union, laid down in the directive 67/548/EEC Annex VI including 8th amendment 96/56/EC<sup>(3)</sup>.

### 1 Acute oral toxicity

Acute oral toxicity is the adverse effect occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24 hours. Rodents (rats and mice) are the usual test animals. The test substance is administered orally by gavage in graduated doses to several groups of experimental animals, one dose being used per group. Subsequently, observations of effects and deaths are made.

The acute oral toxicity was investigated with , a yellowish/amber, clear liquid, in five male and five female mice, strain BOR: NMRI, white, using a limit test. A single dose of 15 ml/kg (undiluted, pH 6.5) was administered orally by gavage to animals that had previously been fasted. No adverse effects were observed during a 14-day post-application (p.a.) observation period. The LD<sub>50</sub> exceeded 15 ml/kg (equivalent to 15 000 mg/kg)<sup>(4)</sup>.

On the basis of this test a classification and labelling of is not necessary.

## **2 Dermal absorption**

Dermal absorption is the process in which a substance is taken up through the skin and hence is systemically available.

Experimental determination of dermal absorption was omitted for the following analogous consideration: in general, invasion via the gastro-intestinal route renders a significantly higher bioavailability compared to the dermal route. As the substance applied orally (a worst-case condition compared to dermal application) beyond the classification limit ( $>> 2000$  mg/kg for the acute toxicity) did not cause any signs of systemic toxicity, this can be deduced also for the cutaneous route of application.

## **3 Dermal irritation**

### **3.1 Dermal primary irritation in the rabbit**

Dermal irritation/corrosion is the production of *reversible/irreversible* inflammatory changes in the skin following the application of the test substance. International standard test method is OECD guideline No. 404 (consistent with EU guideline No. B4): the test substance is applied to one flank of the shaved back skin of rabbits for 4 h. The untreated area of each animal serves as a control.

The acute dermal irritation was tested with a 10-%(v/v) aqueous dilution of (pH 6.7) on the intact skin of 6 rabbits, strain White New Zealand. The contact time under semi-occlusive conditions was 24 hours instead of 4 hours, as required by the OECD guideline. The 1-h and 24/48/72 hours scores were 0 for erythema and oedema in all animals. No irritant effects were observed on the exposed skin area under these stringent conditions<sup>(5)</sup>.

### **3.2 Human Patch test**

Within the scope of a phototoxicity test program, no skin irritation occurred in 10 volunteers after single topical applications of (5-% aqueous dilution) for 24 h (see 3.3).

### 3.3 Photoirritation

Substance-related irritation may occur if skin is exposed simultaneously to sunlight, an exposure scenario which has to be assumed for actives intended for sun care products. In order to detect such effects, primary irritation test procedures have been combined with UV irradiation of the treated skin area, the so-called phototoxicity test. In addition, such a trial includes a control test for primary dermal irritation in the absence of light.

A photo-toxicity test was conducted with (5-% aqueous dilution, v/v) in 10 volunteers: 0.2 ml of the test item was applied under occlusive conditions to two different areas of the forearm one designated as non-irradiated, the other as irradiated test site. After 24-h exposure, one treated site was irradiated with UV-A light (320 – 400 nm) for 15 min, while the other served as non-irradiated control. Skin reactions were scored immediately after termination of light exposure as well as 24 and 48 h later.

No reactions were noted on either the irradiated or non-irradiated site in any subject treated with

In light of this data, the tested preparation at least up to 5-% dilution proved to be non-irritating both in the presence and absence of sun-light and, therefore, can be evaluated as well skin compatible and has not to be classified and labelled with regard to primary skin irritation.

### 4 Mucous membrane irritation (eye irritation)

Eye irritation/corrosion is the production of *reversible/irreversible* changes in the eye following the application of a test substance to the anterior surface of the eye. International standard test method is OECD guideline No. 405 (consistent with EC-guideline No B5): the test substance is introduced into one eye each of at least three rabbits. The untreated eye of each animal serves as a control.

Acute eye irritation was tested with in 6 albino rabbits, strain White New Zealand. 0.1 ml of a 10-% aqueous dilution of the test substance (pH 6.7) was instilled by single application and permanent contact into the left eye of the rabbits, the animals' right eyes served as the experimental controls. The eyes were scored 1, 2, 8 hours and daily up to day 7 post-application. The scores for conjunctival erythema and chemosis, for cornea and for iris were all 0. No test article-dependent findings were observed<sup>(7)</sup>.

In light of the available data, up to at least a 10-% dilution has not to be classified and labelled as "*irritating to the eyes*".

## 5 Skin sensitization

### 5.1 Dermal contact allergy

Skin sensitization (allergic contact dermatitis) is an immunologically mediated cutaneous reaction to a substance. In humans responses may be characterized by pruritis, erythema, oedema, papules, vesicles, bullae or a combination of these. In other species, reactions may differ and only erythema and oedema may be seen. Generally, guinea pigs are used as test animals. The international standard test method is OECD guideline No. 406 (consistent with EU guideline No B6), which also compiles to test protocols generally accepted by the competent authorities. During the induction phase of this test the skin of the test animals is exposed to a concentration of the test substance that is known to have only slightly irritant effects. After a time lag the skin is challenged with the highest non-irritating concentration of the test substance. Signs of hyperreactivity are evaluated 24 and 48 hours post-challenge.

A Guinea Pig Maximization Test according to Magnusson & Kligman was performed with (5-% dilution in water, v/v) to investigate the sensitizing potential with male and female albino guinea pigs, strain Pirbright white. Concentrations for the intracutaneous induction were 5 % in adjuvant and water and 100 % for the epicutaneous induction and challenge.

No signs of irritation and skin reactions indicative of an immune response were seen at the readings 24 and 48 hours after removal of the challenge patch<sup>(8)</sup>.

### 5.2 Photosensitization

Substance-related sensitization may occur if skin is exposed simultaneously to sunlight, an exposure scenario which has to be assumed for actives intended for sun care products. In order to detect such effects, common tests for contact allergy have been combined with UV radiation of the treated skin area, the so-called photoallergy test. This trial includes control tests for primary dermal irritation and contact dermal sensitization in the absence of light.

A photoallergy test with (5-% dilution in water, v/v) was conducted on 29 volunteers: For three weeks, six induction patches with the test item were applied in duplicate to the same site of the skin for 24 h each time, one site subsequently irradiated with UV light (260 – 400 nm) for 15 min each session, while the other site was left non-irradiated. After two weeks, the challenge patch was applied at virgin sites with and without irradiation.

At the challenge phase, no skin reactions were exhibited on either the irradiated or the non-irradiated test material contact site. No reactions were observed on the irradiated, non-treated control site<sup>(9)</sup>.

On the basis of these tests, a sensitizing potential of has to be expected neither in the presence nor in the absence of sun-light and, therefore, a classification and labelling with regard to skin sensitization is not necessary.

## 6 Long-term toxicity

Experimental determination of subchronic toxicity was omitted based on the following considerations:

represents a complex mixture of natural high- and low-molecular weight components including milk constituents, being part of the human diet and considered non-toxic after oral ingestion. In addition, percutaneous bioavailability from topical cosmetic application is expected to be rather limited due to the polar character and – for some substances – high molecular weight of the ingredients.

Therefore, systemic toxic effects after prolonged repeated topical application of are highly unlikely to occur.

## 7 Mutagenicity

Mutagenicity is the capacity to induce a relatively permanent change in the hereditary material of an organism involving changes in the genes ("gene mutations") or chromosomes ("chromosome mutations"). Gene mutations can be investigated in the *Salmonella typhimurium* reversion ("Ames") test. International standard test method is OECD guideline No. 471 (consistent with EU guideline No B14). The mutation is detected by a reversion of histidine-auxotrophic bacteria towards prototrophy.

, a liquid concentrate, was tested with the bacterial tester strains *S. typhimurium* TA 100, TA 1535, TA 1537, TA 98 in the presence and absence of enzymes obtained from the livers of Aroclor 1254 pre-treated rats (S9 mix). Two independent test series were carried out. Aqueous solutions of the test compound were freshly made. The following concentrations were tested in either test runs: 50, 150, 500, 1500 and 5000 µg/plate<sup>(10)</sup>.

did not induce reverse mutations under the above described test conditions, neither with nor without metabolic activation by S9 mix. Thus, no indications exist for to have a potential for gene mutations.

## Conclusion

### *Hydrolyzed Milk Protein*

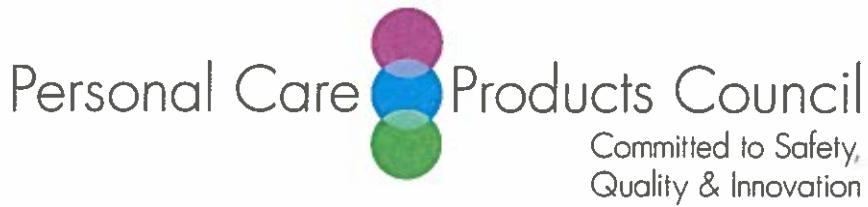
*The toxicological data at hand does not indicate that  might have any hazardous effect on humans considering the intended or foreseeable usage. With respect to the commonly acknowledged toxicological criteria and on the basis of the presented test results  has to be appraised as harmless for the designated use in cosmetics and toiletries.*

10 August 2000

The original signed version is on file at the archives of  s. Versions without signature are also valid due to electronic distribution.

## References:

1. European Commission, Directorate General DG XXIV (1999), Fd. Chem. Tox. 37, 357
2. Organisation for Economic Cooperation and Development - OECD (1997): ENV/MC/CHEM (98)17
3. European Community (1992); Off. J. L154 - 05.06.92
4. Internal data (1990); file no. 5879-502-6
5. Internal data (1990); file no. 5879-502-7
6. Internal data (1990); file no. 5879-503-11
7. Internal data (1990); file no. 5879-502-8
8. Internal data (1990); file no. 5879-502-9
9. Internal data (1990); file no. 5879-503-10
10. Internal data (1995); file no. 5879-501-12



**Memorandum**

**TO:** Lillian Gill, D.P.A.  
Director - COSMETIC INGREDIENT REVIEW (CIR)

**FROM:** Beth A. Jonas, Ph.D.  
Industry Liaison to the CIR Expert Panel

**DATE:** March 2, 2017

**SUBJECT:** Hydrolyzed Casein

Anonymous. 2017. Information concerning Hydrolyzed Casein.

March 2017

## Hydrolyzed Casein

A supplier reports that the Hydrolyzed Casein product is prepared by acidic, alkaline and/or enzymatic hydrolysis of bovine milk until the molecular weight reaches the target range.

	A number average molecular weight	Concentration	Impurities	
			Heavy metals	Arsenic
Hydrolyzed Casein product	600	30% solution in water	Not more than 5 ppm	Not more than 0.5 ppm

### Information concerning Hydrolyzed Casein product

#### Ocular Irritation (HET-CAM)

Non-irritant (active 1.5%, 2006)

- Test System: Fertile, white Leghorn hen's eggs incubated for 10 days  
 Test Concentration: Hydrolyzed Casein product was diluted in distilled water to 5%.  
 Dosing Procedure: 0.3 mL of the test sample was administered to the chorio-allantoic membrane (CAM). 20 seconds later, the test sample was rinsed from CAM with 5 mL of physiological saline.  
 Results: The average of scores was 2.00.  
 Conclusion: Under the conditions of this test, the results indicate that the Hydrolyzed Casein product, at 10%, would have practically no ocular irritation potential *in vivo*.

#### Genotoxicity (Ames test)

Non-mutagenic (as is, 2006, OECD 471)

- Methods: *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and *Escherichia coli* strain WP2uvrA<sup>-</sup> were treated with the test material using the Ames plate incorporation method at five dose levels, in triplicate, both with and without the addition of a rat liver homogenate metabolizing system (10% liver S9 in standard co-factors). The dose range for the range-finding test was determined in a preliminary toxicity assay and was 50 to 5000 µg/plate. The experiment was repeated on a separate day using the same dose range as the range-finding test, fresh cultures of the bacterial strains and fresh test material formulations.
- Results: The vehicle (sterile distilled water) control plates gave counts of revertant colonies within the normal range. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with or without metabolic activation. Thus, the sensitivity of the assay and the efficacy of the S9-mix were validated.  
 The test material caused no visible reduction in the growth of the bacterial

background lawn at any dose level. The test material was, therefore, tested up to the maximum recommended dose level of 5000 µg/plate. No test material precipitate was observed on the plates at any of the doses tested in either the presence or absence of S9-mix.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation.

Dermal Irritation - Human

Non-irritant (Japanese, 20 subjects, as is, 2006)

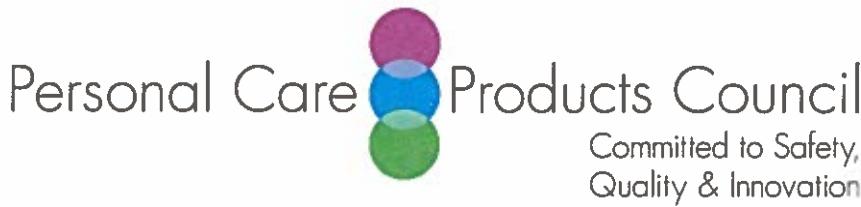
The irritation potential of Hydrolyzed Casein product was investigated in a 24 hours human patch test (occlusive) of 20 subjects (20 females). The backs of the subjects were treated with the test sample (Hydrolyzed Casein product, as is) using Finn Chambers on Scanpor Tape. At 30-60 minutes and 24 hours after removal of the patch, any subjects did not have reaction.

Dermal Sensitization - Human (RIPT)

Non-sensitizer (50 subjects, as is, 2006)

Procedure: 0.2mL of the test material (Hydrolyzed Casein product, as is) is dispensed onto the occlusive, hypoallergenic patch. The patch is applied directly to the skin of the infrascapular regions of the back. After 24 hours, the patch is removed. This procedure is repeated until a series of nine consecutive 24 hours exposures have been made for three consecutive weeks. Subjects are then given a 10-14 day rest period after which a challenge or retest dose is applied once to a previously unexposed test site. The retest dose is equivalent to any one of the original nine exposures. Reactions are scored 24 and 48 hours after application.

Results: During the test, no clinically significant objective reaction was observed in any subjects.



## Memorandum

**TO:** Lillian Gill, D.P.A.  
Director - COSMETIC INGREDIENT REVIEW (CIR)

**FROM:** Beth A. Jonas, Ph.D.  
Industry Liaison to the CIR Expert Panel

A handwritten signature in black ink that reads "Beth A. Jonas".

**DATE:** February 21, 2017

**SUBJECT:** Comments on the Scientific Literature Review: Safety Assessment of Milk Proteins and Protein Derivatives as Used in Cosmetics (release date: February 14, 2017)

The Council has no suppliers listed for Sodium Hydrolyzed Casein and Milk Protein Extract.

### Key Issue

Impurities - It should be made clear that 21CFR700.27 refers to BSE. Milk has never been considered a risk material for BSE. Therefore, it is the source, milk, not just processing that results in milk and milk-derived ingredients not being considered "prohibited cattle materials in cosmetic products".

### Additional Considerations

Impurities - It should also be noted that the *Food Chemical Codex* also has acceptance criteria for the substance itself. For example, acid casein and whey protein isolate protein should contain not less than 90% protein calculated on a dry basis.

Clinical Studies - How many subjects were actually tested with Hydrolyzed Milk Protein (reference 47)?

Summary - The example of a tumor suppression study in the Tumor Suppression section showed an effect in rats fed Hydrolyzed Whey Protein but not Whey Protein itself. The Summary describes an effect for Whey Protein. Perhaps one more example study showing an effect of Whey Protein should be presented in the Tumor Suppression section to better support the paragraph in the Summary.

Figure 1 - If no other molecular weight distributions become available, the title of this Figure should be changed to: "Molecular weight distribution of Hydrolyzed Milk Protein".

Table 5 - It should be made clear that the Hydrolyzed Milk Protein tested in the phototoxicity and photosensitization studies had the molecular weight distribution as shown in Figure 1.