Safety Assessment of Hydrolyzed Wheat Protein and Hydrolyzed Wheat Gluten as Used in Cosmetics

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Cosmetic Ingredient Review

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ABSTRACT

The Cosmetic Ingredient Review (CIR) Expert Panel reviewed the product use, formulation and safety data on hydrolyzed wheat protein and hydrolyzed wheat gluten, which function as skin and hair conditioning agents. The Panel determined that data from clinical and laboratory studies was sufficient to demonstrate that these ingredients will not elicit Type 1 immediate hypersensitivity reactions in sensitized individuals, and will not induce sensitization when the polypeptide lengths of the hydrolysates do not exceed 30 amino acids. 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.

INTRODUCTION

This safety assessment is of hydrolyzed wheat protein (HWP) and hydrolyzed wheat gluten (HWG), which are each mixtures of amino acids and peptides of varying lengths derived from wheat sources. These ingredients function as skin and hair conditioning agents in personal care products. The CIR Expert Panel (Panel) previously reviewed the safety of α-amino acids, animal- and plant-derived amino acids, hydrolyzed collagen, hydrolyzed corn protein, and Triticum Vulgare (wheat) gluten and concluded that these ingredients are safe for use in cosmetic products.[1-7]

CHEMISTRY

The ingredients in this group are interrelated because they each are prepared from wheat proteins by partial hydrolysis to yield cosmetically acceptable raw materials. The definitions of these ingredients are presented in Table 1. Wheat gluten typically represents about 85% of wheat protein, and consists of the water-insoluble fraction of wheat proteins, including gliadins and glutenins.[8] The remaining 15% of wheat proteins consists of water-soluble, non-gluten proteins, including albumins and globulins.

These protein derivatives are prepared by subjecting wheat proteins to enzymatic (e.g., papain) hydrolysis or chemical hydrolyses (e.g., acid, alkaline, or steam hydrolysis). The resulting polypeptide-, oligopeptide-, and peptide-containing products are used as conditioning agents in hair and skin products. Methods used to manufacture protein hydrolysates typically yield broad molecular weight (MW) distributions of peptides, 500-30,000 daltons (Da); however, certain enzymes, such as papain, can routinely yield narrower distributions of 500-10,000 Da.[9-11] For example, if the average MW of an amino acid is assumed to be 135 Da, then, under the broader distribution figures, these ingredients are approximately 4 to 220 amino acids in length (approximately 4 to 74 amino acids in length under the narrower distribution).[12]

Method of Manufacturing

A supplier reported that HWP (MW = 350 Da) may be prepared by both alkaline and enzyme hydrolysis.[13] These processes occur for several hours until the desired MW distribution is reached. The final product is a 25% water solution of HWP. Summary information that includes this data along with additional data from other suppliers can be found in Table 2.

HWP contained in a facial soap that is associated with anaphylaxis reactions in Japan was produced from gluten by partial hydrolysis with hydrogen chloride at 95ºC for 40 min.[14] The MW of the main band of HWP, as determined with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), was 40,000-50,000 Da, which was larger than the main band in gluten.

Water insoluble ("vital") wheat gluten is prepared by washing wheat flour to remove the starch.[15] The gluten remaining is treated with acid to partially deamidate the proteins, which renders them dispersible ("soluble") in water. The resultant proteins have relatively high MWs, which can be hydrolyzed by acid, alkali or protease treatment to yield water soluble proteins, polypeptides, or amino acids, depending on the method and the extent of the hydrolysis. Polypeptides can then be derivatized by quaternization or copolymerization.

There is no standard method for measuring the MWs of the small polypeptides that can be produced by hydrolyzing gluten, for example.[15] The MWs typically are measured by Size-Exclusion High Pressure Liquid Chromatography / Gel Permeation Chromatography (SEHPLC/GPC), GPC / Multi-Angle Laser Light Scattering (MALLS), or SDS-PAGE, and are expressed as weight-average MWs.

Impurities

A supplier of HWP (MW = 350 Da) reported levels of heavy metals and arsenic at $\leq$ 5 ppm and 0.5 ppm, respectively.[13]

USE

Cosmetic

The HWP and HWG addressed in this safety assessment function primarily as hair conditioning agents and skin conditioning agents (miscellaneous) in cosmetic formulations.[16] An additional function may include film former (HWP).

Table 3 presents the current product-formulation data for HWP and HWG. According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), HWP has the most reported uses in cosmetic and personal care products, with a total of 1077; approximately half of those uses are in non-coloring hair products.[17] HWG has a total of 78 uses in cosmetic and personal care products with about half of the uses reported to be hair tints.

In the Personal Care Products Council's (Council) use concentration survey, HWP had a wide maximum use concentration range of 2.0×10^{-5} to 1.7%, with the 1.7% reported in rinse-off non-coloring hair products.[18] HWG had a maximum use concentration range of 0.005% to 0.09%, with 0.09% reported in eye makeup preparations.

HWP is used in cosmetic sprays, including aerosol and pump hair spray products and hair tonics. HWG and HWP may also be used in spray face and neck skin care products and skin fresheners – use in this fashion cannot be confirmed. When used in cosmetic sprays, these ingredients could possibly be inhaled. The maximum concentration of these ingredients reported to be used in a spray product is 0.5% (HWP) in a pump hair spray. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm, with propellant sprays yielding a greater fraction of droplets/particles <10 µm compared with pump sprays.[19,20] 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., able to enter the lungs) to any appreciable amount.[21,22]

HWP and HWG are not restricted from use in any way under the rules governing cosmetic products in the European Union.[23]

Non-Cosmetic

The FDA determined that the use of peptones as direct food substances is generally recognized as safe (GRAS). These GRAS peptones are defined as "the variable mixture of polypeptides, oligopeptides, and amino acids that are produced by partial hydrolysis of casein, animal tissue, soy protein isolate, gelatin, defatted fatty tissue, egg albumin, or lactalbumin (whey protein) (21 CFR §184.1553).

The FDA defines the term "protein" to mean any α-amino acid polymer with a specific defined sequence that is greater than 40 amino acids in size.[24] The FDA considers a "peptide" to be any polymer composed of 40 or fewer amino acids.

The FDA requires allergen labeling when major allergens are included in food. The major allergens include wheat, milk, egg, fish, Crustacean shellfish, tree nuts, peanuts, and soybeans.[25]

TOXICOKINETICS

No published toxicokinetics studies on HWP and HWG were identified by a literature search for these ingredients and no unpublished data were submitted.

TOXICOLOGICAL STUDIES

The proteins that serve as the sources of the HWPs and HWGs that are addressed in this safety assessment are found in the foods we consume daily. HWPs and HWGs are also common food additives. The potential for systemic effects, other than sensitization, from the possible absorption of HWPs and HWGs through the skin is much less than the potential for systemic effects from absorption through oral exposures. This is because the rates of absorption and metabolism of these ingredients in the skin are expected to be negligible compared to the corresponding rates in the digestive tract. Thus, the potential for systemic effects, other than sensitization, are not discussed in detail in this report. This assessment focuses on evaluating the potential for these ingredients to cause sensitization reactions and irritation.

GENOTOXICITY

No published genotoxicity studies on HWP and HWG were identified by a literature search for these ingredients and no unpublished data were submitted.

CARCINOGENICITY

No published carcinogenicity studies on HWP and HWG were identified by a literature search for these ingredients and no unpublished data were submitted.

IRRITATION AND SENSITIZATION

[From the CIR Safety Assessment of α-amino acids][1]: Cysteine HCl and methionine were used as negative controls in in vitro assays to predict potential skin irritants. In separate dermal and ocular studies, arginine (up to 5%), aspartic acid (up to 0.2%), cysteine (up to 5%), glycine (up to 2%), magnesium aspartate (up to 0.1%), serine (up to 0.3%) and tyrosine (up to 1%) did not produce any adverse effects in rats, guinea pigs, or mouse skin models. Glutamic acid was used as a negative control in an in vitro study to identify skin sensitizers. Products containing amino acid ingredients at concentrations up to 2.784% were not dermal irritants or sensitizers in HRIPT studies. In several validation studies for in vitro phototoxicity assays, histidine was used as a negative control. Neither magnesium aspartate up to 0.5% nor 1% tyrosine was phototoxic in assays using yeast.

Irritation

Dermal – Non-Human

In a primary dermal irritation study in 6 New Zealand white rabbits, acid- and enzyme-hydrolyzed HWP was not a primary skin irritant (primary skin irritation score = 0.50; a score of 5+ indicates a primary dermal irritant).[26] The 25% aq. solution (MW = 350 Da) was applied for 24 h to 2.5 cm^2 sites that were clipped, abraded, and occluded.

Dermal - Human

HWP was non-irritating in a human irritation patch test performed in 42 subjects.[27] The HWP was tested at 25% aq. solution (MW = 350 Da), and the subjects received a single dermal dose under occlusive conditions for 48 h.

Ocular – Non-Human

In an ocular irritation study in 6 albino rabbits, HWP (25% aq. solution, MW = 350 Da) was not a primary eye irritant.[28]

Sensitization

Dermal - Non-Human

The possibility of a transdermal pathway for sensitization to gluten and acid-hydrolyzed HWP was studied using BALB/c mice.[14] The HWP was supplied by a manufacturer in Japan and was produced from gluten by partial hydrolysis with hydrogen chloride at 95º C for 40 min. The resultant HWP had a MW of approximately 40,000-50,000 Da. The 7-week-old female mice were shaved and tape-stripped 10 times to remove the stratum corneum, and were then exposed to HWP and gluten (500 µg/mouse), with and without sodium dodecyl sulfate (SDS), or to HWP (20-500 µg/mouse), with SDS, via transdermal patches for 3 to 4 cycles (each cycle consisting of 3 days with the patch on followed by 4 days without the patch), 3 days/week. Active systemic anaphylaxis (ASA) was then induced by intraperitoneal injection of HWP or gluten, respective of the material used during the transdermal exposure. Rectal temperature, scores of anaphylactic responses, and plasma histamine levels were measured. Dose-dependent production of IgE and IgG1 were observed. The i.p. injection of HWP caused ASA in the mice exposed transdermally to HWP, with decreased rectal temperatures, increased anaphylaxis scores, and increased plasma histamine levels. The i.p injection of gluten clearly induced ASA in the mice transdermally exposed to gluten in the presence of SDS, but not in the absence of SDS. When compared to the vehicle control group, the content of HWP-specific IgE and IgG1 was significantly increased in the HWP groups with and without SDS and in the gluten-with-SDS group; IgE in the gluten–without-SDS group was barely increased. The serum content of gluten-specific IgE was significantly increased in the gluten-with-SDS group and both HWP groups, but barely increased in the gluten-

without-SDS group, when compared to the vehicle-control group. The serum content of gluten IgG1with and without SDS and HWP without SDS were also significantly increased, but there were individual differences in the gluten-without-SDS group that showed that SDS had an important role in sensitization by transdermal exposure. Following elicitation of the immediate hypersensitivity reactions, harvested splenocytes were re-stimulated with HWP for 72 h. The secretion of IL-4, IL-5, and IL-10 was increased while that of IL-2 and interferon (IFN)-γ was significantly decreased, demonstrating that transdermal sensitization with HWP was associated with a T helper 2 response.

Dermal - Human

In an occlusive human repeated insult patch test (HRIPT) of 52 subjects, no dermal irritation or sensitization was observed in response to HWP (25% aq. solution, MW = 350 Da) when applied at a volume of 0.2 ml under a 20 mm^2 Webril patch.[29]

A study of sensitization to protein hydrolysates in hair-care products was performed in 3 groups of Finnish patients.[30] The first group, which consisted of 11 hairdressers with hand dermatitis, submitted to scratch and prick tests with 22 trademarked protein hydrolysates, including 2 HWP trademarked hydrolysates (specific chemical characteristics not provided). The second group was comprised of 2160 consecutive adults with suspected allergic respiratory disease: they were subjected to skin prick tests with hydroxypropyl trimonium hydrolyzed collagen, hydrolyzed collagen and/or hydrolyzed milk protein. The third group of 28 adult patients with atopic dermatitis was also tested with 1 to 3 of the hydrolysates tested in group 2 via a skin prick test. Positive reactions were seen in a total of 12 patients (all female with atopic dermatitis) to the hydroxypropyl trimonium hydrolyzed collagen, hydrolyzed collagen and/or hydrolyzed milk protein. No adverse reactions to the HWP trademarked hydrolysates were observed.[30]

Type 1 Hypersensitivity

There have been several reports of Type 1 (i.e., immediate) hypersensitivity reactions to personal care products that contain HWP or HWG, as summarized below. An allergen must have at least 2 IgE-binding epitopes, and each epitope must be at least 15 amino-acid residues long, to trigger a Type 1 hypersensitivity reaction.[31] Type 1 responses can be elicited in sensitized patients when pairs of IgE molecules against a specific allergen are bound to receptors on the surface of mast cells and other cells that mediate immune reactions. The binding of an allergen molecule to two receptor-bound IgE molecules results in the crosslinking of the pair of IgE molecules. The cross-linking of sufficient numbers of IgE pairs bound to the receptors on the surface of a mast cell results in degranulation of the mast cell and the release of vasoactive amines, which are responsible for the Type 1 reaction.

The sera from 5 European patients were studied to determine the reactivity of IgE with hydrolyzed gluten.[32] In 4 of the patients, immediate contact hypersensitivity to HWP (IHHWP) manifested as urticaria in response to either dermal contact with HWP (2 patients) or the ingestion of processed foods containing HWP (2 patients), without sensitivity to traditional wheat food products. The fifth patient (control) exhibited conventional wheat-dependent exercise-induced anaphylaxis (CO-WDEIA) in response to ingesting traditional wheat food products without exhibiting sensitivity to HWP.

The IgE reactivity of sera from the IHHWP patients and the CO-WDEIA patient was characterized using extracts of 4 commercial hydrolyzed gluten preparations (enzymatically- or acid-hydrolyzed), total unmodified wheat protein (UWP), and UWP fractions (i.e., albumins/globulins, gliadins, and glutenins, including high-MW glutenin subunits [HMW-GS] and low-MW glutenin subunits [LMW-GS]). One of the gluten hydrolysates yielded a smear on an SDS-PAGE gel ranging from <6 kDa to about 40 kDa, and the other 3 hydrolysates exhibited large amounts of componenets with MWs similar to those of wheat gluten. The IgE cross-reactivity of the sera was examined from one IHHWP patient with the extracts of one HWP preparation and UWP. Finally, the relative molecular size distributions of two HWP preparations (one the product of acid hydrolysis with a low degree of deamidation and the other the product of enzymatic hydrolysis) was characterized, and the binding of IgE in the serum of one IHHWP patient was determined using the separated polypeptide fractions of two HWP preparations.

The results showed reactivity of serum IgE from the IHHWP patients, especially with the albumins/globulins fraction and less so with the gliadins and LMW-GS fractions, but not with the HMW-GS fraction of UWP. Reactivity of serum IgE from one of the IHHWP patients was observed with the ω5-gliadin of UWP; this patient distinctly exhibited exercise-induced allergic reactions (urticarial) to ingestion of HWP in processed foods. Reactivity of serum IgE from the CO-WDEIA patient was observed with ω5-gliadin and LMW-GS fractions, but not with the HMW-GS fraction of UWP.

Binding patterns of serum IgE from the IHHWP patients to HWP preparations varied by IHHWP patient and by HWP preparation, but in no case did the IgEs bind to HWP polypeptides less than 31,000 Da. The binding of

serum IgE to UWP or to the albumins/globulins fraction of UWP was partially inhibited by HWP. However, the binding of serum IgE to HWP was almost completely inhibited by UWP or HWP. Based on these results, the authors suggested that almost all of the epitopes in the HWP preparation tested were also available in UWP. The molecular-size profiles of two of the HWP preparations ranged from <5,000 Da to > 1,000,000 Da, and both preparations contained substantial amounts of high-MW constituents. Binding of IgE in the serum of the IHHWP patient was greatest to the highest MW fractions of both of these HWP preparations (400,000 Da to 1,000,000 Da), weaker to intermediate molecular-weight fractions (30,000 Da to 400,000 Da), and faint or undetectable to the lowest molecular-weight fractions (< 31,000 Da).

Overall, the authors concluded that most IgE epitopes in UWP are conserved in HWP produced by industrial hydrolysis processes, and the production of new epitopes in the hydrolysates does not appear to contribute substantially to the differences in allergic responses in IHHWP patients compared with CO-WDEIA patients. Additionally, epitopes in UWP appear to be destroyed in HWP polypeptides less than about 30,000 Da. Analysis of HWP fractions under non-reducing, non-dissociating conditions suggested that differences in allergic responses between IHHWP patients and CO-WDEIA patients may be attributable to hydrolysis-induced re-organization in HWP of epitopes that already exist in UWP; re-organization through entanglements, S-S bond interchanges, or non-covalent interactions among the HWP polypeptides may produce relatively soluble, high-MW polypeptide aggregates that can present multiple epitopes efficiently to trigger allergic responses to HWP.[32]

In a Japanese study, wheat protein hydrolysates that were produced by enzymatic hydrolysis had higher concentrations of peptides with MWs greater than 1,050 Da, compared with those produced by acid hydrolysis, which had extremely low concentrations of peptides with MWs greater than 1,050 Da.[33] Investigation of the reactivity of these 2 types of hydrolysates revealed that the acid hydrolysates rarely inhibited IgE binding whereas enzymatic hydrolysates clearly inhibited the binding of IgE to wheat proteins.[33] IgE of patients that had Type 1 hypersensitivity to HWP through percutaneous and/or rhinoconjunctival exposure to a facial soap containing HWP (40,000-50,000 Da) reacted with high-MW polypeptide aggregates.[34] However, an in vitro elicitation test using IgE from different categories of wheat-allergic patients (including patients sensitized to commercial HWP produced by acid hydrolysis, pediatric patients with food allergy to native wheat, adult patients exhibiting WDEIA, and non-atopic healthy adults) revealed that glutens acid-hydrolyzed to various extents retained the ability to activate mast cells in patients sensitized by exposure to commercial acid-hydrolyzed HWP.[35]

A study was performed comparing 5 Japanese women exhibiting both contact allergy (rhinoconjunctival reactions) to HWP (40,000-50,000 Da) in a facial soap and WDEIA reactions to eating “normal wheat products” such as bread, pasta, and pastries (referred to as HWP-WDEIA patients) with 18 Japanese women exhibiting CO-WDEIA reactions.[36] The authors distinguished the 5 Japanese HWP-WDEIA patients from European patients exhibiting IHHWP (see study summarized above), some of whom also exhibited allergic reactions to foods containing HWP, but none with allergic reactions to eating “normal wheat products.”

Positive skin prick tests were obtained for HWP in all 5 of the HWP-WDEIA patients, in contrast to the CO-WDEIA patients. Sera from HWP-WDEIA patients exhibited statistically-significantly elevated IgE reactivity with HWP, compared to reactivity with each of the wheat-protein fractions (i.e., albumins/globulins, gliadins, and glutenins). In contrast, sera from CO-WDEIA patients exhibited statistically-significantly elevated reactivity with the gliadins fraction of wheat proteins, compared to reactivity with HWP.

Sera from the HWP-WDEIA patients exhibited statistically-significantly elevated IgE reactivity with HWP, gluten, wheat flour, and each of the wheat-protein fractions, and statistically-significantly reduced reactivity with recombinant ω5-gliadin, compared to sera from CO-WDEIA patients. Based on these results, the authors suggested that sensitization of HWP-WDEIA patients to components of the gliadins fraction other than ω5-gliadin may help explain the elevated reactivity of sera from HWP-WDEIA patients with the complete gliadins fraction.

Pre-incubation of sera from HWP-WDEIA patients with HWP completely inhibited IgE reactivity with wheat extracts, but pre-incubation with wheat extracts did not inhibit reactivity with HWP. Conversely, pre-incubation of sera from CO-WDIEA patients with HWP only weakly inhibited reactivity with wheat extracts, while pre-incubation with wheat extracts strongly inhibited reactivity with HWP. Based on these results, the authors suggested that the reactivity of sera from CO-WDEIA patients with HWP is attributable to IgE-binding epitopes that survive the hydrolysis of wheat proteins.

Overall, the authors concluded: (1) HWP-WDEIA is a clinical phenotype distinct from CO-WDEIA, as well as from the contact sensitivity to HWP observed in European patients that do not exhibit sensitivity to ingesting “normal wheat products,” (2) the use of a facial soap containing HWP caused both primary contact dermal / rhinoconjunctival sensitization to HWP and, secondarily, WDEIA sensitization to ingested wheat proteins in the HWP-WDEIA patients, and (3) sensitization to gliadins other than ω5-gliadin (e.g., ω1-2-gliadin and γ-gliadin) may

be more important than sensitization to ω5-gliadin in the pathogenesis of HWP-WDEIA, compared with the pathogenesis of CO-WDEIA.[36]

In another study, the allergic reactions of a group of Japanese patients diagnosed with HWP-WDEIA were found likely the result of sensitization primarily through percutaneous and/or rhinoconjunctival exposures to HWP (acid-hydrolyzed UWP; 40,000-50,000 Da) in a facial soap.[8] The authors noted that, by 2010, more than 1300 patients who had used the soap exhibited facial angioedema after use, tested positive for sensitivity to the HWP in skin-prick tests and positive for serum IgE reactivity with the HWP, and developed WDEIA reactions in response to eating natural UWP. Angioedema predominated in the HWP-WDEIA patients, especially angioedema of the eyelids, in contrast to the urticarial wheals predominating in CO-WDEIA patients. The onset of allergic reactions in the HWP-WDEIA patients typically was 1 month to 5 years after starting to use the soap. Many of these patients developed WDEIA in response to eating wheat food products at about the same time as, or subsequent to, the onset of urticarial reactions to the soap.

About half of the HWP-WDEIA patients tested positive in skin-prick tests for sensitivity to wheat and bread. Almost all of the HWP-WDEIA patients tested positive in skin-prick tests for sensitivity to solutions of the soap or the HWP in the soap, in contrast to CO-WDEIA patients, none of whom exhibited sensitivity to these solutions. Only about 7% of HWP-WDEIA patients exhibited serum IgE reactivity with ω5-gliadin, compared to 80% of CO-WDEIA patients. Reactivity with ω5-gliadin among the few positive HWP-WDEIA patients was substantially weaker than the corresponding reactivity among the CO-WDEIA patients. About 17% of HWP-WDEIA patients exhibited serum IgE reactivity with ω5-gliadin and/or HMW-GS, compared to about 94% of CO-WDEIA patients. On the other hand, 70% or more HWP-WDEIA patients exhibited serum IgE reactivity with wheat protein or gluten, compared to only 30% to 40% of CO-WDEIA patients. Sera from HWP-WDEIA patients exhibited IgE binding to HWP polypeptides and to water-soluble and water-insoluble constituents of UWP, but not to purified ω5-gliadin. In comparison, serum IgE from CO-WDEIA patients bound to ω5-gliadin, as well as to the water-soluble and water-insoluble constituents of UWP, but not to the polypeptides of the HWP preparation. Pre-incubation of sera from the HWP-WDEIA patients with solutions of the HWP preparation resulted in concentration-dependent inhibition of the binding of IgE to HWP polypeptides. HWP, but not purified ω5-gliadin, up-regulated the CD203c (an ecto-enzyme on the cell membranes of basophils and mast cells) in HWP-WDEIA patients. However, ω5-gliadin, but not the HWP, up-regulated CD203c in cells from CO-WDEIA patients.

The authors suggested that (1) the hydrophilic constituents of HWP may play an important role in percutaneous and/or rhinoconjunctival sensitization to HWP, (2) production of HWP by acid hydrolysis of UWP will yield charged terminal amino- and carboxyl-groups that increase the water solubility of the HWP, compared to that of UWP, and (3) the surfactants in a soap product will likely facilitate the dermal penetration of the HWP polypeptides, and thereby help to increase the likelihood of sensitization through percutaneous/rhino-conjunctival exposures in people using such products.[8]

Recommendations have been made to individuals with known protein hypersensitivity to minimize dermal exposure to botanical ingredients such as HWP and to not use products that have these constituents that can be incidentally inhaled.[37] Additionally, it has been recommended that manufacturers of personal care products not use known or suspected allergens (including constituents of plants known to produce Type 1 hypersensitivity reactions or of plants that are in the same phylogenetic families as these plants) in products that may be incidentally inhaled (e.g., sprays, shampoos or shower gels, and, presumably, loose powder products as well).

Research on Type 1 hypersensitivity reactions in Japan to products containing HWP is ongoing, as reported by the Japanese Society of Allergology's Special Committee for the Safety of Protein Hydolysates in Cosmetics. Current developments are available at: http://www.jsaweb.jp/modules/en/index.php?content_id=11.

The outbreak in Japan of Type 1 immediate hypersensitivity reactions to a HWG in facial soaps and other products was attributed mainly to the use of a popular soap product (*Cha no shizuku*) containing 0.3% of a HWG called Glupearl 19S (trade name). Glupearl 19S has an average MW of about 50,000 Da.[38] There are presently more than 2100 registered cases of this type of sensitivity across Japan. Data from 547 patients indicated that the signs of sensitization typically appeared 31.5 months (median) after starting to use the soap. The clinical manifestations of sensitization to Glupearl 19S include eyelid edema and contact urticaria during or after using the soap in many, but not all, of the patients. Eating foods containing wheat ingredients caused anaphylactic reactions in about 55% of the patients, including anaphylactic shock in about 25%. Clinical and experimental evidence indicates that the patients have systemic reactions to ingested wheat products because they have been sensitized through percutaneous or permucosal (i.e., through the ocular or nasal mucosae) absorption of Glupearl 19S.[38,39]

Wheat gluten hydrolysates prepared by acid hydrolysis at high temperatures (95ºC or 100 ºC) for 0 to 48 hours have weight-average MWs ranging from < 3000 Da to > 10,000 Da, depending on the duration of the hydrolysis.[38] Regardless of the duration, all of the hydrolysates are about 50% deamidated by the treatment.

Glupearl 19S and hydrolyzed gluten preparations prepared by acid-hydrolysis at 100 °C for 0.5 h exhibited a sensitization potential through dermal exposure in an in vivo mouse model, but gluten that was more extensively hydrolyzed under these conditions for 9 hours exhibited weak sensitization potential in this model.[38,39] The MWs of the hydrolysate preparations, determined by sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE), were ≤ 70,000 Da after 0.5-h acid hydrolysis and ≤ 30,000 Da after 9-h hydrolysis. Glupearl 19S and other gluten hydrolyzates that were acid-hydrolyzed (at 100 °C) for 0, 0.5, 1, 2, 3, 6, 9, 12, 24, or 48 hours were fractionated by size (i.e., three fractions: < 3000 Da, 3000-10,000 Da, and > 10,000 Da fractions) using ultrafiltration spin columns without sodium dodecyl sulfate (SDS) to avoid the effects of SDS binding to the polypeptides, which can reduce the accuracy of MW estimates).[38,39] The fractions were then tested for IgE responses (using serum IgE from an HWP-sensitized patient) by the IgE crosslinking-induced luciferase expression (EXiLE) method. The results showed that the ≤ 3000 Da fractions of Glupearl 19S and the other hydrolysate preparations did not elicit an IgE response, and the 3000-10,000 Da fractions elicited only a weak response, in contrast to both Glupearl 19S and the > 10,000 Da fraction. The authors concluded that the elicitation of a type 1 hypersensitivity response depends on the presence of an epitope with a MW ≥ 3000 Da.[39]

Wheat gluten rendered dispersible by mild acid hydrolysis was further hydrolyzed enzymatically to different extents to yield HWP preparations, including: ~150 Da, ~3000 Da, ~100,000 to 125,000 Da preparations.[15] Some of the ~150 Da, ~3000 Da, and ~100,000 to 125,000 Da preparations were derivatized to yield quaternized peptides, co-polymers, or acylated derivatives. The polypeptides of the ~3000 Da MW preparation did not bind to human anti-gluten (specifically, anti-gliadin) antibodies in vitro in slot blot and western blot analyses, indicating the absence of reactivity of this preparation, in contrast to the gluten and dispersible-gluten preparations that were used as positive controls. All of the 6 wheat IgE-positive patients with conventional wheat allergy tested negative in skin-prick tests with the ~150 Da, ~3000 Da, ~100,000 Da, and ~125,000 Da hydrolysate preparations and a number of derivatives, most of which were derivatives of the ~3000 Da preparation.

Several Danish individuals developed allergic reactions to a dispersible (i.e. rendered "soluble" by mild acid hydrolysis) wheat protein that was used in food products as an emulsifier.[15] This protein was hydrolyzed enzymatically to produce ~150 Da, ~3000 Da, and ~100,000 to 125,000 Da preparations, each of which was tested by Immunospot® or IgE binding using sera from these patients. The ~ 150 Da and ~3000 Da preparations and their derivatives yielded negative results, in contrast to the ~100,000 to 125,000 Da preparation. The authors concluded that the allergic responses in these patients are associated with the partial deamidation of the peptides by acid hydrolysis, and the hydrolysis to ~3000 Da removes the potential for eliciting an allergic response.

Thus, the results of several studies indicate that hydrolysates of gluten with weight-average MWs < 3000 Da exhibit no potential to elicit hypersensitivity reactions in sensitized individuals, in contrast to Glupearl 19S and other hydrolysates with weight-average MWs >10,000 Da.[15,38] Duplicate analysis (GPC MALLS) of two samples of the ~3000 Da hydrolysates that were negative in the in vitro and in vivo studies described above indicated that ~3% of the molar mass of the preparation exceeded 3500 Da, and ~2% exceeded 4000 Da.[40] The authors note that the analyses of the low MW preparations are at the limit of the sensitivity of the method used.

The experimental results support the hypothesis that a polypeptide must be at least 30 amino acids long to have the two IgE-binding epitopes required to elicit Type 1 hypersensitivity reactions.[38] The weight-average MW of the amino acids of wheat protein and wheat gluten is about 119 Da.[15] Thus, polypeptides from wheat protein or wheat gluten that are 30 amino acids long will have a weight-average MW of about 3570 Da.[15] It follows that polypeptides with weight-average MWs of 3500 Da or less do not have the properties required to induce Type 1 hypersensitivity.[15,38]

Phototoxicity

No published phototoxicity studies on HWP and HWG were identified by a literature search for these ingredients and no unpublished data were submitted.

CASE STUDIES

A case of WDEIA in a non-atopic 40-year-old woman was reported in Japan.[8] The patient developed facial wheals and nasal discharge while using an HWP- (Glupearl 19S) containing facial soap (*Cha no shizuku*) over the course of a year (HWP = 40,000 -50,000 Da). Additionally, she suffered multiple episodes of eyelid edema after eating bread or while working or walking during an 11-month period prior to diagnosis. Skin prick tests were positive with a solution of the soap or the HWP, but negative with wheat or bread. The patient also tested positive for WDEIA after ingesting wheat and aspirin together (aspirin, like exercise, is a well-known trigger of allergic reactions). SDS-PAGE and western blotting analyses showed that serum IgE from this patient reacted with

polypeptides, which ranged from 15,000 to 250,000 Da, in a HWP preparation and with both the water-soluble and water-insoluble fractions of UWP, but not with ω5-gliadin.

An additional 3 cases of WDEIA were reported by the same researchers in Japan.[41] The 3 female patients had used the same brand of soap that contained HWP (40,000-50,000 Da). Skin prick tests revealed positive reactions to a 0.1% solution of the soap in physiological saline and to 0.1% HWP in physiological saline. Western blotting of the patients' sera IgE yielded positive reactions with the HWP. The researchers concluded that WDEIA was attributable to cross reactivity to wheat protein induced by HWP exposures in these patients.

A 51- year-old Japanese woman had been using a facial soap containing HWP (40,000-50,000 Da) daily for several years.[42] Approximately 3 months after she started to use the soap, she began to develop angioedema on the eyelids and urticarial rash on the face. She experienced similar episodes many times over a 5-year period when eating wheat-containing food followed by mild exercise, with clinical signs limited to her face. Five years after her initial use of the soap containing HWP, she had an anaphylactic reaction after ingesting normal wheat products and was suspected of having WDEIA. She had no history of atopic dermatitis, food hypersensitivities, or dry skin. The patient developed eyelid angioedema, dyspnea, and a generalized urticarial rash on her entire upper extremity following a skin prick test with the HWP from the soap diluted 1:10,000. An IgE test for wheat and gluten yielded 0.36 UA/ml and 0.40 UA/ml, respectively. Serum ω-5 gliadin-specific IgE antibody titers were within normal limits. The patient did not have a mutation in human filaggrin (FLG), a defect that may disrupt skin barrier function.

In another case study, a 42-year-old woman reported an intense burning sensation over her face, neck, and scalp several hours after applying a moisturizing cream that contained HWP.[43] Specific chemical characteristics of the HWP were not provided. Patch testing with the diluted ingredients of the moisturizing cream resulted in a positive reaction (D2+, D4+) to 50% aq. HWP. No reactions were observed from skin prick testing to standardized wheat extract or contact-urticaria testing with HWP.

Contact urticaria was reported in a 46-year-old woman.[44] The patient developed the clinical signs after applying an eyelid cream and a body moisturizer that contained HWPs for 3 months prior to consulting her physician. Strong positive reactions were observed from the preserved food, wheat gluten that was in the food, the cosmetic creams, and HWP in open application tests and skin prick tests. Further investigation revealed that the HWPs in the cosmetic creams were from the same manufacturer as the gluten in the preserved food. Specific chemical characteristics of the HWP were not provided.

A 27-year-old woman was reported to have a pruritic, erythematous, urticarial rash that became increasingly more intense after subsequent use of a moisturizing body cream that contained HWP.[45] The wheat hydrolysate was not characterized in this study. Skin prick tests with common inhalant allergens, natural rubber latex, and cereal grains, including wheat, were negative. Also negative were the results of prick tests with a series of 21 protein allergens from plant and animal sources that included hen's egg, cow's milk, milk casein, almond, silk protein, aloe gel, papaya fruit, and hydrolyzed collagen. Total serum IgE was slightly elevated. The individual components of the body cream tested negative in an open application test, but a skin prick test was positive (8 mm) to HWP. Further IgE testing revealed that binding occurred specifically to wheat hydrolysate.

In another case study, a 64-year-old woman was reported to have itchy, erythematous, edematous lesions on the eyelids, face, and neck following use of a moisturizing cosmetic cream.[46] The patient was patch tested with the (GEIDC) standard and cosmetics series, the cosmetic cream, and the individual ingredients of the cream. Positive reactions (++) were observed to nickel sulfate, the cosmetic cream (tested neat), and to the HWP ingredient of the cream (10% aq.). Open testing with the HWP (10% aq.) was negative at 30 min. Specific chemical characteristics of the HWP were not provided.

A 23-year-old man with no history of atopy was reported to have a rash that occurred immediately after application of a face cream.[47] The rash included highly pruritic wheals on the face and neck accompanied by bilateral palpebral edema. Other systemic symptoms were not observed. The patient reported a similar reaction previously to a sunscreen and did not report food-induced symptoms or intolerance. A nonblinded skin test with the face cream was negative. Patch testing with the cosmetics True Test panel and the patient's own personal care products resulted in a positive (++) reaction to the patient's face cream at 48 and 96 h; all other readings were negative. Patch testing with the components of the face cream resulted in a positive (++) reaction to 1% HWP in water at 48 and 96 h. Testing in 10 control subjects yielded negative results. The patient underwent further prick tests with flours and cereals, with positive results reported for malt (5 x 4 mm), cereal mix (7 x 5 mm), oats (5 x 5 mm), and hydrolyzed wheat extract (18 x 14 mm). Total IgE was 136 U/ml (reference range = 1-100 U/ml). Results of specific IgE testing to buckwheat, rice, oats, barley, rye, corn, common millet, soy, and wheat were negative. Specific chemical characteristics of the HWP were not provided.

In a case study of a 3-year-old girl with a history of moderate atopic dermatitis, eczema-like skin eruptions were observed following use of an emollient containing HWP.[48] Scaly erythematous lesions were observed on her

knees. No evidence of contact urticaria was observed. Closed patch tests with the European standard series and the emollient were positive (+) for the emollient on days 2 and 3. Additional patch tests with the individual components of the emollient yielded positive results (++) for palmitoyl-HWP on days 2 and 3. Prick test, open test, and open patch test for palmitoyl-HWP were negative, as were prick test and radioallergosorbent test with wheat. Specific chemical characteristics of the HWP were not provided.

Two cases of reactions to HWP were reported in hairdressers.[49] In the first case, the patient, a 23-year-old female with no history of atopy who had been employed as a hairdresser for 2 years, developed watery rhinitis, conjunctivitis, dyspnea, angioedema of the eyelids, asthma-like symptoms at work, contact urticaria, and burning and tingling of the hands and soles when exercising after consumption of wheat-containing foods following long-term use of sprayable hair conditioner and another hairspray that contained laurimonium hydroxypropyl HWP. In the second case, the patient, a 22-year-old female with a history of atopic eczema who had been employed as a hairdresser for 6 months, developed urticarial wheals, work-related sneezing, nasal itching, watery rhinitis, and generalized urticarial and eyelid edema when exercising after consumption of wheat-containing foods following use of spray products also containing laurimonium hydroxypropyl HWP. The exercise-induced symptoms ceased after the second hairdresser switched to a grain-free diet. Skin prick tests with the common aeroallergen series and natural rubber latex were performed with standardized extracts, histamine hydrochloride, and diluent controls. Prick testing was also conducted with wheat, oat, barley and rye flours, gliadin, hair-bleaching agents, paraphenylenediamine, and the products containing HWP and the individual ingredients. Open skin applications tests were performed with the products containing HWP, and specific inhalation challenge or nasal provocation tests were performed with one of the products or the HWP ingredient.

Both patients had strong positive skin prick tests and urticarial reactions in the open skin tests to the products containing HWP. Of the ingredients in these products, laurdimonium hydroxypropyl HWP gave a strong positive reaction in the skin prick test while the remaining ingredients caused no reactions. Three atopic and 4 healthy volunteers were negative to the same HWP. Additionally, the patients were skin-prick-test negative to wheat flour, persulfate salts, and paraphenylenediamine. Occupational asthma was diagnosed in the first patient based on a specific inhalation challenge test with one of the products. This patient also had a rhinitis reaction with itching and marked watery rhinorrhea. In the second patient, nasal provocation with HWP caused marked rhinorrhea with swelling of nasal mucosa. Nasal provocation with HWP in 2 volunteers was negative.[49]

SUMMARY

HWG and HWP function primarily as skin and hair conditioning agents in personal care products. These protein derivatives are prepared by subjecting wheat proteins to acidic, enzymatic or other chemical, partial hydrolyses.

HWP has the most reported uses in cosmetic and personal care products, with a total of 1077; approximately half of those uses are in non-coloring hair products. HWG has 78 reported uses, with about half of the uses reported to be in hair tints.

In the Council's use concentration survey, HWP had a wide maximum use concentration range of 2.0×10^{-5} to 1.7%, with the 1.7% reported in rinse-off non-coloring hair products. HWG had a maximum use concentration range of 0.005% to 0.09%, with the 0.09% reported in eye makeup preparations.

The FDA determined the use of peptones as direct food substances are GRAS.

Ocular and dermal irritation studies of HWP found this ingredient not to be a significant irritant.

In a study of the transdermal pathway for sensitization to gluten and acid-hydrolyzed HWP (40, 000 - 50,000 Da) with and without SDS in BALB/c mice, the i.p. injection of HWP caused ASA, with decreased rectal temperatures, increased anaphylaxis scores, and increased plasma histamine levels. The i.p injection of gluten clearly induced ASA in the presence of SDS, but not in the absence of SDS. The content of HWP-specific IgE and IgG1 was significantly increased in the HWP groups with and without SDS and in the gluten-with-SDS group; IgE in the gluten–without-SDS group was barely increased. The serum content of gluten-specific IgE was significantly increased in the gluten-with-SDS group and both HWP groups, but barely increased in the gluten-without-SDS group. The serum content of gluten IgG1with and without SDS and HWP without SDS were also significantly increased, but there were individual differences in the gluten-without-SDS group that showed that SDS had an important role in sensitization by transdermal exposure. The secretion of IL-4, IL-5, and IL-10 was increased while that of IL-2 and interferon (IFN)-γ was significantly decreased, demonstrating that transdermal sensitization with HWP was associated with a T helper 2 response.

A HRIPT study of a 25% aq. solution of HWP (MW = 350 Da) concluded that this ingredient was not a dermal irritant during the induction phase or sensitizer during the challenge phase of the study.

Multiple cases of allergic reactions, including Type 1 immediate hypersensitivity reactions, were reported in individuals who had used personal care products that contained HWP, most of which were to a facial soap in Japan that contained HWP of 40,000-50,000 Da from acid hydrolysis of gluten at high temperatures. Several studies have been conducted to characterize the cause, manifestations, and mechanisms of these reactions, including tests of serum IgE binding and reactivity to wheat protein, wheat-protein fractions, and HWP and HWG prepared using acid- and/or enzyme-hydrolysis methods yielding products with varied polypeptide size profiles. Hydrolysates with weight-average MWs < 3000 Da exhibit no potential to elicit hypersensitivity reactions in sensitized individuals, in contrast to hydrolysates with weight-average MWs >30,000 Da. Experimental results support the hypothesis that polypeptides with weight-average MWs of 3500 Da or less do not have the potency required to induce Type 1 hypersensitivity.

DISCUSSION

The HWP and HWG ingredients discussed in this safety assessment are protein hydrolysates consisting of polypeptides with average MWs ranging from approximately 500 Da to greater than 30,000 Da, depending on the extent of the hydrolysis. The Panel reviewed data from a raw materials manufacturer and information presented by experts on the potential for exposures to HWP and HWG in cosmetic products to cause Type 1 immediate hypersensitivity reactions. Traditional human repeat insult patch tests (HRIPT) and related tests do not assess the ability of a substance to cause Type 1 reactions.

Production processes involving high-heat acid hydrolysis of wheat protein or wheat gluten can yield partially deamidated high-MW polypeptides with substantial potential to sensitize individuals through percutaneous and permucosal exposures, especially in formulations that contain surfactants. Studies have shown that hydrolysates with weight-average MW of approximately 3000 Da or less exhibit no potential to elicit hypersensitivity reactions in sensitized individuals, in contrast to hydrolysates with weight-average MWs >10,000 Da. Substantial experimental results support the theory that a polypeptide must be at least 30 amino acids long (i.e., MW about 3570 Da, assuming 119 Da/amino acid) to have the two IgE-binding epitopes needed to elicit Type 1 hypersensitivity reactions. Thus, polypeptides with MWs less than 3500 Da do not have the properties required to induce Type 1 hypersensitivity.

The Panel discussed the issue of incidental inhalation exposure to HWP or HWG in aerosol and pump hair spray products. There were no inhalation toxicity data identified or provided. HWP and HWG reportedly are used at concentrations up to 0.5% (HWP) in cosmetic products that may be aerosolized. The Panel noted that 95% – 99% of droplets/particles produced in cosmetic aerosols would not be respirable to any appreciable amount. Coupled with the small actual exposures expected in the breathing zone, the absence of the potential for polypeptides less than 3500 Da from HWP or HWG to induce sensitization, and the generally non-irritating nature of these ingredients, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at http://www.cir-safety.org/cir-findings.

The Panel also addressed concerns about pesticide residues and heavy metals that may be present in botanical ingredients. They emphasized that the cosmetics industry should continue to use the necessary procedures to limit these impurities in the ingredients before blending into cosmetic formulations.

CONCLUSION

The CIR Expert 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.

TABLES

Table 1. Definitions and functions of the ingredients in this safety assessment.[16] (The italicized text below represents additions made by CIR staff.)

Ingredient CAS No.	Definition	Function
Hydrolyzed Wheat Gluten 100684-25-1	Hydrolyzed Wheat Gluten is the *partial* hydrolysate of Triticum Vulgare (Wheat) Gluten derived by acid, enzyme or other method of hydrolysis.	Hair Conditioning Agent; Skin-Conditioning Agent-Misc.
Hydrolyzed Wheat Protein 70084-87-6 100209-50-5 222400-28-4	Hydrolyzed Wheat Protein is the *partial* hydrolysate of wheat protein derived by acid, enzyme or other method of hydrolysis.	Film formers; Hair Conditioning Agent; Skin-Conditioning Agent - Misc.

Table 2. Summary of information from suppliers of hydrolyzed wheat protein.*[50]

Source	Method of Manufacture	Molecular Weight	Nitrogen Content	Gluten Content
1 product defatted wheat germ	3 products enzyme hydrolysis	1 product average MW = 350 Da	1 product 12-15% nitrogen	1 product "gluten-free"
	1 product alkaline and enzyme hydrolysis	1 product average MW = 2200 Da		1 product < 100 ppm gluten
				1 product about 50 ppm gluten

* Information includes data summarized in Anonymous, 2012.[13]

Table 3. Frequency and concentration of use for hydrolyzed wheat gluten and hydrolyzed wheat protein according to duration and type of exposure.[17,18]

	Hydrolyzed Wheat Gluten		Hydrolyzed Wheat Protein	
	# of Uses	**Conc. of Use (%)**	**# of Uses**	**Conc. of Use (%)**
Totals[1]	**75**	**0.005-0.09**	**1069**	**0.00002-1.7**
Duration of Use				
Leave-On	11	0.005-0.09	519	0.00006-1
Rinse-Off	61	0.005-0.01	542	0.00002-1.7
Diluted for (Bath) Use	3	NR	8	0.00002
Exposure Type				
Eye Area	1	0.09	60	0.01-0.9
Incidental Ingestion	NR	NR	20	0.008-0.03
Incidental Inhalation-Spray?[2,6]	6	0.005	287	0.00006-0.4
Reported Spray[3]	NR	NR	NR	0.0003-0.5[a]
Incidental Inhalation-Powder?[4,6]	7	NR	150	0.00006
Reported Powder[5]	NR	NR	NR	0.05
Dermal Contact	21	0.01-0.09	384	0.00002-1
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	16	0.005	522	0.003-1.7
Hair-Coloring	38	NR	91	0.002-0.3
Nail	NR	NR	30	0.002-0.04
Mucous Membrane	12	NR	122	0.00002-0.1
Baby Products	3	NR	2	NR

NR = Not reported

1. 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.
2. It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.
3. Use in a spray product has been reported in response to a survey conducted by the Council.
4. It is possible these products may be powders, but it is not specified whether the reported uses are powders.
5. Use in a powder product has been reported in response to a survey conducted by the Council.
6. Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.

a. 0.03-0.05% in aerosol hair sprays; 0.0003-0.5% in pump hair sprays; and 0.002-0.02% in spray tonics, dressings, and other hair grooming aids.

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