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

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The 2014 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 report was prepared by Christina Burnett, Scientific Analyst/Writer, Bart Heldreth, Ph.D., Chemist CIR, and Ivan Boyer, Ph.D., Toxicologist CIR.

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Memorandum

To:	CIR Expert Panel Members and Liaisons
From:	Christina L. Burnett, Scientific Writer/Analyst
	Ivan Boyer, Senior Toxicologist
Date:	February 21, 2014
Subject:	Draft Final Safety Assessment of Hydrolyzed Wheat Protein and Hydrolyzed Wheat Gluten

At the September 2013 meeting, the Panel issued a tentative safety assessment of hydrolyzed wheat gluten and hydrolyzed wheat protein with the conclusion that these ingredients are safe for use in cosmetics when formulated to minimize peptide lengths greater than 30 amino acids. Additionally, these ingredients should not be used on damaged skin or in products that may come into contact with mucous membranes or may be incidentally inhaled.

The Panel asked that the cosmetics industry continue to provide additional data on manufacturing practices, characterization methods, and composition, including peptide size distributions, to enable better characterization of the nature and variability of these ingredients as used in cosmetic products and to enable the Panel to refine its conclusion. Since the September meeting, no additional new unpublished data were received.

Comments received from the Council prior to the September meeting and on the tentative safety assessment have been considered. These comments are in the panel book package for your review. The Council requested that information on wheat gluten (non-hydrolyzed) be incorporated into this report and that the Panel consider a re-review of the safety assessment of Triticum Vulgare (wheat) protein and Triticum Vulgare (wheat) germ protein.

CIR staff disagrees with the Council's suggestion that the Panel's conclusion for hydrolyzed wheat protein and hydrolyzed wheat gluten indicates that a re-review may be warranted for wheat protein and wheat germ protein, as used in cosmetics. This is because hydrolysis, particularly acid hydrolysis, can be expected to yield products that are significantly different in their chemical properties and potential bioavailability and bioactivity from the starting material (i.e., "whole" or "intact" wheat protein or gluten prepared by extraction methods that typically would be mild compared to hydrolysis). These products will likely differ from the relatively "intact" or "whole" proteins from which they were derived, in water solubility, degree of deamidation, molecular weights, and other properties. Such differences are reflected, for example, in the extensive spreading in electrophoresis gels of the products of hydrolysis, compared with the "whole" or "intact" proteins of the corresponding starting materials in the gels.

Further, the current evidence indicates that the potential for developing wheat-dependent exercise-induced type 1 hypersensitivity through percutaneous and rhinoconjuctival exposures is associated specifically with the use of hydrolyzed wheat protein or hydrolyzed wheat gluten, not with wheat protein and wheat germ protein, in cosmetics or other personal care products. There is no evidence that we could find that topical application of "whole" or "intact" wheat protein or gluten preparations can sensitize people, or elicit hypersensitivity responses from people already sensitized to "whole" or "intact" wheat protein or hydrolyzed wheat gluten. In contrast, all of the available evidence indicates that hydrolyzed wheat protein or hydrolyzed wheat gluten preparations, particularly those with substantial fractions of products having relatively "high" molecular weights (e.g., enzyme hydrolysis products with MW >1050 Da, in one study) appear to have non-negligible capacities to sensitize and elicit hypersensitivity responses from people.

Per the Panel's request for expert insight on reports of type 1 hypersensitivity reactions to hydrolyzed wheat protein/gluten through percutaneous/rhinoconjunctival exposure, two speakers have been scheduled to speak prior to the team deliberations: Dr. Dass Chahal, Research & Technology Director of Sun Care & Biotechnology, Croda Europe, Ltd. and Dr. Kayoko Matsunaga, Professor and Chairperson of the Department of Dermatology at Fujita Health University School of

Medicine. Following the presentations and Q&A of the speakers, the Panel should carefully review the abstract and discussion, and determine if the conclusion is appropriate. The Panel should issue a Final Safety Assessment.

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Hydrolyzed Wheat Protein and Hydrolyzed Wheat Gluten History

May 2012 – Scientific Literature Reviews announced separately for Source Amino Acids and Hydrolyzed Source Proteins.

December 2012 - The CIR Expert Panel combined the 2 reports into 1 and retitled it "plant- and animal-derived amino acids and hydrolyzed proteins". The Panel also removed the ingredient hydrolyzed spinal protein from review as it is a prohibited ingredient. The Panel requested additional data to support the safety of 75 plant- and animal-derived amino acids and hydrolyzed proteins. The additional data needed are: (1) method of manufacturing data for both plant and animal-derived amino acids and hydrolyzed wheat protein; and (2) composition and characterization specifications of plant and animal-derived amino acids and hydrolyzed proteins, including molecular structure and molecular weight ranges from several suppliers to determine if there is a consistency in cosmetic grade plant and animal-derived hydrolyzed proteins, especially hydrolyzed wheat protein.

March 2013 and Post Meeting – The Expert Panel tabled further discussion on animaland plant-derived hydrolyzed proteins to allow CIR staff to reorganize the report and to analyze further data from Japan regarding Type 1 allergic reactions to hydrolyzed wheat protein in a soap product. The staff has decided to group hydrolyzed wheat protein and hydrolyzed wheat gluten in one report in order to facilitate consideration of the concern about hydrolyzed wheat protein in Japan and not dilute the evaluation with other unrelated ingredients. The review of the other animal- and plant-derived hydrolyzed proteins will be performed sometime in the future. Prior to being tabled, the Panel had issued an insufficient data announcement with the following data needs: (1) method of manufacturing data for hydrolyzed wheat protein; and (2) composition and characterization specifications of hydrolyzed wheat protein, including molecular structure and molecular weight ranges from several suppliers to determine if there is a consistency in cosmetic grade hydrolyzed wheat protein.

September 2013 – The Expert Panel issued a tentative safety assessment of hydrolyzed wheat gluten and hydrolyzed wheat protein with the conclusion that these ingredients are safe for use in cosmetics when formulated to minimize peptide lengths greater than 30 amino acids. Additionally, these ingredients should not be used on damaged skin or in products that may come into contact with mucous membranes or may be incidentally inhaled. The Expert Panel asked that the cosmetics industry continue to provide additional data on manufacturing practices, characterization methods, and composition, including peptide size distributions, to enable better characterization of the nature and variability of these ingredients as used in cosmetic products and to enable the Expert Panel to refine its conclusion.

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Hydrolyzed Wheat Protein and Hydrolyzed Wheat Gluten Data Profile* – March 2014							
	Writer, Christina Burnett						
Reported Use Reported Use Composition/ Impurities Manufacturing Manufacturing Animal Animal Case Studies Case Studies							Case Studies
Hydrolyzed Wheat Gluten	Х						
Hydrolyzed Wheat Protein	Х	Х	Х	Х	Х	Х	Х

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

Search Strategy for Hydrolyzed Wheat Protein & Hydrolyzed Wheat Gluten (Performed by Christina Burnett and Ivan Boyer)

<u>February-April 2012</u>: SCIFINDER search for Hydrolyzed Proteins (55 substances, searched under INCI names and CAS No.):

Initial search for "adverse effect, including toxicity" yielded 18 references.

Also performed searches using the following search terms (no limits for reference type): "Hydrolyzed Proteins in Cosmetics" (yield = 30 references); "Skin Sensitization – Hydrolyzed Proteins" (yield = 1 reference); "Bioactive Peptides - Cosmetics" (yield = 18 references); "Skin Irritation – Polypeptides" (yield = 23 references); "Skin Sensitization – Polypeptides" (yield = 11 references); "Biogenic Peptides" (yield = 1 reference); "Bioactive Peptides – Toxicity" (yield = 28 references); "Bioactive Peptides – Skin Irritation" (yield = 1 reference); "Hydrolyzed Proteins Chicken Cells in vitro" (yield = 1 reference); and "Hydrolyzed Protein Irritation" (yield = 7 references).

Many of the references were patents or efficacy reports.

13 references were ordered.

Search updated January 18, 2013.

June-July: SCIFINDER and PubMed search for Hydrolyzed Wheat and CAS Nos. 100684-25-1, 70084-87-6, 100209-50-5, and 222400-28-4

Additional 9 references were ordered.

Search updated January 22, 2014

Additional 2 references were ordered, only 1 was incorporated into the report.

September 9-10, 2013 CIR Expert Panel Meeting

Belsito's Team

DR. BELSITO: Hydrolyzed wheat protein. Okay. That's another one I did on paper. Is this yours, too, Christina?

MS. BURNETT: Yes.

DR. BELSITO: Okay. So you know, I looked at this and I think that really all the issues that we're going to have with these hydrolyzed proteins can be resolved if we say that they have to be less than 30 kilodaltons or less than or equal to 30 amino acids long because at that level they don't seem to bind IgE, which is the major issue and the thing that was the issue in Japan. And then I guess the next issue is should these -- even without restrictions, should they be put on damaged skin where they could get absorbed and would they theoretically sensitize to wheat or gluten? And there was some data in here and it was in rodents where they were actually able to sensitize individuals to wheat. Or was it the Japanese human studies? I can't remember.

MS. BURNETT: There was a sensitization study 24 (inaudible).

DR. BELSITO: Yeah. The dermal --

MR. BOYER: What they are observing in human subjects in both Japan and Europe, but particularly in Japan there's people who are exposed to hydrolyzed wheat protein ingredients through the conjunctiva and through the sinuses and so forth. They apparently can develop sensitization to hydrolyzed wheat protein.

DR. BELSITO: Yeah. And that was the eye drop challenge of one patient that we had.

But yeah, this was a dermal, nonhuman sensitization in tape-stripped mice where they were able to sensitize the hydrolyzed wheat protein. Now, we don't know the molecular weight so, I mean, it's not clear that these were less than 30 kilodaltons or less than 30 amino acids, but I thought, you know, as we originally did, I believe with parabens about not to be used on damaged skin, that that might be a caveat. The other question would be inhalation because here you would not only worry that it would get down to the alveoli; the real inhalation problem would be in the nasopharyngeal area. I thought that we could go safe, not to be used on damaged skin, not to be used in products that could be inhaled, limit to less than 30 kilodaltons, and something the Council raised that I would agree with, a bold label on packaging of any of these hydrolyzed proteins indicating warning, this product contains wheat, gluten, whatever. But that was my thoughts anyway.

DR. LIEBLER: So I basically agree with that. And the only modification I'd make, Don, is about the exclusion of peptides of greater than 30 amino acids. Practically speaking, it's going to be nearly impossible for them to totally exclude that or to know whether they have. So I would change that to say that the mixture should be prepared to minimize components of greater than 30 amino acids in length. It's just like minimizing any other contaminant.

DR. BELSITO: So you're going to say minimize --

DR. LIEBLER: Minimize --

DR. BELSITO: -- peptides greater than 30 amino acids?

DR. LIEBLER: Right.

DR. BELSITO: What about weight? Nothing about weight? Just the amino acid?

DR. LIEBLER: You could say either. Thirty amino acids is a ballpark number that relates in the way you just mentioned to IgE activation. So you could either say 30 amino acids or 30 times 150 to give you whatever kilodaltons that is. But it's easier just to say 30 amino acids or approximately 30 amino acids.

You know, the other point I had, and this is more speculation, but since these issues with the soap in Japan continue to hydrolyze wheat protein involved a soap, it's possible that the issue wasn't necessarily that it had some bad wheat hydrolysate in it, but it might have been the way it was formulated with the other ingredients of soap that made an otherwise relatively innocuous -- a preparation that would have been innocuous in another type of product cause a problem in this product. So perhaps a caution to formulate these to be nonsensitizing or maybe you're coming at the same issue with not to be used in damaged skin.

DR. SNYDER: But I do have a statement that says, "Surfactants in soap facilitate the dermal penetration."

DR. LIEBLER: Yeah. That's what I was thinking. That's what made me think about it. It might have been the way that the wheat proteins were formulated into a product that contains some other things that would have increased the likelihood that they would cause a problem.

DR. BELSITO: But the greatest use of these are in shampoos which are going to have surfactants.

DR. LIEBLER: Right. Yeah.

DR. BELSITO: So if we say not to be used in surfactants, we're going to eliminate their use essentially.

DR. LIEBLER: No, no, I'm not saying that but maybe to be formulated to be nonsensitizing.

DR. BELSITO: Yeah, that's I think too vague. I mean, I think we have data that shows if there's less than 30 amino acids --

DR. LIEBLER: Sure.

DR. BELSITO: -- these aren't an issue and it's a better way of going about it. And then if we say not to be used in products, you know, not intended for use in damaged skin, products that could be inhaled, I think we eliminate, and I guess what Ivan was saying is probably products that would be applied to the eye.

MR. BOYER: Actually, there is facial soap in which apparently people wash their eyelids and so forth with and that contact with the conjunctiva --

DR. SNYDER: (Inaudible) body moisturizer.

MR. BOYER: It seems surface (inaudible) to some extent why some people are getting sensitized.

DR. BELSITO: So not on damaged skin or products intended for application to the eye?

MR. BOYER: That could work.

MR. ANSELL: It would have to be eye area.

DR. BELSITO: Yes.

MR. ANSELL: Cosmetics would not.

DR. BELSITO: Eyelid, eye area.

DR. SNYDER: But then does your size restriction eliminate?

MR. ANSELL: Well, that's kind of, you know, we have good data on the molecular weight.

I also know from our PEG experience that the damaged skin can be very confusing to the community because it doesn't really go to the extent of damage. Does that mean sunburn, abraded, atopic? I'm not sure that the molecular weight distinction -- I mean, how damaged is damaged skin that we'd be worried about main materials which qualified based on the molecular weight?

DR. BELSITO: Well, the studies in the animals were 10 tape strippings. And if I recollect correctly, from the presentation that we had when we did damaged skin, the tape strippings is about what atopic skin is like; was that right?

Because they showed that the parabens weren't an issue on 10 tape-stripped skin and that's why they were when they were put on third degree burns.

But here, 10 tape strippings did allow sensitization of those animals. So basically, damaged skin here is impairment of the stratum corneum because that's all you did with tape stripping. You really don't take much of the living epidermis away. So I think that, yeah, I guess damage is subject to interpretation. You can say, oh, that's a third degree transdermal burn. I don't know how better to say it, you know. Areas deficient in the stratum corneum?

That takes care of all the mucous membranes, right? We don't have to specify eye areas. But that doesn't help the consuming public. Not to be used on eczematous skin? You know --

DR. SNYDER: Where the stratum corneum is not intact or something. They wouldn't know that. I mean, you have to give them some reference of some clinical entity; right? Is that what you're trying to do? Come up with some --

DR. BELSITO: Well, I mean, I guess these aren't guidelines for the public; they're guidelines to industry. So not to be used in products intended for application to areas deficient in the stratum corneum.

DR. LIEBLER: I think damaged skin is okay to use. I mean, I think we're going to really tie ourselves in knots trying to come up with some language that encompasses more specifically the various possibilities for what could constitute damaged skin.

MR. ANSELL: Perhaps in the discussion if we were clear as to the underlying data which resulted in that recommendation.

DR. LIEBLER: Exactly, because we could point to the fact that this study with tape-stripped skin showed sensitization.

DR. BELSITO: Well, I'm just now looking as we're having this discussion. You know, really, it's mucosal applications as well. So is this used in any --

DR. SNYDER: Here.

DR. BELSITO: I'm getting there.

DR. SNYDER: Fifteen mucous membrane products, hydrolyzed wheat gluten and hydrolyzed wheat protein 113 up to 0.1 percent.

DR. BELSITO: Mucous membrane?

DR. SNYDER: Yeah.

DR. BELSITO: It's huge, 118 products total.

MS. BURNETT: That's mostly personal cleanliness products. Bubble bath.

DR. SNYDER: Well, I think if we frame it correctly and the main issue is sensitization, basically peptide contaminants and that they should limit the amount of peptide contamination of (inaudible).

DR. BELSITO: Yeah, but what we know is that less than 30 amino acids won't trigger IgE release. It won't bind to mast cell receptors to trigger IgE release. We don't know whether – we don't know what the hydrolyzed wheat protein that sensitized these mice -- what the composition of that is. So it's entirely possible that a 20 amino acid hydrolyzed product could cause sensitization to the whole molecule. We simply don't have that information. What we know is that if you're sensitized -- even if you're sensitized, less than 30 isn't going to trigger IgE. The converse, whether less than 30 triggers sensitization we don't know.

I think based upon what we have, I think we have to say it should not be used on damaged skin and in products that may contact mucous membranes. And if industry is concerned, if the manufacturers of those 118 products, you know, want to show us some data at a certain level or whatever there's no sensitization occurring with mucous membrane exposure, you know, we'll consider that. But at this point, I mean, the data clearly shows that you can get ocular sensitization and with tape stripping you can get sensitization.

Okay. So to recapitulate where we are, safe, minimize peptides greater than 30 amino acids in length, not on damaged skin or products intended or products intended for application to mucous membranes. Mascaras aren't intended to be applied there. Or products that may contact mucous membranes? Is that a good way of saying it? Not in products that could be inhaled. And then the question is do products that are going to contain these hydrolyzed proteins need a clear warning on them stating like foods do this contains gluten or this contains peanuts or this contains soy?

MR. ANSELL: I would recommend that the panel stay away from labeling. Labels and labeling really cross into a whole regulatory regime that would have to be considered within the context of existing FDA regulation. And --

DR. BELSITO: I thought that was an industry comment that you wanted that kind of label. Did I misread that?

MS. GILL: I thought that was, too.

MR. ANSELL: I can't find that comment.

MR. BOYER: It's wave two and it was from a member.

DR. LIEBLER: Oh, that was the hodge-podge of comments on hydrolyzed proteins.

DR. BELSITO: Right. Yeah.

DR. LIEBLER: Yeah, so I actually am cautious about that for an additional reason. In addition to the point Jay made, which I think are perfectly valid, but I think the additional reason is that once you've digested these down, they're not really gluten or wheat anymore. They're short sequences that have sequence identity to wheat proteins but they're derived from wheat proteins, but it's not like saying it contains wheat or it contains gluten anymore because it really doesn't.

These things are no longer really wheat. They're no longer really gluten.

DR. BELSITO: I will tell you that as a chronic label reader by virtue of what I do, I increasingly see products, for instance, shea butter, you know, sort of surprised me, should not be used by people with tree nut allergy. Or things that have peanut oil should not be used by individuals with known sensitivity to peanuts.

You're seeing that on cosmetic labels already. I'm fine not going the labeling route and let manufactures do what they want to do, but actually, it was an industry point that was brought up about labeling.

MR. ANSELL: Yeah, did it come from us or was it a company?

DR. BELSITO: Yes. It's here. "Please see below for some comments to feedback to the CIR regarding the draft assessment and the use of animal- and plant-derived amino acids." Point 4.

MR. ANSELL: Yeah, from a member company.

MS. BURNETT: Yeah, it wasn't you. It was another company that passed along (inaudible).

MR. BOYER: The other thing to consider, too, is when you're breaking down and when you're preparing hydrolyzed wheat protein, what you may be left with are peptides that are long enough to still represent epitopes. In fact, the research seems to indicate that the epitopes in those hydrozolates are very similar. They react in a very similar manner to the epitopes that you find in intact proteins and intact gluten and so forth.

And in fact, when you break up proteins, you can end up with a mixture. You can end up with a mixture of fairly large polypeptides that can then intermingle. They can basically aggregate and so forth so you end up with epitopes.

DR. LIEBLER: So my interpretation of this, because I did note those same concerns, my interpretation of those studies is that they're all done with hydrolyzed products. Right?

MR. BOYER: I think so.

DR. LIEBLER: They're all done with hydrolyzed products. So the thing that you can't know in that case is whether or not there is residual unhydrolyzed material that produced the observed effect as opposed to attributing it to the hydrolyzed material. And the only way to do that experiment right would be to actually synthesize the shorter pieces and determine with purified synthetic shorter pieces whether or not they produced these effects or not. That would be the right experiment to do. And I don't think that was done. I think it was these hydrolysates that were incompletely characterized in the conclusions. And if I got that as a paper to review that is what I would say as a reviewer. They cannot conclude that these shorter products actually have the biological activities that they attribute to it because they cannot exclude the residual presence of longer products.

MR. BOYER: That's very true. In fact, all of these are brought up from a point of view of a hypothesis.

DR. LIEBLER: Right.

DR. BELSITO: Okay. And the only other comment that I had, Christina, is on page 24 of the PDF. It says a 25 percent aqueous solution. Now, we were told in the beginning that that's how these are supplied, as a 25 percent aqueous solution. So is this 25 percent of 25 percent? Or is this the actual product that is provided to companies to blend into their products?

MS. BURNETT: It would probably be seen as 25 percent solution.

DR. BELSITO: So it's what's actually supplied by the manufactures to companies to then blend in. So it's actually 25 percent, not one-fourth of 25 percent?

MS. BURNETT: Right. I believe so.

DR. BELSITO: Okay.

MS. BURNETT: Without having the data. Yep.

DR. BELSITO: Okay.

DR. SNYDER: So --

DR. BELSITO: So I just put a note to check that. Otherwise, I really didn't have anything. Yeah. No, I think that they're probably what is actually supplied by the manufacturers of hydrolyzed wheat protein because it says someplace in the document that it's – the final product under method of manufacturing. The final product is a 25 percent water solution of hydrolyzed wheat protein and then when you look at here they all say 25 percent aqueous solution.

DR. SNYDER: So (inaudible) it's well below the molecular rate we're saying; right?

DR. BELSITO: Well, it's about where we're setting it, yeah, because 350 kilodaltons was said to be equivalent to approximately 30 amino acids. So that's (inaudible) good too in a sense that it looks like where we want them to be.

DR. SNYDER: And that didn't list it anywhere (inaudible).

DR. BELSITO: Right.

DR. SNYDER: So that's more data to support that that is a good cutoff.

DR. LIEBLER: Wait a second. Thirty amino acids times an average of 150 molecular weight is 4,500. So 4.5 kilodaltons would be your 30 amino acid average cutoff.

DR. SNYDER: (Inaudible) study data that said the polypeptides less than 30 kD could not (inaudible).

DR. BELSITO: Right.

DR. LIEBLER: So I think we should --

DR. SNYDER: Thirty amino acids or 30 --

DR. LIEBLER: Thirty kDs.

DR. BELSITO: They said 30 kD and then they also said less than or equal to 30 amino acids.

DR. LIEBLER: Well, those are very different numbers.

DR. BELSITO: Okay.

DR. LIEBLER: These are very different size molecules you're talking about. Thirty kD, you know, is 30,000.

DR. SNYDER: Right.

DR. LIEBLER: Divided by 150.

DR. SNYDER: Is 20.

DR. LIEBLER: Equals 200 amino acids. Thirty thousand molecular weight divided by 150 is 200.

DR. BELSITO: So that's why amino acids is probably a better number to go with.

DR. LIEBLER: Yeah. That's why I was saying -- and the other thing about that, 30 amino acids is 30 amino acids. If you guess a kilodalton weight, then it depends on what number you assume for the average amino acid weight and

so on. If we decide to use 30 amino acids, we can just use that. If we decide to use a kilodalton equivalent of 30 amino acids it would be 30 times 150, which would be 4.5 kilodaltons.

DR. BELSITO: I like 30 amino acids.

DR. LIEBLER: Yeah.

DR. SNYDER: But it still appeared to me that the ingredient they're using in formulation is well below that.

DR. BELSITO: Well, it's about -- yeah, it's slightly less than 30 amino acids.

DR. LIEBLER: Yeah, that's true. The range is about 5 to about 30 amino acids for these.

DR. SNYDER: So maybe we need to expand the discussion about the manufacturer or the user.

DR. BELSITO: Well, we just point to those studies where at a molecular weight of 350 there were no issues supporting our view that these products should be minimized amino acids greater than -- or peptides greater than 30 amino --

DR. LIEBLER: Right. This is actually -- I put a note in my copy here to Christina – is to suggest that you actually start the discussion with the nature of -- describing the nature of the ingredients that compared to amino acids that these are mixtures of polypeptides ranging from about 4 amino acids to about 30 amino acids in length. Or I'm sorry, about 4 amino acids to up to 200 amino acids in length with a median size of X. And that's the only thing that --

DR. SNYDER: We have that in here.

DR. LIEBLER: That's the Table 2?

DR. SNYDER: No, this is under the chemistry with the average micro weight of amino acids 135 daltons and they range from 4 to 220 amino acids in length.

MS. BURNETT: You're saying put that in the discussion?

DR. LIEBLER: Yeah, I'd say explain that up front so that the reader understands that these are mixtures of peptides of varying lengths but the range is predominately X to Y and the median is approximately Z. And go on to explain that peptides at more than 30 amino acids can participate in type 1 hypersensitivity reactions by cross linking IgEs. Then you can go right into the stuff that you have as the opening text which is the safety of amino acids.

At the end of the current discussion there is the additional data needs on method and manufacturing data and composition and characteristics. I felt that even though what we got was not extensive, the material in table 2 pretty much answers the question for me. So the hydrolyzed wheat proteins are produced by both enzymatic and acid or base hydrolysis. The composition is documented even though it is not for a lot of batches or products. So I would say that my concerns about the lack of information on those two points have been satisfied.

DR. BELSITO: Well, we're specifying now. We don't care how it's manufactured, whether you hydrolyze it or do it enzymatically or however you want it. It should be done to minimize amino acids (inaudible) peptide not integrated in 30 amino acids.

DR. LIEBLER: No, I agree with that. It's just that we didn't have enough information to have an adequate method of manufacture section before and now we do.

MS. GILL: So, Dan, you're suggesting that that last paragraph before the conclusion can come out?

DR. LIEBLER: Yes.

DR. BELSITO: Well, we're deleting -- yeah, the panel requested additional data.

DR. LIEBLER: Right.

DR. BELSITO: We're deleting all of that?

DR. LIEBLER: Correct. Because of size restriction.

DR. SNYDER: Take all that out.

DR. BELSITO: Okay. Well, in the section above where we talk about type 1 and median hypersensitivity can possibly occur following exposure to protein-derived ingredients on tape -- I think we need to add on tape-stripped skin and mucous membranes. And something -- therefore, the panel felt that these products should not -- that hydrolyzed wheat protein and hydrolyzed gluten should not be used in products that may be inhaled or incidentally contact mucous membranes.

DR. SNYDER: So where are we going with this? Are we backing ourselves into a hole here with regards to -- if we had data that says the starting point for the ingredient used in products is below the sensitization level, and we clearly understand what that level is, I'm not certain -- we're on a slippery slope here because can't we get to -- can they get to a level where they're going to be safely used if they don't do that?

DR. BELSITO: Well --

DR. SNYDER: These are going to be safe as long as the hydrolysis is complete enough to minimize the composition of longer than 30 amino acids.

DR. BELSITO: What they would need to do though, you know, quite honestly, to show us that that, in fact, is the case is repeat the mouse tape-stripped studies with the products that --

DR. SNYDER: The 30 molecular weight? 350 molecular weight?

DR. BELSITO: Was clipped, abraded, and occluded. That was irritation. Nonirritating human irritation patch test, ocular. But they didn't look at sensitization. When they looked at sensitization in the next study they tape stripped, so it wasn't irritant on abraded skin but they didn't look long term. So that doesn't really help us. And it doesn't say in this mouse study with tape stripping what they actually used. It just says hydrolyzed wheat protein.

DR. SNYDER: Can we get more information on that? What was specifically used?

MS. BURNETT: I can pull up the study.

DR. BELSITO: I mean, and then you go on and they do this study -- protein hydrolysates in hair care products, three groups of patients. And you look, boom -- 11 hair dressers with hand dermatitis and you get --

DR. SNYDER: Then we go into hydrolyzed collagen and hydrolyzed milk. No reaction to the hydrolyzed wheat hydrolysates were observed.

DR. BELSITO: But you don't know what those individuals were exposed to. They may simply not have been exposed to hydrolyzed wheat. And you don't know why you picked them out. I mean, because hydrolyzed milk protein is used in a lot of hair care products as well.

MS. BURNETT: Mean (inaudible) was 40 or 50 kilodaltons.

DR. SNYDER: Yeah, so it's above.

DR. BELSITO: What?

DR. SNYDER: It was above the sensitized --

MS. BURNETT: Forty to 50 kilodaltons in the mouse study.

DR. BELSITO: So it's above the level we're imposing but we don't know whether below that level would have been negative. So I mean, I think that we can reach a conclusion. And then if industry is concerned they can repeat the mouse study with what we're recommending. A 350 kilodalton product and do it on mucous membranes and tape-stripped skin and show us it doesn't sensitize.

DR. SNYDER: Right. And that's where we're stuck. We know that less than 30 can't trigger the type 1 reaction but we don't know whether --

DR. BELSITO: Yeah, but you could figure that out in the study. You could try and sensitize the --

DR. SNYDER: Yeah, but we don't have the data now.

DR. BELSITO: Right. We don't have the data that tells us.

DR. SNYDER: Right.

DR. BELSITO: Right, but you could easily do the study. I don't know how much money it's going to cost, number one. Number two, the issue is in Europe now you can't market a product as a cosmetic product that's been tested on animals. And I don't know that there's any other use for hydrolyzed proteins other than in cosmetics. If there is you can get away with it. If there isn't, you can't. You're stuck.

DR. SNYDER: And if we had the data then all the other things would go away because we wouldn't have to worry about mucous membrane.

DR. BELSITO: Right. Exactly. If you give me data on tape-stripped skin I'd get rid of the mucous membrane. I'd get rid of the aerosol. And go back to the prior thing that people with known sensitivity shouldn't use it but --

DR. SNYDER: I like that better than where we were going.

DR. BELSITO: Well, but I don't think we have a choice; do you? I mean, how can we say? We have data that shows that you can sensitize. Granted, it's with a 45, 40 kilodalton product. But we don't know that less than or equal or 30 amino acids won't do the same thing. We don't have that data.

MR. ANSELL: So the issue is not elicitation but rather whether it can be induced?

DR. BELSITO: Right.

MR. ANSELL: So don't (inaudible) studies ask for addressing the question of adoption?

DR. BELSITO: I understand, but you're certainly not going to try to induce humans. I mean, human repeated insult patch testing is not for determination of a hazard. It's to confirm safety. And Europe already has a problem with human testing to begin with. I'm not quite sure -- in silico is where they're heading but I think we're stuck with it.

MR. BOYER: It's possible, too, the industry could use some simple in vitro biochemical studies, IgE and so forth, isolated to see whether or not or to determine some cutoffs as to where you're likely to get a sensitization response.

DR. BELSITO: I mean, maybe there is data out in the literature that you can sensitize with a protein of a certain -smaller than a certain peptide. That certainly would also be a way of answering a question if the data is out there already. We haven't searched for it.

MR. ANSELL: And I guess that's my point is that it would be desirable for the panel to iterate a specific question as opposed to also defining what the answer is or how the answer would be determined.

DR. BELSITO: So peptides of bigger or smaller amino acids do not induce type 1hypersensitivity?

MR. ANSELL: So it's insufficient for protein size that induces sensitization?

DR. BELSITO: Yes.

DR. SNYDER: That's probably the first step we should go to rather than all that other stuff about --

MS. GILL: Or is it safe with a limitation?

DR. BELSITO: What?

DR. SNYDER: We don't know that limitation. That's a whole other step. We know the limitations for elicitation but we know the limitation for sensitization.

DR. BELSITO: Okay, so basically where we're at is safe if the hydrolyzed, rehydrolyzed gluten peptides are manufactured in a way to minimize amino acid chain lengths greater than 30. At this point pending data that shows that peptide lengths less than or equal to 30 can or cannot induce sensitization, our recommendation would be not on damaged skin or products that may contact mucous membranes. And not on products that could be inhaled.

DR. LIEBLER: Fine.

DR. SNYDER: We can't do the safe thing yet. We don't know what the size limit is for sensitization, right?

DR. BELSITO: But if we say not to be used on damaged skin, contact mucous membranes or inhaled then we're not worried about that. The sensitization occurred on tape stripped skin and on mucous membranes. So basically, you know, proteins get across in tox stratum corneum. So I'm not so concerned about normal skin.

DR. SNYDER: But in this human study, this hair dresser, hair care, what was that? Those were not damaged skin were they?

DR. BELSITO: Well, if you're a hair dresser you de facto have damaged skin unless you're very elite individual because they're shampooing and hair and cutting wet hair eight hours a day. Plus they had active dermatitis. They have hand dermatitis so they were clearly --

MS. LORETZ: But the damaged skin, going back to the PEG's report when we did the study to look at that and we ended up putting a qualifier on it just because our concern at the start was damaged skin could mean dry skin versus third degree burns. Is there a thought of further defining that or is that later down the road?

DR. BELSITO: Well, in this case, you know, what we got for the PEG's report was simply that when they taped stripped skin it wasn't an issue in terms of absorption that those clinical case studies were due to the fact that it was third degree burns. And essentially you were just -- you might as well be giving the stuff intravenously.

In this case it's ten tape stripped skins which from the presentation that we were given is about the equivalent of patients with atopic dermatitis. And so, I think just saying damaged skin here, I mean I agree with Dan. You know

or you could say skin lacking stratum, an intact stratum corneum barrier including mucous membranes. I don't care how you want to phrase it.

MR. ANSELL: I thought we had discussed that perhaps a more robust discussion in the discussion might be the way to resolve that. That the damaged skin caution arose from data on tape stripped.

DR. LIEBLER: Right. So a little more detailed but not an awful lot.

DR. BELSITO: Right.

DR. LIEBLER: Fair enough?

MR. ANSELL: Sounds great.

DR. LIEBLER: Good.

DR. BELSITO: We'll see what the other group says here.

DR. LIEBLER: All right.

DR. BELSITO: Okeydokey. I think we've chomped that to death.

Marks' Team

DR. MARKS: Okay. The hydrolyzed wheat protein -- where is that on here, is that under hydrolyzed -- yeah, here we go. So, at the March 2013 meeting, we tabled it? The discussion of these, we wanted more information concerning the reports, particularly from Japan of Type 1 allergic reactions. These would be anaphylactictype reactions to the hydrolyzed wheat protein in soap products, so we issued an insufficient data announcement on: One; method of manufacturing data for the hydrolyzed wheat protein; and, two, composition and characterization. Also from the minutes, we decided to split out wheat from other plant proteins such as soy or silk, so it looks like we're going to proceed with doing the plant proteins individually. So, in this report, on the hydrolyzed wheat protein and the hydrolyzed wheat gluten, Rons and Tom, what are the needs? My first question is what's the difference between wheat protein and wheat gluten? Was that mentioned in this report?

DR. BERGFELD: Somewhere, I think.

DR. MARKS: I didn't get a good sense. And why --

DR. SHANK: Wheat gluten would be a protein, and wheat proteins would be a mixture of proteins.

DR. MARKS: Right. So is wheat gluten one protein.

DR. SHANK: That's how I read it.

DR. MARKS: Okay. So, Tom, Rons, needs at that point? The manufacture still, the composition? Method and manufacture, the composition?

MS. EISENMANN: There is some information on, in Table 2. That's what I presume is the ingredient that the industry would like you to assess, the information in Table 2, plus there's information on what the protein, hydrolyzed protein that was causing problems in Japan, with a 40 to 50 kD protein produced by acid hydrolysis over a certain length of time. So you have that information on a bad actor, and then you have the information on the protein from certain suppliers. I think you should assess the safety of the protein that's listed in Table 2.

DR. SLAGA: So we don't need anything.

DR. MARKS: Table 2, what page is that?

MS. BURNETT: Page 30.

DR. MARKS: Pardon?

DR. SLAGA: Page 30.

DR. BRESLAWEC: So I think the point is that we'd like you to focus on size as opposed to specific product.

DR. MARKS: Size. What do you mean by that?

DR. BRESLAWEC: The protein.

DR. SLAGA: Molecular weight?

DR. BRESLAWEC: Molecular --

DR. SLAGA: Range?

DR. BRESLAWEC: Yes.

MS. EISENMANN: Yes.

DR. MARKS: So I see, so, Tom and Rons, does Table 1 suffice for manufacturing? Is that what you're saying, Carol?

MS. BURNETT: Table 2, it's the Table right under it.

DR. MARKS: Table 2. Oh, yeah, here we go. Was that also in written form in the body, did I miss that? Because, normally, we don't, when we read the report, we don't jump to a table and say this is a method of manufacture, there's actual text.

DR. HILL: I think there is. I was just there, I jumped down to the tables, method of manufacturing is on -- sorry, thank you – PDF page 22.

DR. MARKS: Okay.

DR. HILL: And then you have to, because there's sort of preamble information in the chemistry section, you have to use that together with the method of manufacturing side of the section, and probably Table 2 to get the full picture.

DR. MARKS: So this is where you were saying, that's where you got to 40 or 50 kD in the second paragraph of the method and manufacturing. I had that highlighted.

DR. HILL: So your suggestion to concentrate --

DR. MARKS: Larger than the main band in gluten. So how, that was my conundrum is how do you, there are, what, over 1,000 products that contain wheat protein? It's a large number, or 900, whatever it is. How do you make, how do you assure a safe product?

MS. EISENMANN: One suggestion is you limit the molecular weight size of the protein that could be used. Exactly what the limit should be, I mean, there is, one suggestion is it has to be greater than 30 kDs to bind to IgE. But I think some of the industry is using a cut off more of 3 kDs.

DR. MARKS: Three?

MS. EISENMANN: Three.

DR. BRESLAWEC: So anything below 3 is safe?

MS. EISENMANN: Right.

DR. HILL: So the real question is, they started with, does one come from gluten. It doesn't say that -- yes --

DR. MARKS: Yeah.

DR. HILL: -- from gluten by partial hydrolysis, means you shouldn't have anything in there in the first place bigger than gluten, assuming that whatever that was that was larger than gluten and went through the partial hydrolysis, it's hard to imagine why that wouldn't have been -- so, then, it's almost like it might have been a contaminative microbial growth after the fact, after it was produced, I don't know. The real issue is how did it get in there. It's unlikely to have been something that survived the hydrolysis process, to my way of thinking. I don't know what protein would survive the hydrolysis that they're using for gluten and still end up with a 50, 40 to 50 kD molecular size -- molecular weight, excuse me.

MS. EISENMANN: I don't --

DR. HILL: I know you don't know, I can't even conjecture, but I'm thinking the microbial growth happened after manufacture and then it somehow got -- I mean, it's just a guess, this is something that wouldn't normally appear in any of these. An anomaly.

DR. MARKS: Carol, where is the data that support the idea that about 3 kDs, that this is the protein, this is the molecular weight of the presumed allergen, or is that just a theoretical, if it's above 3 kDs, it doesn't bind IgE? Because I don't, there was a similar issue with natural rubber protein, and when the natural rubber protein gloves were manufactured, there was a limit of, like, 230, I think, and once that limit was in place, the Type 1 reactions to natural rubber lay text gloves disappeared, we just don't see it anymore. So that's where I was hoping there would be something, like you say, set a limit, I just couldn't find anything to help me in arriving at that.

MS. EISENMANN: The discussion is in the report under Type 1 hypersensitivity.

DR. MARKS: Yeah, it's on --

MS. BURNETT: Page 25 of the PDF, there's --

DR. MARKS: 25.

MS. BURNETT: -- about midway, there's a few paragraphs about --

DR. MARKS: Yeah, the most IgE epitopes in UWP -- what's the U stand for? The WP is whip. Wheat protein. There's a U on that page.

MS. BURNETT: Unmodified wheat protein.

DR. MARKS: And then what's the H again?

DR. HILL: Hydrolyzed wheat protein.

DR. MARKS: Hydrolyzed.

MS. BURNETT: Starting with the paragraph that says binding patterns of serum IgE.

DR. MARKS: I'm looking at the one, overall, the authors concluded binding pattern. So the one above that. So, in no cases, did the IgE bind to the hydrolyzed wheat protein less than 30 kDs, I see what you're saying.

MS. BURNETT: And then the paragraph starting with, in a Japanese study, which is below that, the last sentence, there arises the size.

DR. MARKS: So you would suggest put the limit of hydrolyzed protein polypeptides less, should be greater than 30 kDs.

MS. BURNETT: Less.

DR. SHANK: Less.

MS. EISENMANN: Less. Or you might choose another one, the 3. I mean, 3, I think, is --

DR. BRESLAWEC: It's the one that your --

MS. EISENMANN: Right, the Japanese study, that's the one they were doing.

DR. MARKS: Where did the 3 come from? You're doing a margin of safety times 10? One tenth of that is 3.

MS. EISENMANN: It's 3 kD.

DR. BRESLAWEC: 3,000 Daltons, 3 kDs.

DR. HILL: So if you read, if you're on page 25 of the PDF, and you ignore that very last three lines, just above that talks about, it's theorized that limiting the size of the proteins or polypeptides. And what comes before that is, they're hypothesizing, probably with some evidence, that they're getting higher molecular weight aggregates from smaller molecular weight fragments by things like disulfide coupling, which is certainly going to happen if you free cystines --

DR. MARKS: Do you feel comfortable, Ron, Ron and Tom, that using maybe that, to me, theorized, if I'm at risk for having an anaphylactic reaction -- I'm not sure theorized, that's just a word, but this 3,000 Dalton, 30 amino acid -- how difficult is this for industry to, are they going to be looking at their ingredients and say, okay, we're going to have no residuals less than 3,000 Daltons.

DR. SLAGA: More.

MS. EISENMANN: I think more and more, they are becoming more concerned about it because of this incident in Japan and other incidents like it. But this is an area where the industry would like you to research this. You may need more time to look at, and we can try to find more references for you, and maybe someone to come in and speak on this issue, if you'd like. This is a concern for the industry right now.

DR. MARKS: I actually like the idea having an allergist, somebody who is an expert in Type 1 allergy come in and speak to this.

DR. SHANK: I would.

DR. SLAGA: Yeah, definitely.

DR. MARKS: Because I don't feel --

DR. SHANK: This is a weak one for me, I'm sorry to say.

DR. HILL: Well, and the reality is that the molecular level understanding of this sort of thing has been coming up very rapidly in the last several years on that. It would make a difference in terms of how one -- I mean, reaction is reaction, but it makes a difference in terms of how one makes predictions and interprets data, I think.

DR. MARKS: So, with that in mind, should we table this and ask that we have an expert in Type 1 allergy come in and discuss it? And it obviously is going to be an allergist who understands not only the molecular biology of this, somebody that's got a basic science background, just not somebody who sort of comes in who, you know, say, a clinician who doesn't understand, perhaps, the basic mechanism and molecular biology. So, we're at the stage now of having a tentative safety assessment of hydrolyzed wheat protein and hydrolyzed wheat gluten. One option would be to table it and ask that an expert come in and address this, does that sound like a reasonable way to proceed? Let's see, who's -- tomorrow, it will be, where is it, hydrolyzed wheat protein -- it's Belsito, but that doesn't matter, we can (laughter) -- so, do you like that idea of tabling?

DR. SLAGA: Table.

DR. SHANK: I do. This is an important issue.

DR. MARKS: Yeah, absolutely. I'm wondering --

DR. SHANK: I think we need to fully understand what's going on, here.

DR. BERGFELD: Do you think that the literature has been searched deeply enough on this subject? Because if there's an expert, he's certainly written on it, and we should probably see that, as well. He or she, excuse me.

DR. HILL: You've got a pretty good series of references, here, but yes.

DR. MARKS: Okay. So I'm going to suggest tomorrow we table it, because I have, I don't feel -- I'm amazed that there's a thousand uses, and it hasn't been seen. You had suggested that it's possibly some manufacturing process in Japan that resulted in this, and that's the same with the -- I mean, it's reproducible in terms of the natural rubber latex in gloves when we had them manufactured in the U.S., we didn't see the problem. Once the HIV epidemic occurred and the demand for gloves outstripped what we could manufacture in the U.S., it was Pacific Rim, they weren't rinsed properly, they weren't processed, and then we had a lot of residual natural rubber protein, and, bammo, then we had the contact urticaria and Type 1 reactions to gloves. So I like the idea of getting an expert in, because, you know, a great majority, it's not an issue with a thousand uses, but we need to understand, in my mind, better what guidance we need to give to industry of how to proceed with this.

DR. SHANK: I agree.

DR. BERGFELD: Does industry have a cap on it currently of 3 kDs?

MS. EISENMANN: Each company has different caps.

DR. BERGFELD: I mean, but what is the highest?

MS. EISENMANN: I don't know that answer, but it's variable.

DR. BRESLAWEC: Probably between 3 to 30.

MS. EISENMANN: Well, I know one goes down to 2.5, too.

DR. MARKS: Yes.

MR. HELDRETH: Just so this isn't something that goes off into table land indefinitely, should we set a time frame on when we expect to have this back in front of you?

DR. MARKS: Yeah, my feeling would be is, if we had the expert at the beginning of the next meeting, we could then have it on the agenda in either the next meeting or the following meeting, then we could proceed with a tentative safety assessment, with the recommendations in terms of dealing with a Type 1. So I should think within one or two meetings, we should be able to do it, I guess, but it depends on the availability of the expert.

DR. BRESLAWEC: Well, we will certainly search an expert out and recommend for CIR to make a decision on which expert they'd like to speak. But my understanding is, when you table something, you don't rewrite the report, you don't redraft the report, it is as stands.

DR. MARKS: Right.

DR. BRESLAWEC: We happen to think that the discussion of the Type 1 allergy is pretty thorough, so we just work from this document and then consider the expertise of the speaker, and then see if you want to amend it at that point.

MS. EISENMANN: Do you have any suggestions for speakers? I mean, we would be also interested, you don't have to say now, but if you have, we would always be interested in your suggestions.

DR. SHANK: Okay. I do, but I'll let you know.

MS. EISENMANN: Okay.

DR. MARKS: Okay. So I'm going to -- depending on what the motion is, let's table. It will be easy, I'll second the motion. If it isn't, I'll make that suggestion, and then we'll see where the panel decides to go with this. Any other comments about the hydrolyzed wheat protein and hydrolyzed wheat gluten? Okay, table. This is the end of the ingredients I have on the agenda, is there anything else, Ron, Ron, Tom, we should cover, Rachel, Halyna, Wilma? If not, I think we can adjourn, then, and we'll read over the ECHA, and then we'll huddle in the morning in terms of how to deal with that. Thank you, everyone.

(Whereupon, the PROCEEDINGS were adjourned.)

Full Panel

DR. BERGFELD: We'll move on then to the next ingredient, which are the wheat proteins. Dr. Belsito?

DR. BELSITO: Yes. Hydrolyzed wheat protein and hydrolyzed wheat gluten. So at the last -- at the March meeting actually, we were asked to look at a whole group of hydrolyzed proteins, and we said, whoa, we just can't do this. So let's split them down and let's start with a hydrolyzed protein of greatest use, which was wheat, which is what we're doing now.

And at that time, we had issued an insufficient data announcement requesting methods of manufacturing, composition, characterization specifications for hydrolyzed wheat protein and when wheat proteins were combined with other amino acids. We're particularly concerned about reports coming out of Japan about hydrolyzed wheat proteins causing reactions in soaps. So we've gotten a lot of new data, including some wave two data from the Council or at least one of the manufacturers pointing out various things.

We looked at this report. We thought it was a very well-written report. And in terms of reactions to wheat proteins, it appears that if the peptide length is less than or equal to 30, it will not cross link and bind IGE and cause reactions. However, it also appeared that if you tape strip skin, at least in mice, you could sensitize them to these wheat proteins. And it wasn't clear the molecular weight or amino acid length of the hydrolyzed wheat proteins that were used in those studies. And it also appeared that when applied to mucus membranes of the eye, you could also sensitize individuals.

So we felt that we could go ahead with a "safe as used," minimize peptide lengths above 30 amino acids long, but that we would need to put a restriction that this not be used on damaged skin, and with a robust discussion in the discussion about the sensitization of mice on tape stripped skin, that it should not be used in products that could contact the mucus membranes, which would actually eliminate a little over 100 currently registered products that could, and not in products that could be inhaled.

To eliminate those restrictions, i.e., damaged skin, mucus membranes, inhalation, what we really need to know is can you sensitize to the hydrolyzed wheat proteins with amino acids that are 30 or smaller in size. And that we don't know because the studies that were done on mice that were sensitizing them were 45 --

DR. SNYDER: Forty to 50.

DR. BELSITO: Four hundred and fifty?

DR. SNYDER: Forty to 50.

DR. BELSITO: Forty to 50 kilodaltons, which are much larger than 30 amino acids. But we don't know whether amino acids will sensitize. So the issue here is that we know that you can apparently eliminate elicitation with a cutoff at 30, but do you eliminate sensitization, and that's the information we need to know to eliminate those restrictions on damaged skin mucus membranes and inhalation.

DR. SHANK: But you still say it's safe?

DR. BELSITO: Safe, but not to be used in those products.

DR. SHANK: With all of that?

DR. BELSITO: Yes. We said formaldehyde formalin was "safe with elimination of hair straightening products." I mean, this can be safely used in a shampoo if they're less than 30 amino acids.

DR. BERGFELD: Dr. Marks?

DR. MARKS: So we struggled with how to deal with this, and we're quite concerned about the type one reactions and anaphylaxis. And we didn't quite understand or felt we had the expertise to come to a conclusion.

So we actually suggested tabling this report and ask that an expert on wheat type one reactions to speak to the Panel and sort through this and help us arrive at a conclusion. There are over a thousand uses. This is very important.

There's been these serious adverse events, particularly in Japan, and so we felt we needed more expertise. But if your team feels that -- comfortable moving forward, that will -- our team will discuss it, although I'm not sure we're prepared to move forward as "safe."

DR. BERGFELD: Ron Shank, any comment?

DR. SHANK: I find the immunotoxicity data confusing, and I don't have sufficient expertise in immunology to tease this out. These ingredients are widely used and have been, but we have not seen outside of Japan apparently this very serious type one response. So before we could go to safety, I would say we need to hear from an expert in this field.

DR. BERGFELD: Comment, Paul?

DR. SNYDER: I don't have a problem with having an expert come in, but I think as Don stated, we labored over this. I think that when you finally look at the data, I think the data on the elicitation is quite good, but there's a cutoff point.

But the question we don't know is, at what point do you get sensitized by peptides, certain peptides? And that's the critical thing that we don't know. And if we knew that, then we could tell them to formulate, to not contain those, and then that would resolve all of our issues.

But I don't have a problem. If it would help others understand the immunology behind it, I'm okay with that.

DR. BERGFELD: Dan?

DR. LIEBLER: So I think the suggestion is not a bad one of having an expert come and speak with us because I think if you look at this, almost everything we have in the report supports safety, as long as the size distribution of the hydrolyzed peptides is kept below some threshold amount. And 30 seems to be -- we're using it as a kind of a magic number, but it's probably somewhere in that neighborhood.

The thing that really throws a wrench in the works in our interpretation is this mouse study, the tape stripping mouse study. And, you know, admittedly we have to, because we're uncertain of the circumstances of those experiments, we're having to kind of work around that in our, you know, proposed assessment here, as Don laid out.

So it might be if we do have an expert come and talk with us, I think one thing we would to do is have that person really give us their input on what they -- how they would asses this mouse study. Now, there might be -- it might not be possible for them to fully assess it if we cannot know what the position of the test material was in that experiment. I think it's a big --

DR. BELSITO: (off mic)

DR. LIEBLER: Well, I don't see it very well, you know, very well laid out. I suspect it's not known well enough to get to our major point, because I think the characterization of the size of the proteins used is not adequate.

So, you know, it's a big unknown. It throws a wrench in the works, and it perhaps would allow time for any more data to emerge if that's possible.

DR. BERGFELD: Ron Hill?

DR. HILL: Yeah, because the interesting complication here is the suggestion that smaller peptides are either via disulfide linkages or some other, they say, entangling. I don't know about that. But I certainly can envision through essentially repolymerization, but through disulfide linkages that we're building up big enough molecules, and then they come to the end where they say if we keep everything below 3,000 molecular weight, theoretically that should solve the problem. I have to think my way through that theory and really read in depth the references list that's here and anything else I can find.

And then, so the characterization issue, that's part of it because on the day of the testing, if that process is actually occurring in a sample, then you really need to know right then by some means what's actually in there to be tested. And I thought on the tape stripping, it sort of is in accord with the data that suggests that on intact skin, in order to get much sensitization, there's some words barely in there for detecting, I think, it was IgE. So I'm a little concerned what does "barely" mean.

But in order to get robust sensitization, it was the SDS, the surfactant, that allowed, I assume, compromising the barrier function of the skin that allowed these things to do something. And I guess this is the first I had really encountered how much -- to what extent if I have a shampoo that has this stuff in it, I rinse it off, is it maybe contacting the mucus membranes in my eye. I even got to thinking, if you're living in a place where the showers don't work as well as they did in this building, you know, that maybe you're leaving more on than if you can rinse well. So that is a little crazy, but not maybe that crazy when we're talking anaphylactic reactions. So there were unknown parameters, I agree.

DR. BERGFELD: So, Don?

DR. BELSITO: Again, you know, the issue in Japan, as you just pointed out, was with soap. And so, you're having that surfactant. You're having damage. I mean, these proteins – stratum corneum is going to an excellent barrier for any protein.

So what you're concerned about is areas of skin with no stratum corneum or a very weak stratum or damaged stratum corneum, and mucosa, which has no stratum corneum. So that's when you're going to start absorbing the proteins.

So, I mean, our restrictions are very, very extensive. And probably industry is, you know, going to hopefully come forward with some data to show us that, you know, with the restricted size length, you can sensitize. But, I mean, quite clearly, you know, in terms of an expert, I'm not sure what an expert is going to tell you that's not in this report. When you get below 30, you can't get, you know, dynameric cross-linking of IgE, and you can't trigger mass cells, period and amen. Above that, you can.

And so, you know, IgE reactions are going to occur with larger molecules. So if you're concerned about the reaction to sensitized individuals, limiting the size will satisfy that.

The question really is, can you sensitize an individual with those smaller molecules, and we don't know. You know, what is the epitope that's coming out and will it sensitize. So that was our issue. And, therefore, since sensitization can only occur when there's no stratum corneum, and we put in the restrictions not undamaged skin, not for inhaled products, and not for products that can contact the mucus membranes.

DR. HILL: But the complication that I think remains sitting in my mind then is the potential for these smaller peptides to grow, again, I guess based on mostly disulfide. I'm not sure what other mechanisms would be involved that could do that --

DR. BELSITO: I can't comment on it.

DR. HILL: I know.

DR. BELSITO: If in formulation these can re- aggregate into longer amino acids, Dan, you may want to comment --

DR. HILL: So what assurances would we want to see in order to conclude, you know, that we have to assure that this won't happen, this is what causes it to happen. And I don't know if you have an expert in on immunology that they're still going to be able to address that particular issue unless they've really thought this through. This is new to me. I haven't encountered this before.

DR. BELSITO: An immunologist wouldn't be able to probably answer whether these can aggregate. Dan might be able to.

DR. LIEBLER: Yeah. I don't really think so. I mean, you know, my lab --

DR. HILL: That's what they're suggesting in here.

DR. LIEBLER: Yeah, I know. My lab does proteomics for a living pretty much. And, you know, we digest proteins, and in some cases we don't reduce and alkylate. We just digest without reduction. And we never have an issue of, you know, forming of larger structures by re-oxidation. So, I mean, I think it's probably -- it would require a very highly oxidizing environment, and that's probably not applicable in this case. So I don't think that's the issue.

I think, you know, quality control in these hydrolysis chemistries, and preparation, and batch checking to make sure that you've actually got the high stuff down below some threshold. We don't know what it is, but it should be very low. And, you know, I think I think that's probably already being done in industry. The capacity exists to assess that.

The problem is with some of these experiments, we don't know what the characterization of the test material was. In this mouse, you know, study, for example, I just don't think it's well enough characterized to allow us to figure out what's going on and how applicable this result is to use of commercial products in humans.

DR. HILL: My gut reaction when I saw that large protein in there was, well, they've got bacterial growth in their raw materials such that it's kicking out, lypo- polysaccharides or something that's causing it, and that was the whole source of the problem. But just a conjecture.

DR. BERGFELD: Jim?

DR. MARKS: Yeah. I'd like to point out that it's not just to the soaps there's been reactions reported to. There was an eyelid cream and body moisturizer, two separate case reports, contact urticaria to that. So I think it's more than just -- and I don't know where the sources -- the one reference actually didn't appear to be Japanese authors, but it looked like it was a secondary report.

DR. BERGFELD: Well, we have two motions that have not been seconded that have to the table. One is to "safe" with great restrictions, and the second is "insufficient." So I'd like to hear a final motion.

DR. MARKS: Table, not "insufficient."

DR. BERGFELD: Oh, excuse me. "Table," thank you.

DR. MARKS: And, again, if I were going to err, I'd prefer to err on the safe side and have somebody who deals with this and understands type one reactions, possibly somebody who can relate back to natural rubber latex, contact urticarian anaphylaxis epidemic which occurred, and then when limits were placed on natural rubber latex gloves, we saw that disappear.

I just would feel more comfortable setting the limits, and perhaps not a whole bunch of restrictions if we understand perhaps a little bit more. We may not get any further, I agree,

Don, but our team felt more comfortable.

DR. BERGFELD: Don?

DR. BELSITO: Well, a couple of things. First of all, I'm not sure that this is comparable to the natural rubber latex. I tried searching because, as you know, with natural rubber latex, it was a series of about six Hev proteins that were shown to be problematic. And, therefore, by removing those Hev proteins from the latex sources, they essentially got rid of the epidemic.

When I tried searching for, you know, key proteins in wheat because I thought of that approach, say, okay, eliminate these, I couldn't find them. Now, I didn't do an extensive and exhaustive search, and that is maybe something that can be done to see if you can identify the epitopes that are sensitizing for the vast majority of people. That's one way of getting around it.

My only concern with tabling this to get an expert in is I'm not sure that they can tell us whether peptides of 30 or less will or will not be sensitizing. And, therefore, we're still going to be missing that critical data that we feel is necessary.

So I would like to go forward with our motion, you know, and get an expert to talk to us. But, I mean, this is the first time we're looking for it, you know. We can table it later on if it appears that we're going to need more time to address issues that we need. But I would just like to see this, you know, progressing because if you read labels and if we're that concerned about the safety of this, you see the number of uses out there.

So, you know, if you're telling me you're concerned, then I think we need to move ahead. And by tabling it, we're not moving ahead. We're just stalling it.

DR. BERGFELD: Response? No response? Is there a motion?

DR. HILL: Could you read that list of restrictions you're proposing again? I think I've got it.

DR. BELSITO: Okay, hold on. So we're saying that these hydrolyzed wheat proteins are safe when they are formulated to minimize peptides greater than or equal to 30 amino acids in length. But they should not be used on damaged skin, on products that could contact mucus membranes, and on products that could be incidentally inhaled.

DR. BERGFELD: Are you making that a motion?

DR. BELSITO: That was my motion originally.

DR. BERGFELD: Well, restating it.

DR. BELSITO: Yeah.

DR. BERGFELD: Is there a second?

DR. SHANK: When you say they can't be used on mucus membranes and inhalation, they being the 30 amino acid links and smaller?

DR. BELSITO: Well, we've already said that what's out there should be 30 amino acids and less. I mean, we're saying anything with significant content above 30 amino acids would be unsafe, or the safety is not known. We're saying that below, you know, 30 or below will not cross-link IgE, will not trigger the anaphylactic reaction, so we're not concerned about those lengths of hydrolyzed wheat proteins in individuals who are already sensitized.

But the question we don't have it, will they induce sensitization in people who are not sensitized who could then be exposed to wheat in food or whatever and develop anaphylactic reactions as a result of sensitization to a cosmetic product?

DR. HILL: So your motion is that restriction and in addition to --

DR. BELSITO: In addition --

DR. HILL: -- damaged skin, mucus membranes, inhalation.

DR. BELSITO: Right.

DR. BERGFELD: Paul, did you want to speak?

DR. SNYDER: Well, I as just going to reiterate. I think, to me, the immunologic data is pretty strong that there's a cutoff for elicitation. It's clear that we've got good data, that less than 30 amino acids in length, you cannot -- even in people who are sensitized, you will not elicit a reaction, a type one reaction.

What we don't have, and we have good data saying that if you have damaged skin and you're exposed to greater than 30 amino acid length hydrolyzed proteins, you will be sensitized. And so, that's the two solid pieces of data we have.

What we don't have is, where is the cutoff for sensitization? And so, we can't link the sensitization with the elicitation cutoff right now. If we got that data that we tell -- advise to not have certain peptide links in the final product, then I'm quite comfortable we would be safe.

DR. BELSITO: I mean, we can move ahead, try and identify an expert who looks at protein sensitization and digests of proteins of albumin, whatever you want, and come in and say, oh, yeah.

I mean, if you have a, you know, a peptide smaller than this, nothing is going to happen immunologically, that'll solve our problem, you know. But at least we're moving ahead.

And, you know, because, again, I think if you're that concerned about the possibility that, you know, there's over 1,000 products out there, then to stall it, I think is a mistake.

DR. BERGFELD: Jim?

DR. MARKS: Second.

DR. BERGFELD: Thank you. Anyone want to make a comment before I call the vote?

DR. LIEBLER: Yes.

DR. BERGFELD: I think the discussion is the main thing.

DR. LIEBLER: So one of the points that was raised last time was a lack of information about how these were prepared and characterized.

You can make -- do enzymatic hydrolysis. You can do modified acid-based hydrolysis. And we didn't have much of anything. Now we have minimal information in table two.

This is an opportunity to get, if there's more information available from industry, on the methods used to characterize these and ensure that the components over 30 amino acids are on the top end of the weight range is minimized, that would be particularly valuable here in helping us formulate our conclusion.

And so, I'd like to emphasize that if it's possible to squeeze harder for that information, we should try and do that.

DR. BERGFELD: Halyna, do you wish to comment at all?

DR. BRESLAWEC: We will certainly look for that information and provide it if we have it.

DR. BERGFELD: All right. Thank you. I'm going to call the question then. I see no one needing to speak. All those in favor with regard to "safe" with all the restrictions as stated? Unanimous. A wonderful discussion. Thank you.

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

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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 on the elicitation of Type I hypersensitivity reactions in sensitized individuals were adequate to support the safety of these ingredients with peptide-length distributions not exceeding 30 amino acids. The Panel concluded that hydrolyzed wheat gluten and hydrolyzed wheat protein are safe in cosmetics when formulated to minimize peptide lengths greater than 30 amino acids. Additionally, these ingredients should not be used on damaged skin or in products that may contact mucous membranes or be incidentally inhaled.

INTRODUCTION

This safety assessment is of hydrolyzed wheat protein (HWP) and hydrolyzed wheat gluten, which are each mixtures of amino acids and peptides of varying length, derived from wheat sources. These ingredients function as skin and hair conditioning agents in personal care products. The CIR Panel (Panel) previously has 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 ingredients.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 other 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, 500-10,000 Da.⁹⁻¹¹ For example, if the average molecular weight of an amino acid is 135 Da, then, under the broader distribution figures, these ingredients are approximately 4 to 220 amino acids in length (and approximately 4 to 74 amino acids in length under the narrower distribution).¹²

Method of Manufacturing

A supplier reported that HWP (MW = 350) may be prepared by both alkaline and enzyme hydrolysis.¹³ These processes occur for several hours until the desired molecular weight 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 95°C for 40 min.¹⁴ The molecular weight of the main band of HWP as determined with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was 40-50 kDa, which was larger than the main band in gluten.

Impurities

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

USE

Cosmetic

The HWP and hydrolyzed wheat gluten function primarily as hair conditioning agents and skin conditioning agents (miscellaneous) in cosmetic formulations.¹⁵ An additional function may include film formers (HWP).

Table 3 presents the current product-formulation data for HWP and hydrolyzed wheat gluten. 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.¹⁶ Hydrolyzed wheat gluten 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.¹⁷ Hydrolyzed wheat gluten 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. Hydrolyzed wheat gluten and HWP may also be used as a spray in 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 HWP confirmed to be used in a spray product is 0.5% in a pump hair spray. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 μ m, with propellant sprays yielding a greater fraction of droplets/particles <10 μ m compared with pump sprays.^{18,19} 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.^{20,21}

HWP and hydrolyzed wheat gluten are not restricted from use in any way under the rules governing cosmetic products in the European Union.²²

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.²³ 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 are milk, egg, fish, Crustacean shellfish, tree nuts, wheat, peanuts, and soybeans.²⁴

TOXICOKINETICS

No published toxicokinetics studies on HWP and hydrolyzed wheat gluten 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 HWP and hydrolyzed gluten that are described in this safety assessment are found in the foods we consume daily. Toxicities from dermal exposure, other than irritation and sensitization, would not be expected to be different from oral exposures and as such not of concern by the Panel. Irritation and sensitization are of concern, and the focus in this report. Data from the previous safety assessment on α -amino acids support that mixtures of amino acids would not likely be irritants or sensitizers.

GENOTOXICITY

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

CARCINOGENICITY

No published carcinogenicity studies on HWP and hydrolyzed wheat gluten 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).²⁵ The 25% aq. solution (MW = 350) was applied for 24 h to 2.5 cm² sites that were clipped, abraded, and occluded.

Dermal - Human

HWP was non-irritating in a human irritation patch test performed in 42 subjects.²⁶ The HWP was tested at 25% aq. solution (MW = 350), 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) was not a primary eye irritant.²⁷

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 95° C for 40 min. The resultant HWP had a MW of approximately 40-50 kDa. The 7-week-old female mice were shaved and tape-stripped 10 times to remove the stratum corneum, and were then exposed to HWP or gluten (500 µg/mouse) 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, with and without sodium dodecyl sulfate (SDS). 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 transfermally 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 were 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 glutenwith-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 IgG1 with 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 restimulated with HWP for 72 h. The secretion of IL-4, IL-5, and IL-10 was increased while that of IL-2 and interferon (FRN)-y were significantly decreased, demonstrating that transdermal sensitization with HWP was associated with Th2-dominant helper T-cell activation.

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) when applied at a volume of 0.2 ml under a 20 mm² Webril patch.²⁸

A study of sensitization to protein hydrolysates in hair-care products was performed in 3 groups of patients.²⁹ 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.²⁹

Type 1 Hypersensitivity

There have been several reports of Type 1 (i.e., immediate) hypersensitivity reactions to personal care products that contain HWP, as summarized below. An allergen must have at least 2 IgE epitopes, and each epitope must be at least 15 amino-acid residues long, to trigger a Type 1 hypersensitivity reaction.³⁰ A patient becomes sensitized when two or more IgE molecules against a specific allergen are bound to receptors on the surface of a mast cell. The cross linking of two or more bound IgE molecules by the allergen results in degranulation of the mast cell and the release of vasoactive amines that elicit the Type I reaction.

The sera from 5 European patients were studied to determine the reactivity of IgE with hydrolyzed gluten.³¹ 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 hydrolyzed gluten preparations (enzymatically- or acid-hydrolyzed), total unmodified wheat protein (UWP), and UWP fractions (i.e., albumins/globulins, gliadins, and glutenins, including high-molecular weight glutenin subunits [HMW-GS] and low-molecular weight glutenin subunits [LMW-GS]). 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 ω 5-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 ω 5-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 30 kDa. 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 kDa to > 1,000 kDa, and both preparations contained substantial amounts of high molecular-weight constituents. Binding of IgE in the serum of the IHHWP patient was greatest to the highest molecular-weight fractions of both of these HWP preparations (400 kDa to 1,000 kDa), weaker to intermediate molecular-weight fractions (30 kDa to 400 kDa), and faint or undetectable to the lowest molecular-weight fractions (< 30 kDa).

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 kDa. 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 molecular-weight polypeptide aggregates that can present multiple epitopes efficiently to trigger allergic responses to HWP.³¹

In a Japanese study, wheat protein hydrolysates that were produced by enzymatic hydrolysis had higher concentrations of peptides with molecular weights greater than 1,050 Da, compared with those produced by acid hydrolysis, which had extremely low concentrations of peptides with molecular weights greater than 1,050 Da.³² 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.³² IgE of patients

that had Type 1 hypersensitivity to HWP through percutaneous and/or rhinoconjunctival exposure to a facial soap containing HWP (40-50 kDa) reacted with high molecular weight polypeptide aggregates.³³ 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 wheat-dependent exercise-induced anaphylaxis (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.³⁴ It is theorized that limiting the size of proteins or polypeptides to no more than approximately 30 amino acid residues (MW=3000 Da) would greatly reduce the potential for causing Type 1 reactions.³⁰

A study was performed comparing 5 Japanese women exhibiting both contact allergy (rhinoconjunctival reactions) to HWP (40-50 kDa) 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.³⁵ The authors distinguished the 5 Japanese HWP-WDEIA patients from European patients exhibiting IHHWP (see study 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 ω 5-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 ω 5-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 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 ω 5-gliadin (e.g., ω 1-2-gliadin and Υ -gliadin) may be more important than sensitization to ω 5-gliadin in the pathogenesis of HWP-WDEIA, compared with the pathogenesis of CO-WDEIA.³⁵

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; 40-50 kDa) 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 ω 5-gliadin, compared to 80% of CO-WDEIA patients. Reactivity with ω 5-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 ω 5-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 ω 5-gliadin. In comparison, serum IgE from CO-WDEIA patients bound to ω 5-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 ω 5-gliadin, up-regulated the CD203c (an ecto-enzyme on the cell membranes of basophils and mast cells) in HWP-WDEIA patients. However, ω 5-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.³⁶ 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 I 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 I hypersentitivy reactions in Japan to products containing HWP is ongoing by the Japanese Society of Allergology's Special Committee for the Safety of Protein Hydolysates in Cosmetics. Current developments are available at: <u>http://www.jsaweb.jp/modules/en/index.php?content_id=11</u>.

Phototoxicity

No published phototoxicity studies on HWP and hydrolyzed wheat gluten 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 = 40-50 kDa). 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 ranging from 15 to 250 kDa in the HWP preparation and with both the water-soluble and water-insoluble fractions of UWP, but not with ω 5-gliadin.

An additional 3 cases of WDEIA were reported by the same researchers in Japan.³⁷ The 3 female patients had used the same brand of soap that contained HWP (40-50 kDa). 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 (40-50 kDa) daily for several years.³⁸ 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.³⁹ 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.⁴⁰ The patient developed the clinical signs after applying an eyelid cream and a body moisturizer that contained HWPs 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.⁴¹ 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.⁴² 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.⁴³ 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.⁴⁴ 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.⁴⁵ In the first case, the patient, a 23-year-old female with no history of atopy who had been employeed 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 employeed 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 standaridized 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 hegative 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.⁴⁵

SUMMARY

Hydrolyzed wheat gluten and HWP function primarily as skin and hair conditioning agents in personal care products. These protein derivatives are prepared by subjecting wheat proteins to 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. Hydrolyzed wheat gluten 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. Hydrolyzed wheat gluten 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.

A HRIPT study of HWP (MW = 350) concluded that this ingredient was not a dermal irritant during induction or sensitizers during challenge.

Multiple cases of allergic reactions, including Type 1 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 40-50 kDa in size. Several studies have been conducted to characterize the cause, manifestations, and mechanisms of these reactions, including tests of serum IgE binding and reactivity wheat protein, wheat protein fractions, and HWP and hydrolyzed gluten prepared using acid- and/or enzymatic-hydrolysis methods yielding products with varied polypeptide size profiles.

DISCUSSION

The hydrolyzed wheat protein and hydrolyzed wheat gluten discussed in this safety assessment are polypeptides ranging from approximately 4 amino acids (approximately 500 Da) to over 220 amino acids (over 30 kDa) in length. It has been suggested that peptides greater than 30 amino acids in length can precipitate Type I hypersensitivity reactions by enabling the crosslinking of IgE in individuals sensitized to hydrolyzed wheat protein or hydrolyzed wheat gluten. Traditional human repeat insult patch tests and related tests do not detect Type I reactions. The Panel felt the data on the elicitation of Type I hypersensitivity reaction in sensitized individuals were adequate to support the safety of hydrolyzed wheat gluten and hydrolyzed wheat protein ingredients with peptide length distributions that do not exceed 30 amino acids. However, no data were available to determine a peptidelength threshold below which sensitization would not be induced in people who are not already sensitized to hydrolyzed wheat gluten or hydrolyzed wheat protein. The Panel noted that a study of mice with tape-stripped skin demonstrated the induction of sensitization to a hydrolyzed wheat protein preparation with a size distribution ranging from about 40 kDa to 50 kDa (or approximately 360-450 amino acids in length). The Panel also noted reports indicating that people using cosmetic products containing hydrolyzed wheat proteins applied to the eye area were sensitized. Unless data can be produced that demonstrate a size-distribution threshold below which sensitization cannot be induced, cosmetics containing hydrolyzed wheat gluten and hydrolyzed wheat protein should not be used on damaged skin or on mucous membranes.

These ingredients should also not be used in products that may be inhaled, including spray products. The Panel discussed the issue of incidental inhalation exposure of potentially sensitizing HWP ingredients from aerosol and pump hair spray products. No inhalation data were identified or provided. This ingredient reportedly is used at concentrations up to 0.5% in cosmetic products that may be aerosolized.

The Panel also expressed concern regarding pesticide residues and heavy metals that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to use the necessary procedures to limit these impurities in the ingredient before blending into cosmetic formulation.

The Panel asked that the cosmetics industry continue to provide additional data on manufacturing practices, characterization methods, and composition, including peptide size distributions, to enable better characterization of the nature and variability of these ingredients as used in cosmetic products and to enable the Panel to refine its conclusion.

CONCLUSION

The CIR Expert Panel concluded that hydrolyzed wheat gluten and hydrolyzed wheat protein are safe in cosmetics when formulated to minimize peptide lengths greater than 30 amino acids (approximately 3.3 kDa). Additionally, these ingredients should not be used on damaged skin or in products that may come into contact with mucous membranes or may be incidentally inhaled.

TABLES

Table 1. Definitions and functions of the ingredients in this safety assessment.¹⁵ (The italicized text below represents additions made by CIR staff.)

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

Table 2. Summary of information from suppliers of hydrolyzed wheat protein.*46

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

* Information includes data summarized in Anonymous, 2012.¹³

	Hydrolyzed V	Hydrolyzed Wheat Gluten		Wheat Protein
	# of Uses	Conc. of Use (%)	# of Uses	Conc. of Use (%)
Totals ¹	78	0.005-0.09	1077	0.00002-1.7
Duration of Use				
Leave-On	13	0.005-0.09	528	0.00006-1
Rinse-Off	62	0.005-0.01	541	0.00002-1.7
Diluted for (Bath) Use	3	NR	8	0.00002
Exposure Type				
Eye Area	1	0.09	67	0.01-0.9
Incidental Ingestion	NR	NR	18	0.008-0.03
Incidental Inhalation-Spray? ^{2,5}	7	0.005-0.06	285	0.00006-0.4
Confirmed Spray ³	NR	NR	NR	0.0003-0.5 ^a
Incidental Inhalation-Powder?4,5	8	0.06	149	0.00006-0.05
Confirmed Powder ³	NR	NR	NR	NR
Dermal Contact	26	0.01-0.09	383	0.00002-1
Deodorant (underarm)-Spray?	NR	NR	NR	NR
Confirmed Spray ³	NR	NR	NR	NR
Not Spray ³	NR	NR	NR	NR
Hair - Non-Coloring	17	0.005	530	0.003-1.7
Hair-Coloring	35	NR	92	0.002-0.3
Nail	NR	NR	28	0.002-0.04
Mucous Membrane	15	NR	113	0.00002-0.1
Baby Products	3	NR	2	NR

Table 3. Frequency and concentration of use for hydrolyzed wheat gluten and hydrolyzed wheat protein according to duration and type of exposure.16,17

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.

It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.
Use has been confirmed by the Council.

4. It is possible these products may be powders, but it is not specified whether the reported uses are powders. 5. 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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2013 FDA VCRP RAW DATA

01A - Baby Shampoos 01B - Baby Lotions, Oils, Powders, and	977016526	HYDROLYZED WHEAT GLUTEN	2
Creams	977016526	HYDROLYZED WHEAT GLUTEN	1
02B - Bubble Baths	977016526	HYDROLYZED WHEAT GLUTEN	2
02D - Other Bath Preparations	977016526	HYDROLYZED WHEAT GLUTEN	1
03G - Other Eve Makeun Prenarations	977016526		1
05A - Hair Conditioner	977016526		4
05F - Shampoos (non-coloring)	977016526		ד 8
051 - Other Hair Preparations	977016526		3
06B - Hair Tinte	977016526		35
10A - Bath Soans and Detergents	977016526		30 8
10F - Other Personal Cleanliness	377010320	III DROETZED WIEAT GEOTEN	0
Products	977016526	HYDROLYZED WHEAT GLUTEN	4
12A - Cleansing	977016526	HYDROLYZED WHEAT GLUTEN	1
12C - Face and Neck (exc shave)	977016526	HYDROLYZED WHEAT GLUTEN	7
12J - Other Skin Care Preps	977016526	HYDROLYZED WHEAT GLUTEN	1
04.0 Other Date: Draduate	077447700		0
01C - Other Baby Products	977117728		2
02B - Bubble Baths	977117728		1
02D - Other Bath Preparations	977117728		1
03A - Eyebrow Pencil	97/11//28	HYDROLYZED WHEAT PROTEIN	1
03B - Eyeliner	977117728		2
U3C - Eye Shadow	977117728	HYDROLYZED WHEAT PROTEIN	1
03D - Eye Lotion	977117728	HYDROLYZED WHEAT PROTEIN	11
03E - Eye Makeup Remover	977117728	HYDROLYZED WHEAT PROTEIN	3
03F - Mascara	977117728	HYDROLYZED WHEAT PROTEIN	26
03G - Other Eye Makeup Preparations	977117728	HYDROLYZED WHEAT PROTEIN	23
04C - Powders (dusting and talcum,	077447700		1
OF A Heir Conditioner	977117720		160
05A - Hair Conditioner	977117720		102
05B - Hair Spray (aerosol lixatives)	977117728		17
05C - Hair Straighteners	977117728		4
05D - Permanent waves	977117728		6
USE - Rinses (non-coloring)	977117728		2
05F - Shampoos (non-coloring) 05G - Tonics, Dressings, and Other Hair	977117728	HYDROLYZED WHEAT PROTEIN	142
Grooming Aids	977117728	HYDROLYZED WHEAT PROTEIN	123
05H - Wave Sets	977117728	HYDROLYZED WHEAT PROTEIN	7
05I - Other Hair Preparations	977117728	HYDROLYZED WHEAT PROTEIN	67
06A - Hair Dyes and Colors (all types			
requiring caution statements and patch			
tests)	977117728	HYDROLYZED WHEAT PROTEIN	75
06B - Hair Tints	977117728	HYDROLYZED WHEAT PROTEIN	1
06C - Hair Rinses (coloring)	977117728	HYDROLYZED WHEAT PROTEIN	10
06D - Hair Shampoos (coloring)	977117728	HYDROLYZED WHEAT PROTEIN	5
06G - Hair Bleaches	977117728	HYDROLYZED WHEAT PROTEIN	1
07A - Blushers (all types)	977117728	HYDROLYZED WHEAT PROTEIN	1
07B - Face Powders	977117728	HYDROLYZED WHEAT PROTEIN	5
07C - Foundations	977117728	HYDROLYZED WHEAT PROTEIN	15
07E - Lipstick	977117728	HYDROLYZED WHEAT PROTEIN	18

07I - Other Makeup Preparations	977117728	HYDROLYZED WHEAT PROTEIN	13
08A - Basecoats and Undercoats	977117728	HYDROLYZED WHEAT PROTEIN	5
08B - Cuticle Softeners	977117728	HYDROLYZED WHEAT PROTEIN	2
08C - Nail Creams and Lotions	977117728	HYDROLYZED WHEAT PROTEIN	1
08E - Nail Polish and Enamel	977117728	HYDROLYZED WHEAT PROTEIN	7
08F - Nail Polish and Enamel Removers	977117728	HYDROLYZED WHEAT PROTEIN	3
08G - Other Manicuring Preparations	977117728	HYDROLYZED WHEAT PROTEIN	10
10A - Bath Soaps and Detergents	977117728	HYDROLYZED WHEAT PROTEIN	67
10E - Other Personal Cleanliness			
Products	977117728	HYDROLYZED WHEAT PROTEIN	20
11A - Aftershave Lotion	977117728	HYDROLYZED WHEAT PROTEIN	3
11G - Other Shaving Preparation			
Products	977117728	HYDROLYZED WHEAT PROTEIN	1
12A - Cleansing	977117728	HYDROLYZED WHEAT PROTEIN	20
12C - Face and Neck (exc shave)	977117728	HYDROLYZED WHEAT PROTEIN	63
12D - Body and Hand (exc shave)	977117728	HYDROLYZED WHEAT PROTEIN	29
12F - Moisturizing	977117728	HYDROLYZED WHEAT PROTEIN	34
12G - Night	977117728	HYDROLYZED WHEAT PROTEIN	15
12H - Paste Masks (mud packs)	977117728	HYDROLYZED WHEAT PROTEIN	12
12I - Skin Fresheners	977117728	HYDROLYZED WHEAT PROTEIN	2
12J - Other Skin Care Preps	977117728	HYDROLYZED WHEAT PROTEIN	29
13B - Indoor Tanning Preparations	977117728	HYDROLYZED WHEAT PROTEIN	1
13C - Other Suntan Preparations	977117728	HYDROLYZED WHEAT PROTEIN	1



Memorandum

TO: Lillian Gill, Ph.D. Director - COSMETIC INGREDIENT REVIEW (CIR)

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

DATE: September 5, 2013

SUBJECT: Comments on the Draft Report on Hydrolyzed Wheat Protein and Hydrolyzed Wheat Gluten Prepared for the September 2013 CIR Expert Panel Meeting

Key Issues

- As this is the first time the CIR Expert Panel has seen this report without the other ingredients, this report should not have included a conclusion. As some information has been provided on method of manufacture and molecular weights, the CIR Expert Panel should assess the safety of those products.
- The Discussion needs to include information on lessons learned from the incident in Japan. The material in the soap, a high molecular weight protein fraction, 40-50 kDa, induced Type 1 reactions and should not be used in cosmetic products. Information provided on lower molecular weight ingredients supports their safety.
- Throughout the report, whenever the protein used in the facial soap in Japan was studied, it should be clearly indicated. For example, it should state: HWP (40-50 kDa).

This report has no page numbers. Therefore, the comments provided below are presented by report section.

Additional Comments

The following reference may also be helpful:

- Hiragun M, Ishii K, Hiragun T, et al. 2013. The sensitivity and clinical course of patients with wheat-dependent exercise-induced anaphylaxis sensitized to hydrolyzed wheat protein in facial soap secondary publication. <u>Allergol Int</u>. July 25 [Epub ahead of print].
- Chemistry As reported by a supplier, alkaline hydrolysis is also used to manufacture Hydrolyzed Wheat Protein.
- Method of Manufacture As a point of reference, please add the molecular weight of wheat gluten to this section.

Sensitization - non-human - Please provide information about the protein used in reference 14.

- Sensitization non-human The following does not make sense: "The serum IgG1 content of gluten..."
- Sensitization human If available, please provide information about the molecular weight or any other characteristics of the "2 HWP trademarked hydrolysates" used in reference 29."
- Case Studies If available, please include information about the molecular weight or any other characteristics of the Hydrolyzed Wheat Protein used in the products to which reactions were reported (references 39, 40, 42, 43, 44).
- Summary The Summary should note characteristics of Hydrolyzed Wheat Proteins to which reactions have been noted and to which reactions have not been observed.
- Discussion The Discussion includes too much information on amino acids. It should include information on the lessons learned from the experience in Japan. As this report just concerns hydrolyzed proteins, the last paragraph should be deleted.
- Reference 22 As of July 2013, the EU Cosmetic Directive has been replaced with the Cosmetic Regulation (Regulation EC No. 1223/2009). Please update this reference.



Memorandum

TO: Lillian Gill, Ph.D. Director - COSMETIC INGREDIENT REVIEW (CIR)

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

Bretance

- **DATE:** October 22, 2013
- SUBJECT: Comments on the Tentative Report on Hydrolyzed Wheat Protein and Hydrolyzed Wheat Gluten

Key Issues

- It does not make sense for CIR to have a safe as used conclusion for Triticum Vulgare (Wheat) Gluten and have a conclusion for Hydrolyzed Wheat Gluten that limits the size of the peptides and the products in which Hydrolyzed Wheat Gluten can be used. Perhaps the CIR Expert Panel needs to reconsider the conclusion on Triticum Vulgare (Wheat) Gluten and consider reviewing the safety of Triticum Vulgare (Wheat) Protein and Triticum Vulgare (Wheat) Germ Protein.
- Somewhere in this report, please mention the Japanese Society of Allergology's Special Committee for the Safety of Protein Hydrolysates in Cosmetics and indicate their purpose (see website at <u>http://www.jsaweb.jp/modules/en/index.php?content_id=11</u>) and note that the research they have been guiding on the ingredient that caused problems in Japan is ongoing.
- There are no page numbers included in the tentative report. Therefore, comments are provided by section heading.

Additional Comments

- Introduction Please indicate the uses and use concentrations reported for Triticum Vulgare (Wheat) Gluten in the original report and the re-review.
- Chemistry As a point of reference, please add the molecular weight range of wheat gluten to this section.
- Cosmetic Use As only one ingredient, Hydrolyzed Wheat Protein, has the function film formers listed, and it is only one additional function, please revise the following sentence. "Additional functions may include film formers (HWP)." The boilerplate language for inhalation exposure still needs to be added to the Cosmetic Use section.

- Irritation and Sensitization (from the amino acids report) Please give some indication of the concentrations tested in the studies of the amino acids.
- Sensitization In the description of the HRIPT (reference 24), please include the amount applied (0.2 ml) and the patch size (20 x 20 mm).
- Summary Please include the average molecular weight of the Hydrolyzed Wheat Protein used in the HRIPT study. The report summarizes one HRIPT study. Therefore. "HRIPT studies" needs to be corrected.
- Discussion The Discussion should make it clear that it has been"theorized" that peptides greater than 30 amino acids in length are needed to elicit Type I reactions. There is also a study (reference 27) that indicates that IgE binding epitopes in wheat protein are destroyed in polypeptides less than about 30 kDa.