Safety Assessment of
Tripeptide-1, Hexapeptide-12, and Related Amides
as Used in Cosmetics

Status: Draft Report for Panel Review
Release Date: February 21, 2014
Panel Meeting Date: March 17-18, 2014

The 2014 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A Hill, 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, D.P.A. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst and Bart Heldreth, Ph.D., Chemist.
Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst
Date: February 21, 2014
Subject: Draft Report on Tripeptide-1, Hexapeptide-12, and Related Amides

The draft report on palmitoyl oligopeptides was tabled at the March 18-19, 2013 CIR Expert Panel meeting, pending reorganization of the safety assessment. During the meeting, the Panel was provided with a letter from the CIR Science and Support Committee, recommending the creation of a new ingredient group consisting of ingredients for which the peptide sequence is known, namely, tripeptide -1, hexapeptide-12 and specific related amides. This has been done. Additionally, at the March meeting, further information was sought to better understand the extent and manner in which solid-phase peptide synthesis is used to create the peptide portion of ingredients included in the safety assessment. To date, this information has not been received.

Included in this package for your review is the draft report, the CIR report history, Literature search strategy, Ingredient Data profile, 2013 FDA VCRP data, Minutes from the March 2013 Expert Panel Meeting, Letter on ingredient group revision from the CIR Science and Support Committee (pcpc1), and use concentration data received from the Council (data1 pdf file).

A new draft safety assessment on tripeptide-1, hexapeptide-12 and related amides was developed. Of these ingredients, two are palmitoyl oligopeptides. The old definition of palmitoyl oligopeptide in the International Cosmetic Ingredient Dictionary and Handbook included amino acids, but the specific peptide sequence was not stated. The new definition in the Dictionary mentions specific amino acid sequences, in that palmitoyl tripeptide-1 (Pal-glycine-histidine-lysine) or palmitoyl hexapeptide-12 (Pal-alanine-proline-glycine-valine-glycine-valine or Pal-valine-glycine-valine-alanine-proline-glycine) is the new name for palmitoyl oligopeptide on an interim basis.

Frequency of use data on palmitoyl oligopeptide (peptide sequence not stated) and palmitoyl tripeptide-1 (known to have gly-his-lys sequence) were provided by FDA, and use concentration data provided by the Personal Care Products Council include data on palmitoyl oligopeptide (peptide sequence not stated) as well as palmitoyl oligopeptide with either of the two peptide sequences. However, it is likely that the peptide sequence of palmitoyl oligopeptide in FDA’s database is glycine-histidine-lysine, alanine-proline-glycine-valine-glycine-valine, or valine-glycine-valine-alanine-proline-glycine. With this in mind, the Panel needs to determine whether the FDA frequency of use and Council use concentration data on palmitoyl oligopeptide – sequence unstated (which could be either of 3 peptide sequences) are useful, taking into consideration the availability of FDA use frequency data on tripeptide-1 and palmitoyl tripeptide-1 and Council use concentration data on palmitoyl oligopeptide (glycine-histidine-lysine sequence), palmitoyl oligopeptide (valine-glycine-valine-alanine-proline-glycine sequence), tripeptide-1, and palmitoyl hexapeptide-12. Furthermore, the data on palmitoyl oligopeptide – sequence unstated may be considered useful if the Panel determines that the biological activity/toxicity of each of the 3 possible peptide sequences is essentially the same. If this determination is made, it could be stated in the report text that, by
definition, palmitoyl oligopeptide in Table 2 is palmitoyl oligopeptide with either of the 3 peptide sequences. This statement would clarify the palmitoyl oligopeptide – sequence unstated entry in Table 2.

It should also be noted that data on Matrixyl 3000, one of the trade names under which palmitoyl oligopeptide is being marketed, are also included in this safety assessment. According to the *International Cosmetic Ingredient Dictionary and Handbook*, palmitoyl tripeptide-1 and palmitoyl tetrapeptide-7 are components of Matrixyl 3000. Furthermore, according to another source, the amino acid sequence of the peptide portion of palmitoyl tetrapeptide-7 in Matrixyl 3000 is glycine-glutamine-proline-arginine. In the absence of information on the % composition of palmitoyl tripeptide-1 versus palmitoyl tetrapeptide-7 in Matrixyl 3000, the Panel needs to determine whether data on Matrixyl 3000 can be used to evaluate the safety of palmitoyl tripeptide-1 or other ingredients included in this safety assessment.

Additionally, after reviewing the available data, the Panel needs to determine whether an insufficient data announcement or tentative report with a safe as used, safe with qualifications, or unsafe conclusion should be issued.
CIR History of:

Palmitoyl Oligopeptides

The Scientific Literature Review on Palmitoyl Oligopeptides was announced in August of 2012.

1st Review, Belsito and Marks Teams/Panel: March 18-19, 2013

The following data on palmitoyl oligopeptides, received from the Personal Care Products Council, are included in the draft report: chemistry (UV-visible spectral analysis, logP, and impurities data included), methods of production, use concentration data, acute oral toxicity, ocular irritation, skin irritation/sensitization (animal and human), and genotoxicity. These data have been incorporated into the draft report. Comments from the Council were also received and have been incorporated.

On day 2 of the Panel meeting, the Panel was provided with comments on the grouping of palmitoyl oligopeptide ingredients from the CIR Science and Support Committee. These comments included the peptide sequence(s) for only 2 ingredients, palmitoyl hexapeptide-12 and palmitoyl tripeptide-1, and it was also noted that tripeptide-1 and hexapeptide-12 are listed in the International Cosmetic Ingredient Dictionary and Handbook. Thus, the CIR Science and Support Committee recommended, in their comments, that the safety assessment be revised to include hexapeptide-12, tripeptide-1, and ingredients in the Dictionary that contain the hexapeptide-12 or tripeptide-1 peptide sequence(s).

The report was tabled by the Panel, pending reorganization of this document. The palmitoyl oligopeptide ingredients were preliminarily grouped together, as they are related structurally by an identical fatty, hydrophobic tail connected to a variable sequence of peptides. The Panel noted, however, that the terminology used for these ingredients does not enable adequate evaluation.

Further information was sought to better understand the extent and manner in which solid-phase peptide synthesis is used to create the peptide portion of such fatty acid peptide ingredients. If additional information enables a better understanding of the amino acid sequences of the peptides of these ingredients than afforded by their definitions in the dictionary, then grouping them together in some fashion may be reasonable.

It was agreed that, if there is a substantial degree of randomness associated with the peptides of these ingredients, then it would be important for the Panel to consider how that might influence the safety evaluation. For example, some small peptides are potent stimulators of angiogenesis. The potential for such an activity to promote tumor growth and metastasis in people with undiagnosed skin cancer might then be an issue. Given the present uncertainties, grouping a large number of these ingredients together might be inappropriate.

2nd Review, Belsito and Marks Teams/Panel: March 17-18, 2014

Of the ingredients included in the draft report that was reviewed at the March 2013 Panel meeting, current definitions in the International Cosmetic Ingredient Dictionary and Handbook include the peptide sequence for only 2, namely, palmitoyl hexapeptide-12 (either val-gly-val-ala-pro-gly, or ala-pro-gly-val-gly-val) and palmitoyl tripeptide-1 (gly-his-lys). Thus, the draft report has been revised to include palmitoyl hexapeptide-12, palmitoyl tripeptide-1, hexapeptide-12, tripeptide-1, and other ingredients in the Dictionary that contain the hexapeptide-12 or tripeptide-1 peptide sequence(s).

At the March 2013 panel meeting, further information was sought to better understand the extent and manner in which solid-phase peptide synthesis is used to create the peptide portion of such fatty acid peptide ingredients. To date, this information has not been received.

<table>
<thead>
<tr>
<th>Palmitoyl Oligopeptide</th>
<th>Acute toxicity</th>
<th>Repeated dose toxicity</th>
<th>Irritation</th>
<th>Sensitization</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

- **Tripeptide-1**
  - X
  - X
  - X

- **Palmitoyl Tripeptide-1**
  - X

- **Palmitoyl Hexapeptide-12**
  - X

- **Biotinoyl Tripeptide-1**
  - X

- **Copper Tripeptide-1**
  - X

- **Acetyl Tripeptide-1**
  - X

- **Azelaoyl Tripeptide-1**
  - X

- **Bis(Tripeptide-1)**
  - X

- **Copper Acetate**
  - X

- **Manganese Tripeptide-1**
  - X

- **Myristoyl Tripeptide-1**
  - X

- **Hexapeptide-12**
  - X

- **Myristoyl Hexapeptide-12**
  - X

- **Aminolevulinoyl Tripeptide**
  - X

- **Anacardoyl Tripeptide-1**
  - X

- **Caffeoyl Tripeptide-1**
  - X

- **Coumaroyl Tripeptide-1**
  - X

- **Mevalonoyl Tripeptide-1**
  - X

- **Nicotinoyl Tripeptide-1**
  - X

- **Quinoyl Tripeptide-1**
  - X

- **Retinoyl Tripeptide-1**
  - X

- **Thioctoyl Tripeptide-1**
  - X

- **Ursoloyl Tripeptide-1**
  - X
New Search Strategy
(Tripeptide-1, Hexapeptide-12, and Related Amides – 9/18/2013 and 1/9/14)

SciFinder Searches

- Palmitoyl Oligopeptide (2013 and 2014 only)
- Tripeptide-1
- Acetyl Tripeptide-1
- Azelaoyl Tripeptide-1

- Bis(Tripeptide-1) Copper Acetate
  CAS No. 130120-57-9

- Copper Tripeptide-1
- Manganese Tripeptide-1
- Palmitoyl Tripeptide-1

- Myristoyl Glycine/Histidine/Lysine Polypeptide
- Myristoyl Tripeptide-1 (Interim New Name)

- Hexapeptide-12
- Myristoyl Hexapeptide-12
- Palmitoyl Hexapeptide-12
- Aminolevulinoyl Tripeptide-1
- Anacardoyl Tripeptide-1
- Biotinoyl Tripeptide-1
- Caffeoyl Tripeptide-1
- Coumaroyl Tripeptide-1
- Mevalonoyl Tripeptide-1
- Nicotinoyl Tripeptide-1
- Quinoyl Tripeptide-1
- Retinoyl Tripeptide-1
- Thioctoyl Tripeptide-1
- Ursoloyl Tripeptide-1

PepBank Search (amino acid sequences)

- Gly-his-lys
- Val-gly-val-ala-pro-gly
- Ala-pro-gly-val-gly-val
Palmitoyl Oligopeptides

Anything else? Okay, palmitoyl oligopeptides. The day has come. I think that this is the first time we're looking at this. We do have some data -- safety test data on a few ingredients.

But the issue I had was that these peptide residues vary in chain length. We're told that the order of the amino acids within the residue can be very different despite having the same name. Clearly, some of these are biologically active, not only in terms of things that might not give me concern, like collagen synthesis in getting rid of wrinkles, but muting proinflammatory cytokines like IL6.

So one of the questions I would have is, what happens if you put this over a skin cancer, like a melanoma, and you reduce immune response, do you enhance the risk of metastases?

My safety issues with this group went on and on, and I really thought that sort of at the end of the day maybe -- let me see if I can see all the comments.

It's really difficult, and I almost felt that there were only two products -- the nanofiber gel and the biocide, nanofiber gel and the biopeptide CL -- that maybe had enough data, but even then you had to go set up with trade name because the other ones might have totally different amino acid sequences as far as my understanding.

So I had a real hard time wrapping myself around this family of ingredients and even thinking that we could do palmitoyl oligopeptides as a family.

So, with that as an overview, I'll turn it over to -- I had a lot of comments throughout the document, but I'll turn that over to Dan and Paul.

DR. LIEBLER: Okay. So, this one -- I had a lot of comments on it.

It seems more complicated than it really is, just from a chemistry perspective. But these products are all peptides, and the end terminus is modified by palmitic acid. So it's an N-palmitoyl version of each of these peptides.

The ingredients have a nomenclature that's confusing at first because a palmitoyl dipeptide, for example, could have a number of different ingredients. But the number after the dipeptide is apparently a code for which amino acids are in the dipeptide or in the tripeptide or in the tetrapeptide and so forth.

And I think table 1 provides some of that information, but it's confusing to me because when I read the method of manufacturer it indicates that most of these and almost all of these are produced by solid-phase peptide synthesis, which generally means you put on specifically one amino acid; then you put on another amino acid; then you put on another and another. And so, that will produce peptide sequences of high purity.

And of course, the last step -- you put on the palmitic acid on the end terminus, and you're done. That generally produces peptides of high purity in a very well defined composition sequence.

Now the table 1 is confusing because it has entries that indicate this tripeptide, for example, may contain lysine and glycine, for example, or two other amino acids. At least three amino acids with at least two of these -- I mean -- I'm sorry. A tripeptide with at least two of these amino acids in any order.

The in-any-order part, which appears in the table, seems to be inconsistent with the way solid-phase peptide synthesis works unless the synthesis actually took as the first amino acid a pool of the possible amino acids, added that, and then in the second step used a pool instead of a pure amino acid and added that.

And I can't tell -- I mean, I don't know how it's done. I could imagine that that could be done.

I'm not sure why you would do it except to have more diversity in the structures and only have to do one batch process to make the compound.

So I think we need to have more information about that because the method of synthesis suggests logically that these should be defined sequences whereas the table information suggests that these are semi-randomized sequences of a least defined composition in terms of the
amino acids.

DR. BELSITO: Well, we're talking about table 1, Wilbur. There were three ingredients where you say monograph development in progress. Is this a REACH dossier? What is this monograph that's in process?

DR. ANDERSEN: Something new.
DR. BRESLAWE: It's an INCI monograph.
DR. BELSITO: INCI monograph.
DR. BRESLAWE: Nomenclature.
DR. BELSITO: Nomenclature, okay.
DR. ANDERSEN: Something new is being added to the dictionary.
DR. BELSITO: Okay.
DR. ANDERSEN: Dan, what's the counterpart methodology that would yield a gemisch of ordering?

DR. LIEBLER: Well, if you did pools, if you used the solid-phase method and you did pools instead of -- for example, let's say you were making -- I'm trying to find an example. Here's palmitoyl peptide-4, which is page 33 of the PDF file. It's got lysine-threonine, lysine-serine as the four amino acids. You could start with a pool of all of serine, lysine, threonine, and then the first residue would be a mixture of those, proportional to what's in the pool. And then in the next you could use another pool. But then you wouldn't get a defined sequence that is portrayed here.

It seems to me that something is not correct in the way that this is described. It could be that these are actually randomized sequences that contain the named amino acids, but it might be that they're not really randomized. So I don't know.

That information should be available. It's just a matter of asking the precise of the manufacturers about how it's actually done and what these do contain.

DR. EISENMANN: I think they've had difficult naming these ingredients over the years as I think you've noticed. So they've started -- one of the first ones that was ever named is this palmitoyl oligopeptide.

DR. LIEBLER: Right.
DR. EISENMANN: And when they gave it a name, I think they gave a name to cover more than one peptide.

DR. LIEBLER: Yes, right. That sounds like a catch-all name.
DR. EISENMANN: Right, right. It's a catch-all name, and now -- right now -- one company is using that name for two sequences.

And then there are other names in there where they -- and then they've gone to this naming, you know, palmitoyl peptide, dipeptide-2. So the next peptide will get the next number.

DR. LIEBLER: Right.
DR. EISENMANN: And, unfortunately, the name is not sequence-specific, and I'm not sure why the INCI committee decided to do it this way. But it seems like the ingredients are sequence-specific based on the information that's been coming in.

DR. LIEBLER: Yes. So, I mean, if the decision has been taken not to make the name sequence-specific, that's out of our hands. We can still deal with these. It just makes it a little trickier.

But what we need is the table 1, essentially, to be a more accurate look-up representation. So, if we look at palmitoyl oligopeptide-6 and we look to table 1, we can see that that's, you know, valine-lysine, valine-histamine, or something like that. We can find it.

DR. EISENMANN: Right. They're just putting them in alphabetical order in the definition, but the information that's been coming in made them telling the sequence of what they actually are.

DR. LIEBLER: So, if these are defined sequence, table 1 currently suggests that they're semi-random.

DR. EISENMANN: Correct.
DR. LIEBLER: So that probably isn't correct. So we need to fix table 1 and then just add the defined sequence.

DR. EISENMANN: Unfortunately, that's the definition.
And I don't know if you've read the memo -- that we're suggesting that you cut back on these ingredients, not to do all the peptides together, that you should pick a peptide in addition to like palmitoyl.

DR. BELSITO: When did we get that memo?
DR. LIEBLER: I might not have gotten that memo.
DR. EISENMANN: I put it on your (inaudible) this morning.
DR. LIEBLER: Oh, I didn't look at that.
DR. EISENMANN: From CIR SSC.
DR. LIEBLER: So what do you want to do?
DR. BRESLAWEC: Well, our proposal is --
DR. EISENMANN: Do a peptide instead of basing it on palmitoyl because, unfortunately, the two peptides that are in palmitoyl oligopeptide also have other names. They're also tripeptide-1 and hexapeptide-12.
DR. LIEBLER: Okay. So we respectfully suggest that CIR Expert Panel table as before so that the panel and staff can consider the following suggestions and develop reasonable science-based strategy for grouping.

So I definitely agree with the table again at this point.
DR. EISENMANN: So then the focus would be for this report just to do tripeptide, which is this three sequence. It's lysine-lysine and the other one is valine- glysine, valine-alanine-lysine. That's the two --
DR. BELSITO: So do a specific amino acid sequence.
DR. EISENMANN: Correct.
DR. BELSITO: And say that specific sequence --
DR. EISENMANN: Correct.
DR. BELSITO: -- is okay.
DR. EISENMANN: Right. That sequence would be sold under these names, under more than one name, unfortunately, currently.
DR. LIEBLER: Well, that would be the least of our problems.
DR. BELSITO: But would there be -- I guess I'm less concerned about the same thing having different names than different things having the same name.
DR. BRESLAWEC: But that's a situation that exists with the current naming.
DR. BELSITO: How is that going to be rectified?
DR. EISENMANN: You would have to say that your judgment is on this sequence, and if something else is being sold under this name --
DR. BELSITO: So, in other words, the report could not be titled The Safety of Ingredient X. It would have to be The Safety of Amino Acid ABCD Palmitoyl and Amino Acid. I mean, how are you going to do that?
DR. EISENMANN: In some ways it's similar to when you do botanicals. For some botanicals, you say the safety you're assessing is of the extract that was tested.

So that also would be true here. It would be the safety of the sequence for which you have data.

DR. BRESLAWEC: What this does is it takes it out of the basic review on the palmitoyl component and focusing on the peptide.
DR. BELSITO: Yes, which is where we need to focus.
I mean, I don't have a problem with that. I don't have a problem looking over the data again. I think that would certainly make it much easier -- to look at a specific amino acid sequence and see what data are out there for it.

I guess I just want to go on record; if one of them is the one that down-regulates IL6, I'm very concerned about a cosmetic product that now is having a potentially significant biological effect in terms of immune responses.

That might be very beneficial for the rosacea patient who has erythema, but I'm concerned about the patient who has a skin cancer who's throwing something on that's going to down-regulate proinflammatory immunity. Just, my big concern with these.
DR. LIEBLER: Yes.
DR. BELSITO: I'm not concerned about sensitization. I'm not concerned about irritation.
DR. LIEBLER: I do think that those claims are being somewhat oversold when I look at the references that supposedly support these kinds of biospecific effects based on these peptides.

I mean, we'll have to come back to that when we have specific ingredients in the table and consider that. It's a potentially important consideration, but I do believe from what I saw that some of that stuff is hype. For example, in a couple of spots in the report, some of these sequences were referred to as being part of a certain collagen or a certain antibody, but with a di or tripeptide sequence that doesn't mean squat, you know, when it comes down to biological activity.

And those sequences are also parts of many other proteins, especially when you're down to a tri or tetrapeptide sequence. They're highly -- appear in many other proteins.

DR. BELSITO: Okay. I mean, I guess the only other comment -- I know this is probably meaningless given the fact that we're going to table this, and I certainly agree with it. You know, since these are small peptides, when you look at the skin irritation and sensitization studies in table 3, page 41 of the Panel Book and you start looking, early on you see a lot of reports of slight erythema, slight erythema, slight erythema, erythema -- all of which were considered to be nonirritant. But it just makes me a little bit concerned were these urticarial reactions to these peptides.

So, I mean, just as we look at it again I would just like everyone to keep that in mind.

Those are my two major issues.

DR. LIEBLER: So I also was going to suggest removing the palmitoylated hydrolyzed plant and animal proteins as being a bridge too far, and that's, I guess, the last paragraph of this suggestion from the council.

And then these potentially other derivatives, like the acetyl tripeptide, azole tripeptide and the copper complexes and manganese complexes and so forth -- I think we do need to have some further discussion and consideration of how to make this grouping more rationale.

DR. SNYDER: So how easy is it going to be -- if we go for the peptide, how easy is it going to be to then know exactly what in the dictionary is encompassed under the peptide designation rather than the lead ingredient, palmitoyl?

So, I mean, how are we going to capture -- are we going to be able to capture all the ingredients?

DR. BRESLAWEC: First of all, I would be glad to bring the editor of the INCI dictionary to provide a briefing about the nomenclature and how it's applied to peptides. I'm not sure it's going to clear everything up, but I think it might provide a better framework for the discussion.

We're not suggesting that from now on every single protein, or peptide, be looked at separately. Our suggestion is based on the fact that this is a new type of ingredient for the panel and work through a more limited sort of report, identify the issues that are critical and then consider, or reconsider, different grouping mechanisms.

So we're not suggesting every peptide should be reviewed on its own from now on.

DR. BELSITO: I totally agree. My thought as I tried to wrap my arms around this family -- I just didn't see it as a family.

So I applaud PCPC for coming up with that approach and also having us look at defined amino acid groups rather than saying, well, this is four amino acids that can be arranged any way you want.

DR. BRESLAWEC: Then again, I don't know. Maybe at the end of your discussion you'll determine that, gee, that's okay.

DR. BELSITO: Right.

DR. BRESLAWEC: But I'm not sure that you can reach that conclusion now.

DR. BELSITO: Right.

DR. BRESLAWEC: And we certainly can't.

DR. BELSITO: Mm-hmm.

DR. LIEBLER: So the idea would be that we would do a focused report with a couple of ingredients and then once we've got our bearings either reopen the report or do another report with more ingredients?
DR. BRESLAWEC: I think that's an administrative question --
DR. LIEBLER: Okay.
DR. BRESLAWEC: -- and can be handled pretty much a lot of different ways.
DR. LIEBLER: Okay, but I agree with that strategy.
DR. BELSITO: But don't you think the order of the amino acid is going to have very significant effects on their biological activities, or you think these are so small and they're linked to this palmitoyl group that it really doesn't matter?
DR. LIEBLER: I favor the latter. I mean, in other words, I don't think that these -- I don't think that we're going to be running into magic sequences that have profound biological activities with these very small peptides.

My only concern is when we actually get into larger peptides from hydrolyzed proteins. That's when we might actually get into antigenic epitopes that would be likely to produce allergic responses. I'm not sure that we would be getting, you know, profound mimicry of biological signaling molecules with these peptides, for example, certainly not with dipeptides, tetrapeptides, those kinds of things here.
DR. BELSITO: Anything else? Okay, if not, tabled.
DR. ANDERSEN: Tabling it is easy. Thinking about what the right groupings should be is a step that I'm not comfortable that I understand what's being proposed by the council. So I think we've got a lot of work ahead of us to figure out just what such a grouping would look like, strategically. I wish it was clear to me now, but it's not.
DR. BRESLAWEC: We also wish we could propose a clear path forward here, and I'm not sure that we can. We just think it deserves, you know, a little more time and consideration.

MR. JOHNSON: I just have one question regarding one of the comments provided, you know, stating that the substances in the report do not have INCI names and they should be deleted from the safety assessment.
DR. BELSITO: They don't have cosmetic uses. I don't know that they should be deleted. I think it's the purview of the panel, you know, as to whether we think it contributes to the understanding of the safety of the ingredients that are actually used as cosmetics.
DR. EISENMANN: Well, if the report now is going to be on a specific sequence, then data on other sequences are not necessary.
DR. BELSITO: Right. That would be --
DR. EISENMANN: So that's those other -- some sequences they're developing just to simulate the immune system, and those aren't relevant to --
DR. BELSITO: No, no, no. We're going to look at two or three specific amino acid sequences, and all of this other -- any data on other sequences should not be included in the report.

DR. BRESLAWEC: I guess my question is, did you decide then to go ahead with this study but limited to the one ingredient which has two different specific peptides in it and table the rest?
Are you planning on moving ahead --
DR. BELSITO: No, we're going to table the whole thing.
DR. BRESLAWEC: The whole thing, okay.
DR. BELSITO: Yes. I mean, I think it will be easier to look at the data again --
DR. LIEBLER: Right.
DR. BELSITO: -- when all the extraneous stuff has been removed and we know what we're looking at.
DR. BRESLAWEC: Okay.
DR. ANDERSEN: My inclination at this point -- until we better understand the proposal on the flipside here of keeping palmitoyl oligopeptide and adding a whole bunch of things that weren't previously in the report, it's hard to assess that without doing some homework. I could see just backing the report off to the oligopeptide with its two incarnations.
DR. EISENMANN: See, but those two incarnations have other names. That's the thing. The peptide part of it is tripeptide-1 or hexapeptide-12.
DR. ANDERSEN: Okay.
DR. EISENMANN: So that's why I suggested some of these other components.
DR. LIEBLER: Yes, I think having, you know, palmitoyl oligopeptide as sort of the blanket name and then there are other ingredients that are chemically defined, with different names, doesn't really pose a big problem for us for reviewing those.

There could be a problem more on the end of the definitions in the dictionary and how council deals with it. That's another issue really.

But I think particularly for this prototype, if we end up dealing with two or three chemically well defined substances, then I think we're back in business.

DR. ANDERSEN: Okay. So let me feed it back to you, Carol, and see if I understand the rationale.

Palmitoyl oligopeptide is an old INCI name that is, in truth, two more specific INCY names -- palmitoyl tripeptide-1 and palmitoyl hexapeptide-12.

DR. EISENMANN: Correct.

DR. ANDERSEN: Okay. So you would add those two. And then taking off on that, any safety assessment of palmitoyl tripeptide-1 would legitimately address tripeptide-1 by itself. Any review of palmitoyl hexapeptide-12 would naturally address hexapeptide-12. And as long as you're going to focus on tripeptide-1, why not look at manganese tripeptide-1?

DR. EISENMANN: Right, and I don't know where I cut it, or we cut it -- you know, these other -- I didn't think these other ones in the bottom group were necessarily appropriate to look at, but I just wanted to be inclusive and include every tripeptide-1 that was in the dictionary at this point.

DR. ANDERSEN: I understand, but in a limited fashion --

DR. EISENMANN: Correct.

DR. ANDERSEN: -- the logic is oligopeptide is actually, again, an old definition that includes two specific newly identified names.

So, in a formulation, a company could use either palmitoyl tripeptide-1 or palmitoyl oligopeptide and be kosher as it now stands?

DR. EISENMANN: Probably both. If one company has the name palmitoyl oligopeptide and if you probably bought the ingredient from them, you would use -- so if you bought it from another company, you would probably use palmitoyl tripeptide-1.

DR. ANDERSEN: But for purposes of constructing a family, I think get the logic represented in this first grouping.

DR. LIEBLER: So, Alan, are you suggesting that this construction include the palmitoyl versions of these peptides as well as the non-palmitoyl versions of these peptides?

DR. BELSITO: That would be more reasonable, yes.

DR. ANDERSEN: I think so because I can't picture that the palmitoyl moiety is going to be the issue.

DR. LIEBLER: Well, it's going to change the properties of these a lot.

DR. EISENMANN: Frequently, the peptides -- as I understand it, the peptides themselves aren't necessarily used. They've all been added to the dictionary as part of the naming process. So, if somebody just comes in and wants the palmitoyl peptide, they will also name the peptide itself now, so whether or not the peptide itself is used.

DR. ANDERSEN: It's a building block.

DR. LIEBLER: So that's my concern. If the -- because I think the palmitoyl versions of these short peptides are going to have very different properties than the nonmodified versions and these short peptides are going to really fall into our short peptide analysis family. I'm wondering if they could be included in the other reports we're doing on short peptides or hydrolyzed proteins.

DR. BELSITO: Well, why don't we see what's there?

DR. LIEBLER: Yes.

DR. BELSITO: We don't even know what's in the dictionary. It may turn out that there's only palmitoyl for these, I mean.

DR. EISENMANN: No, they're listed --

DR. LIEBLER: They're listed at the bottom.

DR. BELSITO: So, I mean, let's look at them. We can always delete the ingredients, but I'm certainly more comfortable using the framework of the amino acid sequence and then look at what's added to it rather than palmitoyl with any old amino acid sequence.
DR. LIEBLER: Okay. I mean, we're going to go through another kind of --
DR. BELSITO: We're going to table it again anyway.
DR. LIEBLER: Yes, we're going to go through another round of thinking about
this and another round of discussion, and we may end up backtracking a little bit on some things
that we come up with today. That's fine.
DR. BELSITO: Okay. Anything else?
MR. JOHNSON: Dr. Belsito, I have one question.
DR. BELSITO: Yes.
MR. JOHNSON: Carol mentioned two palmitoyl oligopeptides. I know on
Panel Book page 14 under Composition and Impurities there are two different CAS numbers listed
for palmitoyl oligopeptide. And I was wondering, are those indicative of the two different names
that you were referring to earlier?
Those two, okay.
DR. BELSITO: We will also see structures next time and have very specific
molecules to work with.
DR. EISENMANN: They provided sequences.
DR. BELSITO: Yes.
DR. EISENMANN: All right.
Palmitoyl Oligopeptides

Dr. Marks: Are there any other comments? If not, we'll move on to the next ingredient. Wilbur, you're up again. This is the palmitoyl oligopeptides, and this is the first time we're looking at this group of ingredients. We received a memo dated March 18 on paper. This morning I found it on my desk here from the CIR Science and Support Committee of the Personal Care Products Council suggesting that this group of ingredients should be tabled. There are some issues in terms of nomenclature and also a suggestion as to what should be included. Do you want to address that memo? I think that's important. Have you seen this memo, Tom, Ron, Ron? Are you reading it now?

Dr. Johnson: I think our primary objection is that the family is generated by the palmitoyl moiety when we don't think the palmitoyl moiety is going to drive the physiologic activity. We think it would be more appropriate from a toxicologic standpoint to generate the families based on the protein loyalty which is more likely to be the driver. We're not suggesting that the family is wholly inappropriate or that it needs to be done material by material, but we would slice and dice this group quite differently and not based on alphabetization but, rather, generated on chemistry and physiology and therefore are not really prepared to suggest how the grouping should be formed today but that it be tabled and reexamined.

Dr. Marks: I see Ron Hill shaking his head yes, nonverbally communicating he likes that idea. Ron Shank, Tom?

Dr. Shank: I have other concerns. There are several pages in the report on cellular activity. These compounds are capable of stimulating collagen synthesis, angiogenesis and others, and I wonder does that not make them drugs? And if the answer is, no, it does not, could the Council please address that for us? And if it does, should these be reviewed as a cosmetic ingredient? Also I think there is a strong data need because of this that we need reproductive and developmental toxicity data. In Wave 2 we got some information on penetration of these compounds and it shows that they penetrate into the dermis. But then the author concluded it would not therefore to into lymph and blood and I don't quite understand that. If it gets into the dermis I think it would get into the lymph and blood. I have several concerns about the safety not just the grouping.

Dr. Marks: Thank you, Ron. I was going to ask for a preview of where we would be going with these ingredients. You had also endorsed tabling it, but I raise these concerns at this point so that the next time we see these ingredients these issues may be addressed.

Dr. Shank: Yes. It's going to a scientific committee of the Council? If they could consider not only how to group these chemically but also are these drugs as well as cosmetic ingredients?

Dr. Ansell: I think the CIR Support Committee would be more than happy to reply suggesting a more appropriate grouping. We would be happy to address any questions. As it relates to the physiologic activity as it relates to its designation as a drug or cosmetic, I would suggest that would be directed to the FDA liaison as to its regulatory status.

Dr. Slaga: Do these have a type of effect in vivo if the doses are used in cosmetics? I agree with Ron that the cellular effects are quite pronounced in some cases, but what I'm suggesting is does this really occur in vivo as a cosmetic?

Dr. Shank: I could see advantages in cosmetics to stimulate collagen synthesis.

Dr. Slaga: But not angiogenesis.

Dr. Shank: Not angiogenesis, no, but certainly collagen synthesis for removing wrinkles.

Dr. Bergfeld: That's very significant. Was there significance in the paper?

Dr. Shank: Yes.

Ms. Lorentz: May I comment that that gets to the whole grouping question too, again the driver not being the palmitoyl.

Dr. Shank: Using the driver is maybe not the right term because it's the palmitoyl that drives the peptide into the skin. Just the peptide itself would be absorbed
think rather slowly, but when you add a fatty acid to it, that makes penetration much more likely.

DR. HILL: Not just into the skin, but into cells once it gets inside. The other thing I picked up on this was that it also increases the interest in impurities if you have for example a palmitoyl pentapeptide which perhaps in and of itself isn't inactive, you would want to know if there was a tripeptide or tetrapeptide impurity for example based on the ways that peptide synthesis is done. It's challenging to get 100 percent pure peptides. Then you need information about those impurity levels and some biological activity, so that would be another related issue to this in my mind. I was looking for ingredient-by-ingredient information if we were going to go forward with these as they were.

DR. ANSELL: We're not suggesting what the conclusion would be. We're certainly not suggesting ingredient-by-ingredient reviews. But we do think that if we pulled the family apart and reassemble it into smaller groups, perhaps they would lend themselves to building new and larger families but not based solely on the derivation but, rather, a look at both sides.

MS. WEINTRAUB: I also noted the lack of reproductive and developmental toxicity data, also no carcinogenicity data and absorption, distribution, metabolism and excretion wasn't found either. So there seems to be a lot of data needed in addition to other concerns.

DR. MARKS: Tom, were you concerned about carcinogenicity of these compounds?

DR. SLAGA: No.

DR. MARKS: With the lack of data, the reason you weren't, again going forward? Or you thought there was enough in here in answering Rachel's concern?

DR. SLAGA: On carcinogenicity? I didn't have a concern about carcinogenicity. I do believe that we had some genotoxicity data and that was sufficient.

DR. SHANK: But there is a data need for reproductive and developmental.

DR. SLAGA: Yes.

DR. MARKS: That was clear. Rachel was anticipating my next question which was were there any other obvious data needs? Obviously since we're tabling it we're not going to sort through the compounds. That was one of the things I had printed out, the long list of compounds or ingredients.

DR. HELDRETH: I wanted to add that if we're going to look at these in a different grouping fashion based on the protein portion of the molecule, you'll note in the definition that there's a great amount of possible variability of what the amino acids are and what order they're in. So if we're going to base it on that part of the molecule, it would be nice if the support committee could provide us with which actual compounds are designated, say, palmitoyl tetrapeptides because that can be a number of different actual ingredients that if we're looking at the protein function or protein side of it are quite different.

DR. MARKS: Is there any comment about that, Jay?

DR. ANSELL: Yes. There was a change in nomenclature from the parent ingredient to nomenclature of other naming conventions later on in the INCI process that are perhaps better and would certainly try to point out the correct INCI nomenclature.

DR. HILL: One thing I wanted to follow-up on to Dr. Slaga's comment is while there is nothing indicative of carcinogenicity, I don't remember if it's in the first submission or in Wave 2, there is language to suggest that there might be changes in invasiveness of cells depending on the particular peptide so that's something that needs some attention. It's different than tumorigenicity and any of that, but it's changing the invasive character of the cells, then that's important.

DR. SLAGA: That relates to the antiogenesis that Ron has concern about.

DR. MARKS: Tom, going back to Rachel's question, it doesn't change your concern.

DR. SLAGA: No, it doesn't change anything about the genesis.

DR. MARKS: But the question is could it enhance the invasion if were metastatic?

DR. SLAGA: Angiogenesis inhibitors are used a lot in treatment of malignancy.

DR. HILL: Angiogenesis and cell invasion are related, but distinct phenomena as well.
DR. MARKS: We have some significant issues to resolve with these ingredients in terms of its biologic activity and back to originally is this a drug or a cosmetic. Perhaps we could dodge it if we say it's a drug, but I don't think we can do that. We have to address this. Rachel?

MS. WEINTRAUB: I have one question for Tom about genotoxicity that nanofiber gel CS was found to be genotoxic but not without metabolic activation in certain strains.

DR. SLAGA: What you have to do is take the total data for all of genotoxicity and in general it's more predominantly negative. That was the only positive and that happens now and again. I can't argue that that's not true, but generally since the majority are negative, I don't have a concern.

DR. MARKS: Anticipating when this is resubmitted, the different ingredients, was there any concern of several of these ingredients having hydrolyzed collagen? You were talking about the peptide variability. Is there any concern that these have collagen? You, Ron Shank, mentioned right in the beginning that they can increase collagen synthesis, but if you look at sodium palmitoyl hydrolyzed collagen, there is palmitoyl hydrolyzed collagen and then there are these proteins also. If we hadn't looked at this group I was going to say did you want to split any of these things out because of the difference there. But what's your sense with those? Did that raise any concerns or not, different than the peptide?

DR. SHANK: No concerns other than the other report which is on hydrolyzed amino acids and proteins.

DR. MARKS: Presumably tomorrow I'll be seconding a motion that these ingredients be tabled and I'll raise the concerns that were raised here, the need for repro and developmental toxicity. And I'll raise the thorny question whether this is a drug or a cosmetic or at least mention it because of the activity of increased collagen synthesis, the angiogenesis, and obviously the grouping is the main driver.

DR. BERGFELD: I want to add a comment that in many cosmetic products that have antioxidants in them there is increased collagen being formed and there is a biological activity of the epidermis and the upper portions of the dermis and that is still considered a cosmetic. It's dependent on concentration of the antioxidant and there are some that are released only to physicians which are higher concentrations and to my knowledge there is no prescription item in the antioxidant group.

DR. HILL: Are you talking about something like kojic acid?

DR. BERGFELD: We have a lot of antioxidants that fall into the fruit acid groups and the retinoids in a whole line of cosmetics now, but they all have biological activity and we can even see it on histology as well as clinical improvement of skin texture and wrinkles.

DR. HILL: I brought up sunscreens at the last meeting in a moment of lapse, but I had read just a few weeks before that the very extended version of what's a drug, a medical device and a cosmetic and there are clearly gray areas and maybe this is a case where we need some further clarification, and it may not be forthcoming anytime soon I guess.

DR. MARKS: I'll mention it tomorrow, but as Wilma you've said, we have other ingredients that have had biologic effects like alteration of the immune system and perhaps one of them that we're discussing today can have an impact. It should be raised and then we'll see where we go with it as we review the ingredient.

DR. BERGFELD: Ron, as to localized effects versus a systemic effect, would that make a difference for you between cosmetic and drug?

DR. SHANK: Certainly if it's systemic, yes. As to local, I'm not so much concerned.

DR. BERGFELD: I think these are mainly local.

DR. ANSELL: At very low-use concentrations with some products as well at ten-thousandth of a percent.

DR. MARKS: Are there any other comments about these palmitoyl oligopeptides?

DR. JOHNSON: I have one question, Dr. Marks, with respect to the Wave 2 data. Is it agreeable that all of those data will be incorporated into the safety assessment?

DR. MARKS: I guess if it's applicable to the ingredients in this new presentation. We'll see in the next draft report. Is there anything else about these ingredients?
DR. HILL: Wilbur, I assume you would like whatever commentary, thoughts or feedback on that, you would like that along with the actual report.
DR. JOHNSON: Yes.
DR. HILL: It will be forthcoming. I just wanted to be sure.
Palmitoyl Oligopeptides

DR. SNYDER: Yes, the Palmitoyl Oligopeptide is a -- the document, the first time we've seen this, the document comprises of 45 ingredients based upon a scientific literature research that was conducted in August of 2014. We had quite a lengthy discussion about this ingredient, also, and our team came to the conclusion that we wanted to make a motion to table this ingredient to identify the correct groupings and have the groupings be based upon the peptide, not based upon what the peptide is bound to. We thought that would be a better way to look at these ingredients and maybe to bring other ingredients into the mix. And, so, we would make a motion to table this ingredient.

DR. MARKS: Second.

DR. BERGFELD: Motion's been made and a second. There's no comment on that. All those in favor at the table?

DR. MARKS: Well --

DR. BERGFELD: Okay, it's approved. To table and comments now.

DR. MARKS: Yes, our team discussed some needs. Even though we tabled it, we wanted to alert interested parties that we were concerned about reproductive and developmental toxicity and we needed data on that. And then we actually had a fairly robust discussion about the potential drug effects of these particularly increased angiogenesis from these compounds. So, again, we wanted to delve into the cellular activity of these compounds and get some more --

DR. SLAGA: No, the cellular effects and cell culture, that's where this data came from being stimulating angiogenesis and really the critical thing is does this occur in vivo?

DR. BERGFELD: Ron Shank?

DR. SHANK: Well, and also it has many biological activities and I questioned whether these were actually drugs and should be reviewed as cosmetic ingredients at all. I think when there is a discussion, that has to be handled somehow. And that's why we felt a need for reproductive and developmental toxicity data.

DR. BERGFELD: Paul?

DR. SNYDER: We would agree with that assessment.

DR. BERGFELD: All right, any other discussion points that need to be put on the table for the minutes?

Dan?

DR. LIEBLER: One other point was just some clarification of the actual chemical composition of whichever of the Palmitoyl Oligopeptides are advanced for a consideration in the future. Most of these appear to be made by solid phased peptide synthesis, which should produce defined sequences, mixtures are pretty high purity, but the table one indicates that they're semi-random mixtures.

So, I mean, I suppose you could do a pooled approach to solid phase synthesis where you use a pool of amino acids for the first cycle, a pool for the second cycle. I'm not sure what's done, so, that needs to be clarified and the naming conventions, well, there isn't any, but it is completely unsatisfactory for these, so, it's really hard to tell what we're reviewing.

So, whether it's industry or whether it's the CIR staff or some interaction between those to come up with information to tell us exactly what it is that we're looking at. It's important and it's particularly going to be important if we're considering any biological activities of these compounds because if they are specific to sequences, then, obviously, we need to know the sequences involved.

DR. BERGFELD: Ron Hill?

DR. HILL: Yes, in follow-up to that, it's very well-known that when you do peptide synthesis of any kind, be it solid phase or liquid phase, certain steps are more problematic and sometimes they don't go to 100 completion. So, then what you have a fraction, however small, depending on how the analytical chemistry is done where there's a missing amino acid, and,
so, then if it does on to the next step, suddenly, you've got a sequence that's different, it's shorter, and sometimes that can be 10 percent of the mixture and then that's typically handled at the end with purification.

So, that's the kind of information that would be needed particularly when we're looking at biological activity and I was trying to do a search here because they tried to use -- I didn't know whether this came from staff or from industry or who, but they tried to use DEREK to justify lack of hits and I think that's an entirely inappropriate use of that program in this particular case, and, so, I wouldn't really buy that without a lot more detail.

DR. SNYDER: I'll throw one more piece on the pile here.

DR. BERGFELD: Okay.

DR. SNYDER: The only acceptable method for characterizing compounds like this these days is mass spectrometry.

DR. BERGFELD: Thank you. All right, we'll move ahead then. This has been tabled.
Safety Assessment of
Tripeptide-1, Hexapeptide-12, and Related Amides
as Used in Cosmetics

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INTRODUCTION

The safety of tripeptide-1, hexapeptide-12, and related amides used in cosmetics is reviewed in this safety assessment. These ingredients function primarily as skin and hair conditioning agents, skin protectants, and antioxidants in cosmetic products. However, other functions include: skin bleaching agents (anacardoyl tripeptide-1, coumaroyl tripeptide-1, and quinoyl tripeptide-1), antiacne agents (caffeoyl tripeptide-1), and chelating agents (nicotinyl tripeptide-1). The ingredient name, palmitoyl oligopeptide listed in the International Cosmetic Ingredient Dictionary and Handbook has been retired, and is now represented by the palmitoyl tripeptide-1 or palmitoyl hexapeptide-12 ingredient names in the dictionary on an interim basis. Most of the toxicity data included in this safety assessment are on a trade name material (Matrixyl 3000) containing palmitoyl oligopeptide and palmitoyl tetrapeptide-7, and other trade name materials in which palmitoyl oligopeptide (Val-Gly-Val-Ala-Pro-Gly or Gly-His-Lys sequence) is the only oligopeptide component. The Val-Gly-Val-Ala-Pro-Gly sequence (a.k.a. one of the two sequences of hexapeptide-12) is an elastin peptide and the Gly-His-Lys sequence (a.k.a. tripeptide-1) is a liver growth factor peptide and a fragment of type I collagen. Data on the biological activity of these peptide sequences are also included.

CHEMISTRY

The ingredients in this report are related structurally by bearing one of three distinct peptide sequences, either tripeptide-1 (Glycine-Histidine-Lysine) or hexapeptide-12 (Valine-Glycine-Valine-Alanine-Proline-Glycine or Alanine-Proline-Glycine-Valine-Glycine-Valine). The remaining ingredients reviewed in this safety assessment include one of these three peptide sequences with a different group attached through an amide linkage.

Definition and Structure

A generic structure for palmitoyl oligopeptides [palmitoyl = N-(1-oxohexadecyl); oligopeptides = a chain of 2 or more amino acids linked through a peptide bond (i.e., carboxylic acid of one amino acid reacts with the β-position amine of another amino acid to form an amide (with loss of water)] is shown in Figure 1.

![Figure 1. A generalized structure](image-url)

The ingredient name, palmitoyl oligopeptide in the International Cosmetic Ingredient Dictionary and Handbook has been retired and is represented by the palmitoyl tripeptide-1 (palmitoyl-glycine-histidine-lysine) or palmitoyl hexapeptide-12 (palmitoyl-alanine-proline-glycine-valine-glycine-valine or palmitoyl-valine-glycine-valine-alanine-proline-glycine) ingredient name on an interim basis. The old definition of palmitoyl oligopeptide (retired, amino acid sequence not stated) was: Palmitoyl oligopeptide is the product obtained by the reaction of palmitic acid with a synthetic peptide consisting of two or more of the following amino acids: alanine, arginine, aspartic acid, glycine, histidine, lysine, proline, serine, or valine.

Now that the sequences intended by the ingredient name palmitoyl oligopeptide are known, the structures are as follows:
According to another source, palmitoyl oligopeptide (Pal-GHK; palmitoyl tripeptide-1) is one of 2 active ingredients in the skin care ingredient Matrixyl 3000.2 Data on Matrixyl 3000 are included in this safety assessment. Palmitoyl oligopeptide consists of a short chain of 3 amino acids (also known as GHK peptide [fragment of type I collagen] or glycine-histidine-lysine) that is connected to palmitic acid. The other active ingredient is palmitoyl tetrapeptide-7 (Pal-GQPR), which consists of a short chain of four amino acids (also known as GQPR peptide or glycine-glutamine-proline-arginine) connected to palmitic acid. The tetrapeptide portion is a natural fragment of the IgG immunoglobulin.

Physical and Chemical Properties

Palmitoyl Oligopeptide

A chemical supplier provided data on palmitoyl oligopeptide, identified as CAS No. 147732-56-7 and CAS No. 171263-26-6.3 Palmitoyl oligopeptide (CAS No. 147732-56-7) is also known as Pal-GHK (Pal-Gly-His-Lys-OH) and L-Lysine, N-(1-oxohexadecyl)glycyl-L-histidyl. It is a white powder and has a molecular weight of 578.80 and a log P of 4.81. The ingredient BIOPEPTIDE-CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH [CAS No. 147732-56-7]) has a density of 1.13.

Palmitoyl oligopeptide (CAS No. 171263-26-6) is also known as Pal VGVAPG (Pal-Val-Gly-Val-Ala-Pro-Gly-OH) and Glycine, N-(1-oxohexadecyl)-L-valylglycyl-L-valyl-L-alanyl-L-propyl. It is also a white powder and has a molecular weight of 737.00 and a logP of 5.09.3

Hexapeptide-12 (Val-Gly-Val-Ala-Pro-Gly)

A study was performed to obtain quantitative data on the nature and yields of oxidation products formed by a prototypic oxidant system (HO’/O2) on small peptides, including Val-Gly-Val-Ala-Pro-Gly.4 Study results indicated that hydroperoxide formation occurred nonrandomly (Pro > Val > Ala > Gly) and that the formation of hydroperoxide was inversely related to carbonyl yields (both peptide-bound and released). Multiple alcohols were generated at both side-chain and backbone sites. Summation of the product concentrations provided clear evidence for the occurrence of chain reactions in peptides exposed to HO’/O2, with the overall product yields exceeding that of the initial HO’ generated.
Method of Manufacture

**Palmitoyl Oligopeptides**  
**Copper Tripeptide-1, and Hexapeptide-12**

The following general information relating to the synthesis of peptides coupled to palmitic acid was found in the published literature: Peptides have been synthesized by solid phase fluorenylmethoxycarbonyl chemistry using an Advanced Chemtech MPS 350 synthesizer. Palmitic acid was coupled to the deprotected amino-terminus of the resin-bound protected peptides both manually and by using the peptide synthesizer employing the same reaction conditions used in standard amino acid coupling. Peptides and monopalmitic acid-peptide conjugates were cleaved from the resin, deprotected, and purified using standard procedures.

Several strategies for the synthesis of lipidated peptides, both in solution and on solid support, have been developed. Regarding peptides with longer amino acid chains, synthesis on solid support has practically always been performed. Shorter peptides have been synthesized both in solution and on solid support. Particularly, hexa- and heptapeptides corresponding to the Ras- and Rab-C-termini, respectively, have been synthesized in solution.

Specifically, palmitoyl oligopeptide (CAS No. 147732-56-7) is synthesized via stepwise peptide synthesis (specifically, palmitoyl tripeptide-1). The C-terminal amino acid (Lys) is protected on its acidic function, after which each protected amino acid (Gly, His) is coupled. A last coupling procedure is realized with palmitic acid instead of an amino acid. The protected peptide is deprotected on the lateral function of lysine and histidine and on the C-terminal acidic function of Lys.

Palmitoyl oligopeptide (CAS No. 171263-26-6; one of the palmitoyl hexapeptide-12 sequences) is produced via stepwise acid phase peptide synthesis. The C-terminal amino acid (Gly) is protected on its acid function, after which each protected amino acid (Val-Gly-Val-Ala-Pro-) is coupled. A last coupling procedure is realized with palmitic acid instead of an amino acid. The protected peptide is deprotected to remove the protecting groups present on the lateral function of proline, alanine, valine, glycine, and valine and on the C-term function (Gly) of the peptide.

**Copper Tripeptide-1**

Copper Tripeptide-1 (Glycyl-L-histidyl-L-lysine-Cu$^{2+}$) is prepared by the combination of purified glycyl-L-histidyl-L-lysine with equimolar cupric acetate, followed by neutralization with 0.1 N sodium hydroxide and centrifugation (at 5000 g for 30 minutes at 3°C) to remove insoluble material, usually excess copper (II) as its hydroxide.

**Palmitoyl Tripeptide-1**

Palmitoyl tripeptide-1 (palmitoyl-Gly-L-His-L-Lys) has been produced via solid phase synthesis, yielding a peptide of high purity (> 97%).

**Hexapeptide-12 (Val-Gly-Val-Ala-Pro-Gly)**

The synthetic peptide valine-glycine-valine-alanine-proline-glycine, which contains the recognition sequence for the elastin receptor, has been produced using an automated synthesizer. Reverse-phase HPLC was used for further purification.

**Composition/Impurities**

**Palmitoyl Oligopeptide**

The impurities content of palmitoyl oligopeptide (CAS No. 147732-56-7) and palmitoyl oligopeptide (CAS No. 171263-26-6) has been described as follows: acetate (< 5%), palmitic acid (< 5%), and water (< 5%).

**Tripeptide-1 (Glycine-Histidine-Lysine)**

Commercial glycyl-L-histidyl-L-lysine-Cu$^{2+}$ is approximately 95% pure, but often includes small amounts of mildly neurotoxic materials, as measured by behavior after intracranial injection, tail flick assays, and gripping ability of mice on spinning disks. Most of the neurotoxic materials can be removed by dissolving glycyl-L-istidyl-L-lysine in glass-distilled water (50 mg/ml), centrifuging at 20,000 g for 1 h at 3°C, and then lyophilizing the supernatant.
Copper Tripeptide-1 (Glycyl-L-Histidyl-L-Lysine-Cu\textsuperscript{2+})

Glycyl-L-histidyl-L-lysine-Cu\textsuperscript{2+} is prepared by the combination of purified glycyl-L-histidyl-L-lysine with equimolar cupric acetate, followed by neutralization with 0.1 N sodium hydroxide and centrifugation (at 5000 g for 30 minutes at 3\textdegree) to remove insoluble material, usually excess copper (II) as its hydroxide.\textsuperscript{12} The supernatant (in a solvent of glass-distilled water) is passed through a G-10 column, and the elution peak absorbing at 600 nm is collected and lyophilized to obtain glycyl-L-histidyl-L-lysine-Cu\textsuperscript{2+}.

Crystalline glycyl-L-histidyl-L-lysine-Cu\textsuperscript{2+} is prepared by dissolving glycyl-L-histidyl-L-lysine-Cu\textsuperscript{2+} (30 mg, 88 \textmu mol) in an aqueous copper(II) acetate solution (0.3 ml, 0.3 M). Ethanol (1.26 ml) is added and the vessel walls are then scratched to initiate crystallization of dark blue-purple crystals. The mother liquor is decanted and the crystals are dissolved by adding distilled water. Ethanol (0.4 ml) is then introduced to reach a cloud point. After standing, dark purple-blue octahedral crystals are formed.\textsuperscript{12}

USE

Cosmetic

The ingredients reviewed in this safety assessment function primarily as skin and hair conditioning agents, skin protectants, and antioxidants in cosmetic products.\textsuperscript{1} Additional functions include: skin bleaching agents (anacardoyl tripeptide-1, coumaroyl tripeptide-1, and quinoyl tripeptide-1), antiacne agents (caffeoyl tripeptide-1), and chelating agents (nicotinyl tripeptide-1). According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) in 2013, the following palmitoyl oligopeptides are being used in cosmetic products:\textsuperscript{13} palmitoyl oligopeptide, tripeptide-1, palmitoyl tripeptide-1, biotinoyl tripeptide-1, and copper tripeptide-1.

Results from a survey of ingredient use concentrations provided by the Personal Care Products Council in 2013 indicate that, collectively, the ingredients reviewed in this safety assessment are being used at concentrations ranging from 0.0000001% (palmitoyl oligopeptide (glycine-histidine-lysine-OH [GHK]) and palmitoyl oligopeptide (valine-glycine-valine-alanine-proline-glycine-OH [VGVAPG] ) to 1% (palmitoyl oligopeptide [GHK]). The highest concentration of 1% relates to ingredient use in leave-on products. VCRP data on ingredient use frequencies and use concentration data provided by the Council are summarized in Table 2.

Cosmetic products containing tripeptide-1, hexapeptide-12 and related amides may be applied to the skin and hair, or, incidentally, may come in contact with the eyes and mucous membranes. Products containing these ingredients may be applied as frequently as several times per day and may come in contact with the skin or hair for variable periods following application. Daily or occasional use may extend over many years.

Palmitoyl oligopeptide (sequence unstated) is used in body and hand sprays (maximum use concentration = 0.001%), and biotinoyl tripeptide-1 is used in pump hair sprays (0.0002% to 0.0006%). Because palmitoyl oligopeptide and biotinoyl tripeptide-1 are used in products that are sprayed, they could possibly be inhaled. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 \textmu m, with propellant sprays yielding a greater fraction of droplets/particles below 10 \textmu m, compared with pump sprays.\textsuperscript{14,15,16,17} Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.\textsuperscript{14,15}

Non-Cosmetic

A palmitoyl-tailed sequential oligopeptide carrier (SOC\textsubscript{n}-II) for engineering immunogenic conjugates has been developed.\textsuperscript{18} The authors noted that the main guideline in designing effective immunogens as vaccine candidates capable of eliciting potent and specific immune responses is to combine B/T cell epitopes and adjuvants as immunostimulators on the same carrier that links the major histocompatibility complex with T cell receptors. With the goal of contributing to the development of carriers for human usage, SOC\textsubscript{n}-II was formed by the repeating peptide unit (Aib-Lys-Aib-Gly\textsubscript{n}), \textit{n} = 2-7, elongated from the amino-terminus by the palmitoyl group, which is known for its adjuvanticity. Aib in the amino acid sequence represents \textit{\alpha}-aminoisobutyric acid.
TOXICOKINETICS

In Vivo Studies

Tripeptide-1 (Glycyl-L-Histidyl-L-Lysine)

Glycyl-L-histidyl-L-lysine (1% in saline; dose = 10 mg/kg) was injected into the tail vein of male rats (number and ages not stated). Blood samples were collected prior to dosing and for up to 60 minutes post-dosing. Plasma concentration-time profiles of glycyl-L-histidyl-L-lysine and its L-histidyl-L-lysine metabolite indicated that both were not detected in pre-dose plasma samples. However, after i.v. injection, glycyl-L-histidyl-L-lysine was rapidly degraded to L-histidyl-L-lysine, which was rapidly eliminated from circulating blood. It has been reported that glycyl-L-histidyl-L-lysine is unstable in human plasma and is rapidly degraded by aminopeptidases.

In Vitro Studies

Tripeptide-1 (Glycyl-L-Histidyl-L-Lysine)

In an enzyme assay, the liver growth factor Gly-His-Lys was hydrolyzed by an aminotripeptidase purified from rat brain cytosol.

TOXICOLOGY

Acute Oral Toxicity

Palmitoyl Oligopeptide

The acute oral toxicity of the ingredient BIOPEPTIDE-CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was evaluated using 10 Sprague-Dawley rats (5 males, 5 females; ages not stated). The test substance was administered by gavage at a dose of 2,000 mg/kg. Dosing was followed by a 14-day observation period, after which necropsy was performed. Dosing had no effect on general behavior or body weight gain, and none of the animals died. There were no apparent abnormalities at necropsy. BIOPEPTIDE-CL was classified as nontoxic (LD50 > 2,000 mg/kg).

Repeated Dose Toxicity

Palmitoyl Oligopeptide

There were no clinical signs or mortalities in a cumulative skin irritation study on BIOPEPTIDE CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) involving guinea pigs. In the guinea pig maximization test on BIOPEPTIDE-CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH), the test substance was evaluated at a concentration of 75% in a saline vehicle. Clinical signs were not observed and none of the animals died during the study. Additionally, body weight gain was unaffected by test substance administration.

Ocular Irritation

In Vivo

Palmitoyl Oligopeptide

The ocular irritation potential of the ingredient BIOPEPTIDE-CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was evaluated using 3 male New Zealand White rabbits. The test substance (0.1 ml) was instilled into the conjunctival sac of the left eye of each animal, and the eyes were not rinsed. Ocular reactions were scored at approximately 1 h, 24 h, 48 h, and 72 h post-instillation, and then on days 5 and 8. On day 1, very slight conjunctival
reactions (chemosis and redness) were observed in all 3 animals. No other ocular reactions were observed for the duration of
the study. It was concluded that BIOPEPTIDE-CL was a slight irritant in this study (maximum ocular irritation index = 4.7).

BIOPEPTIDE EL (contains 100 ppm palmitoyl oligopeptide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH) was instilled as
a single dose (0.1 ml) into the left eye of each of 3 male New Zealand White rabbits. Eyes were not rinsed, and reactions
were scored at 24 h, 48 h, and 72 h post-instillation. Moderate or slight conjunctival irritation (chemosis [score = 2] and
redness [score = 1 or 2]) was observed in all animals for up to 4 days post-instillation. Neither iridial irritation nor corneal
opacity was observed. BIOPEPTIDE EL was considered a non-irritant when instilled into the eyes of rabbits. This
conclusion was based on the observation that the mean scores for chemosis, redness, and degree of corneal opacity in 2 of
the 3 animals did not reach the criteria values for irritation under the experimental conditions of the testing facility.

In Vitro

Palmitoyl Oligopeptide

The ocular irritation potential of the ingredient MAXI-LIP (contains 1,000 ppm palmitoyl oligopeptide, as Pal-Gly-
His-Lys-OH) was evaluated in the hen’s egg chorioallantoic membrane in vitro assay. Details relating to the assay protocol
were not included. Sodium dodecyl sulfate (0.5% w/v) served as the positive control. MAXI-LIP was classified as slightly
irritating, but was considered "well tolerated". The positive control was classified as an ocular irritant.

The hen’s egg chorioallantoic membrane in vitro assay was also used to evaluate the ocular irritation potential of
Dermaxyl (contains 200 ppm palmitoyl oligopeptide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH). The test substance was diluted
to 50% (w/v) in distilled water prior to testing. The score for each egg was determined by the sum of the notations of
hyperemia, hemorrhage, and coagulation (coagulation = opacity and/or thrombosis). The notation for the test substance
corresponded to the arithmetic mean, rounded off to one decimal of the scores obtained for 4 eggs. Sodium dodecyl sulfate
(0.5% w/v) served as the positive control. The mean irritation index was 0.8 for diluted Dermaxyl and 12.0 for the positive
control. The test substance was classified as practically non-irritating.

Dermaxyl ocular irritation potential was also evaluated in the SIRC fibroblastic cell line using the neutral red
releasing method. Sodium dodecyl sulfate and sodium chloride served as positive and negative controls, respectively. The
IC50, defined as the test substance concentration that inhibited 50% of the cell survival and growth, was > 50%, and the %
mortality at 50% dilution was 37.9%. It was concluded that the test substance caused negligible cytotoxicity.

Skin Irritation and Sensitization

The following skin irritation and sensitization data on palmitoyl oligopeptide are summarized in Table 3.

Animal

Palmitoyl Oligopeptide

The ingredient BIOPEPTIDE CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was evaluated
for its skin irritation potential using 3 male New Zealand White rabbits. BIOPEPTIDE CL was applied to scarified or non-
scarified skin of the flank (0.5 ml on 6 cm² area, clipped free of hair), using an occlusive hypoallergenic dressing, for 24 h.
Reactions were scored at 24 h and 72 h post-application. At 24 h post-application, slight erythema was observed on both
flanks of 2 rabbits. These were the only reactions observed during the study. BIOPEPTIDE CL was classified as a non-
irritant (PII = 0.3).

A cumulative skin irritation study on BIOPEPTIDE CL was performed using 10 guinea pigs (5 males, 5 females; ages
not stated). The test substance was applied to the left flank (0.05 ml on 2 cm x 2 cm area, clipped free of hair) once
daily for 14 consecutive days. The right flank was treated with purified water (control). The test site was not covered with a
dressing during the application period. Reactions were evaluated immediately prior to each application and approximately 24
h after the last application by comparing the reactions on both flanks. The animals were killed and cutaneous samples were
removed from treated sites. Cutaneous reactions were not observed during the study. However, a very slight beige
coloration of the skin was observed in each animal. It was concluded that BIOPEPTIDE CL was a non-irritant in guinea
pigs (maximum weekly mean irritation index = 0).
The skin sensitization potential of BIOPEPTIDE CL was studied using 30 guinea pigs (ages not stated) in the maximization test. The test group consisted of 20 animals (10 males, 10 females) and the control group consisted of 10 animals (5 males, 5 females). During induction day 1, test animals were injected intradermally with the test substance (1% in 0.9% isotonic saline vehicle [injection volume = 0.1 ml]) in the presence of Freund’s complete adjuvant. The test substance (0.5 ml) was cutaneously applied to test animals on induction day 8. The control group was treated only with vehicle during the induction period. The challenge phase was initiated after a 12-day non-treatment period. A dry compress containing the test substance (75% in saline vehicle [0.5 ml]) was applied, under an occlusive dressing, to the right flank, and vehicle only (0.5 ml) was applied to the left flank of all animals. The compress and occlusive dressing were removed at the end of the 24-h application period. Challenge reactions were evaluated at 24 h and 48 h after removal. The animals were then killed and cutaneous samples were obtained from challenge sites. Microscopic examination was not performed on cutaneous samples. Cutaneous reactions were not observed during the challenge phase. It was concluded that no cutaneous reaction attributable to the sensitization potential of BIOPEPTIDE-CL at the maximal non-irritant concentration of 75% was observed in guinea pigs.

BIOPEPTIDE EL (contains 100 ppm palmitoyl oligopeptide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH) was evaluated in a skin irritation study involving 3 male New Zealand White rabbits (ages not stated). A dry compress containing the test substance was applied (0.5 ml on 6 cm² area, clipped free of hair) for 4 h under a semi-occlusive dressing. Reactions were scored at 24 h, 48 h, and 72 h post-removal. Moderate cutaneous reactions (erythema, but no edema) were observed, and these reactions were reversible within 24 h or 48 h. Cutaneous reactions were not observed on days 3 and 4. BIOPEPTIDE EL was considered a non-irritant (mean erythema score < 1.0).

Human

Palmitoyl Oligopeptide

The skin irritation potential of the ingredient MAXI-LIP (contains 1,000 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was evaluated using 10 healthy adult volunteers. The ingredient was applied to dorsal skin (~ 0.02 ml on 50 mm² area), using an occlusive patch (Finn chamber on Scanpor), for 48 h. Untreated sites (covered with occlusive patch) served as negative controls. Reactions were scored 30 min after patch removal. Neither irritation nor significant cutaneous intolerance was observed (primary irritation index [PII] = 0). There was also no evidence of a secondary effect. MAXI-LIP was classified as "very well tolerated".

The skin sensitization potential of MAXI-LIP was evaluated in a human repeated insult patch test (HRIPT) using 52 subjects. The study was initiated with 57 subjects (16 to 79 years old), 5 of whom withdrew for reasons unrelated to ingredient application. During induction, patches (type not stated) were applied 3 times per week for a total of nine 24-h induction applications. Non-treatment periods during the induction phase were described as 24 h following each Tuesday and Thursday patch removal and 48 h following each Saturday removal. The challenge phase was initiated following a 2-week non-treatment period. Challenge patches were applied for 24 h to a new test site that was adjacent to the induction patch site. Reactions were scored 24 h and 72 h after patch application. Barely perceptible (+) to moderate (2+) reactions were observed during induction and/or challenge phases. However, it was noted that these transient, low-level responses were considered clinically insignificant. It was concluded that MAXI-LIP did not indicate a clinically significant potential for dermal irritation or allergic contact sensitization.

The ingredient DERMAXYL (contains 200 ppm palmitoyl oligopeptide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH) was evaluated for skin irritation potential using 10 adult volunteers. A single 48-h application of the test substance (diluted to 50%) was made, under an occlusive patch, to dorsal skin. Neither irritation nor significant cutaneous intolerance was observed (primary irritation index [PII] = 0). There was also no evidence of a secondary effect. Diluted Dermaxyl was considered very well tolerated.

An HRIPT on DERMAXYL was performed using 53 healthy adult volunteers. The test substance was diluted to a concentration of 50% prior to application. The test procedure involved 48-h occlusive patch applications of the diluted test substance (area of application not stated). Eight induction applications were made, followed by challenge patch application. Neither skin irritation (mean irritation index [induction] = 0.04) nor sensitization indicative of cutaneous intolerance was observed.
Manganese Tripeptide-1 (Glycyl-L-Histidyl-Lysine)

The use of manganese tripeptide-1 in the treatment of various signs of cutaneous facial photodamage was evaluated using 14 female subjects (40 to 70 years old) with moderate photodamage and hyperpigmentation of the face. Individuals with a history of reactions to skin care products or who were undergoing concurrent topical and/or systemic drug therapy for skin disorders were excluded from the study. All participants were required to discontinue use of retinoids, alpha and beta hydroxyl acids, and other topical skin care products. At 4 weeks prior to initiation of the study, the participants were required to discontinue direct facial sun exposure. A facial serum formulation containing manganese tripeptide-1 (formulated in a non-irritating facial serum base; concentration not stated) was applied by each subject twice daily for up to 12 weeks. The formulation was well tolerated. Only one of the 14 subjects had mild erythema, and there was one instance of tightness and drying associated with application of the formulation. According to the clinical evaluator, treatment with the manganese peptide complex produced a significant improvement in the appearance of mottled hyperpigmentation, sallowness, lentigines, and surface roughness/dryness.

Other Skin Studies

Palmitoyl Tripeptide-1

The anti-wrinkle effect, due to increased collagen synthesis, of palmitoyl tripeptide-1 (palmitoyl-Gly-His-Lys) was evaluated in a blind, vehicle-controlled test involving 15 female subjects (44 to 59 years old). Essentially, wrinkles are due to reduced collagen-packing in the dermis. Both a cream containing the tripeptide (3 ppm) and a placebo cream were applied around the eye zones twice daily for 4 weeks. On days 0 and 28, skin replicas were taken on both sides of the face and analyzed using an image analysis system. The following measurements were made, and their variations analyzed with respect to day 0 and the placebo: 39% decrease in wrinkle length, 23% decrease in wrinkle depth, and a 17% decrease in overall skin roughness at the end of the 4-week period. The placebo cream had no significant effect. All differences between skin treated with the tripeptide versus the placebo cream were statistically significant.

Both a vehicle (not identified) and palmitoyl tripeptide-1 (palmitoyl-Gly-L-His-L-Lys, 4 ppm in vehicle) were applied to the skin of 23 healthy female volunteers for 4 weeks. Skin layer thickness was monitored using ultrasound echography. A small, but statistically significant, increase in skin thickness (~ 4%, compared to vehicle alone) was observed at the site treated with palmitoyl tripeptide. This value was not considered negligible, because it was noted that the thinning of aging skin occurs at a rate of approximately 6% every 10 years.

Palmitoyl Oligopeptide

The peptide palmitoyl oligopeptide, modeled on repair signaling sequences, has been marketed as a cosmetic ingredient that enhances skin rejuvenation. The extracellular matrix (ECM) in the basement membrane that separates the epidermis from the dermis also serves as a mediator of receptor-induced interactions between cells, guiding growth and differentiation. Damage to the ECM leads to repair that is initiated through processes such as protein synthesis and cell differentiation and proliferation. Most of these functions are related to signaling by peptides that are released from the ECM to cells through cell membrane receptors. Over time, aged skin is characterized by decreased production of new collagen and increased proteolytic activity, resulting in increased collagen degradation. In senescent fibroblasts, there is decreased synthesis of type I collagen, and these cells proliferate at a much slower rate when compared to fibroblasts in young skin. Peptides modeled on repair signaling sequences have been claimed to be cosmetic ingredients that enhance skin rejuvenation.

An in vivo study on the skin rejuvenating effect of Matrixyl™ 3000 (palmitoyl oligopeptide + palmitoyl tetrapeptide-7) was performed. Panel 1 (Matrixyl™ 3000 vs. placebo) consisted of 24 volunteers with a mean age of 56.1 years. The test substance and excipient were tested at a concentration of 3% in a cream formulation. Each cream formulation was applied to one-half of the face (on different sides) in the morning and at night for 2 months, in the absence of all other anti-wrinkle, reparative, restructuring, or regenerating products. Skin rejuvenation was assessed using profilometry and image analysis, photography, and cutometry. After 56 days, a statistically significant decrease in deep wrinkles and skin roughness resulted from application of Matrixyl™ 3000 (p < 0.01) when compared to results at day 0. For a similar comparison involving the excipient cream, there were no statistically significant differences in results at day 0 vs. those at day 56. Also, after 56 days, a statistically significant increase in skin elasticity and tone resulted from application of Matrixyl™ 3000 (p < 0.01) when compared to results at day 0.
Immunosuppression and Hepatocellular Effects

Tripeptide-1 (Gly-His-Lys)

The immunosuppressive activity of the Gly-His-Lys tripeptide was evaluated using CBA mice and Wistar rats (animal numbers not stated). The tripeptide (in sterile isotonic NaCl) was administered i.p. ten times at the following doses before, during and after immunization: 0.5, 1.5, 5, 50, 150 and 450 mg/kg. The dose volumes were 0.1 ml (mice) and 0.2 ml (rats), and the interval between doses was 24 h. The animals were killed one day after the last injection. Liver sections were examined morphologically and the mitotic index of hepatocytes was calculated. Sheep erythrocytes served as the antigen. Humoral response intensity was estimated by the number of antibody-producing cells in the spleen at 5 days after immunization. The delayed-type hypersensitivity (DTH) reaction in rats was assayed by the difference between the weights of regional (site of antigen administration) and contralateral (popliteal) lymph nodes and counts of nucleated cells in these lymph nodes. A marked increase in the mitotic index of hepatocytes was observed at doses of ≥ 1.5 mg/kg. The 0.5 mg/kg dose had no effect on the mitotic index. Signs of liver degeneration were observed at doses of 150 and 450 mg/kg, and these changes were more pronounced at the higher dose. Doses of the tripeptide ≥ 1.5 mg/kg also suppressed the humoral immune response; however, this effect was not observed at a dose of 0.5 mg/kg. This immunosuppressive effect was described as dose-dependent. The effects of the tripeptide on the DTH and humoral immune response were similar.

CELLULAR EFFECTS

Stimulation of Angiogenesis

Palmitoyl Hexapeptide-12

Palmitoyl Hexapeptide-12 (Pal-Val-Gly-Val-Ala-Pro-Gly), an elastin sequence, enhanced angiogenesis in the chick chorioallantoic membrane by promoting endothelial cell migration and tubulogenesis through upregulation of membrane-type metalloproteinase-1 (MT1-MMP), a matrix metalloproteinase (MMP). In the in vivo angiogenesis assay, the chick chorioallantoic membrane was exposed to allow direct access. On day 6 of embryonic development, angiogenic areas were delimited with a silicon ring containing phosphate-buffered saline (PBS, control) or palmitoyl hexapeptide-12 (50 ng) in a final volume of 20 µl. Embryos were then placed in an incubator to induce spontaneous angiogenesis and were treated daily. Treated areas were photographed daily on days 6 to 10 of embryonic development.

Hexapeptide-12 (Val-Gly-Val-Ala-Pro-Gly)

In vitro organ culture with rings from normal human coronary artery was used to demonstrate the angiogenic role of the elastin peptide Val-Gly-Val-Ala-Pro-Gly in human vascular smooth muscle cells. After 3 days in culture (Val-Gly-Val-Ala-Pro-Gly, 100 µg/ml), the vascular rings in the collagen gel containing elastin peptide elaborated metalloproteinase activity and sprouted and grew. The authors noted that these results suggest that Val-Gly-Val-Ala-Pro-Gly peptide generated at the site of proteolysis during vascular injury may have angiogenic activity.

Stimulation of Collagen and Fibronectin Synthesis

Palmitoyl Oligopeptide

Normal human fibroblasts were cultured in Dulbecco’s modified eagle medium in the presence of fetal calf serum. After cell confluence was achieved, the culture medium was replaced and the fibroblasts were incubated (without serum) for 72 h in the presence of vitamin C and palmitoyl oligopeptide (up to 7.5 ppm) or Matrixyl™ 3000 (palmitoyl oligopeptide + palmitoyl tetrapeptide-7) (up to 11 ppm). Control media consisted of the culture medium alone or with a positive control (transforming growth factor beta (TGFβ)). Matrix proteins (collagen 1 and fibronectin) were assayed using the enzyme-linked immnosorbant assay (ELISA) method and hyaluronic acid was assayed using a colorimetric method. A dose response for collagen 1 synthesis was observed following incubation with Matrixyl™ 3000, but not palmitoyl oligopeptide. Matrixyl™ 3000 yielded values for collagen 1 synthesis greater than those that would be expected on the basis of simple addition. Incubation with the positive control resulted in 102% stimulation of collagen synthesis.

Palmitoyl Tripeptide-1

The stimulation of collagen synthesis by palmitoyl tripeptide-1 (pamitoyl-Gly-L-His-L-Lys) in human fibroblasts in vitro was studied. Collagen synthesis was monitored by the incorporation of tritiated proline, considered to be a strong
signal of collagen synthesis. Results indicated that this strong signal of collagen synthesis was observed at a concentration of 0.5 µM/liter. In another experiment, skin samples (human biopsies [abdominal tissue]) from plastic surgery were irradiated with daily doses of UVA light for one week. Microscopic examination revealed strong degradation of dermal collagen. Following irradiation, the skin samples were treated with retinoic acid (500 ppm) or palmitoyl tripeptide (5 ppm) during the same week. Treatment with either compound resulted in almost total preservation and/or recovery of high density of collagen in the skin samples.

A dose response for de novo synthesis of fibronectin and hyaluronic acid was observed in the presence of Matrixyl™ 3000, but not palmitoyl oligopeptide. Matrixyl™ 3000 induced a 164% increase in fibronectin synthesis; this ingredient also stimulated hyaluronic acid synthesis by 179%, whereas the value for palmitoyl oligopeptide was 14%.2

Growth Promotion

Tripeptide-1(Glycyl-L-Histidyl-L-Lysine)

It has been found that the human plasma tripeptide, glycyl-L-histidyl-L-lysine alters the growth rate or state of differentiation of a wide variety of cultured cells and organisms (e.g., hepatocytes, neurons, mycoplasma, fungi, and Ascaris larvae), and may mediate a functional or nutritional need that is common to diverse organisms.40,41,42,43 Studies on the growth-promotion activity of glycyl-L-histidyl-L-lysine are summarized below.

Effect on Growth Factor Production

Copper Tripeptide-1(Glycyl-L-Histidyl-L-Lysine)

The effect of copper tripeptide-1 on normal and keloid-producing dermal fibroblasts was studied using a serum-free in vitro model.44 Primary cultures of dermal fibroblasts were established from excisional biopsies of 3 different keloid and 3 different normal facial skin specimens obtained from 5 patients. Copper tripeptide-1 was added to cultures at a concentration of 1 x 10⁻⁹ mol/L. The cellular response was described in terms of viability and secretion of basic fibroblast growth factor (bFGF) and transforming growth factor-β1 (TGF-β1). Cell viability was between 86% and 96% (mean = 92%) throughout the experiment. Phosphate-buffered saline served as the vehicle control. No different bFGF secretion pattern was observed in normal fibroblasts or keloid-producing fibroblasts when compared to controls. At 24 h, normal and keloid-producing dermal fibroblasts treated with copper tripeptide-1 secreted less TGF-β1 when compared to controls (p < 0.05), suggesting a possible clinical use for decreasing excessive scar formation.

Chemotactic Activity

Hexapeptide-12 (Val-Gly-Val-Ala-Pro-Gly)

The extracellular matrix contains elastic fibers, which provide elasticity and resilience to tissues that require the ability to deform repetitively and reversibly. The valine-glycine-valine-alanine-proline-glycine hexapeptide (Val-Gly-Val-Ala-Pro-Gly or VGVAPG) is known for its chemotactic activity against monocytes, fibroblasts, and tumor cells.45,46,47 The Val-Gly-Val-Ala-Pro-Gly peptide (VGVAPG), amino acid sequence is a repeating peptide in tropoelastin, and study results have demonstrated that tropoelastin and elastin-derived peptides are chemotactic for fibroblasts and monocytes.48,49,50 A study was performed to identify the chemotactic activity of VGVAPG using fetal bovine ligament nae fibroblasts and human mononuclear peripheral blood cells.45 Chemotaxis was assayed using a double micropore membrane system in modified Boyden chambers. Study results indicated that VGVAPG was chemotactic for fibroblasts and monocytes, and optimal activity was noted at a concentration of ~10⁻⁸ M. The authors noted that the following results support the possibility that at least part of the chemotactic activity of tropoelastin and elastin peptides is contained in VGVAPG sequences: (1) polyclonal antibody to bovine elastin selectively blocked the fibroblast and monocyte chemotactic activity of both elastin-derived peptides and VGVAPG; (2) monocyte chemotaxis to VGVAPG was selectively blocked by preexposing the cells to elastin peptides; and (3) undifferentiated (nonelastin producing) bovine ligament fibroblasts, capable of chemotaxis to platelet-derived growth factor, did not show chemotactic responsiveness to either VGVAPG or elastin peptides until after matrix-induced differentiation and the onset of elastin synthesis.
Enzyme Upregulation/Release

Hexapeptide-12 (Val-Gly-Val-Ala-Pro-Gly)

The valine-glycine-valine-alanine-proline-glycine hexapeptide is also known for its ability to activate metalloproteinases.51,52

Soluble kappa-elastin peptides have been shown to stimulate the expression of matrix metalloproteinase-2 (gelatinase A, MMP-2), but not metalloproteinase-9 (MMP-9) by human fibrosarcoma HT-1080 cells, both at the protein and mRNA levels; the maximal effect was observed at 25 µg/ml of kappa-elastin.51 The valine-glycine-valine-alanine-proline-glycine hexapeptide was found to mimick this stimulatory effect on kappa elastin MMP-2 secretion, described as 1.6-fold over the control value, at a concentration of 200 µg/ml.

The treatment of cultured human skin fibroblasts with tropoelastin or with heterogenic peptides, obtained after organo-alkaline or leukocyte elastase hydrolysis of insoluble elastin, induced a high expression of pro-collagenase-1 (pro-MMP-1)).52 The identical effect was achieved after stimulation with a valine-glycine-valine-alanine-proline-glycine synthetic hexapeptide (200 µg/ml).

The effects of the Val-Gly-Val-Ala-Pro-Gly hexapeptide on polymorphonuclear leukocytes (PMNLs) were studied in vitro.53 PMNLs were obtained from 20 healthy volunteers (< 30 years old). Val-Gly-Val-Ala-Pro-Gly is a repeated sequence in the elastin molecule, and polymorphonuclear leukocyte (PMNL) stimulation by elastin peptides results in several responses that are normally associated with inflammation, such as, migration, aggregation, degranulation, and generation of oxygen radicals. The results of this study indicated that, when compared to non-treated cells, Val-Gly-Val-Ala-Pro-Gly stimulated superoxide anion production (p < 0.001); 2.5 x 10^{-5} M was the most effective concentration. Val-Gly-Val-Ala-Pro-Gly also had the following effects: stimulatory effect on H2O2 production (p < 0.01), when compared to the non-stimulated basic value, significantly (p < 0.05) enhanced the release of elastase, significantly (p < 0.01) increased intracellular free calcium (Ca^{2+}), when compared to the basic value of 6 µmol/min, and significantly (p < 0.01) increased the release of myeloperoxidase (enzyme of neutrophilic granulocytes) activity.

Effect on Cell Adhesion

Hexapeptide-12 (Ala-Pro-Gly-Val-Gly-Val)

A strategy for designing and synthesizing thermostable biologically active proteins was proposed, and involved combining a rigid and extremely hydrophobic peptide unit with a biologically active peptide unit.54 The cell adhesive peptide sequence Arg-Gly-ASP (RGD), as a functional peptide unit, was incorporated into an elastin-based rigid polyhexapeptide with a repeating unit of Ala-Pro-Gly-VAL (APGVGV). The fusion gene designed was expressed in E. coli, and the resulting protein was designated ER4. The authors noted that the ER4-coated cell culture plate demonstrated sufficient cell adhesive activity through the RGD sequence on the surface of ER4. The thermostability of ER4 was determined by estimating the remaining cell adhesive activity after autoclaving (120°C) for 20 minutes. When compared to native ER4, the ER4 synthesized retained more than 90% of the cell adhesive activity.

Hexapeptide-12 (Val-Gly-Val-Ala-Pro-Gly)

The synthetic peptide Val-Gly-Val-Ala-Pro-Gly inhibited the adhesion of freshly isolated rabbit vascular smooth muscle cells to α-elastin at concentrations of 0.01 to 1 mM.11 There was no inhibitory activity on the adhesion of cells in the synthetic state at a concentration of 0.01 mM. It was noted that, during the early stages of atherosclerosis, arterial smooth muscle cells undergo a transition from a contractile to a synthetic phenotype that is characterized by the loss of myofilaments and the formation of extensive rough endoplasmic reticulum and a large Golgi complex.

OTHER EFFECTS

Effect on Wound Healing

Copper Tripeptide-1 (Glycyl-L-Histidyl-L-Lysine-Cu^{2+})

Glycyl-L-histidyl-L-lysine-Cu^{2+} is a growth factor that has been isolated from human plasma. The peptide portion of this complex has an amino acid structure that is similar to the copper ion transport site on human albumin, and, thus, has
an affinity for copper(II) that is equivalent to that of the copper transport site on albumin. This peptide sequence is that of the peptide moiety of palmitoyl oligopeptide (Pal-GHK).

The term, matrikine is proposed to designate extracellular matrix-derived peptides that regulate connective tissue cell activity, and glycyl-histidyl-lysine complexed with copper is a well-studied matrikine. The expression and activation of matrix metalloproteinases in a model of experimental wound healing in rats and their modulation by glycyl-L-histidyl-L-lysine-Cu(II), a potent activator of wound repair, were investigated using groups of 6 male Sprague-Dawley rats. The rats were anesthetized by i.p. injection with sodium pentobarbital (40 mg/kg), and dorsal skin was clipped free of hair. Full thickness skin incisions were made perpendicular to the spine through the pannicus carnosus to the fascial plane. Wound chambers were inserted under the skin, and the incisions were closed. Glycyl-L-histidyl-L-lysine-Cu2+ (2 g, in phosphate-buffered saline) (3 test groups) or the same volume of saline (3 control groups) was injected serially into the chambers. The animals were killed on day 3, 7, 12, 18, or 22 after chamber implantation. Wound fluid was immediately collected by aspiration with a 1-ml syringe, centrifuged, and then stored until zymography. The solid material deposited in the chambers was collected and remained frozen until the time of analysis. The wound fluid and the neosynthetized connective tissue deposited in the chambers were collected and analyzed for matrix metalloproteinase expression and/or activity. For histologic examination, wound chambers were immediately immersed, after collection in 10% formalin (in phosphate-buffered saline). After fixation of chamber contents (no specific definition of tissues), tissues were embedded in paraffin and 4 µm thick sections were stained.

Throughout the experiment, interstitial collagenase activity increased progressively in the wound fluid; L-histidyl-L-lysine-Cu2+ treatment did not alter the activity of this enzyme. Matrix metalloproteinase-9 (gelatinase B) and matrix metalloproteinase-2 (gelatinase A) were the 2 main gelatinolytic activities expressed during the healing process. During the early stages of wound healing (day 3) pro-matrix metalloproteinase (pro-form of matrix metalloproteinase-9) was strongly expressed. In the wound fluid, pro-matrix metalloproteinase decreased rapidly and disappeared after day 18. Pro-matrix metalloproteinase-2 was expressed at low levels at the beginning of the healing process, having increased progressively until day 7 and decreased until day 18. In the wound tissue, matrix metalloproteinase-9 expression persisted in the glycyl-L-histidyl-L-lysine-Cu2+-injected chamber until day 22. Activated matrix metalloproteinase-2 was present in the wound fluid and wound tissue, having increased until day 12 and then decreased progressively. Glycyl-L-histidyl-L-lysine-Cu2+ injections increased pro-matrix metalloproteinase-2 and activated matrix metalloproteinase-2 during the later stages of healing (days 18 and/or 22). Together with biochemical analysis, histologic examination of chamber contents confirmed that glycyl-L-histidyl-L-lysine-Cu2+ injection increased cell invasion and extracellular matrix deposition in the wound chambers. The authors noted that these study results demonstrated that various types of matrix metalloproteinases are selectively expressed or activated at various periods of wound healing. They also noted that glycyl-L-histidyl-L-lysine-Cu2+ modulated the expression of these enzymes and may, thereby, significantly affect wound remodeling.

**REPRODUCTIVE AND DEVELOPMENTAL TOXICITY**

Data on the reproductive and developmental toxicity palmitoyl and the other oligopeptides reviewed in this safety assessment were not found in the published literature.

**GENOTOXICITY**

**Palmitoyl Tripeptide-1**

Ames test results for palmitoyl tripeptide-1 (MAXI-LIP and BIOPEPTIDE-CL trade name materials) were negative with and without metabolic activation in *Salmonella typhimurium* bacterial strains.

The genotoxicity of MAXI-LIP (contains 1,000 ppm palmitoyl oligopeptide, as Pal-Gly-Lys-OH) was evaluated, with and without metabolic activation, using the following *Salmonella typhimurium* strains: TA98, TA100, TA1535, and TA1538. The test material (0.1 ml in ethanol solution) was non-genotoxic. In another assay, the genotoxicity of BIOPEPTIDE-CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was evaluated, with and without metabolic activation, using the following *Salmonella typhimurium* strains: TA98, TA102, TA1535, and TA1537. At doses up to 5,000 µg/plate, the test material was classified as non-genotoxic.
Effect on DNA

Tripeptide-1 (Glycyl-L-Histidyl-L-Lysine)

The effects of glycyl-L-histidyl-L-lysine on Morris hepatoma 7777 cells were studied. The cells were incubated with glycyl-L-histidyl-L-lysine at concentrations ranging from 0.2 ng/ml to 20 ng/ml. A glycyl-L-histidyl-L-lysine concentration of 2 ng/ml had the greatest stimulatory effect on \(^{3}H\)-thymidine and \(^{3}H\)-leucine incorporation. The incorporation of \(^{3}H\)-thymidine into DNA in randomly proliferating cells increased by 50%. Also, in randomly proliferating cells, the incorporation of \(^{3}H\)-leucine into protein increased by 29%. Additionally, synergistic effects were noted when insulin and glucagon were included in the incubation mixture along with glycyl-L-histidyl-L-lysine. The results of experiments involving cells rendered quiescent by serum starvation indicated that cells in the G1 phase of the cell cycle were more sensitive to glycyl-L-histidyl-L-lysine stimulation. Also, in experiments involving quiescent cells, \(^{3}H\)-thymidine incorporation increased earlier and peaked at a higher value when compared to control cells. The authors noted that this finding suggests that glycyl-L-histidyl-L-lysine may play a role in stimulating quiescent cells to re-enter the cell cycle.

Gene Activation

Palmitoyl Oligopeptide

Reportedly, molecular biology methods have enabled access to intracellular, functional, and morphological changes induced by substances after cell layer (fibroblasts or keratinocytes) or tissue (epidermis and synthetic epidermis) exposure. With this in mind, it is possible to define the profile of the method of action of a substance in relation to the genes activated or repressed, and compare the findings with those for a control cell culture or tissue. The gene activation profile for palmitoyl oligopeptide (Pal-glycine-histidine-lysine) has been determined using a bank of 450 genes. Palmitoyl oligopeptide activated few genes, however, its profile was more specifically oriented toward keratinocyte anchoring (alpha-catenin and laminin receptor) and differentiation (keratin 10). Additionally, this oligopeptide increased the synthesis of extracellular matrix (syndecan and heparin sulfate glycoprotein). The profile characterized by the genes activated in fibroblasts indicated that palmitoyl oligopeptide stimulated numerous genes. Additional details were not provided.

CARCINOGENICITY

Data on the carcinogenicity of palmitoyl and other oligopeptides reviewed in this safety assessment were not found in the published literature.

Effect on Normal and Cancer Cell Growth

Tripeptide-1 (Glycyl-L-histidyl-L-lysine)

Glycyl-L-histidyl-L-lysine was studied to determine its growth-promoting potential using human KB cells (subline of human HeLa tumor cell line), HeLa cells, and WI-38 cells (human diploid cell line, derived from normal embryonic lung tissue) in serum-free medium, serum-limited medium (dialyzed fetal calf serum [DFCS]), and cell medium supplemented with bovine serum albumin (BSA). Glycyl-L-histidyl-L-lysine stimulated the growth of KB and HeLa cells, but not WI-38 cells. When compared to cells grown in serum-free medium, there was no significant difference in the cellular growth ratio between cells grown in media supplemented with glycyl-L-histidyl-L-lysine or BSA. However, when a combination of BSA and GHL was present in the 0.5% DFCS medium, the growth-promoting activity of GHL was observed. The rate of growth of cells in the serum-limited medium containing BSA and glycyl-L-histidyl-L-lysine was not significantly different when compared to cells grown in medium containing 5% DFCS. The concentration of glycyl-L-histidyl-L-lysine that was required for optimal growth of cells in serum-limited medium containing BSA (6 mg/l) was in the range of 250 to 500 ng/ml. The concentration of BSA that was required for optimal growth in serum-limited media containing glycyl-L-histidyl-L-lysine (500 ng/ml) was 6 mg/ml. BSA concentrations of > 6 mg/ml caused a decrease in the growth-promoting activity of the medium.
Chemotactic Activity and Metastasis

Hexapeptide-12 (Val-Gly-Val-Ala-Pro-Gly)

Tumor cell interactions with elastin and implications relating to pulmonary metastasis were studied using tumor cell lines of murine origin, namely, M27 Lewis lung carcinoma cells and H59 Lewis lung carcinoma cells. Elastin surrounds microvessels in the pulmonary circulation and may pose a barrier to the extravasation of metastatic tumor cells. Lung-colonizing murine melanoma cells are the source of enzymatic activity that degrades elastin, and, additionally, the elastin fragments liberated by enzymatic digestion of insoluble elastin stimulate tumor cell chemotaxis. The results of this study indicated that Val-Gly-Val-Ala-Pro-Gly, a synthetic peptide that is a repeat sequence in the elastin molecule, displayed tumor cell chemotactic activity. It was postulated that the ability to migrate in response to elastin fragments may facilitate tumor cell invasion of elastin-rich pulmonary tissue.

In another study, it was noted that the M27 and H59 variants of Lewis lung carcinoma differ in their responsiveness to Val-Gly-Val-Ala-Pro-Gly. M27 cells, selected for metastasis to the lung, are highly responsive to a positive gradient of Val-Gly-Val-Ala-Pro-Gly. H59 cells, selected for metastasis to the liver, do not migrate in response to Val-Gly-Val-Ala-Pro-Gly.

SUMMARY

The safety of tripeptide-1 and hexapeptide-12, and related amides in cosmetics is reviewed in this safety assessment.

According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) in 2013, the following ingredients are being used in cosmetic products: palmitoyl oligopeptide, tripeptide-1, palmitoyl tripeptide-1, biotinoyl tripeptide-1, and copper tripeptide-1. Results from a survey of ingredient use concentrations provided by the Personal Care Products Council in 2013 indicate that, collectively, the ingredients reviewed in this safety assessment are being used at concentrations ranging from 0.0000001% (palmitoyl oligopeptide (glycine-histidine-lysine-OH [GHK]) and palmitoyl oligopeptide (valine-glycine-valine-alanine-proline-glycine-OH [VGVAPG])) to 1% (palmitoyl oligopeptide [GHK]). The highest concentration of 1% relates to ingredient use in leave-on products. The ingredients reviewed in this safety assessment function primarily as skin and hair conditioning agents, skin protectants, and antioxidants in cosmetic products. However, other functions include: skin bleaching agents (anacardoyl tripeptide-1, coumaroyl tripeptide-1, and quinoyl tripeptide-1), antiacne agents (caffeoyl tripeptide-1), and chelating agents (nicotinyl tripeptide-1).

The peptide sequences in ingredients reviewed in this safety assessment have been produced by solid phase synthesis.

The impurities content of both palmitoyl oligopeptide (CAS No. 147732-56-7) and palmitoyl oligopeptide (CAS No. 171263-26-6) has been described as follows: acetate (< 5%), palmitic acid (< 5%), and water (< 5%). Commercial glycyll-histidyl-lysine-Cu is approximately 95% pure, but often includes small amounts of mildly neurotoxic materials. Most of the neurotoxic materials can be removed by dissolving glycyll-histidyl-lysine in glass-distilled water (50 mg/ml), centrifuging at 20,000 g for 1 h at 3°C, and then lyophilizing the supernatant.

After i.v. injection, glycyll-histidyl-lysine was rapidly degraded to L-histidyl-L-lysine, which was rapidly eliminated from circulating blood. It has been reported that glycyll-histidyl-L-lysine is unstable in human plasma and is rapidly degraded by aminopeptidases. In an enzyme assay, the liver growth factor Gly-His-Lys was hydrolyzed by an aminotripeptidase purified from rat brain cytosol.

BIOPEPTIDE-CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was nontoxic (LD50 > 2,000 mg/kg) in an acute oral toxicity study involving rats. Studies designed to evaluate the repeated dose toxicity of the ingredients reviewed in this safety assessment were not found in the published literature. However, neither treatment-related clinical signs/mortalities were observed in cumulative skin irritation/sensitization studies on BIOPEPTIDE-CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH; guinea pigs tested) and 75% BIOPEPTIDE-CL (guinea pigs tested).

Palmitoyl tripeptide-1 (BIOPEPTIDE-CL, contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was slightly irritating to the eyes of rabbits. Palmitoyl hexapeptide-12 (BIOPEPIDE EL, contains 100 ppm palmitoyl
oligopeptide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH) was non-irritating to the eyes of rabbits. In the hen’s egg chorioallantoic membrane in vitro assay for evaluating ocular irritation potential, MAXI-LIP (contains 1,000 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was classified as an irritant and DERMAXYL (contains 200 ppm palmitoyl oligopeptide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH) was practically non-irritating. In the in vitro neutral red release assay for evaluating ocular irritation potential, DERMAXYL caused “unimportant cytotoxicity”.

In skin irritation studies (single application) involving rabbits, BIOPEPTIDE CL and BIOPEPTIDE EL were classified as non-irritants. BIOPEPTIDE CL was also classified as a non-irritant in a cumulative skin irritation study involving guinea pigs. BIOPEPTIDE CL did not induce skin sensitization at a challenge concentration of 75% in the maximization test.

In human skin irritation studies (single application), MAXI-LIP and DERMAXYL (50%) were classified as non-irritants. HRIPT results for MAXI-LIP and DERMAXYL (50%) were negative for skin irritation and sensitization.

A facial serum formulation containing manganese tripeptide-1 was applied by each of 14 subjects with moderate photodamage and hyperpigmentation twice daily for up to 12 weeks. The formulation was well tolerated; one subject had mild erythema.

A cream containing 3 ppm palmitoyl tripeptide-1 (palmitoyl-Gly-His-Lys) was applied around the eyes of 15 female subjects twice daily for 4 weeks. Application resulted in a statistically significant anti-wrinkle effect, in that decreased wrinkle length, and depth and a decrease in overall skin roughness were observed. The application of palmitoyl tripeptide-1 (4 ppm in vehicle) to the skin of 23 female subjects for 4 weeks caused a statistically significant increase (4% increase) in skin thickness. A study evaluating the skin rejuvenating effect of Matrixyl

[m]3000 (palmitoyl oligopeptide + palmitoyl tetrapeptide-7) was performed using 24 subjects. The cream formulation was applied to the face twice daily for 2 months. A statistically significant decrease in both deep wrinkles and skin roughness and a statistically significant increase in skin elasticity and tone were reported.

Dose-dependent suppression of the humoral immune response was observed in CBA mice and Wistar rats at i.p. doses of ≥ 1.5 mg/kg tripeptide-1 (Gly-His-Lys). The doses tested ranged from 0.5 to 450 mg/kg.

The stimulation of collagen synthesis by palmitoyl tripeptide-1 in human fibroblasts in vitro was studied. A strong signal of collagen synthesis was noted at a concentration of 0.5 µM/liter. In the same study, human skin samples were irradiated with daily doses of UVA light for one week, resulting in degradation of dermal collagen. Treatment with palmitoyl tripeptide-1 (5 ppm) during the same week caused almost total preservation and/or renewal of collagen. In another study, normal human fibroblasts were incubated in the presence of vitamin C and palmitoyl oligopeptide (up to 7.5 ppm) or palmitoyl oligopeptide + palmitoyl tetrapeptide-7 (up to 11 ppm). A dose response for collagen 1 synthesis and the de novo synthesis of fibronectin and hyaluronic acid was not observed.

Palmitoyl hexapeptide-12 (Pal-Val-Gly-Val-Ala-Pro-Gly) enhanced angiogenesis in the chick chorioallantoic membrane (in an in vivo model) by promoting endothelial cell migration and tubulogenesis through upregulation of membrane-type metalloproteinase-1 (MT1-MMP), a matrix metalloproteinase. Results from an in vitro assay using human vascular smooth muscle cells suggested that hexapeptide-12 may have angiogenic activity. After 3 days in culture, the vascular rings in the collagen gel containing the peptide elaborated metalloproteinase activity and sprouted and grew. According to another study, various types of matrix metalloproteinases are selectively expressed or activated during various periods of wound healing. Other peptide-induced cellular effects were as follows: stimulation of collagen synthesis (palmitoyl oligopeptide and palmitoyl tripeptide-1), alteration of growth rate or state of differentiation of hepatocytes and neurons (tripeptide-1), reduced secretion of human dermal fibroblast growth factors (copper tripeptide-1), chemotactic activity for fetal bovine ligament nuchae fibroblasts and human monocytes (hexapeptide-12), stimulation of pro-collagenase-1 expression in human skin fibroblasts (hexapeptide-12), and stimulation of elastase and myeloperoxidase release from human polymorphonuclear leukocytes (hexapeptide-12).

Ames test results for palmitoyl tripeptide-1 (MAXI-LIP and BIOPEPTIDE-CL trade name materials) were negative with and without metabolic activation in Salmonella typhimurium bacterial strains. In another assay, a glycyl-L-histidyl-L-lysine concentration of 2 ng/ml had the greatest stimulatory effect on 3H-thymidine and 3H-leucine incorporation into the DNA of proliferating Morris hepatoma 7777 cells. The gene activation profile for palmitoyl oligopeptide (Pal-glycine-histidine-lysine) has been determined using a bank of 450 genes. Palmitoyl oligopeptide activated few genes; however, its profile was more specifically oriented toward keratinocyte anchoring (alpha-catenin and laminin receptor) and differentiation (keratin 10). Additionally, this oligopeptide increased the synthesis of extracellular matrix (syndecan and heparin sulfate glycoprotein).
Data on the carcinogenicity or reproductive and developmental toxicity of the ingredients reviewed in this safety were not found in the published literature. However, data from other studies indicated that tripeptide-1 (glycyl-L-histidyl-L-lysine) stimulated the growth of human KB and HeLa tumor cells, but not normal human WI-38 cells, and that hexapeptide-12 (Val-Gly-Val-Ala-Pro-Gly) displayed tumor cell chemotactic activity, which may facilitate metastasis.
Table 1. Definitions, structures and functions of the ingredients in this safety assessment. 1, CIR staff

<table>
<thead>
<tr>
<th>Ingredient CAS No.</th>
<th>Definition &amp; Structure</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitoyl Oligopeptide [171263-26-6 and 147732-56-7]</td>
<td>Palmitoyl Oligopeptide is the product obtained by the reaction of palmitic acid with either a tripeptide consisting of gly-his-lys, or a hexapeptide consisting of val-gly-val-ala-pro-gly or ala-pro-gly-val-gly-val. The INCI Name, palmitoyl oligopeptide, originally developed in 1994, was designated with a retired status in 2013. Trade name assignments formerly published with the name Palmitoyl Oligopeptide will be retained in the retired monograph, and also published with the new name assignment as either palmitoyl tripeptide-1 or palmitoyl hexapeptide-12, for an interim period.</td>
<td>Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents</td>
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<tr>
<td>Tripeptide-1 [1269107-24-5]</td>
<td>Tripeptide-1 is the synthetic peptide consisting of gly-his-lys.</td>
<td>Skin Protectants; Skin Conditioning Agents - Miscellaneous</td>
</tr>
</tbody>
</table>
Table 1. Definitions, structures and functions of the ingredients in this safety assessment.  

<table>
<thead>
<tr>
<th>Ingredient CAS No.</th>
<th>Definition &amp; Structure</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitoyl Tripeptide-1</td>
<td>Palmitoyl Tripeptide-1 is the reaction product of palmitic acid and tripeptide-1.</td>
<td>Skin-Conditioning Agents - Miscellaneous</td>
</tr>
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<td>Palmitoyl Hexapeptide-12</td>
<td>Palmitoyl Hexapeptide-12 is the product of the reaction of palmitic acid and hexapeptide-12.</td>
<td>Antioxidants</td>
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<tr>
<td>Biotinoyl Tripeptide-1 [299157-54-3]</td>
<td>Biotinoyl Tripeptide-1 is the reaction product of biotin and tripeptide-1.</td>
<td>Hair Conditioning Agents</td>
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<tr>
<td>Copper Tripeptide-1 [89030-95-5]</td>
<td>Copper Tripeptide-1 is a complex formed by copper and tripeptide-1.</td>
<td>Skin-Conditioning Agents - Miscellaneous</td>
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</tbody>
</table>
### Table 1. Definitions, structures and functions of the ingredients in this safety assessment

<table>
<thead>
<tr>
<th>Ingredient CAS No.</th>
<th>Definition &amp; Structure</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl Tripeptide-1</td>
<td>Acetyl Tripeptide-1 is product obtained by the acetylation of tripeptide-1.</td>
<td>Skin-Conditioning Agents - Miscellaneous</td>
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<tr>
<td>Azelaoyl Tripeptide-1</td>
<td>Azelaoyl Tripeptide-1 is the product obtained by the reaction of azelaic acid and tripeptide-1.</td>
<td>Antioxidants; Hair Conditioning Agents; Skin Protectants</td>
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<tr>
<td>Bis(Tripeptide-1) Copper Acetate [130120-57-9]</td>
<td>Bis(Tripeptide-1) Copper Acetate is acetate salt of the product of the reaction of tripeptide-1 with copper chloride.</td>
<td>Skin-Conditioning Agents - Miscellaneous</td>
</tr>
</tbody>
</table>
Table 1. Definitions, structures and functions of the ingredients in this safety assessment.\textsuperscript{1, CIR staff}

<table>
<thead>
<tr>
<th>Ingredient CAS No.</th>
<th>Definition &amp; Structure</th>
<th>Function</th>
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<tbody>
<tr>
<td>Myristoyl Tripeptide-1</td>
<td>Myristoyl Tripeptide-1 is the product obtained by the reaction of myristic acid and tripeptide-1.</td>
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<td><img src="structure1.png" alt="Myristoyl Tripeptide-1 Structure" /></td>
<td>Skin-Conditioning Agents - Miscellaneous</td>
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<tr>
<td>Hexapeptide-12</td>
<td>Hexapeptide-12 is the synthetic peptide consisting of either val-gly-val-ala-pro-gly, or ala-pro-gly-val-gly-val.</td>
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<td><img src="structure2.png" alt="Hexapeptide-12 Structure" /></td>
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<td><img src="structure3.png" alt="Hexapeptide-12 Structure" /></td>
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<td>OR</td>
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<tr>
<td>Myristoyl Hexapeptide-12</td>
<td>Myristoyl Hexapeptide-12 is the reaction product of myristic acid and hexapeptide-12.</td>
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<td><img src="structure4.png" alt="Myristoyl Hexapeptide-12 Structure" /></td>
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<td><img src="structure5.png" alt="Myristoyl Hexapeptide-12 Structure" /></td>
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<td>Ingredient CAS No.</td>
<td>Definition &amp; Structure</td>
<td>Function</td>
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<tr>
<td>Aminolevulinoyl Tripeptide-1</td>
<td>Aminolevulinoyl Tripeptide-1 is the product obtained by the reaction of aminolevulinic acid and tripeptide-1.</td>
<td>Antioxidants; Hair Conditioning Agents; Skin Protectants</td>
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<td>Anacardoyl Tripeptide-1</td>
<td>Anacardoyl Tripeptide-1 is the product obtained by the reaction of anacardic acid and tripeptide-1.</td>
<td>Antioxidants; Skin Bleaching Agents; Skin Protectants</td>
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<td>Caffeoyl Tripeptide-1</td>
<td>Caffeoyl Tripeptide-1 is the product obtained by the reaction of caffeic acid and tripeptide-1.</td>
<td>Antiacne Agents; Antioxidants; Skin Protectants</td>
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<td>Coumaroyl Tripeptide-1</td>
<td>Coumaroyl Tripeptide-1 is the product obtained by the reaction of coumaric acid and tripeptide-1.</td>
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<td>Mevalonoyl Tripeptide-1</td>
<td>Mevalonoyl Tripeptide-1 is the product obtained by the reaction of mevalonic acid and tripeptide-1.</td>
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<td>Ingredient CAS No.</td>
<td>Definition &amp; Structure</td>
<td>Function</td>
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<td>Nicotinoyl Tripeptide-1</td>
<td>Nicotinoyl Tripeptide-1 is the product obtained by the reaction of niacin with tripeptide-1.</td>
<td>Antioxidants; Chelating Agents; Skin Protectants</td>
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<td>Quinoyl Tripeptide-1</td>
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<td>Antioxidants; Hair Conditioning Agents; Skin Bleaching Agents; Skin Protectants</td>
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<td>Retinoyl Tripeptide-1</td>
<td>Retinoyl Tripeptide-1 is the product obtained by the reaction of retinoic acid and tripeptide-1.</td>
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<td>Thioctoyl Tripeptide-1 is the product obtained by the reaction of thioctic acid and tripeptide-1.</td>
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<td>Ingredient/CAS No.</td>
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<td>Function</td>
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<td>Ursoloyl Tripeptide-1</td>
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1. CIR staff
Table 2. Current Frequency and Concentration of Use According to Duration and Type of Exposure^{1,12,13}

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<thead>
<tr>
<th>Exposure Type</th>
<th>Palmol Oligopeptide (no sequence)</th>
<th>Palmol Oligopeptide (GHK)</th>
<th>Palmol Oligopeptide (VGVAPG)</th>
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<tbody>
<tr>
<td></td>
<td># of Uses</td>
<td>Conc. (%)</td>
<td># of Uses</td>
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<td>Eye Area</td>
<td>109</td>
<td>0.00001-0.0002</td>
<td>NR</td>
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<td>Incidental Ingestion</td>
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<td>0.0015-0.0009</td>
<td>NR</td>
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<td>Incidental Inhalation- Sprays</td>
<td>203</td>
<td>0.001</td>
<td>NR</td>
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<td>Incidental Inhalation- Powders</td>
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Table 2. Current Frequency and Concentration of Use According to Duration and Type of Exposure\(^{13,62,63}\)

<table>
<thead>
<tr>
<th>Duration</th>
<th>Rinse-off Product Uses</th>
<th>Leave-on Product Uses</th>
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<tbody>
<tr>
<td>NR</td>
<td>NS</td>
<td>Totals</td>
</tr>
</tbody>
</table>

NR = Not Reported; NS = Not Surveyed; Totals = Rinse-off + Leave-on Product Uses.

*It is possible that these products may be powders, but it is not specified whether the reported uses are powders.

**It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays.

Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.
<table>
<thead>
<tr>
<th>Test Substance</th>
<th>Animals/Subjects</th>
<th>Doses/Concentrations Tested</th>
<th>Procedure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIOPEPTIDE CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH)</td>
<td>3 male New Zealand White rabbits (ages not stated)</td>
<td>0.5 ml on 6 cm² area of flank</td>
<td>Applied for 24 h under occlusive hypoallergenic dressing</td>
<td>Slight erythema in 2 rabbits (both flanks). Classified as non-irritant (primary irritation index [PII] = 0.3)³⁰</td>
</tr>
<tr>
<td>BIOPEPTIDE CL</td>
<td>10 male and female guinea pigs (strain not stated)</td>
<td>0.05 ml on 4 cm² area on left flank</td>
<td>Applied (uncovered) once daily for 14 consecutive days</td>
<td>Non-irritant (maximum weekly mean irritation index = 0)²⁴</td>
</tr>
<tr>
<td>BIOPEPTIDE CL</td>
<td>20 male and female guinea pigs (strain and ages not stated)</td>
<td>Intradermal injection with 1% (0.1 ml) and cutaneous application of undiluted ingredient during induction. 24-h challenge with 75% [maximal non-irritant concentration] under occlusive dressing</td>
<td>Maximization test</td>
<td>Non-sensitizer²⁵</td>
</tr>
<tr>
<td>BIOPEPTIDE EL (contains 100 ppm palmitoyl oligopeptide, as Pal-Val-glu-Val-Ala-Pro-Gly-OH)</td>
<td>3 male New Zealand White rabbits (ages not stated)</td>
<td>0.5 ml on 6 cm² area of flank</td>
<td>Applied for 4 h under semi-occlusive dressing</td>
<td>Moderate erythema, reversible within 24 h or 48 h. Classified as non-irritant (mean erythema score of &lt; 1)³¹</td>
</tr>
<tr>
<td>MAXI-LIP (contains 1,000 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH)</td>
<td>10 adults (ages not stated)</td>
<td>~ 0.02 ml on 50 mm² area of dorsal skin</td>
<td>Applied for 48 h under occlusive patch (Finn chamber)</td>
<td>Non-irritant (PII = 0)²⁸</td>
</tr>
<tr>
<td>MAXI-LIP</td>
<td>52 subjects (16 to 79 years old)</td>
<td>Undiluted ingredient applied during induction and challenge</td>
<td>Human repeated insult patch test (HRPT). 24-h induction applications. 24-h challenge.</td>
<td>Barely perceptible (+ reaction) to moderate (2 reaction) during induction and/or challenge phases. No clinically significant potential for skin irritation or sensitization³²</td>
</tr>
<tr>
<td>DERMAXYL (contains 200 ppm palmitoyl oligopeptide, as Pal-Val-Gly-Val-al-a-Pro-Gly-OH)</td>
<td>10 adults (ages not stated)</td>
<td>Test concentration of 50% on dorsal skin</td>
<td>Applied for 48 h under occlusive patch</td>
<td>Non-irritant when diluted to 50%²⁴</td>
</tr>
<tr>
<td>DERMAXYL</td>
<td>53 adults (ages not stated)</td>
<td>Test concentration of 50% applied during induction and challenge</td>
<td>HRPT. Eight 48-h induction applications, followed by challenge</td>
<td>Non-irritant (mean irritation index = 0.04) and non-sensitizer³³</td>
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<tr>
<td>MATRIXYXL (contains 100 ppm palmitoyl pentapeptide-4)</td>
<td>10 adult subjects (ages not stated)</td>
<td>0.02 ml on 50 m² area on dorsal skin</td>
<td>Applied for 48 h under occlusive patch (Finn chamber)</td>
<td>Very slight erythema in 1 subject. Classified as non-irritant (PII = 0.10)⁶⁴</td>
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<tr>
<td>MATRIXYXL</td>
<td>51 male and female subjects (19 to 78 years old)</td>
<td>Undiluted ingredient applied during induction and challenge</td>
<td>HRPT (protocol not stated)</td>
<td>Non-irritant and non-sensitizer⁶⁵</td>
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References


### 2013 FDA VCRP Data

**Palmitoyl Oligopeptide**

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
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<tr>
<td>03B</td>
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<tr>
<td>03C</td>
<td>Eye Shadow</td>
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<tr>
<td>03D</td>
<td>Eye Lotion</td>
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<td>03G</td>
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<td>Tonics, Dressings, and Other Hair Grooming Aids</td>
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<td>07B</td>
<td>Face Powders</td>
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**Tripeptide-1**

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**Biotinoyl Tripeptide-1**

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**Copper Tripeptide-1**

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<td>03G - Other Eye Makeup Preparations</td>
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**Palmitoyl Tripeptide-1**

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

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

DATE: September 27, 2013

SUBJECT: Concentration of Use by FDA Product Category: Palmitoyl Oligopeptide, Tripeptide-1 ingredients and Hexapeptide-12 ingredients
## Concentration of Use by FDA Product Category*

**Palmitoyl Oligopeptide (sequence unstated; GHK or VGVAPG)**

- **Anacardyl Tripeptide-1**
- **Biotinyl Tripeptide-1**
- **Caffeoyl Tripeptide-1**
- **Coumaroyl Tripeptide-1**
- **Mevalonoyl Tripeptide-1**
- **Nicotinoyl Tripeptide-1**
- **Quinoyl Tripeptide-1**
- **Retinoyl Tripeptide-1**
- **Thiocystyl Tripeptide-1**
- **Ursoloyl Tripeptide-1**

**Palmitoyl Oligopeptide (VGVAPG)**

- **Hexapeptide-12**
- **Palmitoyl Hexapeptide-12**
- **Myristoyl Hexapeptide-12**

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<tr>
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<td>Other eye makeup preparations</td>
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<td>Palmitoyl Oligopeptide (sequence unstated)</td>
<td>07C</td>
<td>Foundations</td>
<td>0.0011%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (sequence unstated)</td>
<td>07E</td>
<td>Lipstick</td>
<td>0.0015-0.009%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (sequence unstated)</td>
<td>11A</td>
<td>Aftershave lotions</td>
<td>0.00002%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (sequence unstated)</td>
<td>12C</td>
<td>Face and neck products not spray</td>
<td>0.00001-0.0002%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (sequence unstated)</td>
<td>12D</td>
<td>Body and hand products not spray</td>
<td>0.00005-0.0004% 0.001%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (sequence unstated)</td>
<td>12F</td>
<td>Moisturizing products not spray</td>
<td>0.0003%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (sequence unstated)</td>
<td>12G</td>
<td>Night products not spray</td>
<td>0.0002%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (sequence unstated)</td>
<td>13C</td>
<td>Other suntan preparations</td>
<td>0.02%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (GHK)</td>
<td>03D</td>
<td>Eye lotion</td>
<td>0.0001-0.2%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (GHK)</td>
<td>07C</td>
<td>Foundations</td>
<td>0.0001-1%</td>
</tr>
<tr>
<td>Product Description</td>
<td>Code</td>
<td>Material Use</td>
<td>Concentration</td>
</tr>
<tr>
<td>----------------------------------------------------------</td>
<td>------</td>
<td>-------------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (GHK)</td>
<td>07E</td>
<td>Lipstick</td>
<td>0.001-1%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (GHK)</td>
<td>07F</td>
<td>Makeup bases</td>
<td>0.0002%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (GHK)</td>
<td>12A</td>
<td>Skin cleansing (cold creams, cleansing lotions, liquids and pads)</td>
<td>0.0008%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (GHK)</td>
<td>12C</td>
<td>Face and neck products not spray</td>
<td>0.0001-0.02%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (GHK)</td>
<td>12D</td>
<td>Body and hand products not spray</td>
<td>0.000001-0.005%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (GHK)</td>
<td>12G</td>
<td>Night products not spray</td>
<td>0.0001-0.01%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (GHK)</td>
<td>12H</td>
<td>Paste masks and mud packs</td>
<td>0.0001%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (GHK)</td>
<td>12J</td>
<td>Other skin care preparations</td>
<td>0.001-0.01%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (GHK)</td>
<td>13A</td>
<td>Suntan products not spray</td>
<td>0.25%</td>
</tr>
<tr>
<td>Tripeptide-1</td>
<td>03F</td>
<td>Mascara</td>
<td>0.00002%</td>
</tr>
<tr>
<td>Tripeptide-1</td>
<td>03G</td>
<td>Other eye makeup preparations</td>
<td>0.00002%</td>
</tr>
<tr>
<td>Tripeptide-1</td>
<td>05G</td>
<td>Tonics, dressings and other hair grooming aids</td>
<td>0.0001%</td>
</tr>
<tr>
<td>Tripeptide-1</td>
<td>12A</td>
<td>Skin cleansing (cold creams, cleansing lotions, liquids and pads)</td>
<td>0.00003%</td>
</tr>
<tr>
<td>Tripeptide-1</td>
<td>12C</td>
<td>Face and neck products not spray</td>
<td>0.0001-0.001%</td>
</tr>
<tr>
<td>Tripeptide-1</td>
<td>12D</td>
<td>Body and hand products not spray</td>
<td>0.0003%</td>
</tr>
<tr>
<td>Tripeptide-1</td>
<td>12G</td>
<td>Night products not spray</td>
<td>0.00025%</td>
</tr>
<tr>
<td>Biotinoyl Tripeptide-1</td>
<td>03B</td>
<td>Eye liner</td>
<td>0.0006%</td>
</tr>
<tr>
<td>Biotinoyl Tripeptide-1</td>
<td>03F</td>
<td>Mascara</td>
<td>0.00005-0.00066%</td>
</tr>
<tr>
<td>Biotinoyl Tripeptide-1</td>
<td>05A</td>
<td>Hair conditioners</td>
<td>0.00002-0.0002%</td>
</tr>
<tr>
<td>Biotinoyl Tripeptide-1</td>
<td>05B</td>
<td>Hair sprays pump sprays</td>
<td>0.0002-0.0006%</td>
</tr>
<tr>
<td>Biotinoyl Tripeptide-1</td>
<td>05F</td>
<td>Shampoos (noncoloring)</td>
<td>0.00002-0.0002%</td>
</tr>
<tr>
<td>Biotinoyl Tripeptide-1</td>
<td>05G</td>
<td>Tonics, dressings and other</td>
<td>0.0006%</td>
</tr>
<tr>
<td>Ingredient</td>
<td>Code</td>
<td>Category</td>
<td>Concentration</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>------</td>
<td>-----------------------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Biotinoyl Tripeptide-1</td>
<td>05I</td>
<td>Other hair preparations (noncoloring)</td>
<td>0.0006%</td>
</tr>
<tr>
<td>Biotinoyl Tripeptide-1</td>
<td>12C</td>
<td>Face and neck products not spray</td>
<td>0.0002%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (VGVAPG)</td>
<td>03C</td>
<td>Eye shadow</td>
<td>0.01%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (VGVAPG)</td>
<td>03D</td>
<td>Eye lotion</td>
<td>0.2%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (VGVAPG)</td>
<td>07C</td>
<td>Foundations</td>
<td>0.0001%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (VGVAPG)</td>
<td>07E</td>
<td>Lipstick</td>
<td>0.5%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (VGVAPG)</td>
<td>07F</td>
<td>Makeup bases</td>
<td>0.01%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (VGVAPG)</td>
<td>08B</td>
<td>Cuticle softeners</td>
<td>0.01%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (VGVAPG)</td>
<td>12A</td>
<td>Skin cleansing (cold creams, cleansing lotions, liquids and pads)</td>
<td>0.001%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (VGVAPG)</td>
<td>12C</td>
<td>Face and neck products not spray</td>
<td>0.0018-0.1%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (VGVAPG)</td>
<td>12D</td>
<td>Body and hand products not spray</td>
<td>0.0000001-0.01%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (VGVAPG)</td>
<td>12F</td>
<td>Moisturizing products not spray</td>
<td>0.0004%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (VGVAPG)</td>
<td>12G</td>
<td>Night products not spray</td>
<td>0.01%</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide (VGVAPG)</td>
<td>12I</td>
<td>Skin fresheners</td>
<td>0.01%</td>
</tr>
<tr>
<td>Palmitoyl Hexapeptide-12</td>
<td>12C</td>
<td>Night products not spray</td>
<td>0.002%</td>
</tr>
</tbody>
</table>

*Ingredients included in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported.
†Product category codes used by FDA

Information collected in 2013
Table prepared: September 27, 2013
Memorandum

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

FROM: CIR Science and Support Committee of the Personal Care Products Council

DATE: March 18, 2013

SUBJECT: Comments on the Grouping of the Palmitoyl Oligopeptide Ingredients

The grouping of ingredients included in the report titled “Safety Assessment of Palmitoyl Oligopeptides as Used in Cosmetics” by the presence of the palmitoyl group is inappropriate as the peptide portion of the ingredient determines the functionality. We respectfully suggest that the CIR CIR Expert Panel table this report so that the CIR Expert Panel and staff can consider the following suggestions and develop a reasonable, science-based strategy for grouping the peptide ingredients.

The lead ingredient (ingredient with the most uses reported to the VCRP) is Palmitoyl Oligopeptide. According to the only supplier listed for this INCI name, this ingredient is actually two different specific peptide sequences bound to palmitic acid (Palmitoyl-Glycine-Histidine-Lysine-OH and Palmitoyl-Valine-Glycine-Valine-Alanine-Proline-Glycine-OH). Palmitoyl Oligopeptide is an older INCI name with a definition of: “the product obtained by the reaction of palmitic acid with a synthetic peptide consisting of two or more of the following amino acids: alanine, arginine, aspartic acid, glycine, histidine, lysine, proline, serine, or valine.” The Palmitoyl Oligopeptide INCI name was not changed when the peptide naming conventions were changed.

The focus of this CIR report should be only on the two peptide sequences sold under the INCI name Palmitoyl Oligopeptide.

After the Palmitoyl Oligopeptide name was assigned, the INCI committee has used different naming conventions for peptide ingredients. For example, the INCI name Myristoyl Glycine/Histidine/Lysine Polypeptide is still included in the Dictionary and appears to include the same amino acid sequence as one sequence sold under the INCI name Palmitoyl Oligopeptide.

Under the most recent peptide naming conventions, the INCI Committee has assigned the name Palmitoyl Tripeptide-1 to palmitic acid bound to Tripeptide-1 (a peptide containing glycine, histidine and lysine), and the name Palmitoyl Hexapeptide-12 to palmitic acid bound to Hexapeptide-12 (a peptide containing alanine, glycine, proline and valine). Based on their definitions, Palmitoyl Tripeptide-1 and Palmitoyl Hexapeptide-12 appear to be the same sequences as ingredients sold under the name Palmitoyl Oligopeptide. It should also be noted that the peptide ingredients themselves
(Tripeptide-1 and Hexapeptide-12) are also included in the Dictionary.

Continuing to keep Palmitoyl Oligopeptide as the lead ingredient, and if it is confirmed that ingredients sold under the names Tripeptide-1 and Hexapeptide-12 correspond to the sequences sold under the name Palmitoyl Oligopeptide, we recommend that this report should include the following ingredients:

- Palmitoyl Oligopeptide
- Tripeptide-1*
- Acetyl Tripeptide-1*
- Azelaoyl Tripeptide-1*
- Bis(Tripeptide-1) Copper Acetate*
- Copper Tripeptide-1*
- Manganese Tripeptide-1*
- Palmitoyl Tripeptide-1
- Myristoyl Glycine/Histidine/Lysine
- Polypeptide*
- Hexapeptide-12*
- Myristoyl Hexapeptide-12*
- Palmitoyl Hexapeptide-12

The ingredients marked with an asterisk are not included in the current draft report on palmitoyl peptides.

Other Tripeptide-1 ingredients that could be considered for inclusion in this report are:

- Aminolevulinoyl Tripeptide-1
- Anacardoyl Tripeptide-1
- Biotinoyl Tripeptide-1
- Caffeoyl Tripeptide-1
- Coumaroyl Tripeptide-1
- Mevalonoyl Tripeptide-1
- Nicotinoyl Tripeptide-1
- Quinoyl Tripeptide-1
- Retinoyl Tripeptide-1
- Thioctoyl Tripeptide-1
- Ursoloyl Tripeptide-1

The remaining palmitoyl peptide ingredients in the draft report should be reviewed based on the number of uses reported to the FDA VCRP.

If the CIR Expert Panel decides to continue to group these ingredients based on the presence of the palmitoyl group, please consider removing the palmitoyl plant- and animal-derived hydrolyzed protein ingredients (Palmitoyl Hydrolyzed Collagen, Palmitoyl Hydrolyzed Milk Protein, Palmitoyl Hydrolyzed Wheat Protein, Potassium Hydrolyzed Corn Protein, Potassium Palmitoyl Hydrolyzed Oat Protein, Potassium Hydrolyzed Rice Protein, Potassium Palmitoyl Hydrolyzed Sweet Almond Protein, Potassium Palmitoyl Hydrolyzed Wheat Protein, Sodium Palmitoyl Hydrolyzed Collagen, Sodium Palmitoyl Hydrolyzed Wheat Protein) from this report. These ingredients would be more appropriately reviewed with the specific hydrolyzed protein ingredient as suggested in the memo concerning the grouping of ingredients in the CIR report on plant- and animal-derived hydrolyzed proteins and amino acids.