Safety Assessment of Vitis Vinifera (Grape)-Derived Ingredients as Used in Cosmetics
Enclosed is the Safety Assessment of Vitis Vinifera (Grape)-Derived Ingredients as Used in Cosmetics (Draft Final). This assessment evaluates the safety of 24 cosmetic ingredients. At the June meeting, the Panel issued a Tentative Report with a conclusion of safe for use in cosmetic formulations in the present practices of use and concentration.

Updated VCRP data are now incorporated into the cosmetic use section and the frequency and concentration of use table (Table 7). The new information previously was distributed to the Panel as a June meeting Wave 2 document and presented to the Panel at the meeting.

At the June meeting, the Panel included hydrolyzed grape fruit and hydrolyzed grape skin to this safety assessment. These two ingredients are obtained from crude materials that are hydrolyzed and not specifically protein hydrolysates. The Panel considered that the existing data apply to the safety of these ingredients. Additionally, some unpublished data were received previously on hydrolyzed grape skin. These data have been added to the document, as indicated below.

The unpublished data listed below have been incorporated into the report, and vertical lines on the right and left margins (when possible) or underlining is used to designate this in the document. The data submissions are included in the data tab of this report package. As stated above, some of the included submissions are data received previously on hydrolyzed grape skin; the other data were received after the last version of the document was prepared for the June Panel meeting:

1. Personal Care Products Council. Monograph proof on Vitis Vinifera (Grape) Shoot Extract. (July 3, 2012). (see Table 1, p 11).
2. Information: Hydrolyzed Grape Skin. (Dec 19, 2009)
   a. Phenbiox SRL. 2011. Technical data sheet UVIOX (hydrolyzed grape skin). (see Table 2, p 12).
   b. Phenbiox SRL. 2011. Safety data sheet UVIOX (hydrolyzed grape skin). (see Table 2, p 12).
   c. ABICH S.r.l. 2009. In vitro evaluation of the eye irritation potential of hydrolyzed grape (Vitis vinifera) fruit skin. Report No. REL/0444/2009/IRRO/ELB. (see the Ocular Irritation section, p 8)
3. Personal Care Products Council. Updated concentration of use by FDA product category: grape-derived ingredients. (June 14, 2012). (see Table 8, p 20).
5. Studies of products containing Vitis vinifera (grape) seed extract. (May 9, 2012)
   a. Institute for In Vitro Sciences. 2006. Bovine corneal opacity and permeability assay with optional histology (aftershave lotion containing 0.15% Vitis vinifera (grape) seed extract). Study Number: 06AF67.350049. (see the Ocular Irritation section, p 7)
b. Clinical Research Laboratories, Inc. 2006. An in-use safety evaluation to determine the dermal irritation potential of a cosmetic product or toiletry (aftershave lotion containing 0.15% Vitis vinifera (grape) seed extract). CRL Study Number: CRL66106. (see Table 12, p 28).

c. TKL Research. 2006. Summary report: Repeated insult patch test of an aftershave lotion containing 0.15% Vitis vinifera (grape) seed extract. TKL Study No.: DS103906-5. (see Table 12, p 29).

It is expected that the Panel will issue a Final Safety Assessment at this meeting.
*The CIR Staff notifies of the public of the decision not to re-open the report and prepares a draft statement for review by the Panel. After Panel review, the statement is issued to the Public.

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

△ Document for Panel Review

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| **Public Comment** | **CIR** | **Expert Panel** | **Re-Reviews** | **Report Color** |
| Draft Priority List | Draft Priority List | DRAFT PRIORITY LIST | Re-review to Panel | Buff Cover |
| Priority List | INGREDIENT | PRIORITY LIST | | |
| 60 day public comment period | | | YES | Buff Cover |
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* The CIR Staff notifies of the public of the decision not to re-open the report and prepares a draft statement for review by the Panel. After Panel review, the statement is issued to the Public.

** If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.
February 21, 2012: Scientific Literature Review

The unpublished data listed below were received and incorporated into the SLR:

1. **Product information: Vitis Vinifera (Grape) Leaf Extract and Vitis Vinifera (Grape) Fruit Extract**

2. **Information: Vitis Vinifera (Grape) Fruit Extract**
   a. Arch Personal Care Products LP. 2011. Toxicological Summary Blend 3EL - New contains 3% Vitis Vinifera (Grape) Fruit Extract (water extract).
   b. BioScreen Testing Services Inc. 2009. Evaluation of one sample Blend 3EL-New (contains 3% Vitis Vinifera (Grape) Fruit Extract (water extract)) utilizing the ocular irritation test method.
   c. BioScreen Testing Services Inc. 2009. Evaluation of one sample Blend 3EL-New (contains 3% Vitis Vinifera (Grape) Fruit Extract (water extract)) utilizing the dermal irritation test method.
   d. BioScreen Testing Services Inc. 2009. 100 Human Subject repeat insult test patch test skin irritation/sensitization valuation of Blend 3EL-New (contains 3% Vitis Vinifera (Grape) Fruit Extract (water extract))
   e. Arch Personal Care Products LP. 2011. Toxicological Summary NAB® Grape Extract Vitis Vinifera (Grape) Fruit Extract (water extract).
   h. AMA Laboratories. 2002. 50 Human subject repeat insult patch test skin irritation/sensitization evaluation (occlusive patch) NAB® Grape Extract contains 10% Vitis Vinifera (Grape) Fruit Extract (water extract). AMA Reference No.: MS 02-RIPT.C3070.O.50.APAC

As an additional note – unpublished data on Hydrolyzed Grape Skin were submitted to the CIR. However, since this ingredient is not included in this report, these data are not included.

June 11-12, 2012: Draft Report

The following data were received after the SLR was announced and are included in the draft report:

1. **Product information: Vitis Vinifera (Grape) Seed Extract.**
   a. Symrise. 2010. Product information Neo Actipone® Grape Seed (Vitis Vinifera (Grape Seed Extract).

2. **Concentration of Use by FDA Product Category:** Grape-Derived Ingredients.

3. **HR IPT of a Product Containing Vitis Vinifera (Grape) Juice.**
   a. Clinical Research Laboratories Inc. 2010. Repeated insult patch test of a make-up primer containing 0.1% Vitis Vinifera (Grape) Juice. CRL Study Number: CRL84410.

4. **Summaries of HRIPTs of Products Containing Grape-Derived Ingredients.**
   a. Clinical Research Services. 2006. Summary of an HRIPT of a hair styling product containing 0.5% Vitis Vinifera (Grape) Juice Extract (row 1 [irritation results] and 3 [sensitization results] of the table)
   b. RCTS, Inc. 2007. Summary of an HRIPT of a body lotion containing 0.0002% Vitis Vinifera (Grape) Seed Extract (Row 2 [irritation results] and 4 [sensitization results] of the table)

5. **HRIPTs: Vitis Vinifera (Grape) Seed Extract and Vitis Vinifera (Grape) Fruit Extract.**
   a. Product Investigations, Inc. 2010. Determination of the irritating and sensitizing propensities of a hair conditioner containing 0.1% Vitis Vinifera (Grape) Seed Extract (10% dilution tested).
   c. Product Investigations, Inc. 2007. Determination of the irritating and sensitizing propensities of a foundation containing 0.0239% Vitis Vinifera (Grape) Fruit Extract (tested neat).

Additionally, prior to the meeting, updated VCRP data were received from the FDA and presented to the Panel. This information has since been incorporated.
The Panel added hydrolyzed grape fruit and hydrolyzed grape skin to this safety assessment. These 2 ingredients are obtained from crude materials that are hydrolyzed and not specifically protein hydrolysates. The Panel considered that the existing data apply to the safety of these ingredients.

The Panel issued a Tentative Report with a conclusion of safe as used.

September 10-11, 2012: (Draft) Final Report
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“X” indicates that data were available in the category for that ingredient.
Vitis Vinifera (Grape) Ingredients

Created Keep Me Posted for:
Vitis Vinifera grape and toxicity and dermal
Vitis Vinifera grape and mutagenicity or genotoxicity

PubMed Search – July 2012
((VITIS AND VINIFERA) OR GRAPE) AND HYDROLYZED AND (FRUIT OR SKIN) – 6 hits/ 0 useful

TOXNET Search – Jan 30, 2012
(((VITIS AND VINIFERA) OR GRAPE) AND (((SEED OR BUD OR FLOWER OR FRUIT OR JUICE OR LEAF OR SEED OR SKIN OR ROOT OR VINE) AND EXTRACT) OR (FRUIT AND (POWDER OR WATER)) OR JUICE OR (LEAF AND (OIL OR WATER OR WAX)) OR SEED OR (SEED AND POWDER) OR (SKIN AND POWDER) OR (VINE AND SAP))) OR 84929-27-1 OR 85594-37-2 OR 8016-21-5 OR ENOCIANINA - 1202 hits

Grape and Dermal Effects; Vinifera and Dermal Effects (no patents) – 75 hits

Ordered 55 papers (1/31/12)

Feb. 2, 2012

ChemPortal
84929-27-1 – no pertinent findings
85594-37-2 - no pertinent findings
8016-21-5 - no pertinent findings
enocianina – no pertinent findings

OECD – no hits

HPVIS – no hits

IARC – no pertinent findings

NTP – some relevant information found

Dr. Duke – constituent info

NTIS – no hits

JECAF/AWHO - no pertinent findings

FCC – no pertinent findings

USP - no pertinent findings

EU – most in EU database

SciFinder
Vitis Vinifera grape and toxicity and dermal
Vitis Vinifera grape and mutagenicity or genotoxicity

Ordered an additional 7 papers
We're moving on to the next ingredient with Dr. Marks presenting, and this is the vitis group which is grape.

DR. MARKS: This is the first time we've seen this draft report on the safety of vitis vinifera derived ingredients which are listed in Panel Book page 7 from bud, flower, fruit, juice, leaf, et cetera. We felt that these ingredients could be considered safe and that we could move on with a draft tentative report with a conclusion as safe.

DR. BERGFELD: Is there a motion?

DR. MARKS: Yes.

DR. BERGFELD: Is there a second?

DR. BELSITO: Yes and no. Our group also felt we could go ahead with a safe as used, but we wondered by the hydrolyzed fruit and the hydrolyzed skin were not added and felt that they could be added to this report and found safe as used.

DR. BERGFELD: Is the Marks team agreeable with those additions?

DR. MARKS: Yes.

DR. BERGFELD: Alan?

DR. ANDERSEN: My only concern is that the game plan as we've been looking at it was to separately focus later on a group of hydrolyzed materials that would raise all of the questions related to extent of hydrolysis, what's the product, in a way that we couldn't do if they're just add-ons to the regular group. I don't expect that there are any significant issues here and arguably adding them is a way of dealing with the source material hydrolyzed or unhydrolyzed, it's just the hydrolyzed as a group seemed to us to be a more efficient way of getting at that question.

DR. BERGFELD: Don?

DR. BELSITO: Then I guess the question from our panel is on the panax ginseng root there are three hydrolyzed products, hydrolyzed ginseng root, ginseng root extract and ginseng saponins, are we going to delete those from that report? I think we just need a little internal consistency particularly at this meeting.

DR. LIEBLER: I think the idea of separating out hydrolyzed ingredients for separate review is driven by the consideration of proteins and amino acids and it makes perfect sense in that context, but in this case, these ingredients are much more complicated than proteins. If this were for example grape protein extract and then hydrolyzed grape protein extract, then I think it would make a lot of sense to accept the game plan. But in the case of a crude material that's hydrolyzed, it's protein, it's all the carbohydrates and everything else and it depends on the hydrolysis conditions what you end up with. I think we needn't feel like we're violating a perfectly reasonable game plan in this context, so I think that it's really okay.

DR. ANDERSEN: Bart, do you want to fight about it?

MR. HELDRETH: No. If you want to increase our inefficiency, we'll take it.

DR. BERGFELD: Thank you. It's my understanding then we'll just add these hydrolyzed ingredients. Is there any other discussion?

DR. MARKS: Tom, do you want to comment about the geno-tox data? There was some concern yesterday about that.

DR. SLAGA: There's a big history mutagenicity especially in eggs and different food products. In this there was some grape juice plus raw extracts of grapes that had either potent or strong activity, but if you look at in more detail which MTP has, this is more related to bacterial mutagenesis. When you go to mammalian mutagenesis this falls out, or in vivo mutagenesis, and some of the bad players that are bacterial mutagenic are some of the flavonoids which have been later tested for carcinogenic activity and are negative.

DR. BELSITO: That should go in the discussion because the in vitro data is very different from the in vivo data. The other thing as we questioned about whether it should go in the discussion or be completely ignored is Panel Book page 13 where they did some UVI-induced skin pigmentation and found it was reduced when the group was fed grape extract. However, the difference was not significant. We felt that since the difference was not significant that it really shouldn't even come up in the discussion, and that study was somewhat tenuous anyway we thought.
DR. BERGFELD: Are you deleting that paragraph or you're just not discussing it?

DR. BELSITO: We're not deleting it, but we're not discussing it because the finding was not significant. The only other thing that we obviously have to put in the discussion is the heavy metal pesticide usual boilerplate.

DR. BERGFELD: Are there any other additions, suggestions or edits? Seeing none, I'll for the question. All those in favor of safe approval of this ingredient? Thank you. Unanimous.

Belsito Team – June 11, 2012

DR. BELSITO: Yeah. Okay. So these are the grape-derived ingredients. This is the first time we're seeing it, 22 grape-derived ingredients. A whole bunch of data and Alan just wants me to point out that they did not add hydrolyzed grape skin, but would add it should we feel it necessary. And so I'd like to hear from our industry colleagues as to whether industry would support or not support the addition of the grape skin and also if FDA or the consumer groups have issues with that. I see smiles over there.

DR. EISENMANN: We don't care either way. It just didn't seem consistent to have hydrolyzed ingredients in one report and not the other. That was all I was trying to understand why is it okay to have a hydrolyzed ginseng root and not hydrolyze grape root. What's the difference? I just don't understand it.

DR. BRESLAWEC: Yeah, we would certainly defer to whatever --

DR. BELSITO: Well, it's hydrolyzed grape skin, not grape root.

DR. EISENMANN: I know. I know. Hydrolyzed Panax root is in the Panax report.

DR. BELSITO: Right.

DR. EISENMANN: Why is not okay to have hydrolyzed --

DR. BRESLAWEC: Skin.

DR. EISENMANN: -- skin in this report? I don't understand the difference.

DR. BELSITO: Well, isn't the skin where all the tannins are? And, therefore, de facto, that might be a significantly different chemical composition than the fruit and the (inaudible) and the --

DR. EISENMANN: Well, there's two hydrolyzed -- the hydrolyzed fruit, too, that's not in the grape report. There are two hydrolyzed grape ingredients that are not in the report. I just don't understand the logic.

DR. BELSITO: What other hydrolyzed -- so there's a hydrolyzed fruit extract?

DR. EISENMANN: Yes, I believe so, or hydrolyzed fruit. I don't know if it's an extract.

DR. BELSITO: Well, I guess the only issue that I would have is, I see -- and I'm just seeing this now for the first time, I don't know how I missed it, is I assume that it was decided to leave out the skin because the skin would have all these tannins that the rest of the plant parts wouldn't. But then I see we're looking at leaf-seed-skin extract, which is the whole megillah. And so that's like --

DR. EISENMANN: Well, skin extract is in here.

DR. BELSITO: Yeah, that's what I mean. Yeah.

MS. FIUME: Dr. Belsito? The issue wasn't the skin. It's just in the past -- I think this is on -- we've had the hydrolyzed ingredients that we've taken out waiting for the hydrolyzed proteins to be discussed. So that is why the hydrolyzed ingredients were not added to this report.

DR. EISENMANN: Why are they in the ginseng report? That's my question.

MS. FIUME: I don't know the answer to that. I'm sorry.
DR. BELSITO: Okay. To hydrolyze or not to hydrolyze? That is the question. Panel members?

MS. WEINTRAUB: Is this a hydrolyzed protein? It's just hydrolyzed, right?

DR. BELSITO: Right.

MS. WEINTRAUB: It's not a protein.

DR. BELSITO: I don't think it is.

DR. LIEBLER: If you take the whole fruit and hydrolyze it, then it will have hydrolyzed protein in it along with hydrolyzed other chemical classes. So it will have a lot of different hydrolyzed chemicals. It's not -- hydrolyzed anything is not in this current 22 ingredient list, right? And that seems like a good place to leave it.

DR. BELSITO: But then your point is, we looked at hydrolyzed ginseng root, so why are we doing that? Should we go -- do we want to rethink and go back and delete hydrolyzed from the ginseng classification? I agree. We've got to be consistent here.

DR. LIEBLER: So let's just be practical. This hydrolyzed -- is there a hydrolyzed anything from grape that we need to consider as a ingredient? Or at least possibly considering --

DR. BELSITO: Hydrolyzed skin extract.

DR. EISENMANN: And fruit, I think, there's two. I remember there's two, but I can't -- I think it's fruit and skin.

DR. LIEBLER: And so they weren't -- why were they not included in this originally?

DR. BELSITO: Because we don't do hydrolyzed.

DR. LIEBLER: Okay. So we have --

DR. KLAASSEN: And why don't we do hydrolyzed?

MS. FIUME: We were doing some hydrolyzed. Some of our lists had hydrolyzed ingredients in it before, and we knew that CIR was going to do a hydrolyzed protein group later on. So the decision had been made, I believe it was last year, to pull out the hydrolyzed ingredients until that was done. Bart would be the person that can explain it better, but I think he's involved in the other room.

But that had been the decision, is to leave out the hydrolyzed ingredients until that large hydrolyzed protein report was completed. If I can get it to come up -- that's in the process, and I can look and see exactly what's in there. And then it was after that point that we would add hydrolyzed ingredients into the list.

DR. LIEBLER: Okay. So I think the reason to do the proteins and amino acid continuum that way is sensible. And it's not because the hydrolyzed products would necessarily have some unique adverse effects. It's more that they constitute a whole different, more complex mixture of molecules to consider and they don't fit that well with the amino acids or the synthetic amino acid mixtures.

So I think the fact that we're doing that doesn't mean that we can't consider any other sort of crude material hydrolysates, because I think the CIR's already reviewed lots of crude material hydrolysates of one sort or another, right? Haven't there been some hydrolyzed this or that that have been done?

DR. BRESLAWEC: Yeah.

DR. LIEBLER: Okay. Because in this case, if you took hydrolyzed grape skin or hydrolyzed fruit, you would not only have -- depending on the conditions of hydrolysis, you'd not only have hydrolyzed proteins, you'd have a hydrolyzed flavonoid carbohydrate conjugates, you'd have hydrolyzed nucleic acids, you'd have lots of different things that are present in a fragment. It would be a very complex mixture, but a lot of these are already very complex mixtures. I don't see any reason not to have hydrolyzed stuff along with this other stuff, which is every bit as complex.

DR. BERGFELD: So you're adding them? You're adding it?

DR. LIEBLER: I don't see a reason not to add them, particularly if they are relevant to this group and, you know, should be considered along with them.
DR. BERGFELD: Do you add the extract of it or what do you do? What part of this?

DR. LIEBLER: It depends on what the options are on the menu.

DR. BERGFELD: Okay.

DR. BELSITO: So Dan, you want to add the two hydrolyzed --

DR. LIEBLER: I'm fine with that. I don't see a reason to think that those would be unique in their properties in terms of our safety assessment..

DR. KLAASSEN: I agree with that..

DR. BELSITO: Okay. So we are asking that the two hydrolyzed and any other hydrolyzed forms of grapes that PCPC can identify be added to this report?

DR. SNYDER: Skin and fruit --

DR. BELSITO: Add hydrolyzed. Okay. Okay, so then -- do I hear anyone commenting that there are any conclusions that we can reach other than safe as used, in which case we need to then start looking at discussion? Does anyone have any concerns with that conclusion, safe as used?

DR. LIEBLER: I'm fine with it.

DR. BELSITO: Okay, because if you didn't, then I would not allow any wine at dinner tonight. Okay, we're going to go safe as used.

Discussion obviously pesticides and heavy metals, important with this. And then the only issue I had was the skin lightening properties, which I didn't know if we should add. And I'm trying to look, I mean, I wrote this down. I reviewed this so long I got a --

DR. SNYDER: Page 7.

DR. BELSITO: Yeah, page 7. Okay, yeah. A decrease in the melanin index and UV-induced pigmented skin throughout the study as compared to control values and then they were not statistically significant. So I didn't know what to make with that.

And the other thing that I had in here is that -- and I didn't know how to interpret this -- the paragraph above it, it says, "Using a Pen-Ray lamp to areas of the back on male and female brownish guinea pigs who were irradiated twice a week for 3 weeks with 900 millijoules per centimeter squared." I'm assuming it's 900 millijoules per centimeter squared of UVA, and hence the name Pen-Ray refers to the fact that this is UVA, in which case, I think that needs to be specified. UVA is usually reported in joules per centimeter squared, so I would change it to 0.9 joules because if -- UVB is usually reported in millijoules, and 900 millijoules would fry any animal I know of with UVB. So again, I'm assuming Pen-Ray is a UVA only, and I would specify that.

MS. FIUME: Okay. I'll check that.

DR. BELSITO: Okay. So safe as used. So you want to just strike that whole melanin index or you just want to keep it and it's not significant and we don't discuss it? I mean, I've never even heard of that.

DR. BERGFELD: We had some of that with vitamin C.

DR. BELSITO: Yeah, I mean, is it an antioxidant effect or something? I mean, I guess you could leave it in and -- I mean, in the end it says it's not significant and not even included in the discussion. Anything else? No? Okay.

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**Marks Team – June 11, 2012**

DR. MARKS: So this is the first time we've seen this draft report. And of course, the two things are the data needs. Are there any data needs? And then, are the ingredients which are included in this -- which vary from the bud to the flower to the fruit to the leaf to the root. It looks like it's the seed, the skin, we've got everything, even sap. So, there's no part of the grape that's ignored.

Is there a reason -- I wanted to ask Monice, is there a reason why we excluded hydrolyzed grape skin?
MS. FIUME: We have not been doing the hydrolyzed because we were going to wait until they were all done and sort of -- is that correct, with that sound? Fairly characterized?

MS. BURNETT: They hydrolyzed protein?

MS. FIUME: All the hydrolyzed protein -- the hydrolyzed have been kept out of the reports because of the hydrolyzed portion until we do the hydrolyzed proteins in groups such as that.

DR. ANDERSEN: Yes. That's the short answer.

DR. MARKS: Okay. Rons, Tom?

DR. SLAGA: The only thing on the genotoxicity. It states that fractions of raw grapes, which I'm not sure what that really means other than no extract, that the potent mutagen and then even under grape juice it says it's mutagenic. Is there any reference for that? Or is that -- I looked even in the unpublished and I couldn't see where --

DR. MARKS: You're on what page, Tom?


DR. MARKS: Okay.

MS. FIUME: For the fractions it was a mutagenicity screened of foods, by Stultz, et al., in 1984. And for grape juice --

DR. SLAGA: And it has grape juice, too, two lines below that.

MS. FIUME: Reference 79, Patrenelli, et al., in 1996. And I know the first study. If I remember correctly, they were large studies where they were examining mutagenicity of foods and they were huge group studies.

DR. SLAGA: Well, you know, we have to deal with that either in the discussion or some -- because it sounds like something in raw grapes which then you wonder if it's going to carry over to any of the fractions.

DR. SHANK: Especially in the extracts, which would be concentrated. So I thought we needed genotox on the fruit extract.

DR. ANDERSEN: Well, the follow-up study is informative. Contribution of phenols, quinones, and reactive oxygen species to the mutagenicity of white grape juice. So, it's pretty clear the direction that that's going.

So, the question of which components are of concern, they've identified it. You know, I don't know what that second paper actually says in terms of how serious a contribution comes from phenols and quinones, versus reactive oxygen species. But that second paper would seem to be the more relevant one, Reference 80.

So, if you take a look at report page 21, which is Table 7, the description in the results column for Reference 80 talks about glutathione making the effect go away, which argues for reactive oxygen species.

DR. SHANK: Or metabolized of the quinone and phenol.

DR. ANDERSEN: Let's see. Polyphenol oxidase mediated oxidation of grape juice phenolics, generates species that can induce mutations. So, I think Monice has captured the thrust of that second paper. Is that a mechanism of action that's going to be hugely relevant to cosmetics?

DR. MARKS: Tom?

DR. SLAGA: Well, you know actually myself have worked with grape seed extracts and skin extracts before, and I never had a concern for this. That popped up and I never did ever see any data make that kind of statement of being that potent. I don't have any concern with overall other than we have to deal with -- if that's under genotoxicity in the tables, we have to deal with -- for some reason, about it --

DR. ANDERSEN: Interpretation of --

DR. SLAGA: -- in the discussion.
DR. MARKS: So is that how you would like to deal with it? Ron Shank mentioned needing more data.

DR. SLAGA: Well, maybe we could ask for further data here, too?

DR. MARKS: That would be an insufficient data notice.

DR. SLAGA: Could we have one for the --

DR. SHANK: Fruit extract, genotox on the fruit extract, that's used --

DR. SLAGA: In further data on the fractions of raw grapes and grape juice.

DR. MARKS: So it should be an insufficient data. You would like to go with insufficient data notice?

DR. SLAGA: Oh.

DR. SHANK: Yeah, the seed extract was a sensitizer.

DR. MARKS: Actually, I had irritation sensitization was okay.

DR. SHANK: Okay.

DR. MARKS: But we can go back. Let's finish up with the tumor business, because we wanted an insufficient data notice? That's where we would go from here, and then we'll do the sensitivity..

DR. SLAGA: The thing is that we don't have fractions of raw grapes here, do we?

DR. MARKS: Demonstrated.

DR. SLAGA: I mean, that statement --

DR. MARKS: Well, you would think when you extract from any of these they would be probably raw. I don't know. Presumably what they're talking about, fractions of raw grapes are just crushed, physically crushed. But we don't know that either, do we?

DR. SLAGA: No, the details of that I don't know. And the grape juice it just says "grape juice," it doesn't say a fraction.

MS. FIUME: Some of it was commercial. I'll pull both of those papers tonight and look at them.

DR. SLAGA: Okay, so we can look at it in the morning? Okay, good.

DR. MARKS: So do you want me to hold off, then since I'm going to be making a motion, do you want me to hold off on the insufficient data?

DR. SLAGA: Check with Ron about the one. He said --

DR. SHANK: Well, I said the seed extract was a sensitizer and now I can't find it.

DR. MARKS: Well, let's go back to this. How do you want me to handle, though, the genotox issue here? I mean, it's pretty strong statements.

DR. SLAGA: Yeah, I know.

DR. MARKS: Potent mutagenic activity.

DR. SLAGA: See, generally we want the genotoxicity and if it's positive we want the carcinogenicity of certain aspects. Seed extracts, anyway, are really negative. I mean, because they have a carcinogenic activity.

DR. MARKS: I guess the question here would be, can you handle that disconnect in the discussion? And then we could put a personal communication. Tom Slaga has worked with this in the lab, not had problems.
DR. SLAGA: No, no.

MR. ANSELL: How about, there's extensive human experience showing that grape juice is not carcinogenic.

DR. SLAGA: I think normally that it refers to (inaudible).

DR. MARKS: Well, we think. There's certainly, I think, epidemiologically there's very little association if any between human cancer and grapes, but --

DR. SLAGA: Other than the alcohol part.

DR. MARKS: Yeah. So, how would you like to proceed, Tom?

DR. SLAGA: Let's see the stuff in the morning, then we'll --

DR. MARKS: Okay. You'll tell me. So, that's page under the genotox. I'm going to put a question here. Tom, genotox, insufficient --

DR. SLAGA: I think we can handle it in the discussion, okay.

DR. MARKS: -- data? Discussion.

DR. SLAGA: Because the carcinogenicity is --

DR. SHANK: But that's on the seed extract.

DR. SLAGA: Yeah, I know, only on the seed.

DR. MARKS: Who would have thought grape was so controversial?

DR. SLAGA: Well, grape juice. I mean, give me a break. All the kids drinking grape juice.

DR. MARKS: That's because this hasn't made it out to the public yet.

SPEAKER: Yeah, we'll fix that. (Laughter)

DR. MARKS: No more grapes. Probably Welch's knew this years ago but it's been a proprietary secret.

Okay, so we're going to delay this, Tom, until the morning? We're going to delay at least that, the genotox to the morning whether it's insufficient data? If it were insufficient, what would you want? You can tell me that in the morning, too.

DR. SLAGA: Repeat.

DR. MARKS: And then, we'll handle it into the discussion.

Okay, Ron? Shall we move on to the sensitivity or did you want any more about the genotox?

DR. SHANK: Well, actually it's irritant rather than sensitization.

DR. MARKS: Yeah.

DR. SHANK: So, I got that wrong.

DR. MARKS: And you were under --

DR. SHANK: Well, we don't have any data for a lot of these others: Bud, flower, leaf, root.

DR. MARKS: Yeah.

DR. SHANK: Shoot, skin, vine, sap.
DR. MARKS: I thought the irritation, to me, sensitization was okay.

DR. SHANK: Okay.

DR. MARKS: When I looked down there, there was some high concentrations. They had HRIPT, there was a fruit extract, the grape juice extract, seed extract, neither irritants or sensizers. So, I kind of just took that -- if I looked at the -- I took that as being representative and read across when I -- you know, I would have thought something in the case reports might have come out as, you know, among grape workers. Either in the wineries or in the fields, there would have been an occupational sensitivity, and none of that's come out. So, I was willing to take what we had in here as being sufficient for irritation sensitivity.

MR. HILL: Well, yeah. I mean, the ones that I flagged we have nothing on leaf oil, nothing on root extract, nothing on vine sap. If you look at chemical constituency of root, roots don't exist apparently because there are no significant chemicals of constituency. Serious.

DR. MARKS: Yeah. No, I hear you.

DR. SLAGA: Well, no one has looked, that's why.

MR. HILL: No, the root is here and it has got eight things listed but they all say "non-significant" and parts per million, and then we move on to something else. So, seed.

So, and the essential oil is very different, I think. But we do have tox information for a couple of the major components in here. It seems pretty clean, I guess. I just wonder if there are no reported uses, they're quite different in terms of character.

We wouldn't expect any case reports if they're not being used, I'm not sure that grape workers are all that exposed to whatever is in root extract. Vine sap, yeah, probably. Leaf oil, probably.

DR. MARKS: And the big ones -- the big uses in terms of numbers are seed extract with close to 500, and fruit extract which is 238. Let me see where the root is in here. Do we have any use of root?

MR. HILL: No.

DR. MARKS: No root use. Yeah, that doesn't surprise me. Okay, so --

MR. HILL: No leaf oil.

DR. MARKS: Any other needs? Any impurities? Was that left out? I highlighted that. Do we have impurities? Anything that talks about that? Do we need it?

I mean, obviously we'll have the pesticides, metals boilerplate, but do we need anything that says anything about impurities in here?

DR. ANDERSEN: As a botanical it contains just about everything anyway.

DR. MARKS: Yeah.

DR. ANDERSEN: So, what's an impurity.

MR. HILL: That was my reaction to that question.

DR. MARKS: Yeah, okay. I just want to be --

MR. HILL: He's talking about pesticides, and we have that covered, right?

DR. MARKS: Yes, that should be in the discussion.

MS. FIUME: Do you want composition to be composition/impurities or just leave it as composition?

DR. MARKS: I'll ask my colleagues. Ron? Obviously this was not a concern with them, I just -- that section was, to me, conspicuously missing and I wanted to be sure to bring it up that we didn't end up --
DR. SLAGA: There's not a section in here, Ron, for impurities.

DR. MARKS: Correct.

DR. SHANK: We most likely will be pesticides.

DR. MARKS: Yes, exactly. Do you think heavy metals?

DR. SHANK: No.

DR. MARKS: They don't use heavy metals as an insecticide? Well, that's an insecticide.

DR. SHANK: Not on grapes.

DR. MARKS: Yeah, okay. And it wouldn't be grown in land that had heavy metals, say, that could be absorbed in, whatever?

DR. SLAGA: Not in California..

DR. MARKS: Okay. So what did we decide about impurities? Have a section in that just include the pesticide boilerplate? Okay.

MS. FIUME: And actually on Panel Book page 9 for grape seed oligomeric proanthocyanidins it does state what the USP --

DR. MARKS: Oh, yes.

MS. FIUME: What their needs are. No more than 10 parts per million heavy metals, what their restrictions are.

DR. SHANK: Are we going to use NMT as an acronym now throughout?

MS. FIUME: I'm sorry.

DR. SHANK: No more than? NMT?

MS. FIUME: Do you want me to write it out?

DR. SHANK: No, just asking. Because now all of a sudden it's in several reports.

MS. FIUME: The problem --

DR. SHANK: NMT?

MS. FIUME: I didn't define it.

DR. SHANK: Yeah, it's yours..

MS. FIUME: I did define it before I used it, but I can put no more than if --

DR. SHANK: No, I was just asking if this is what we're going to be using. It's fine with me..

MS. FIUME: Either way.

DR. MARKS: Do you want any separate impurities and just put the pesticides boilerplate in that?

DR. SHANK: That would be fine.

DR. MARKS: Or do you just want to put it in the discussion and not even have an impurities section?

DR. SHANK: I'd put it in the discussion.

DR. MARKS: In the discussion, okay.
DR. SHANK: With grape viniculture there have been occupational reproductive toxicity problems applying pesticides.

DR. MARKS: Yes.

DR. SHANK: With grape plants, so we should just say the discussion of pesticide should not be in cosmetic ingredients.

DR. MARKS: Right. Now there was -- any other -- so far it only sounds like a potential data need is for genotox data, or at least a clarification of what's going on there.

DR. SHANK: Probably more of clarification --

DR. MARKS: Page 14.

DR. SHANK: Because common sense would come in pretty clear and say --

DR. MARKS: You weren't listening this morning. That was bullet 3, common sense is supposed to be applied to botanicals.

DR. SHANK: It was pointed out to us.

DR. MARKS: The other question I had is there was a previous report last year on -- well, that was the hydrogenated grape-seed oil so we should keep that separate. There's no reason to combine these. Okay, I just wanted to be sure that we addressed that while we had the opportunity.

Okay, so I get the sense we're leaning towards safe, but, Tom, you're going to give me the go-ahead tomorrow morning whether we go safe or not and then handle the genotox issue on page 14 in the discussion. And we'll handle the pesticides in the discussion also as an impurity. Okay, anything else? Ron, you're okay now with the irritation?

DR. SHANK: Yes, yes.

DR. MARKS: Okay. Ron Hill? Okay. So we all can be reassured tonight as we have our glass of wine that it's safe.

DR. HILL: I would like to know what's in root extract since again, everything listed is not significant.

DR. SLAGA: We could -- actually we could throw that out.

DR. MARKS: Remember we have that in present use and concentration, so one wouldn't think root extract is going to be that much different.

DR. HILL: I wouldn't expect a whole lot showing up in root that didn't show up in seed.

DR. MARKS: Right. Is that enough --

DR. HILL: But I'm not a botanist either.

DR. MARKS: Most of the plants I've studied and only relevant to allergens, they're distributed throughout the plant. So if you're okay with the leaf or the stem, you're going to be okay with the root, too, meaning -- or if you're not, you're going to get an allergy from that, too. So that wasn't a -- particularly since it's not used -- and we always cover it within the present use and concentration. And now we have the caveat that it would have the same similar array of chemicals. Does that sound okay to you, Ron and Tom?

DR. HILL: Well, as I was saying, the trouble is that there are no chemicals listed in roots. That's all I'm saying. There's a list of chemicals, but they're not --

DR. MARKS: Does that sound okay?

DR. SLAGA: Yes.

DR. MARKS: Okay, good. So I'm going to circle safe, and since I'm making the motion, Ron, when the genotox comes up, I'm going to defer to you. But at any rate, we'll move forward. Tentatively at this point, unless it changes tomorrow morning with more data that you have, Monice, we'll go with safe.
Safety Assessment of
Vitis Vinifera (Grape)-Derived Ingredients
as Used in Cosmetics

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The 2012 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel Liebler, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Monice M. Fiume, Senior Scientific Analyst/Writer.
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ABSTRACT

The Expert Panel assessed the safety of 24 *Vitis vinifera* (grape)-derived ingredients and found them safe for use in the present practices of use and concentration in cosmetics. These ingredients are most frequently reported to function in cosmetics as skin conditioning agents. Some of these ingredients are reported to function as antioxidants, flavoring agents, and/or colorants. The Panel reviewed the available animal and clinical data to determine the safety of these ingredients. Some constituents of grapes have been assessed previously for safety as cosmetic ingredients; others are compounds that have been discussed in previous CIR safety assessments.

INTRODUCTION

This report is a safety assessment of the following 24 *Vitis vinifera* (grape)-derived ingredients for use in cosmetic formulations:

- Vitis Vinifera (Grape)
- Vitis Vinifera (Grape) Bud Extract
- Vitis Vinifera (Grape) Flower Extract
- Vitis Vinifera (Grape) Fruit Extract
- Vitis Vinifera (Grape) Fruit Powder
- Vitis Vinifera (Grape) Fruit Water
- Vitis Vinifera (Grape) Juice
- Vitis Vinifera (Grape) Juice Extract
- Vitis Vinifera (Grape) Leaf Extract
- Vitis Vinifera (Grape) Leaf Oil
- Vitis Vinifera (Grape) Leaf/Seed/Skin Extract
- Vitis Vinifera (Grape) Leaf Water
- Vitis Vinifera (Grape) Leaf Wax
- Vitis Vinifera (Grape) Root Extract
- Vitis Vinifera (Grape) Seed Extract
- Vitis Vinifera (Grape) Seed Powder
- Vitis Vinifera (Grape) Shoot Extract
- Vitis Vinifera (Grape) Skin Extract
- Vitis Vinifera (Grape) Skin Powder
- Vitis Vinifera (Grape) Vine Extract
- Vitis Vinifera (Grape) Vine Sap
- Hydrolyzed Grape Fruit
- Hydrolyzed Grape Skin

These ingredients are reported to have many functions in cosmetics; they are reported most frequently to function as skin conditioning agents. Some of these ingredients are reported to function as antioxidants, flavoring agents, and/or colorants. In the Food and Drug Administration (FDA) Food Labeling regulations (21CFR101) subpart C, which addresses Specific Nutrition Labeling Requirements and Guidelines, grapes are listed as one of the 20 most frequently consumed raw fruits.

The safety of *Vitis Vinifera* (Grape) Seed Oil and Hydrogenated Grapeseed Oil was reviewed previously in 2011 by the Cosmetic Ingredient Review (CIR) Expert Panel in the Safety Assessment of Plant-Derived Fatty Acid Oils as Used in Cosmetics, at which time the Panel concluded that these ingredients are safe as used in cosmetics. Consequently, these two ingredients are not included in this safety assessment.

The detailed chemical composition of *Vitis vinifera* is given later in this assessment. Some of the constituents of grape, such as ascorbic acid, biotin, malic acid, etc., are cosmetic ingredients for which a CIR safety assessment is available; others are compounds that have been discussed in previous CIR safety assessments.

Many studies have been conducted with *Vitis vinifera* (grape)-derived ingredients with regard to health claims, anti-oxidant activity, and so forth. This safety assessment only includes studies and study-types that relate directly to the safety of the cosmetic use of these ingredients.

Note: In many of the published studies, it is not known how the substance being tested compares to the cosmetic-grade ingredient. Therefore, if it is not known whether the ingredient being discussed is a cosmetic ingredient, the test substance will be identified as “grape…” (e.g. grape seed extract); if it is known that the substance is a cosmetic ingredient, the terminology “Vitis Vinifera (Grape)…” (e.g. Vitis Vinifera (Grape) Seed Extract) will be used.

CHEMISTRY

**Definition**

The definitions of the *Vitis vinifera* (grape)-derived ingredients are provided in Table 1. *Vitis vinifera* is also known as wine grape, European grape, and grapevine.

**Chemical and Physical Properties**

Chemical and physical property data are provided in Table 2.
Composition

A detailed list of chemical constituents by plant part is presented in Table 3, and a more focused listing of constituents of *Vitis vinifera* is provided in Table 4. Table 5 provides the conclusions from CIR safety assessments that exist for some of the constituents of grape. Table 6 includes information on the toxicity of some constituents.

Grapes contain fruit acids, and the unripe fruit contains 34 ppm oxalic acid. Grape seeds contain 6-20% oil. Phenols are the third most-abundant constituent in grapes; carbohydrates and fruit acids are the most- and second most-abundant, respectively. The total extractable phenolics in grapes are present at ≤10% in the pulp, 60-70% in the seeds, and 28-35% in the skin.

The amount of a constituent present in the plant varies with the region in which it is grown. For example, fruit of grapes from Africa and Asia contained 50.0 μg β-carotene equivalents per 100 g of fruit while elsewhere trace β-carotene equivalent were present in the fruit. The cultivar, climate condition, and degree of maturation also affect the composition, as does whether the grapes are red or white.

It has also been shown that the amount of a constituent present in an extract is dependent on the medium used during extraction and the variety of *Vitis vinifera* used. For example, a red grape methanolic extract, red grape water extract, white grape methanolic extract, and white grape water extract each contained 0.22, 0.04, 0.01, and 0.02 mg/g trans-resveratrol, respectively; 0.9, 0.35, 2.25, and 4.09 mg/g (+)-catechin, respectively; 1.1, 0.32, 1.08, and 2.10 mg/g (-)-epicatechin, respectively; and 0, 0.13, 0.04, and 0.03 mg/g quercetin, respectively.

Melatonin (N-acetyl-5-methoxytryptamine) is present in grapes. Depending on variety and location, levels of melatonin in grape skin have ranged from 0.005-1.2 ng/g. The stage of growth also affects the amount present. Studies have indicated that melatonin may also be present in the flesh and seeds of grapes.

**Vitis Vinifera (Grape) Fruit Extract**

Fruit acids, sugars, minerals, pectin, tannins, proteins, anthocyanins, waxes, flavonoids, xanthophylls, carotene, vitamins, polysaccharides, aromatic substances, and procyanidins are part of the composition of Vitis Vinifera (Grape) Fruit Extract.

**Vitis Vinifera (Grape) Juice**

A commercial brand grape juice contained 4.4 mg/L quercetin and 6.2 mg/L myricetin.

**Vitis Vinifera (Grape) Leaf Extract**

Potassium and calcium bitartrate, calcium malate, fruit acids, sugar, flavonoids, and tannins are part of the composition of Vitis Vinifera (Grape) Leaf Extract.

**Vitis Vinifera (Grape) Seed Extract**

The main constituents of grape seeds are reported to be phenolic compounds. Those phenolic compounds from standardized grape seed extracts are reported to be 92-95% oligomeric proanthocyanidins. Proanthocyanidin structures vary depending upon the source of the flavanol(s) building blocks (monomer units), the degree of oligomerization (how many flavanol repeat units), and the presence of modifications (such as esterification) of the 3-hydroxyl group. The most prominent grape seed extract proanthocyanidin is depicted in Figure 1. Catechin, epicatechin, and taxifolin are the primary flavanols present in grape seeds, and comprise the majority of the remaining phenols in grape seed extracts. Heating of oligomeric proanthocyanidins, under acidic conditions, leads to the release of anthocyanins, and in turn, flavanols. Accordingly, the length of oligomeric proanthocyanidins and the concentration of flavanols in grape seed extracts are highly dependent on the extraction techniques used.

![Figure 1. Grape seed proanthocyanidin](image-url)
Grape seed oligomeric proanthocyanidins (United States Pharmacopeia [USP]-grade for dietary supplements) contain no more than 10 ppm heavy metals, no more than 19.0% catechin and epicatechin on the anhydrous basis, no more than 8.0% water, and no more than 2% water-insoluble fraction.12

Vitis Vinifera (Grape) Seed Extract, as the trade name ActiVin, contains 54% dimeric, 13% trimeric, and 7% tetrameric oligomeric proanthocyanidins and a small amount of catechin derivatives, flavonoids, and other oligomeric proanthocyanidins.13

Vitis Vinifera (Grape) Skin Extract
Grape skin extract (enocianina) is an approved food color additive exempt from batch certification. The FDA describes the color additive as containing the common components of grape juice: anthocyanins, tartaric acid, tannins, sugars, and minerals (21CFR73.170). A small amount of residual sulfur dioxide may be present following aqueous (aq.) extraction in the presence of sulfur dioxide. The grape anthocyanins are usually either monoglycerides or diglycosides.14 The Food Chemicals Codes states the primary color components of grape skin extract are anthocyanins, such as the glucosides of malvidin, peonidin, petunidin, delphinidin, or cyanidin. Food-grade grape skin extract is to contain no more than 1 mg/kg arsenic and no more than 5 mg/kg lead.

Preparation/Extraction

Vitis Vinifera (Grape) Fruit Extract
A product information sheet on a mixture that contains Vitis Vinifera (Grape) Fruit Extract states that the solvent of extraction is glycerin.7 The resulting composition of the mixture is 75-100% glycerin, 50-75% Vitis Vinifera (Grape) Fruit Extract, and 10-25% water, and the ratio of extract to botanical is 2:1. Potassium sorbate and sodium benzoate, 0.3% each, are used as preservatives. The extract is filtered clear after preparation.

Vitis Vinifera (Grape) Leaf Extract
A product information sheet on a mixture that contains Vitis Vinifera (Grape) Leaf Extract states that the solvent of extraction for this product is also glycerin.9 The resulting composition of the mixture is 75-100% glycerin, 10-25% water, and 5-10% Vitis Vinifera (Grape) Leaf Extract. As above, potassium sorbate and sodium benzoate, 0.3% each, are used as preservatives and the extract is filtered clear after preparation.

Another source reported the extraction of grape leaves with a propylene glycol solution.15 The composition of this extract was not provided.

Vitis Vinifera (Grape) Seed Extract
One manufacturer reported that Vitis Vinifera (Grape) Seed Extract is prepared as a concentrated extract by separating the seeds from the fruit, cleaning and comminuting the seeds, extracting with alcohol, and then filtering the extract.16 The filtrate is concentrated by distillation, and then spray-dried. The ratio of fresh plant material to extract is 133:1.

USP-grade grape seed oligomeric proanthocyanidins (dietary supplement) is a fraction of an extract of ripe Vitis vinifera seeds.12 The extract is prepared using alcohol, methanol, acetone, ethyl acetate, water or mixtures of these solvents. The extract is then further enriched in oligomeric proanthocyanidins by fractionation with ethyl acetate or by other means.

Vitis Vinifera (Grape) Skin Extract
Grape skin extract (enocianina), the FDA-approved color additive, is prepared by the aq. extraction (steeping) of the fresh deseeded marc remaining after grapes have been pressed to produce grape juice or wine (21CFR73.170). During the steeping process, sulfur dioxide is added and most of the extracted sugars are fermented to alcohol. The extract is concentrated by vacuum evaporation, during which practically all of the alcohol is removed.
**USE**

**Cosmetic**

The *Vitis vinifera* (grape)-derived ingredients included in this safety assessment are reported to have many possible functions in cosmetic formulations. *Vitis Vinifera* (Grape) Seed Extract is reported to function as an anti-caries agent, anti-dandruff agent, anti-fungal agent, anti-microbial agent, antioxidant, flavoring agent, light stabilizer, oral care agent, oral health care drug, and sunscreen agent. Many of the other *Vitis vinifera* (grape) ingredients are reported to function as skin conditioning agents, and a few are reported to function as antioxidants. Five of the ingredients - the seed extract, the fruit powder, the juice, the juice extract, and the skin extract – are reported to function as flavoring agents and four of those five (all except the seed extract), as well as the skin powder, are reported to function as colorants. The *International Cosmetic Ingredient Dictionary and Handbook* does not list the functions for *Vitis Vinifera* (Grape) and *Vitis Vinifera* (Grape) Leaf Wax. A listing of all the reported functions for each ingredient is provided in Table 1.

The FDA collects information from manufacturers on the use of individual ingredients in cosmetics as a function of cosmetic product category in its Voluntary Cosmetic Registration Program (VCRP). VCRP data obtained from the FDA in 2012 indicate that *Vitis Vinifera* (Grape) Seed Extract is used in 495 cosmetic formulations, *Vitis Vinifera* (Grape) Fruit Extract is used in 238 cosmetic formulations, and *Vitis Vinifera* (Grape) Leaf Extract is used in 80 cosmetic formulations. The other in-use *Vitis vinifera* (grape)-derived ingredients are used in less than 15 formulations, and 11 *Vitis vinifera* (grape)-derived ingredients are not reported to be used.

The *Vitis vinifera* (grape)-derived ingredients are used at low concentrations in cosmetic formulations. *Vitis Vinifera* (Grape) Leaf Extract is included at up to 3% in leave-on formulations (perfumes); *Vitis Vinifera* (Grape) Fruit Extract and *Vitis Vinifera* (Grape) Juice are included at up to 2% in rinse-off skin cleansing products and paste masks and mud packs, respectively. All others are used at <1% in formulation. no reported uses were received in the VCRP for *Vitis Vinifera* (Grape) Shoot Extract, but use concentration data were provided in the industry survey. It should be presumed that *Vitis Vinifera* (Grape) Shoot Extract is used in at least two cosmetic formulations.

Frequency and concentration of use data categorized by exposure and duration of use are provided in Table 7, and the ingredients for which no uses are reported are listed in Table 8.

Products containing *Vitis vinifera* (grape)-derived ingredients may be applied to the eye area or mucus membranes or could be incidentally ingested. Additionally, *Vitis Vinifera* (Grape) Fruit Extract, *Vitis Vinifera* (Grape) Fruit Water, *Vitis Vinifera* (Grape) Juice, *Vitis Vinifera* (Grape) Leaf Extract, and *Vitis Vinifera* (Grape) Seed Extract are used in cosmetic products that could possibly be inhaled; concentrations of use for ingredients used in products that could be inhaled range from 0.00002% *Vitis Vinifera* (Grape) Seed Extract in pump hairsprays to 3% *Vitis Vinifera* (Grape) Leaf Extract in perfumes. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm. 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) in any appreciable amount.

All of the *Vitis vinifera* (grape)-derived ingredients named in this safety assessment, with the exception of hydrolyzed grape skin, are listed in the European Union inventory of cosmetic ingredients.

**Non-Cosmetic**

*Vitis Vinifera* (Grape) Seed Extract

Grape seed extracts are used as nutritional supplements.

*Vitis Vinifera* (Grape) Skin Extract

Grape skin extract (enocianina) is a food color additive exempt from batch certification that can be used for coloring only still and carbonated drinks and ades, beverage bases, and with restrictions, alcoholic bases (21CFR73.170). According to the evaluation of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the acceptable daily intake (ADI) of grape skin extract is 0-2.5 mg/kg bw.

**TOXICOKINETICS**

It has been reported that most phenolic compounds in grapes are readily metabolized by the gut flora, producing metabolites that potentially can be absorbed into the bloodstream by passive diffusion or active transport systems. A number of factors may play a role in the bioavailability of polyphenols, but maximum plasma values are generally reached between 5 min and 2 h after administration. Oligomeric procyanidins and other higher molecular weight phenols are not appreciably absorbed, but they can release monomer and dimer units and epicatechin that can be absorbed.
TOXICOLOGICAL STUDIES

Dermal

Vitis Vinifera (Grape) Seed Extract

The acute dermal toxicity of Vitis Vinifera (Grape) Seed Extract (trade name ActiVin; a water-ethanol extract) was evaluated in five male and five female albino rats. A single dose of 2 g/kg moistened with 0.3 ml deionized water was applied to the clipped intact dorsal skin of each animal for 24 h, and the dose covered approximately 5-6% of the total body surface. The test site was covered with a gauze bandage that was secured with tape, and collars were placed on the animals to avoid ingestion. The animals were observed for 14 days. None of the animals died during the study, and there were no test material-related clinical findings, body weight changes, or findings at necropsy. Very slight to slight erythema and desquamation was observed in all animals; these dermal responses subsided in all but three animals by day 12. One male rat had edema from day 6 to day 9. The dermal LD50 of Vitis Vinifera (Grape) Seed Extract in albino rats was >2 g/kg; this dose was also the no-observed effect level (NOEL) for systemic toxicity in this dermal study.

Oral

Vitis Vinifera (Grape) Seed Extract

Five male and five female albino rats were given a single dose of 5 g/kg Vitis Vinifera (Grape) Seed Extract (trade name ActiVin) by gavage. The animals were observed for 14 days. One female died on day 1 of the study. Matting and test material around the mouth, hypoactivity, and ocular discharge were noted for some animals; all animals appeared normal by day 3. The oral LD50 of Vitis Vinifera (Grape) Seed Extract in albino rats was >5 g/kg.

The acute oral toxicity of a grape seed extract (extracted in water and ethanol) containing 89.3% proanthocyanidins was determined using groups of 5 male and 5 female F344/DuCrj rats. The extract was dissolved in purified water, and the animals were dosed by gavage with 0, 2, or 4 g/kg of the extract at a rate of 10 ml/kg bw. None of the animals died, and the LD50 of the grape seed extract was >4 g/kg.

Vitis Vinifera (Grape) Seed/(Grape) Skin Extract

The acute oral toxicity of a mixed grape seed and grape skin extract (extracted in ethanol) containing 76% total polyphenols was determined in a litmus test using female Wistar rats. Three rats were given a single oral dose by gavage of 5 g/kg in saline at a rate of 10 ml/kg. Three negative control rats were dosed with saline only. There were no signs of toxicity for up to 14 days after dosing, and no gross lesions were observed at necropsy. The LD50 of the mixed grape seed/skin extract was >5 g/kg.

Repeated Dose Toxicity

Dietary repeated dose toxicity studies are presented in Table 9.

In a 3-wk study in which female SKH-1 hairless mice were fed a diet containing 0, 0.2, or 0.5% grape seed extract containing 89.3% proanthocyanidins for 3 wks, no signs of toxicity were reported. In 90-day dietary repeated dose studies in rats, the NOAELs of grape seed extract and grape skin extract were approximately 2150 and 1780 mg/kg bw/day for male and female rats, respectively. No toxic effects were observed in female B6C3F1 mice after 6 mos of dietary administration of up to 500 mg/kg bw/day Vitis Vinifera (Grape) Seed Extract or in male rats fed 100 mg/kg bw/day Vitis Vinifera (Grape) Seed Extract for 12 mos. Dietary administration of 7.5 or 15% of a grape colour extract to Beagle dogs for 90 days resulted in a statistically significant decrease in body weight gains in the high dose group; no other significant changes were observed.

Skin Lightening Effect

Vitis Vinifera (Grape) Seed Extract

The lightening effect of the oral administration of a grape seed extract (extracted in water and ethanol) containing 89.3% proanthocyanidins on UV-induced pigmentation of guinea pig skin was examined. The extract did not contain resveratrol or other phenolic compounds, such as anthocyanidins and flavonols. Using a PEN-RAY lamp (UV containing UVA and UVB, peak at 366 nm), two areas on the backs of male and female brownish guinea pigs were irradiated 2x/wk for 3 wks with 0.9 J/cm² UV. One wk after the final UV exposure, groups of 5 irradiated animals were fed a diet containing 1% of the grape seed extract or a standard diet for 8 wks. The lightening effect was determined every 2 wks by measuring the L*-value (lightness) and the melanin index at the two irradiated sites and an unexposed site. The L*-value was measured with a reflectance spectrophotometer, and the melanin index was calculated using these data. After 8 wks of dosing, blood samples were taken from each animal and the animals were then killed. Skin samples were taken from UV-irradiated and a non-treated sites and evaluated for 3,4-dihydroxyphenylalanine (DOPA)-positive melanocytes and markers of oxidative DNA damage.

There were no differences in body wts between the groups. The UV-induced skin pigmentation was reduced in the group fed grape seed extract, as indicated by the increase in L*-value and the decrease in melanin index in UV-induced pigmented skin throughout the study as compared to control values; these differences were not statistically significant. These parameters were similar for both groups in un-irradiated skin. The number of DOPA-positive melanocytes in the grape seed extract group was decreased compared to the control group. The number of melanin 8-hydroxy-2'-deoxyguanosine (8-OHdG)-positive cells, melanin-Ki-67-positive cells, and melanin proliferating cell nuclear antigen (PCNA)-positive cells in irradiated skin also decreased in
the grape skin extract group compared to controls; the decrease observed with melanin-Ki-67-positive cells was statistically significant.

**REPRODUCTIVE AND DEVELOPMENTAL TOXICITY**

Published reproductive and developmental toxicity data were not found for *Vitis Vinifera* (Grape)-derived ingredients. A reproduction study on grape color extract is described below. Information on estrogenic activity of some of the constituents of *Vitis vinifera* is provided in Table 6.

**Grape Color Extract**

A two-generation reproductive study on grape color extract was performed using Sprague-Dawley rats.29 (The Code of Federal Regulations (21CFR73.169) states that the color additive grape color extract is an aq. solution of anthocyanin grape pigments made from Concord grapes (*Vitis labruscra*) or a dehydrated water soluble powder prepared from the aq. solution. The aq. solution is prepared by extracting the pigments from precipitated lees produced during the storage of Concord grape juice. It contains the common components of grape juice, namely anthocyanins, tartrates, malates, sugars, and minerals, etc., but not in the same proportion as found in grape juice. The dehydrated water soluble powder is prepared by spray drying the aq. solution containing added malto-dextrin). Groups of 25 male and 25 female rats (F₀ generation) were fed diets containing 0, 7.5, or 15% (w/w) grape color powder or a diet containing 9% by wt malto-dextrin for 3 wks; after 3 wks, the rats were mated within their respective groups. Female F₀ rats, which were allowed to deliver, were fed the test diets throughout mating, gestation, and lactation. Each litter (the F₁ generation) was culled to 10 pups (5 males and 5 females if possible) on day 4. On day 21 of lactation, two F₁ males and two F₁ females were selected for a subsequent 13-wk study followed by a reproduction study. The F₀ parents and the remaining offspring were killed.

The selected F₁ animals were fed the same dietary levels of grape color extract as their parents. After 13 wks of dosing, the rats were mated within their respective groups. The F₁ rats were also allowed to deliver and were fed the test diets throughout mating, gestation, and lactation. The F₂ generation litters were culled as described above. On day 21 of lactation, all F₁ parents and F₂ pups were killed.

All animals, except one F₁ male of the malto-dextrin group, survived until scheduled termination. Dietary administration of up to 15% grape color powder had no effect on reproductive parameters or fertility. Body weights of the F₁ and F₂ pups of both test groups were statistically significantly decreased compared to controls at day 21 of lactation. Also, compared to controls, the body weights of F₀ pups of the high-dose group were statistically significantly decreased on day 4, while the body weights of F₁ pups of both test groups were statistically significantly decreased at birth. No microscopic lesions were reported in any of the groups.

In the F₁ animals fed the test diets for 13 wks prior to dosing, the group mean body weight gain was statistically significantly decreased in the high dose females. Statistically significant differences in several clinical chemistry parameters were observed between groups after 6 wks of dosing; the values were comparable at the end of 13 wks of dosing. The following statistically significant differences were recorded at necropsy regarding body and organ weights of the F₁ animals: body weights of the high dose animals were decreased; absolute and relative liver weights were decreased in males and females of both test groups; absolute adrenal gland weights were decreased in males of both test groups and high-dose females; and relative thyroid gland weights were decreased in males of both test groups.

**GENOTOXICITY**

Genotoxicity testing on grape-derived extracts is summarized in Table 10. (Table 6 includes information on the genotoxic potential of some of the constituents of *Vitis vinifera*).

*In vitro*, mixed results were reported in the genotoxicity of *Vitis vinifera* (grape)-derived ingredients but *in vivo*, mostly negative results were obtained. Fractions of raw grapes demonstrated potent mutagenic activity in an Ames test,40 and water and ethanol extracts of red and white grapes enhanced mitomycin-C (MMC)-induced sister chromatid exchanges (SCEs) in a SCE assay in human lymphocytes, but there was no effect on SCEs without MMC.6 Grape juice was also mutagenic *in vitro*, as demonstrated in the Ames test.31,32 However, grape seed extract was not mutagenic *in vitro* in an Ames test or chromosomal aberration assay,27 nor *in vivo* in the mouse micronucleus test.27,33 A mouse micronucleus test with grape skin extract was negative.33 In vitro, grape seed/grape skin extract was weakly mutagenic in an Ames test but not genotoxic in a chromosomal aberration assay, and the mixed extract demonstrated a statistically significant increase in micronuclei after 48 h, but not after 72 h.26

**CARCINOGENICITY**

**Oral**

*Vitis Vinifera* (Grape) Seed Extract

In a photocarcinogenicity study (described later in this report in Table 10), a group of 20 SKH-1 hairless mice were fed a diet containing 1% grape seed extract that contained 89.3% proanthocyanidins for 30 wks to determine whether dietary grape seed extract alone had any effect on skin tumor formation.34 No skin tumors formed.
Inhibition of Tumor Promotion

The inhibition of tumor promotion by *Vitis vinifera* has been assessed in many studies; some of these studies are summarized in Table 11.

Seed polyphenols and extracts in particular were shown to inhibit 7,12-dimethylbenz[a]anthracene (DMBA)-initiated and 12-O-tetradecanoylphorbol-13-acetate (TPA)-promoted tumors in mouse skin; dermal application and dietary administration both had significant inhibitory activity.35-39 Dietary grape seed extract also inhibited UV-initiated, UV-promoted, or UV-initiated and promoted skin tumors in hairless mice,34 and it inhibited the formation of azoxymethane (AOM)-induced aberrant crypt foci (ACF) in the intestines of rats.40 Some of the studies summarized in Table 11 examined the effect of applying DMBA to mice and then later either treating the animals topically or in the diet with grape seed extract without TPA.35,36,61 Mice did not develop tumors when dosed dermally or orally with grape seed extract after initiation with DMBA.

IRRITATION AND SENSITIZATION

Skin Irritation/Sensitization

Dermal irritation and sensitization data are presented in Table 12.

In *in vitro* testing, a product containing 3% *Vitis Vinifera* (Grape) Fruit Extract was predicted to be a non-irritant in a dermal irritancy test in human skin, a product containing 10% *Vitis Vinifera* (Grape) Fruit Extract was predicted to be non-irritating/ minimal in an Epiderm MTT viability assay, and hydrolyzed grape skin was predicted to be non-irritating in an MTT assay. In a single-dose study in NZW rabbits, *Vitis Vinifera* (Grape) Seed Extract applied neat was classified as moderately irritating; in a human 2-wk use study, a formulation containing 0.15% *Vitis Vinifera* (Grape) Seed Extract was not an irritant. In an in *in vitro* assay of pro-sensitizing potential, hydrolyzed grape skin did not increase the expression of the investigated markers and did not show any stimulating potential of the immune cellular response mediated by monocytes/macrophages. In clinical testing, products containing up to 10% *Vitis Vinifera* (Grape) Fruit Extract, a formulation containing 0.1% *Vitis Vinifera* (Grape) Juice, cosmetic formulations containing 0.5% *Vitis Vinifera* (Grape) Juice Extract, and *Vitis Vinifera* (Grape) Seed Extract tested at a maximum concentration of 1% in raw material were not irritant or sensitizers in human repeated insult patch testing (HRPIPTs).

Occupational Exposure

A skin prick-to-prick test was performed on vineyard workers to assess the prevalence of sensitization to grapes with occupational exposure.42 Three groups of vineyard workers, 120/group, were tested: harvesters (Group A), workers in grape selection (Group B), and workers operating de-stemming/crushing/pressing machines (Group C); a group of 120 office employees (Group D) was used as a negative control group. The test, the needle was inserted into a cleaned grape and then inserted into the skin. Normal saline was used as a negative control. Eight harvesters in Group A (6.7%) and five grape selection workers in Group B (4.2%) had positive prick-to-prick tests to grapes; an additional 15 workers in Group A and 9 workers in Group B had weak positive reactions that were considered negative in this study. None of the workers in the other two groups had positive reactions. (Workers in Groups A and B had greater exposure to grapes than did workers in Groups C or D.) The reported sensitization to grapes was asymptomatic; none of the employees tested had any reported history or symptoms upon exposure.

Case Report

A female grape farmer presented with an eczematous dermatitis of the hand.43 The genus and species of grape were not stated. Patch testing with a crushed bud that had not been exposed to gibberellin (a vegetable hormone she applied to the grapes), an ethanol extract of a bud, a crushed leaf, an ethanol extract of a leaf, and with gibberellin was performed using Finn chambers, as was patch testing with standard allergens and several photoallergens. The only positive reactions were to the crushed and ethanol-extracted bud preparations. Irradiation with 0.7 J/cm² ultraviolet A (UVA) and 15 mJ/cm² UVB light increased the erythema and edema. The minimal response dose of UVA was >1.4 J/cm² and the minimal erythema dose of UVB was 45 mJ/cm². In similar testing of 22 farmers, a weak positive reaction to the bud and/or leaf was observed in 6 subjects. The reactions did not increase with UV irradiation and subsided within 96 h.

Ocular Irritation

*Vitis Vinifera* (Grape) Fruit Extract

A product containing 3% *Vitis Vinifera* (Grape) Fruit Extract was predicted to be a minimal ocular irritant.44 The ocular irritation potential of a single sample of a blend containing 3% *Vitis Vinifera* (Grape) Fruit Extract, extracted in water, was evaluated in a standard volume-dependent dose-response study using the ocular irritation test method. The irritation Draize equivalent scores ranged from 4.5 to 6.4 for neat samples of the product tested at volumes ranging from 25 -125 μl.

The irritancy classification for a product containing 10% *Vitis Vinifera* (Grape) Fruit Extract was non-irritating/minimal.45 An EpiOcular MTT viability assay was performed to determine the ocular irritation potential of a product containing 10% *Vitis Vinifera* (Grape) Fruit Extract that was extracted with water. The tissue samples were treated with neat test article for 16, 64, and 256 min. The ET₅₀ was >256 min.
**Vitis Vinifera (Grape) Seed Extract**

A product containing 0.15% *Vitis Vinifera* (Grape) Seed Extract was classified as a mild ocular irritant during in vitro testing. A bovine corneal opacity and permeability assay (BCOP) was performed with undiluted samples of an after shave lotion containing 0.15% *Vitis Vinifera* (Grape) Seed Extract; the extract was prepared with the extraction solvents butylene glycol and water. Sterile deionized water served as the negative control and ethanol as the positive control. The in vitro score for the test article was 1.0. (Test materials with in vitro scores of 0-25 are classified as mild irritants). The positive control had an in vitro score of 43.2; test materials with in vitro scores of 25.1-55 are classified as moderate irritants.

**Hydrolyzed Grape Skin**

Hydrolyzed grape skin was predicted to be non-irritating to eyes in a cytotoxicity assay evaluating ocular irritation potential. A neutral red uptake (NRU) assay using fibroblast cultures was performed with 0.15 – 5 mg/ml hydrolyzed grape skin. Sodium lauryl sulfate (SLS) was used as a positive control. The IC₅₀ value (i.e., the concentration of test compound that induces a 50% decrease of cell growth/survival) for hydrolyzed grape skin was >5 mg/ml. The IC₅₀ value for the positive control was 0.063 mg/ml.

**Non-Human**

**Vitis Vinifera (Grape) Seed Extract**

The ocular irritation potential of *Vitis Vinifera* (Grape) Seed Extract (trade name ActiVin) was evaluated in six female NZW rabbits. The test article, 85 mg, was instilled into the conjunctival sac of the right eye, the eyelid was held closed for 1 sec, and the eye was not rinsed. The contralateral eye served as an untreated control. The eyes were scored for irritation using the Draize method at 1, 24, 48, and 72 h and 4, 7, and 14 days after instillation of the test article. Conjunctival irritation was observed in all animals, four animals had iridal reactions, and three had corneal reactions. The irritation was reversible and completely subsided by day 14. The Maximum Average Score (MAS) at 24 h for *Vitis Vinifera* (Grape) Seed Extract was 16.7/110.

**Summary**

This report addresses the safety of 24 *Vitis Vinifera* (Grape)-derived ingredients as used in cosmetics. These ingredients are reported to have many functions in cosmetics, but the most frequently reported function of the *Vitis Vinifera* (Grape) ingredients is as a skin conditioning agent. According to VCRP data obtained from the FDA, *Vitis Vinifera* (Grape) Seed Extract is used in 495 cosmetic formulations, *Vitis Vinifera* (Grape) Fruit Extract is used in 238 cosmetic formulations, and *Vitis Vinifera* (Grape) Leaf Extract is reported to be used in 80 cosmetic formulations; nine other *Vitis vinifera*-derived ingredients are reported to be in use, and they are used in less than 15 formulations.

Fruit acids and trans-resveratrol are constituents of *Vitis vinifera*, and polyphenols are found in all parts of the plant. The main constituents of grape seeds are reported to be phenolic compounds, and standardized grape seed extracts are reported to contain 92-95% oligomeric proanthocyanidins. Grape skin extract contains anthocyanins, tartaric acid, tannins, sugars, and minerals.

Grapes are one of the 20 most frequently consumed raw fruits. The oral LD₅₀ values of grape seed extract and grape skin extract in rats were > 4-5 and >5 g/kg, respectively, and the dermal LD₅₀ (and NOEL for systemic toxicity) in albino rats was >2 g/kg.

In a 3-wk dietary study in which female SKH-1 hairless mice were fed a diet containing 0, 0.2, or 0.5% grape seed extract containing 89.3% proanthocyanidins for 3 wks, no signs of toxicity were reported. In 90-day dietary repeated dose studies in rats, the NOAELs of grape seed extract and grape skin extract were approximately 2150 and 1780 mg/kg bw/day for male and female rats, respectively. No toxic effects were observed in female B6C3F1 mice after 6 mos of dietary administration of up to 500 mg/kg bw/day *Vitis Vinifera* (Grape) Seed Extract or in male rats fed 100 mg/kg bw/day *Vitis Vinifera* (Grape) Seed Extract for 12 mos. Dietary administration of 7.5 or 15% of a grape colour extract to Beagle dogs for 90 days resulted in a statistically significant decrease in body weight gains in the high dose group; no other significant changes were observed. Grape seed extract reduced UV-induced skin pigmentation in guinea pigs, but the difference was not statistically significant when compared to controls that did not receive grape skin extract.

A two-generation reproductive study in which 7.5 or 15% grape colour extract was fed in the diet was performed using Sprague-Dawley rats. The only statistically significant effects observed were decreases in the body weights of F₁ and F₂ pups of both test groups and in body weights and liver, adrenal gland, and thyroid gland weights in F₁ animals fed the test article for 30 days prior to mating.

In vitro, mixed results were reported in the genotoxicity of *Vitis vinifera* (grape)-derived ingredients but in vivo, mostly negative results were obtained. Fractions of raw grapes demonstrated potent mutagenic activity in an Ames test, and water and ethanol extracts of red and white grapes enhanced mitomycin-C (MMC)-induced sister chromatid exchanges (SCEs) in a SCE assay in human lymphocytes, but there was no effect on SCEs without MMC. Grape juice was also mutagenic in vitro, as demonstrated in the Ames test. However, grape seed extract was not mutagenic in vitro in an Ames test or chromosomal aberration assay, nor in vivo in the mouse micronucleus test. A mouse micronucleus test with grape skin extract was negative. In vitro, grape seed/grape skin extract was weakly mutagenic in an Ames test but not genotoxic in chromosomal aberration assay, and the mixed extract demonstrated a statistically significant increase in micronuclei after 48 h, but not after 72 h.
Vitis vinifera, the seed extract in particular, was shown to inhibit DMBA-initiated and TPA-promoted tumors in mouse skin; dermal application and dietary administration both had significant inhibitory activity. Dietary grape seed extract also inhibited UV-initiated, UV-promoted, or UV-initiated and promoted skin tumors in hairless mice. The formation of AOM-induced ACF in the intestines of rats was also inhibited by dietary grape seed extract. Dietary administration of 1% grape seed extract for 30 wks did not produce skin tumors in mice, and grape seed extract and grape seed powder were not tumor promoters when applied dermally to mice following initiation with DMBA.

In in vitro testing, a product containing 3% Vitis Vinifera (Grape) Fruit Extract was predicted to be a non-irritant in a dermal irritation test in human skin, a product containing 10% Vitis Vinifera (Grape) Fruit Extract was predicted to be non-irritating/minimal in an Epiderm MTT viability assay, and hydrolyzed grape skin was predicted to be non-irritating in an MTT assay. In a single-dose study in NZW rabbits, Vitis Vinifera (Grape) Seed Extract applied neat was classified as moderately irritating; in a human 2-wk use study, a formulation containing 0.15% Vitis Vinifera (Grape) Seed Extract was not an irritant. In an in vitro assay of pro-sensitizing potential, hydrolyzed grape skin did not increase the expression of the investigated markers and did not show any stimulating potential of the immune cellular response mediated by monocytes/macrophages. In clinical testing, products containing up to 10% Vitis Vinifera (Grape) Fruit Extract, a formulation containing 0.1% Vitis Vinifera (Grape) Juice, cosmetic formulations containing 0.5% Vitis Vinifera (Grape) Juice Extract, and Vitis Vinifera (Grape) Seed Extract tested at a maximum concentration of 1% in a raw material were not irritant or sensitizers in human repeated insult patch testing (HRRIPTs). Some asymptomatic sensitization reactions were seen in an occupational setting in vineyard workers who had substantial exposure to grapes. One case study was found that reported positive reactions to grape bud preparations.

Products containing 3 and 10% Vitis Vinifera (Grape) Fruit Extract were predicted to be minimal ocular irritants in in vitro testing. In a non-human study using rabbits, the MAS at 24 h for Vitis Vinifera (Grape) Seed Extract was 16.7/110. A product containing 0.15% Vitis Vinifera (Grape) Seed Extract was classified as a mild ocular irritant during a BCOP assay, and hydrolyzed grape skin was predicting to be non-irritating to eyes in a NRU study.

**DISCUSSION**

Most of the irritation and sensitization testing performed on the *Vitis vinifera*-derived ingredients included in this report demonstrated that these ingredients are not dermal irritants or sensitizers, with the exception of one 4-h semi-occlusive study of Vitis Vinifera (Grape) Seed Extract that reported moderate irritation using rabbits. Because all the other irritation and sensitization test results were negative, including a human study using up to 10% Vitis Vinifera (Grape) Fruit Extract in a product, the CIR Expert Panel was of the opinion that the one study was an outlier and that the weight of evidence supports the view that these ingredients are not irritants or sensitizers.

The Panel discussed the findings of mutagenic activity of grape and grape juice in some of the bacterial mutagenicity tests. The Panel is aware that there is a history of positive Ames tests with some foods, including grape. Although positive results for mutagenicity occur in bacterial assays, it is known that constituents of foods such as grapes, e.g., flavonoids, do not appear to be genotoxic to mammals in vivo. Additionally, *Vitis vinifera*-derived extracts have demonstrated an inhibition of tumor promotion. Therefore, the mutagenic effects in bacterial systems were not considered relevant to the safety of these cosmetic ingredients.

The *Vitis vinifera* plant parts contain a number of constituents and some of the constituents, such as ascorbic acid, biotin, and malic acid, are cosmetic ingredients for which a CIR safety assessment is available. Others are compounds that have been discussed in previous CIR assessments. For example, *Vitis vinifera*, and therefore derived extracts, contains a variety of phytochemicals, all present at relatively low concentrations. The Panel has discussed in previous CIR safety assessments that although some of these phytochemicals could exert significant biological effects (e.g., isoflavones), the low levels preclude significant effects. Also, although no dermal absorption data were available, in the Panel’s experience, phytosterols and phytosterol esters are not significantly absorbed and do not result in systemic exposure and extensive data are available showing that these phytosterol constituents are not estrogenic, are not reproductive toxicants, are not genotoxic, and are not carcinogenic.

Because some of the *Vitis vinifera* (grape)-derived ingredients are reported to be used in preparations which may be aerosolized, with concentrations ranging from 0.00002% Vitis Vinifera (Grape) Seed Extract in pump hairsprays to 3% Vitis Vinifera (Grape) Leaf Extract in perfumes, the Panel discussed the issue of incidental inhalation exposure. The Panel considered that the preponderance of the data indicate that incidental inhalation exposures to these ingredients in aerosolized cosmetic products would not cause adverse health effects, as follows. In the absence of inhalation data, the Panel noted that the *Vitis vinifera* (grape)-derived ingredients did not produce systemic toxicity in oral single-dose or long-term (up to 12 mos) repeated dose studies; grape color extract was not a reproductive or developmental toxicant; *Vitis vinifera* (the seed extract in particular) inhibits the promotion of tumors; and the *Vitis vinifera* (grape)-derived ingredients do not appear to be irritants or sensitizers. Further, these ingredients are reportedly used at low concentrations in cosmetic products that may be aerosolized. The Panel noted that 95% – 99% of droplets/particles produced in cosmetic aerosols would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concern based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel’s approach to...
evaluating incidental inhalation exposures to ingredients in cosmetic products that may be aerosolized is available at [http://www.cir-safety.org/cir-findings](http://www.cir-safety.org/cir-findings).

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

**CONCLUSION**

The CIR Expert Panel concluded the Vitis vinifera (grape)-derived ingredients listed below are safe for use in the present practices of use and concentration in cosmetics.

<table>
<thead>
<tr>
<th>Vitis Vinifera (Grape);</th>
<th>Vitis Vinifera (Grape) Leaf Wax;*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitis Vinifera (Grape) Bud Extract;</td>
<td>Vitis Vinifera (Grape) Root Extract;*</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Flower Extract;*</td>
<td>Vitis Vinifera (Grape) Seed;</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Fruit Extract;</td>
<td>Vitis Vinifera (Grape) Seed Extract;</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Fruit Powder;</td>
<td>Vitis Vinifera (Grape) Seed Powder;</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Fruit Water;</td>
<td>Vitis Vinifera (Grape) Shoot Extract;</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Juice;</td>
<td>Vitis Vinifera (Grape) Skin Extract;*</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Juice Extract;</td>
<td>Vitis Vinifera (Grape) Skin Powder;*</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Leaf Extract;</td>
<td>Vitis Vinifera (Grape) Vine Extract;</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Leaf Oil;*</td>
<td>Vitis Vinifera (Grape) Vine Sap;*</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Leaf/Seed/Skin Extract;*</td>
<td>Hydrolyzed Grape Fruit;*</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Leaf Water;*</td>
<td>Hydrolyzed Grape Skin.*</td>
</tr>
</tbody>
</table>

Were ingredients in this group not in current use (as indicated by *) to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in the group.
### Table 1. Definitions, Functions, and Chemical Class

<table>
<thead>
<tr>
<th>Ingredient (CAS No.)</th>
<th>Definition</th>
<th>Reported Function(s)</th>
<th>Chemical Class</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vitis vinifera</em> (Grape) (85594-37-2)</td>
<td>a plant material derived from the whole plant, <em>Vitis vinifera</em></td>
<td>not reported</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Bud Extract (85594-37-2)</td>
<td>the extract of the buds of <em>Vitis vinifera</em> (grape)</td>
<td>skin conditioning agent - misc</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Flower Extract (85594-37-2)</td>
<td>the extract of the flowers of <em>Vitis vinifera</em></td>
<td>skin conditioning agent – emollient; fragrance ingredient</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Fruit Extract (84929-27-1; 85594-37-2)</td>
<td>the extract of the fruit of <em>Vitis vinifera</em></td>
<td>skin conditioning agent – misc; antioxidant</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Fruit Powder (85594-37-2)</td>
<td>the powder obtained from the dried, ground fruit of <em>Vitis vinifera</em></td>
<td>skin conditioning agent – misc; antioxidant; colorant; flavoring agent</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Juice Extract (85594-37-2)</td>
<td>the liquid expressed from the fresh pulp of the grape</td>
<td>skin conditioning agent – misc; antioxidant; colorant; flavoring agent</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Juice Extract (85594-37-2)</td>
<td>the extract of the juice of <em>Vitis vinifera</em></td>
<td>antioxidant; colorant; flavoring agent</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Leaf Extract (84929-27-1; 85594-37-2)</td>
<td>the extract of the leaves of <em>Vitis vinifera</em></td>
<td>skin conditioning agent – misc</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Leaf Oil 8016-21-5</td>
<td>the essential oil derived from the leaves of the grape, <em>Vitis vinifera</em></td>
<td>fragrance ingredient</td>
<td>essential oils and waters</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Leaf/Seed/Skin Extract (85594-37-2)</td>
<td>the extract of the leaves, skin, and seeds of <em>Vitis vinifera</em></td>
<td>antioxidant</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Leaf Water (85594-37-2)</td>
<td>an aq. solution of the steam distillate obtained from the leaves of <em>Vitis vinifera</em></td>
<td>skin conditioning agent – misc</td>
<td>essential oils and waters</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Leaf Wax (85594-37-2)</td>
<td>a wax obtained from the vine leaf of <em>Vitis vinifera</em></td>
<td>not reported</td>
<td>waxes (natural and synthetic)</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Root Extract (84929-27-1; 85594-37-2)</td>
<td>the extract of the roots of <em>Vitis vinifera</em></td>
<td>skin conditioning agent – misc</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Seed (85594-37-2)</td>
<td>the seed of <em>Vitis vinifera</em></td>
<td>skin conditioning agent – misc</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Seed Extract (84929-27-1; 85594-37-2)</td>
<td>the extract of the seeds of <em>Vitis vinifera</em></td>
<td>anti-caries agent; anti-dandruff agent; antioxidant; skin protectant; anti-fungal agent; anti-microbial agent; light stabilizer; oral care agent; oral health care drug; sunscreen agent</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Seed Powder (85594-37-2)</td>
<td>the powder obtained from the dried, ground seeds of <em>Vitis vinifera</em></td>
<td>abrasive; exfoliant</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Shoot Extract (85594-37-2)</td>
<td>the extract of the shoots of the vines of <em>Vitis vinifera</em></td>
<td>antioxidant, skin protectant</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Skin Extract (85594-37-2)</td>
<td>extract of the skin of the grape, <em>Vitis vinifera</em></td>
<td>antioxidant; colorant; flavoring agent</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Skin Powder (85594-37-2)</td>
<td>the powder obtained from the dried, ground skin of <em>Vitis vinifera</em></td>
<td>skin conditioning agent – misc; antioxidant; binder; colorant</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Vine Extract (85594-37-2)</td>
<td>the extract of the vine of <em>Vitis vinifera</em></td>
<td>skin conditioning agent – misc</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grape) Vine Sap</td>
<td>the sap obtained from the vines of <em>Vitis vinifera</em></td>
<td>skin conditioning agent – misc</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td>Hydrolyzed Grape Fruit</td>
<td>the hydrolysate of the fruit of <em>Vitis vinifera</em> derived by acid, enzyme or other method of hydrolysis</td>
<td>cosmetic astringent; skin protectant, skin conditioning agent-misc</td>
<td>botanical products and botanical derivatives</td>
</tr>
<tr>
<td>Hydrolyzed Grape Skin</td>
<td>the hydrolysate of the skin of <em>Vitis vinifera</em> derived by acid, enzyme or other method of hydrolysis</td>
<td>antioxidant; light stabilizer; skin protectant; skin conditioning agent-emollient</td>
<td>botanical products and botanical derivatives</td>
</tr>
</tbody>
</table>
### Table 2. Chemical and Physical Properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vitis Vinifera (Grape) Fruit Extract</strong></td>
<td>Mixture containing 75-100% glycerin (solvent), 50-75% Vitis Vinifera (Grape) Fruit Extract, and 10-25% water</td>
<td></td>
</tr>
<tr>
<td>appearance</td>
<td>clear yellow liquid with a faint fruity odor</td>
<td>7</td>
</tr>
<tr>
<td>density</td>
<td>1.225-1.245</td>
<td>7</td>
</tr>
<tr>
<td>refractive index</td>
<td>1.445-1.465</td>
<td>7</td>
</tr>
<tr>
<td>pH</td>
<td>4.0-5.0</td>
<td>7</td>
</tr>
<tr>
<td>solubility</td>
<td>in water clear soluble</td>
<td>7</td>
</tr>
<tr>
<td><strong>Vitis Vinifera (Grape) Leaf Extract</strong></td>
<td>Mixture containing 75-100% glycerin (solvent), 5-10% Vitis Vinifera (Grape) Leaf Extract, and 10-25% water</td>
<td>9</td>
</tr>
<tr>
<td>appearance</td>
<td>dark brownish-red colored liquid with a faint herbal odor</td>
<td>9</td>
</tr>
<tr>
<td>density</td>
<td>1.215-1.235</td>
<td>9</td>
</tr>
<tr>
<td>refractive index</td>
<td>1.445-1.465</td>
<td>9</td>
</tr>
<tr>
<td>pH</td>
<td>4.0-5.0</td>
<td>9</td>
</tr>
<tr>
<td>solubility</td>
<td>in water clear soluble</td>
<td>9</td>
</tr>
<tr>
<td><strong>Vitis Vinifera (Grape) Seed Extract</strong></td>
<td>Appearance red to brown powder</td>
<td>16</td>
</tr>
<tr>
<td>water content</td>
<td>8% (upper limit)</td>
<td>16</td>
</tr>
<tr>
<td><strong>Vitis Vinifera (Grape) Skin Extract</strong></td>
<td>Appearance red to purple powder or liquid</td>
<td>49</td>
</tr>
<tr>
<td>appearance in solution</td>
<td>purplish-red liquid</td>
<td>4</td>
</tr>
<tr>
<td>purplish-red liquid, lump, powder, or paste with a characteristic odor</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>solubility</td>
<td>soluble in water</td>
<td>49</td>
</tr>
<tr>
<td><strong>Hydrolyzed Grape Skin</strong></td>
<td>Appearance ruby red aq. solution</td>
<td>51</td>
</tr>
<tr>
<td>odor</td>
<td>characteristic, fruity</td>
<td>51,52</td>
</tr>
<tr>
<td>boiling point</td>
<td>98-102°C (760 mm Hg)</td>
<td>52</td>
</tr>
<tr>
<td>density</td>
<td>$\approx 1 \ g/cm^3$</td>
<td>52</td>
</tr>
<tr>
<td>pH</td>
<td>2.6 – 3.5</td>
<td>51</td>
</tr>
<tr>
<td>2.8-4</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>solubility</td>
<td>completely soluble in water; soluble in alcohol and acetone</td>
<td>52</td>
</tr>
<tr>
<td>dry residue</td>
<td>$\geq 1.5%$ w/w</td>
<td>51</td>
</tr>
<tr>
<td>water content</td>
<td>$\geq 90%$</td>
<td>52</td>
</tr>
<tr>
<td>phenol content</td>
<td>700 – 1500 mg/kg</td>
<td>51</td>
</tr>
</tbody>
</table>

### Table 3. Chemical constituents by plant part

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Amount (ppm)</th>
<th>Plant</th>
<th>Chemical</th>
<th>Amount (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,6-dimethyl-trans-oct-2,7-dien-1,6-diol-beta-d-glucopyranoside</td>
<td>NS</td>
<td>oleic-acid</td>
<td>230-1183</td>
<td></td>
</tr>
<tr>
<td>delphinidin</td>
<td>NS</td>
<td>petunidin-3-caffeoylglucoside</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>leucocyanidin</td>
<td>NS</td>
<td>riboflavin</td>
<td>0.5-2</td>
<td></td>
</tr>
<tr>
<td>limonene</td>
<td>NS</td>
<td>stigmasterol</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>malic acid</td>
<td>NS</td>
<td>vitispirane</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>2,2,6-trimethyl-8-(1-hydroxy-ethyl)-7-oxa-bicyclo-(4,3,0)-nona-4,9-diene</td>
<td>NS</td>
<td>lutein</td>
<td>0.7-7</td>
<td></td>
</tr>
<tr>
<td>2,6-dimethyl-trans,trans-octa-2,6-dien-1,8-diol</td>
<td>NS</td>
<td>lutein-5,6-epoxide</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>2,6-dimethyl-trans-octa-2,7-dien-1,6-diol-6-o-alpha-d-arabinofuranosyl-beta-d-beta-d-glucopyranoside</td>
<td>NS</td>
<td>lutein-5,8-epoxide</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>3,7-dimethyl-oct-1-ene-3,6,7-triol</td>
<td>NS</td>
<td>luteoxanthin</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>3,7-dimethyl-oct-1-ene-3,7-diol</td>
<td>NS</td>
<td>lycopene</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>3,7-dimethyl-octa-1,5,7-trien-3-ol</td>
<td>NS</td>
<td>lysine</td>
<td>150-772</td>
<td></td>
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### Table 3. Chemical constituents by plant part

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Table 3. Chemical constituents by plant part

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Table 3. Chemical constituents by plant part

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<td>NS</td>
<td>oleic-acid</td>
<td>22,200-74,000</td>
</tr>
<tr>
<td>epicatechin-3-gallate</td>
<td>NS</td>
<td>palmitic-acid</td>
<td>3300-11000</td>
</tr>
<tr>
<td>fat</td>
<td>60,000-200,000</td>
<td>protein-acid</td>
<td>89,000</td>
</tr>
<tr>
<td>linoleic-acid</td>
<td>33,000-110,000</td>
<td>stearic-acid</td>
<td>1440-4800</td>
</tr>
</tbody>
</table>

**Hull Husk**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>gentisic-acid</td>
<td>NS</td>
<td>syringic-acid</td>
<td>NS</td>
</tr>
<tr>
<td>o-hydroxybenzoic acid</td>
<td>NS</td>
<td>vanillic-acid</td>
<td>NS</td>
</tr>
<tr>
<td>p-hydroxybenzoic acid</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Petiole**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>oenin</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
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</table>

NS – not specified

Table 4. Additional constituent data

**Polyphenols**

- Cinnamic acids: coumaric, caffeic, ferulic, chlorogenic, and neochlorogenic acid
- Benzoic acids: p-hydroxybenzoic acid; protocatechuic, vanillic, and gallic acid
- trans-Resveratrol (trans-3,5,40-trihydroxystilbene)

**Fruit**

**Polyphenols**

- Flavones: quercetin (traces) and quercitrin; quercetin-, kaempferol-, and myricetin-3-monoglusides; quercetin-glucuronoside; astilbin; engeletin
- Catechins: catechin; epicatechin, gallo catechin, epicatechingallage
- Anthocyanins: delphinidin-, petunidin-, malvidin- (41.2%), cyanidin-, and peonidin-3-monoglusides; 3-glucosides; 3-acetylglycosides; 3-coumaroylglycosides; 3-cafeoylglycosides; 3,5-diglucosides; 3-acetyl-5-diglucosides; 3-coumaroyl-5-diglucosides; and 3-cafeoyl-5-diglucosides of cyanidin, delphinidin, peonidin, petunidin, and malvidin
- Procyanidins: procyanidin B1, B2, B3, B4, B5, B7, B8; 15 acylated procyanidins that are esters of gallic acid; 14 dimeric, 11 trimeric, and one tetrameric procyanidin

α-Hydroxy acids: tartaric, citric, and malic acids
Esters: containing cinnamic and tartaric acids
Aldehydes: vanillic; protocatechuic; cinnamic and coniferyl aldehydes
Vitamins: C, B group, PP
Carotene
Sugars: Fructose, Glucose
Polysaccharides: containing galactose, mannose, arabinose, rhamnose, galacturonic acid
Proteins
Volatile constituents
Waxes
Pectin
Table 4. Additional constituent data

Seeds
Polyphenols (5-8 by wt%: 60-70% of grape polyphenols are found in grape seeds; they are flavan-3-ol derivatives)
- Catechins: (+)-catechin; (-)-epicatechin; (-)-epicatechin-3-O-gallate
- Procyanidins: procyanidin B1, B2, B3, B4, B5, B7, B8; procyanidins C1; procyanidins B5-3’-gallate
- Proanthocyanidins (mostly hexamers)
- Flavonoids (4-5%): kaemperferol-3-O-glucosides; quercetin-3-O-glucosides; quercetin; myricetin
Proteins (7-10%): containing arginine, cystine, leucine (11.4%), valine, phenylalanine
Triglycerides (6-20%): containing palmitic, stearic, oleic (37%), and linoleic (55%) acids
Unsaponifiables (0.5-1%): phytosterols: b-sitosterol
Phospholipids: phosphatidylserine, phosphatidylinositol, lecithin, cephalin, cerebrosides, phosphatidic acid
Vitamin E

Leaves
Polyphenols
- Anthocyanins
- Catechins: catechin; epicatechin; gallocatechin; epicatechin-3-O-gallate
- Ellagitannins: brevilagin-1; vitilagin; isovitilagin
- Flavones: traces of quercitrin, quercetin, kaempferol, rutin, iso-quercitrin, luteolin
Organic Acids: tartaric, malic, oxalic, fumaric, succinic, citric, and glyceric acids
Phenol acids: o- and p-hydroxybenzoic acid; protocatechuic, gallic, vanillic, syringic, and ellargic acids
Esters: containing cinnamic acids and tartaric acid
Vitamins: C, PP, B group, folic acid
Carotenoids
Volatile constituents
Waxes
Proteins
Mineral salts (5-7%)

Table 5. Conclusions of CIR safety assessments on ingredients that are constituents of Vitis vinifera (grape)

<table>
<thead>
<tr>
<th>Component Reviewed</th>
<th>Conclusion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic Acid</td>
<td>safe as used (≤0.0004% in leave-ons; ≤0.3% in rinse-offs)</td>
<td>54</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>safe as used (≤10% in leave-ons; ≤5% in rinse-offs)</td>
<td>55</td>
</tr>
<tr>
<td>Benzoic Acid</td>
<td>safe as used (≤5% in leave-ons; ≤5% in rinse-offs; 0.08% in diluted for (bath) use formulations)</td>
<td>56</td>
</tr>
<tr>
<td>Benzylic Alcohol</td>
<td>safe as used (≤3% in leave-ons; ≤10% in rinse-offs; ≤0.9% in diluted for (bath) use formulations)</td>
<td>56</td>
</tr>
<tr>
<td>Biotin</td>
<td>safe as used (≤0.6% in leave-ons; ≤0.01% in rinse-offs)</td>
<td>57</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>safe as used as used (≤5% in leave-ons; ≤1% in rinse-offs)</td>
<td>58</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>safe as used (≤4% in leave-ons; ≤10% in rinse-offs; ≤39% in diluted for (bath) use formulations)</td>
<td>59</td>
</tr>
<tr>
<td>Fumaric Acid</td>
<td>safe as used (≤0.2% in leave-ons; ≤0.2% in rinse-offs; ≤5% in diluted for (bath) use formulations)</td>
<td>60</td>
</tr>
<tr>
<td>Lactic Acid</td>
<td>safe for use at ≤10%, final formulation pH ≥3.5; when formulated to avoid increasing sun sensitivity or when directions for use include the daily use of sun protection; safe for use in salon products at ≤30%, final formulation pH ≥3.0, in products designed for brief, discontinuous use followed by thorough rinsing from the skin, when applied by trained professionals, and when application is accompanied by directions for the daily use of sun protection</td>
<td>61</td>
</tr>
<tr>
<td>Malic Acid</td>
<td>safe for use as a pH adjuster; insufficient for other uses</td>
<td>62</td>
</tr>
<tr>
<td>Myristic Acid</td>
<td>safe as used (≤10% in leave-ons; ≤19% in rinse-offs)</td>
<td>63</td>
</tr>
<tr>
<td>Niacin</td>
<td>safe as used (≤0.1% in leave-ons)</td>
<td>64</td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>safe as used (≤20% in leave-ons; ≤19% in rinse-offs)</td>
<td>65,66</td>
</tr>
<tr>
<td>Palmitic Acid</td>
<td>safe as used (≤16% in leave-ons; ≤20% in rinse-offs)</td>
<td>65,66</td>
</tr>
<tr>
<td>Pantothenic Acid</td>
<td>safe as used (≤0.01% in leave-ons: 0.00001% in rinse-offs)</td>
<td>66,67</td>
</tr>
<tr>
<td>Salicylic Acid</td>
<td>safe as used when formulated to avoid skin irritation and when formulated to avoid increasing the skin’s sensitivity to sun, or, when increased sun sensitivity would be expected, directions for use include the daily use of sun protection (≤3% in leave-ons; ≤3% in rinse-offs)</td>
<td>68</td>
</tr>
<tr>
<td>Stearic Acid</td>
<td>safe as used (≤22% in leave-ons; ≤43% in rinse-offs)</td>
<td>69,66</td>
</tr>
<tr>
<td>Succinic Acid</td>
<td>safe as used (≤0.2% in leave-ons; ≤26% in rinse-offs)</td>
<td>69</td>
</tr>
<tr>
<td>Tocopherol</td>
<td>safe as used (≤2% in leave-ons; ≤0.4% in rinse-offs; ≤0.8% in products diluted for use)</td>
<td>70</td>
</tr>
<tr>
<td>Component</td>
<td>Toxicity information</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>Polyphenol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resveratrol</td>
<td>- in rats given daily oral administration of resveratrol (300, 1000, 3000 mg/kg for 28 days), nephrotoxicity and other signs of toxicity was observed at the high dose level, dehydration and loss of body wt were observed at the mid-dose level, and the NOAEL was 300 mg/kg/day; in several mammary cancer cell lines, resveratrol showed mixed estrogen agonist/antagonist activities, whereas in the presence of 17β-estradiol, it was an anti-estrogen; progesterone receptor (PR) protein expression was induced with the compound alone, but when combined with estradiol, the expression was suppressed; exhibited estradiol antagonist activity for estrogen receptor (ER)-α with select estrogen response elements and no such activity with ER-β; in vivo, resveratrol was not an agonist at the ER; when resveratrol and 17β-estradiol were administered in combination, a synergistic effect was observed; oral or subcutaneous (s.c.) administration of trans-resveratrol (produced no estrogenic response in the uterine tissue of the animals; trans-resveratrol was not mutagenic in an Ames test, induced dose-dependent chromosome aberrations in the Chinese hamster lung, and induced micronuclei, poly nuclei, and karyorrhectic cells in a sister chromatid exchange assay - not genotoxic in a mouse or rat micronucleus test or in an Ames test - not an ocular or dermal irritant in rabbits; not a sensitizer in a local lymph node assay (≤25% w/v in dimethyl formamide); not mutagenic in an Ames test, was clastogenic in a chromosomal aberrations assay in human lymphocytes, non-genotoxic in an in vivo bone marrow micronucleus test in rats, not adverse effect in rats in repeated dose studies (up to 90 days with up to 700 mg/kg bw/day); 750 mg/kg bw/day was not embryotoxic in rats; readily absorbed, metabolized, and excreted in rats - concentrations of 1 nM - 100 μM trans-resveratrol in DMSO, evaluated in a yeast estrogen screen, did not have estrogenic activity at any of the concentrations tested; when the same concentrations were measured for estrogenic activity in CHO-K1 cells, concentration-dependent ERα and ERβ agonist activity was observed and ERβ showed greater activation; compared to estradiol, resveratrol had weaker activity, and the agonist activity was inhibited by 4-hydroxytamoxifen</td>
<td></td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>do not appear to be readily absorbed or metabolized; low acute oral toxicity; weight-of-evidence analysis indicates anthocyanins are not genotoxic</td>
<td></td>
</tr>
<tr>
<td>Carotenoids</td>
<td>no evidence of adverse biological activity</td>
<td></td>
</tr>
<tr>
<td>Lutein/Esters</td>
<td>single-dose, 4-wk, and 13-wk oral studies found no evidence of toxicity</td>
<td></td>
</tr>
<tr>
<td>Chlorogenic Acid</td>
<td>- an antioxidant that inhibited tumor promotion by phorbol esters in mice; some controversy exists over allergic reactions in green coffee beans, but it was accepted that chlorogenic acid was not the allergen - in mice, 2% (20,000 ppm) chlorogenic acid in the diet for 96 weeks induced papillomas and carcinomas of the forestomach, alveolar type II-cell tumors of the lung, and renal cell adenomas; few toxic effects resulted from acute exposure; subchronic dietary exposures did not induce clinical symptoms of toxicity, however, reduced kidney and adrenal wts and hyperplasia of the forestomach were observed; some genotoxic effects seen in vitro but not in vivo</td>
<td></td>
</tr>
<tr>
<td>Coumarin</td>
<td>limited evidence in experimental animals for carcinogenicity; not classifiable as to its carcinogenicity in humans (IARC)</td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>epidemiological studies implicated high dietary intake levels of flavonoids in heart disease, but a study of cancer risk failed to find a link; some evidence of genotoxicity in bacterial assays, but a European Organization of Cosmetic Ingredients Industries and Services (UNITIS) report stated that flavonoids do not appear to be genotoxic to mammals in vivo; flavonoids are not considered allergens</td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>- genotoxic in vitro but not in vivo; some evidence for carcinogenicity (renal tumors) was found in one of several studies, in one species (rat), in one gender (male); antioxidant properties noted; estrogenic properties, similar to other flavonoids, were noted; overall conclusion by the Council of Europe Committee of Experts on Cosmetic Products was that quercetin did not present potential risks for human health, but that skin effects and dermal penetration data were needed to complete a toxicological profile; a weight of evidence approach supported a finding that at estimated dietary levels of as a dietary supplement (200-1200 mg/d), adverse health effects would not be produced; reduced histamine release from antigen-induced human basophil cells - quercetin alone, 100 μM, increased the spontaneous number of sister chromatid exchanges (SCEs) in human lymphocytes; however, 50 and 100 μM inhibited mitomycin C (MMC)-induced SCEs in a dose-dependent manner</td>
<td></td>
</tr>
<tr>
<td>(+)-Catechin; (-)-Epicatechin</td>
<td>no effect on SCEs in human lymphocytes in the presence or absence of MCC</td>
<td></td>
</tr>
<tr>
<td>Kaempferol</td>
<td>increased the frequency of sister chromatid exchanges in cultured hamster cells; shown to mutate and transform human and mouse cells in culture</td>
<td></td>
</tr>
<tr>
<td>Monoterpenes</td>
<td>these chemicals may be skin irritants</td>
<td></td>
</tr>
<tr>
<td>Phenolic Acids</td>
<td>- in a MMC-induced SCE assay in human lymphocytes, 100 μM caffeic acid enhanced MMC-induced SCEs by 55%; 100 μM caffeic acid alone enhanced MMC-induced SCEs by 26% - caffeic acid is reported to penetrate skin and have UV photoprotective activity; an IARC report stated that there was sufficient evidence for carcinogenicity in animals, but no data on carcinogenicity in humans – caffeic acid was possibly carcinogenic to humans</td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Toxicity information on some components of Vitis Vinifera (grape)
### Table 6. Toxicity information on some components of *Vitis Vinifera* (grape)

<table>
<thead>
<tr>
<th>Component</th>
<th>Toxicity information</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>- the carcinogenic potency of caffeic acid, estimated based on an average human intake of 1 mg/kg bw/day, was less than 1000 cancer cases per 1,000,000 individuals; in rats 1 or 2% (10,000 or 20,000 ppm) caffeic acid in the diet for 51 weeks to 2 years induced papillomas of the forestomach and renal adenomas; one study in which rats were exposed to 2% (20,000 ppm) caffeic acid in the diet for 2 yrs showed treatment-induced carcinomas of the forestomach, whereas two studies with shorter exposure durations showed no such effect; caffeic acid was shown to exert strong promotion activity for forestomach carcinogenesis; chronic exposure to caffeic acid in the diet induced hyperplasia of the forestomach (mice, rats, and hamsters), hyperplasia of the kidney (mice and rats), and increased liver and kidney wts (rats); few toxic effects resulted from acute exposure; subchronic dietary exposures did not induce clinical symptoms of toxicity, however, hyperplasia of the forestomach was observed; some genotoxic effects seen in <em>vitro</em> but not in vivo</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Ferulic Acid</strong></td>
<td>- in an SCE assay, ferulic acid did not affect SCEs in the presence of absence of MMC</td>
</tr>
<tr>
<td></td>
<td>- this acid is reported to penetrate skin and have UV photoprotective activity</td>
</tr>
<tr>
<td><strong>Phytosterols</strong></td>
<td>oral studies demonstrate that phytosterols and phytosterol esters are not significantly absorbed and do not result in systemic exposure; small amounts did appear in the ovaries; well-defined phytosterols and phytosterol esters are not estrogenic and do not pose a hazard to reproduction; phytosterols were not mutagenic in bacterial and mammalian systems</td>
</tr>
<tr>
<td><strong>Tannins</strong></td>
<td>IARC has concluded that tannins are not classifiable to their carcinogenicity</td>
</tr>
<tr>
<td><strong>Leucocyanidin</strong></td>
<td>without stating any details, a review source stated this substance has been reported to be toxic to some laboratory animals; symptoms included cardiac failure and hepatic lesions</td>
</tr>
<tr>
<td><strong>Terpene Alcohols</strong></td>
<td></td>
</tr>
<tr>
<td><strong>- percutaneous absorption, 954 µg/cm²/h through human cadaver skin; ocular irritant in rabbit eyes (undiluted)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>citronellol</strong></td>
<td>- dermal LD₅₀ in rabbits, 2650 mg/kg; oral LD₅₀ in rats, 3450 mg/kg; dietary NOAEL in rats in a 12 wk study, 50 mg/kg bw/day; inhalation NOAEC in rats in a 100 day inhalation study, 0.3 mg/m³; not mutagenic in an Ames assay with activation, a rec-assay, or a host-mediated assay; undiluted, dermal irritant in guinea pigs and rabbits in most tests; mostly not an irritant in clinical testing at up to 40%, irritation was reported in a study at 32% in acetone; not a sensitizer in a Buehler (2.5-25%) or maximization (max.) test (10%) in guinea pigs, positive reaction at 50% (but not ≤25%0 in mice; not a sensitizer in an HRIPT at 25%</td>
</tr>
<tr>
<td><strong>D,L-citronellol</strong></td>
<td>- dermal LD₅₀ in rabbits, &gt;5000 mg/kg; oral LD₅₀ in rats, 3600 mg/kg; no adverse effects in rats in dietary studies with ≤1000 mg/kg bw/day for up to 16 wks and with 100 mg/kg bw/day for 27 wks; not mutagenic in an Ames test or rec-assay, equivocal results with regard to polyplody in one chromosome aberration test at up to 0.125 mg/ml in DMSO and inconclusive results in another at up to 156.3 µg/ml, and not genotoxic in a bone marrow micronucleus assay; undiluted was a dermal irritant in rabbits in most single application tests and a primary irritation study and 30 and 100% in ethanol caused irritation in a primary irritation study in guinea pigs; mixed irritation results in clinical studies, but generally &lt;10% was not irritating; ocular irritant in rabbit eyes (12.5% and undiluted); mixed results in LLNA assays, but mostly sensitizing at 30 and 50, and mixed results in guinea pig sensitization studies, with both positive and negative results at 10%; not a sensitizer in multiple HRIPTs at 2-12.5%, 20 positive reactions in a max. study at 5% in pet. in 25 subjects, 2 positive reactions in a modified Draize test at 10% in alcohol in 73% volunteers, not a sensitizer in other chronic max. studies with 5-6% in pet; not phototoxic at 5% in pet. in clinical testing</td>
</tr>
<tr>
<td><strong>geraniol</strong></td>
<td>- dermal LD₅₀ in rabbits, &gt;5000 mg/kg; oral LD₅₀ in rats, 4500 mg/kg; some erythema (+ rxn in 2 and ± rxn in 8/314 subjects) with up to 0.5%; ocular irritant in rabbit eyes (undiluted); not a sensitizer in guinea pigs at up to 4%; not a sensitizer at 4% in pet. in a clinical max. study</td>
</tr>
<tr>
<td><strong>nerol</strong></td>
<td>- dermal LD₅₀ in rabbits, &gt;5000 mg/kg; oral LD₅₀ in rats, 4500 mg/kg; some erythema (+ rxn in 2 and ± rxn in 8/314 subjects) with up to 0.5%; ocular irritant in rabbit eyes (undiluted); not a sensitizer in guinea pigs at up to 4%; not a sensitizer at 4% in pet. in a clinical max. study</td>
</tr>
<tr>
<td><strong>α-terpineol</strong></td>
<td>- oral LD₅₀ in mice, 2830 mg/kg; 1000 mg/kg bw/day for 2 wks caused reduced body wt gains and an increase in serum cholesterol; not mutagenic in an Ames test or mouse lymphoma assay; did not induce pulmonary tumors in mice given i.p. injections; a derma irritant in animals studies, but not a dermal irritant in a 4-h clinical study; not a sensitizer in guinea pigs; in clinical patch tests, 5% in pet. had 1/1606 positive and 11/1606 questionable reactions in one study and 2/1200 positive reactions in another</td>
</tr>
<tr>
<td><strong>Cyclic</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Triterpene Alcohols</strong></td>
<td></td>
</tr>
<tr>
<td><strong>- hepatoprotective and anti-carcinogenic activity has been suggested for lupeol; no toxicity data were available; triterpene alcohols were considered to have intermediate risk</strong></td>
<td></td>
</tr>
<tr>
<td>Table 7. Frequency and concentration of use according to duration and type of exposure</td>
<td></td>
</tr>
<tr>
<td>--------------------------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td><strong>Vitis Vinifera (Grape)</strong></td>
<td><strong>Vitis Vinifera (Grape) Bud Extract</strong></td>
</tr>
<tr>
<td><strong># of Uses</strong></td>
<td><strong>Max. Conc. of Use (%)</strong></td>
</tr>
<tr>
<td>Totals*</td>
<td></td>
</tr>
<tr>
<td><strong>Duration of Use</strong></td>
<td></td>
</tr>
<tr>
<td>Leave-On</td>
<td>4</td>
</tr>
<tr>
<td>Rinse Off</td>
<td>1</td>
</tr>
<tr>
<td>Diluted for (Bath) Use</td>
<td>NR</td>
</tr>
<tr>
<td><strong>Exposure Type</strong></td>
<td></td>
</tr>
<tr>
<td>Eye Area</td>
<td>NR</td>
</tr>
<tr>
<td>Incidental Ingestion</td>
<td>NR</td>
</tr>
<tr>
<td>Incidental Inhalation-Spray</td>
<td>NR</td>
</tr>
<tr>
<td>Incidental Inhalation-Powder</td>
<td>NR</td>
</tr>
<tr>
<td>Dermal Contact</td>
<td>3</td>
</tr>
<tr>
<td>Deodorant (underarm)</td>
<td>NR</td>
</tr>
<tr>
<td>Hair - Non-Coloring</td>
<td>1</td>
</tr>
<tr>
<td>Hair-Coloring</td>
<td>NR</td>
</tr>
<tr>
<td>Nail</td>
<td>NR</td>
</tr>
<tr>
<td>Mucous Membrane</td>
<td>NR</td>
</tr>
<tr>
<td>Baby Products</td>
<td>NR</td>
</tr>
<tr>
<td><strong>Vitis Vinifera (Grape) Fruit Powder</strong></td>
<td></td>
</tr>
<tr>
<td><strong># of Uses</strong></td>
<td><strong>Max. Conc. of Use (%)</strong></td>
</tr>
<tr>
<td>Totals*</td>
<td></td>
</tr>
<tr>
<td><strong>Duration of Use</strong></td>
<td></td>
</tr>
<tr>
<td>Leave-On</td>
<td>2</td>
</tr>
<tr>
<td>Rinse Off</td>
<td>NR</td>
</tr>
<tr>
<td>Diluted for (Bath) Use</td>
<td>2</td>
</tr>
<tr>
<td><strong>Exposure Type</strong></td>
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</tr>
<tr>
<td>Eye Area</td>
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<td>Incidental Inhalation-Spray</td>
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<tr>
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<tr>
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<td>NR</td>
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<td>Hair-Coloring</td>
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<tr>
<td>Nail</td>
<td>NR</td>
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<td>Mucous Membrane</td>
<td>2</td>
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<tr>
<td>Baby Products</td>
<td>NR</td>
</tr>
<tr>
<td><strong>Vitis Vinifera (Grape) Juice Extract</strong></td>
<td></td>
</tr>
<tr>
<td><strong># of Uses</strong></td>
<td><strong>Max. Conc. of Use (%)</strong></td>
</tr>
<tr>
<td>Totals*</td>
<td></td>
</tr>
<tr>
<td><strong>Duration of Use</strong></td>
<td></td>
</tr>
<tr>
<td>Leave-On</td>
<td>1</td>
</tr>
<tr>
<td>Rinse Off</td>
<td>6</td>
</tr>
<tr>
<td>Diluted for (Bath) Use</td>
<td>NR</td>
</tr>
<tr>
<td><strong>Exposure Type</strong></td>
<td></td>
</tr>
<tr>
<td>Eye Area</td>
<td>NR</td>
</tr>
<tr>
<td>Incidental Ingestion</td>
<td>NR</td>
</tr>
<tr>
<td>Incidental Inhalation-Spray</td>
<td>NR</td>
</tr>
<tr>
<td>Incidental Inhalation-Powder</td>
<td>NR</td>
</tr>
<tr>
<td>Dermal Contact</td>
<td>1</td>
</tr>
<tr>
<td>Deodorant (underarm)</td>
<td>NR</td>
</tr>
<tr>
<td>Hair - Non-Coloring</td>
<td>5</td>
</tr>
<tr>
<td>Hair-Coloring</td>
<td>1</td>
</tr>
<tr>
<td>Nail</td>
<td>NR</td>
</tr>
<tr>
<td>Mucous Membrane</td>
<td>NR</td>
</tr>
<tr>
<td>Baby Products</td>
<td>NR</td>
</tr>
</tbody>
</table>
### Table 7. Frequency and concentration of use according to duration and type of exposure

<table>
<thead>
<tr>
<th>Duration of Use</th>
<th>Vitis Vinifera (Grape) Seed Extract</th>
<th>Vitis Vinifera (Grape) Seed Powder</th>
<th>Vitis Vinifera (Grape) Shoot Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># of Uses</td>
<td>Max. Conc. of Use (%)</td>
<td># of Uses</td>
</tr>
<tr>
<td>Leave-On</td>
<td>495</td>
<td>0.00002 -0.2</td>
<td>1</td>
</tr>
<tr>
<td>Rinse Off</td>
<td>369</td>
<td>0.00002- 0.2</td>
<td>1</td>
</tr>
<tr>
<td>Diluted for (Bath) Use</td>
<td>8</td>
<td>0.002-0.003</td>
<td>NR</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exposure Type</th>
<th>Vitis Vinifera (Grape) Seed Extract</th>
<th>Vitis Vinifera (Grape) Seed Powder</th>
<th>Vitis Vinifera (Grape) Shoot Extract</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Exposure Type</th>
<th># of Uses</th>
<th>Max. Conc. of Use (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye Area</td>
<td>19</td>
<td>0.0002-0.09</td>
</tr>
<tr>
<td>Incidental Ingestion</td>
<td>18</td>
<td>0.0002</td>
</tr>
<tr>
<td>Incidental Inhalation-Spray</td>
<td>28</td>
<td>pump spray: 0.00002 0.0002-0.02</td>
</tr>
<tr>
<td>Incidental Inhalation-Powder</td>
<td>4</td>
<td>0.0002</td>
</tr>
<tr>
<td>Dermal Contact</td>
<td>411</td>
<td>0.0002-0.2</td>
</tr>
<tr>
<td>Deodorant (underarm)</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Hair - Non-Coloring</td>
<td>62</td>
<td>0.00002-0.1</td>
</tr>
<tr>
<td>Hair-Coloring</td>
<td>1</td>
<td>NR</td>
</tr>
<tr>
<td>Nail</td>
<td>1</td>
<td>0.001</td>
</tr>
<tr>
<td>Mucous Membrane</td>
<td>60</td>
<td>0.0002-0.02</td>
</tr>
<tr>
<td>Baby Products</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vitis Vinifera (Grape) Vine Extract</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Totals*</th>
<th># of Uses</th>
<th>Max. Conc. of Use (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11</td>
<td>0.004</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Duration of Use</th>
<th>Vitis Vinifera (Grape) Vine Extract</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Duration of Use</th>
<th># of Uses</th>
<th>Max. Conc. of Use (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leave-On</td>
<td>10</td>
<td>0.004</td>
</tr>
<tr>
<td>Rinse Off</td>
<td>1</td>
<td>NR</td>
</tr>
<tr>
<td>Diluted for (Bath) Use</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exposure Type</th>
<th>Vitis Vinifera (Grape) Vine Extract</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Exposure Type</th>
<th># of Uses</th>
<th>Max. Conc. of Use (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye Area</td>
<td>2</td>
<td>NR</td>
</tr>
<tr>
<td>Incidental Ingestion</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Incidental Inhalation-Spray</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Incidental Inhalation-Powder</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Dermal Contact</td>
<td>10</td>
<td>0.004</td>
</tr>
<tr>
<td>Deodorant (underarm)</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Hair - Non-Coloring</td>
<td>1</td>
<td>NR</td>
</tr>
<tr>
<td>Hair-Coloring</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Nail</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Mucous Membrane</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Baby Products</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

* Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types my not equal the sum of total uses
NR – not reported
* Includes suntan preparations, and it t is not known whether or not those product are sprays
* It is not known whether or not this product is a pump or a spray

### Table 8. Ingredient Not Reported to be Used

- Vitis Vinifera (Grape) Flower Extract
- Vitis Vinifera (Grape) Leaf Oil
- Vitis Vinifera (Grape) Leaf/Seed/Skin Extract
- Vitis Vinifera (Grape) Leaf Water
- Vitis Vinifera (Grape) Leaf Wax
- Vitis Vinifera (Grape) Root Extract
- Vitis Vinifera (Grape) Skin Extract
- Vitis Vinifera (Grape) Skin Powder
- Vitis Vinifera (Grape) Vine Sap
- Hydrolyzed Grape Fruit
- Hydrolyzed Grape Skin
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Extraction Solvent</th>
<th>Animals/Group</th>
<th>Study Duration</th>
<th>Dose/Concentration</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>not specified</td>
<td>SKH-1 hairless mice, 20F</td>
<td>3 wks</td>
<td>0, 0.2, or 0.5%</td>
<td>- no significant difference in body weights or other signs of toxicity; no gross differences observed in the organs of treated and untreated mice</td>
<td>34</td>
</tr>
<tr>
<td>as above</td>
<td>water and ethanol</td>
<td>F344/DuCrj rats, 10M/10F</td>
<td>90 days</td>
<td>0, 0.02, 0.2, or 2%</td>
<td>- no mortality in any of the grps; - no clinical signs of toxicity; - the few small but statistically significant changes in organ weights noted, primarily in the 0.2% group, were not dose-dependent; - no treatment-related microscopic changes were observed</td>
<td>37</td>
</tr>
<tr>
<td>grape seed extract containing 89.3% proanthocyanidin</td>
<td>not stated</td>
<td>Sprague-Dawley rats, 20M/20F</td>
<td>90 days</td>
<td>0, 0.62, 1.25, or 2.50%; mean test article intake was 434, 860, and 1788 mg/kg bw/day for males; 540, 1052, and 2167 mg/kg bw/day for females</td>
<td>- no mortality; - a mild head-tilt in 6 of 20 female rats in the 2.5% grp; the researchers remarked that it was doubtful this observation was treatment-related; - a small but statistically significant increase in feed consumption by males of the 2.5% grp from day 7 until study termination; similar increases were observed for males of the 1.25% grp, but the occurrence was at irregular intervals; - body wts and body wt gains were similar for treated and control grps; - a decrease in heart/body wt ratio in females of the 1.25% grp was not considered treatment-related; - no gross or microscopic lesions were reported at necropsy; - the NOAEL was ~2150 mg/kg bw/day for female rats and 1780 mg/kg bw/day for male rats</td>
<td>34</td>
</tr>
<tr>
<td>grape seed extract composed of ~90.5% total phenols</td>
<td>not stated</td>
<td>Sprague-Dawley rats, 20M/20F</td>
<td>90 days</td>
<td>0, 0.5, 1.0, or 2.0%; extract intake was 348, 642, and 1586 mg/kg bw/day for males; 469, 883, and 1928 mg/kg bw/day for females</td>
<td>- no mortality and no clinical signs of toxicity; - feed consumption was increased in test grps compared to controls; increases by males of the 2.0% grp reached statistical significance, with no corresponding increase in body wts or body wt gains; - no differences in organ wts between the test and control groups; - differences in clinical chemistry and hematology parameters between the test and control grps were not considered to be toxicologically significant; - no test-article related gross or microscopic lesions were observed</td>
<td>35</td>
</tr>
<tr>
<td>grape seed extract that contained &lt;5.5% catechin monomers</td>
<td>water</td>
<td>Sprague-Dawley rats, 20M/20F</td>
<td>90 days</td>
<td>0, 0.5, 1.0, or 2.0%; extract intake was 348, 642, and 1586 mg/kg bw/day for males; 469, 883, and 1928 mg/kg bw/day for females</td>
<td>- no mortality; - no significant changes in body wt or physical appearance; - no significant differences in BUN levels or ALT and CK activity between treated and control animals; - no gross or microscopic lesions were observed</td>
<td>35</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract (as ActiVin)</td>
<td>water-ethanol</td>
<td>female B6C3F1; no/group not specified</td>
<td>6 mos</td>
<td>0, 100, 250, or 500 mg/kg bw/day</td>
<td>- no treatment-related mortality; - no significant changes in body wt or physical appearance; - no significant differences in BUN levels or ALT and CK activity between treated and control animals; - no gross or microscopic lesions were observed</td>
<td>13</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract (as ActiVin)</td>
<td>water-ethanol</td>
<td>male B6C3F1; no/group not specified</td>
<td>12 mos; animals were killed at 90-day intervals</td>
<td>100 mg/kg bw/day</td>
<td>- no treatment-related mortality; - no significant changes in body weight or physical appearance; - no significant differences in BUN levels or ALT and CK activity between treated and control animals; - no gross or microscopic lesions; - hepatic genomic DNA fragmentation was monitored as an index of oxidative DNA damage; no significant changes were observed</td>
<td>13</td>
</tr>
</tbody>
</table>
### Table 9. Repeated Dose Toxicity Studies

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Extraction Solvent</th>
<th>Animals/Group</th>
<th>Study Duration</th>
<th>Dose/Concentration</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>grape skin extract containing 87.3% total</td>
<td>not specified</td>
<td>Sprague-Dawley rats, 20M/20F</td>
<td>90 days</td>
<td>2.5%; mean test</td>
<td>- no mortality; no clinical signs of toxicity</td>
<td>84</td>
</tr>
<tr>
<td>phenols expressed as gallic acid equivalents</td>
<td></td>
<td></td>
<td></td>
<td>article intake was</td>
<td>small but statistically significant increase in feed consumption by treated male however, body wts and body wt gains were similar for</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1788 mg/kg bw/day</td>
<td>treated and control grps</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>for males and 2167</td>
<td>statistically significant changes in some hematology measurements were noted at study termination, but none were considered clinically</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mg/kg bw/day for</td>
<td>relevant</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>females</td>
<td>statistically significant decrease in absolute and relative heart wt of female test animals was not considered treatment-related</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- no gross lesions were reported</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- a common renal cortical inflammation of minimal severity, comprised predominately of lymphocytic interstitial filtrates, was observed in 11 of</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>the male test animals; this was stated to be a common lesion seen in male rats and not considered treatment-related</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- the NOAEL was approximately 2150 mg/kg bw/day for female rats and 1780 mg/kg bw/day for male rats</td>
<td></td>
</tr>
<tr>
<td>grape color extract consisting of 40% of the</td>
<td>---</td>
<td>Beagle dogs, 4M/4F</td>
<td>90 days</td>
<td>0, 7.5, or 15% (w/w)</td>
<td>- physical appearance and behavior were normal for all dogs</td>
<td>86</td>
</tr>
<tr>
<td>naturally occurring grape-color extract in a</td>
<td></td>
<td></td>
<td></td>
<td>(w/w)</td>
<td>- body wt gains in the high dose grp were statistically significantly decreased compared to the controls, while feed consumption was</td>
<td></td>
</tr>
<tr>
<td>malto-dextrin carrier</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>comparable for test and control animals</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- no significant differences in absolute or relative organ wts between treated and control animals</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- no significant differences in ophthalmic, clinical chemistry, hematology, or urinary parameters between the group</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- no gross or microscopic lesions were noted</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** ALT = serum alanine aminotransferase; BUN = blood urea nitrogen; CK = serum creatinine kinase; grp = groups; NOAEL = no-observed adverse effect level; wt = weight
Table 10. Genotoxicity studies

<table>
<thead>
<tr>
<th>Concentration/Vehicle</th>
<th>Procedure</th>
<th>Test System</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grape Fruit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IN VITRO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fractions of raw grapes (concentration not specified);</td>
<td>Ames test</td>
<td><em>Salmonella typhimurium</em> TA 98 and TA100, with and without metabolic activation; grapes were washed, peeled, trimmed, and seeded; 250 g sample was blended with 500 ml water and fractionated; fractions were obtained with chloroform and n-butanol (fraction 5), water (fraction 7), methanol (fraction 3) or hexane (fraction 4)</td>
<td>was mutagenic in TA98 and TA100 without metabolic activation for all fractions except fraction 7</td>
<td>30</td>
</tr>
<tr>
<td>75-350 μg/ml methanolic extracts of red grapes</td>
<td>SCE assay; MMC-induced</td>
<td>human lymphocytes</td>
<td>enhanced MMC-induced SCEs in a dose-dependent manner; no effect on SCEs without MMC</td>
<td>6</td>
</tr>
<tr>
<td>75-350 μg/ml water extracts of red grapes</td>
<td>SCE assay; MMC-induced</td>
<td>human lymphocytes</td>
<td>statistically significant increase in MMC-induced SCEs at 300 μg/ml; no effect on SCEs without MMC</td>
<td>6</td>
</tr>
<tr>
<td>75-350 μg/ml methanolic extract of white grapes</td>
<td>SCE assay; MMC-induced</td>
<td>human lymphocytes</td>
<td>enhanced MMC-induced SCEs in a dose-dependent manner; no effect on SCEs without MMC</td>
<td>6</td>
</tr>
<tr>
<td>75-350 μg/ml water extract of white grapes</td>
<td>SCE assay; MMC-induced</td>
<td>human lymphocytes</td>
<td>enhanced MMC-induced SCEs in a dose-dependent manner; no effect on SCEs without MMC</td>
<td>6</td>
</tr>
<tr>
<td><strong>Grape Juice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>grape juice fractions (genus and species not stated) from canned or bottled juice in DMSO</td>
<td>Ames test</td>
<td><em>S. typhimurium</em> TA 98 and TA100, with and without metabolic activation</td>
<td>marked mutagenic activity</td>
<td>31</td>
</tr>
<tr>
<td>0.25-1.0 ml commercially available white grape juice (genus and species not stated)</td>
<td>Ames test</td>
<td><em>S. typhimurium</em> TA97, TA98, TA100, TA102, TA104, and TA1530 with and without metabolic activation</td>
<td>without metabolic activation, a positive mutagenic response was observed in all strains except TA102; toxicity was observed with TA102; TA104 was the most sensitive; metabolic activation did not affect response; response was not due to histidine</td>
<td>32</td>
</tr>
<tr>
<td>0.25-1.0 ml of 3 commercial brands of white grape juice (genus and species not stated)</td>
<td>Ames test</td>
<td><em>S. typhimurium</em> TA104 without metabolic activation</td>
<td>positive response with all 3 brands, but there was considerable difference in the potency of the response that was not attributable to the amount of solids</td>
<td>32</td>
</tr>
<tr>
<td>0.25-1.0 ml fresh grape juice (genus and species not stated)</td>
<td>Ames test</td>
<td><em>S. typhimurium</em> TA104 without metabolic activation</td>
<td>concentration-dependent mutagenic response</td>
<td>32</td>
</tr>
<tr>
<td>white grape juice (genus and species not stated)</td>
<td>examined the role of phenols, quinones, and reactive oxygen species in the mutagenicity of white grape juice in the Ames test</td>
<td></td>
<td>mutagenicity was markedly suppressed by reduced glutathione, but was not influenced by superoxide dismutase or catalase; polyphenol oxidase-mediated oxidation of grape juice phenolics generates species that can induce mutations</td>
<td>87</td>
</tr>
<tr>
<td><strong>Grape Seed Extract</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-1250 μg/plate; extracted with water and ethanol; extract contained 89.3% proanthocyanidins</td>
<td>Ames test</td>
<td><em>S. typhimurium</em> TA 98 and TA100, with and without metabolic activation</td>
<td>negative</td>
<td>27</td>
</tr>
<tr>
<td>156-5000 μg/plate; extracted with water and ethanol; extract contained 89.3% proanthocyanidins</td>
<td>Ames test</td>
<td><em>S. typhimurium</em> TA1535 and TA1537, with and without metabolic activation</td>
<td>negative</td>
<td>27</td>
</tr>
</tbody>
</table>
### Table 10. Genotoxicity studies

<table>
<thead>
<tr>
<th>Concentration/Vehicle</th>
<th>Procedure</th>
<th>Test System</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.4-37.5 μg/ml; extracted with water and ethanol; extract contained 89.3% proanthocyanidins</td>
<td>chromosomal aberration assay</td>
<td>CHL cells exposed for 24-48 h without metabolic activation</td>
<td>negative</td>
<td>27</td>
</tr>
<tr>
<td>18.8-75 μg/ml; extracted with water and ethanol; extract contained 89.3% proanthocyanidins</td>
<td>chromosomal aberration assay</td>
<td>CHL cells exposed for 18 h without metabolic activation</td>
<td>negative</td>
<td>27</td>
</tr>
<tr>
<td>18.8-300 μg/ml; extracted with water and ethanol; extract contained 89.3% proanthocyanidins</td>
<td>chromosomal aberration assay</td>
<td>CHL cells exposed for 6 h with metabolic activation</td>
<td>negative</td>
<td>27</td>
</tr>
<tr>
<td>1, 4, or 20 µM; extract contained 95% proanthocyanidins</td>
<td>comet assay</td>
<td>3 murine keratinocytes cell line were pretreated with the extract</td>
<td>protective effect; comet length decreased in a dose-dependent manner</td>
<td>38</td>
</tr>
<tr>
<td><strong>Grape Seed/Grape Skin Extract</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-5000 μg/plate; extracted with ethanol; extract contained 76% of total phenols</td>
<td>Ames test</td>
<td>S. typhimurium TA1535, TA1537, TA98 and TA100, with and without metabolic activation</td>
<td>weakly mutagenic</td>
<td>26</td>
</tr>
<tr>
<td>9.7 and 19.5 μg/ml; extracted with ethanol; extract contained 76% of total phenols</td>
<td>chromosomal aberration assay</td>
<td>human lymphocytes</td>
<td>negative</td>
<td>26</td>
</tr>
<tr>
<td><strong>PHOTOMUTAGENICITY – IN VITRO</strong></td>
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<tr>
<td><strong>Grape Skin</strong></td>
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<tr>
<td>0.001-10 mg/ml grape skin color (Vitis vinifera or Vitis labrusca) in PBS</td>
<td>Ames test of irradiated color: the color was irradiated with 4 black light bulbs (FL15BL-B) that emit light between 300-400 nm; most of the UVB was filtered; the bacterial suspension was irradiated for 30 min with 1.25 J/cm² UVA</td>
<td>S. typhimurium TA98, TA100, and TA102 with and without metabolic activation</td>
<td>no significant increase in mutations compared to irradiated suspension with grape skin color; 10 mg/ml non-irradiated grape-skin color was not mutagenic</td>
<td>88</td>
</tr>
<tr>
<td>0.01-1 mg/ml grape skin color (Vitis vinifera or Vitis labrusca) in PBS</td>
<td>photocytotoxicity; cell survival was measured before UVA, 1 h after UVA, and after 1 h UVA irradiation and 24 h incubation</td>
<td>WTK-1 cells</td>
<td>delayed cytotoxicity was observed with 1 mg/ml following 24 h incubation after UVA exposure</td>
<td>88</td>
</tr>
<tr>
<td><strong>IN VIVO</strong></td>
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<tr>
<td><strong>Grape Seed Extract</strong></td>
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<tr>
<td>0, 0.5, 1, or 2 g/kg in distilled water; extracted with water and ethanol; extract contained 89.3% proanthocyanidins</td>
<td>micronucleus test</td>
<td>5 or 6 mice were dosed orally; dose was repeated after 24 h</td>
<td>negative</td>
<td>27</td>
</tr>
<tr>
<td>0, 0.5, 1, or 2 g/kg in 0.5% aq. CMC; extract contained 90.5% total phenols by wt (genus and species not stated)</td>
<td>micronucleus test</td>
<td>6 male mice/group were dosed by gavage at a volume of 20 ml/kg; 24 h harvest for all doses; 48 h harvest for 0 and 2 g/kg groups</td>
<td>1 high-dose animal found dead 1h after dosing; cytotoxic (statistically significant decrease in the PCE:NCE ratio) at the 2 g/kg - 48-h harvest; no other cytotoxic effects were observed; not clastogenic</td>
<td>33</td>
</tr>
<tr>
<td><strong>Grape Seed/Grape Skin Extract</strong></td>
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</tr>
<tr>
<td>2 g/kg in saline; extracted with ethanol; extract contained 76% of total phenols</td>
<td>micronucleus test</td>
<td>6 female Wistar rats; blood samples were taken after 48 and 72 h</td>
<td>statistically significant increase in micronuclei after 48 h, but not after 72 h</td>
<td>26</td>
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<tr>
<td><strong>Grape Skin Extract</strong></td>
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<tr>
<td>0, 0.5, 1, or 2 g/kg in 0.5% aq. CMC; extract contained 87.3% total phenols by wt (genus and species not stated)</td>
<td>micronucleus test</td>
<td>6 male mice/group were dosed by gavage at a volume of 20 ml/kg; 24 h harvest for all doses; 48 h harvest for 0 and 2 g/kg groups</td>
<td>no clinical signs of toxicity; not cytotoxic or clastogenic</td>
<td>33</td>
</tr>
</tbody>
</table>

Abbreviations:  CMC = carboxymethylcellulose; DMSO – dimethyl sulfoxide; MMC = mitomycin C; PBS = phosphate-buffered saline; PCE:NCE = polychromatic erythrocyte: normochromatic erythrocyte; SCE = sister chromatid exchange;
<table>
<thead>
<tr>
<th>Test Article</th>
<th>Dose/Vehicle</th>
<th>Animals/Group</th>
<th>Procedure</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DERMAL APPLICATION</strong></td>
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<td><strong>Grape</strong></td>
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<tr>
<td>total extract of <em>Vitis vinifera</em> (all active ingredients of the plant); ethanolic fraction was used</td>
<td>5 and 10 mg/kg 20 Swiss albino female mice</td>
<td>- DMBA-initiation (40 µg/0.2 ml acetone) - after 2 wks, TPA-promotion (5 µg/0.2 ml acetone) - extract topically applied 1 h prior to TPA - applications made 2x/wk for 20 wks</td>
<td>time of appearance of first tumor was delayed by 3 wks (wk 9 vs. wk 6); dose-dependent inhibition of skin tumorigenesis; the number of mice with tumors was inhibited 40-50% and the number of tumors per mouse (tumor multiplicity) was inhibited 16-27%</td>
<td>39</td>
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<tr>
<td>Grape Seed</td>
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<tr>
<td>grape seed polyphenol as a lyophilized powder containing 95% (w/w) polyphenols; extracted with ethyl acetate</td>
<td>0, 0.5, and 1.5 mg/mouse applied in 0.1 ml acetone 20 female SENCAR mice</td>
<td>- DMBA-initiation (10 µg/0.1 ml acetone); 1 wk after initiation: Group 1 – 0.1 ml acetone applied Group 2 – 0.5 mg grape seed powder in acetone Group 3 – 1.5 mg grape seed powder in acetone - 30 min after application, TPA promotion (2 µg/0.1 ml acetone) in groups 1-3; applications were made 2x/wk for 19 wks Group 4 – 0.1 ml acetone applied; no DMBA initiation Group 5 – 1.5 mg grape seed powder applied, starting 1 wk after DMBA initiation, 2x/wk for 19 wks</td>
<td>Groups 1-3: time of appearance of the tumor in Groups 2 and 3 was delayed by 1 and 2 wks, respectively, compared to Group 1; grape seed powder significantly inhibited TPA tumor promotion in a dose-dependent manner as evidenced by a reduction in tumor incidence (35 and 60% inhibition), total number of tumors (61-83% inhibition), and tumor volume per mouse (48 and 63% decrease); tumor growth was not significantly inhibited Group 4: no skin tumors were observed when grape seed powder was evaluated as a promoter - there were no differences in wt gain between animals exposed to grape seed powder and those that were not</td>
<td>35</td>
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<tr>
<td>grape seed polyphenolic fraction</td>
<td>0, 5, 10, or 20 mg in 0.4 ml acetone 20 female CD-1 mice</td>
<td>- DMBA-initiation (50 µg/0.2 ml acetone) - 2 wks later, grape seed was topically applied - 20 min after application, TPA promotion (5.2 µg/0.2 ml acetone) - applications were made 2x/wk for 15 wks</td>
<td>tumor incidence was inhibited by 30, 40, and 60% with 5, 10, or 20 mg grape pre-treatment, respectively; tumor multiplicity was significantly reduced 63, 51, and 94%, respectively; the % of tumors classified as papillomas was 94, 88, 97, and 100% in the 0, 5, 10, and 20 mg groups, and the remaining tumors were carcinomas</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>grape seed polyphenolic fraction</td>
<td>0 or 20 mg in 0.4 ml acetone 10 female CD-1 mice</td>
<td>- DMBA initiation, as above - 2 wks later, acetone or grape seed extract was applied derrmally 2x/wk for 15 wks - no TPA promotion</td>
<td>no tumors were observed in animals of either group</td>
<td>36</td>
<td></td>
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<tr>
<td><strong>Grape Seed Extract</strong></td>
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<tr>
<td>grape seed extract containing 95% proanthocyanidins</td>
<td>0, 1, 2.5, or 5 µmol in 0.2 ml acetone female SENCAR mice, no. per group not specified</td>
<td>- DMBA (0.1 µmol in 0.2 ml acetone) applied topically 2x/wk for 4 wks - extract applied 20 min prior to DMBA</td>
<td>DMBA alone induced dermal hyperplasia, increasing epidermal thickness by 4.6 times the normal average; grape seed extract inhibited DMBA-induced hyperplasia in a dose-dependent manner; DMBA induced mutations in the Ha-ras oncogene; the extract had a dose-dependent inhibitory effect on the number of animals with Ha-ras mutations</td>
<td>38</td>
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<tr>
<td>grape seed extract containing 95% proanthocyanidins</td>
<td>0, 1, and 2.5 µmol female SENCAR mice, no. per group not specified</td>
<td>- DMBA (0.1 µmol in 0.2 ml acetone) applied topically 2x/wk for 4 wks - extract applied 20 min prior to DMBA</td>
<td>DMBA alone increased epidermal thickness 5x as well as the PCNA level; application of the extract statistically significantly inhibited both increases in a dose-dependent manner</td>
<td>39</td>
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<tr>
<td><strong>Grape Fruit Powder/Grape Seed Extract</strong></td>
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<tr>
<td>freeze-dried grape powder (from fresh red, green, and blue-black Cal. grapes; genus/species not stated); powdered grape seed extract containing 95% proanthocyanidins</td>
<td>1, 2, or 4 mg each 15 female SENCAR mice</td>
<td>- DMBA (0.1 µmol; vol. 0.2 ml), 2x/wk for 4 wks - 30 min after DMBA application, grape test article was applied - 5 mice/group were killed 2 days, 4 wks, or 8 wks after dosing - some animals were dosed for 24 wks</td>
<td>DMBA treatment produced epidermal hyperplasia, and both grape test substances inhibited the hyperplasia; % PCNA-positive cells decreased in a dose-dependent manner, and the change was statistically significant with 4 mg topical powder for the animals killed after 24 wks, there was clear reduction in the number of papillomas in animals dosed with 2 mg grape powder</td>
<td>41</td>
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</tr>
<tr>
<td>Test Article</td>
<td>Dose/Vehicle</td>
<td>Animals/Group</td>
<td>Procedure</td>
<td>Results</td>
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<tr>
<td><strong>DIETARY ADMINISTRATION</strong></td>
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<tr>
<td><strong>Grape Fruit Powder</strong></td>
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<td>15 female SENCAR mice</td>
<td>mice were given treated feed 2 wk prior to DMBA for up to 12 wks - DMBA (0.1 μmol; vol. 0.2 ml), 2x/wk for 4 wks - some animals were given treated feed for 24 wks</td>
<td>DMBA treatment produced epidermal hyperplasia, dietary grape powder inhibited the hyperplasia; % PCNA-positive cells decreased in a dose-dependent manner with treated feed, and the change was statistically significant with 2 and 5% powder in feed for 12 wks for the animals dosed for 24 wks, there was clear reduction in the number of papillomas in animals fed the grape powder</td>
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</tr>
<tr>
<td><strong>Grape Seed Extract</strong></td>
<td></td>
<td>female SENCAR mouse, no. per group not specified</td>
<td>- rats were fed the extract in the diet - after 2wks of treated diet, DMBA (0.1 μmol in 0.2 ml acetone) applied topically 2x/wk for 4 wks</td>
<td>DMBA alone increased epidermal thickness 5x and increased the PCNA level; dietary exposure to the extract statistically significantly inhibited both increases in a dose-dependent manner</td>
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<tr>
<td><strong>as above</strong></td>
<td></td>
<td>20 female C3H/HeN mice</td>
<td>DMBA-initiation (0.4 μmol/0.2 ml acetone) - after 1 wk, TPA promotion (0.01 μg/0.1 ml acetone); 2x/wk for 27 wks - treated diet was started with TPA application</td>
<td>time of appearance of first tumor was delayed by 4 wks (0.2% group) and 10 wks (0.5% group); tumor incidence decreased 20% in the 0.2% group (not statistically significant) and 35% in the 0.5% group (statistically significant) (12, 8 and 5 mice of the 0, 0.2, and 0.5% groups each had tumors); number of tumors per group decreased by 43% (0.2% group) and 70% (0.5% group); tumor size was significantly decreased in both test groups; 20% of the mice given untreated feed developed carcinoma, while only 5% of the mice of the 0.2% group and none in the 0.5% group developed carcinoma</td>
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<tr>
<td><strong>as above</strong></td>
<td></td>
<td>10 female C3H/HeN mice</td>
<td>DMBA initiation as above - after 1 wk, fed treated diet for 27 wks; no TPA promotion - a control group for spontaneous tumors was treated with 0.2 ml acetone 2x/wk</td>
<td>no tumors were observed in animals of either group</td>
<td></td>
</tr>
<tr>
<td><strong>as above</strong></td>
<td>0.5% in feed</td>
<td>5 female C3H/HeN mice</td>
<td>- mice were fed treated feed - either 1 wk later, a single application of 5 μg TPA was made and the mice were killed after 6, 12, or 24 h or TPA was applied 3x on alternate days and the mice were killed 6 h after the last application - skin edema was measured using skin punches and bi-fold skin thickness measurements</td>
<td>- TPA caused an increase in mean epidermal thickness and vertical thickness of epidermal cell layers - grape seed extract significantly reduced the epidermal thickness after multiple TPA applications and in mice killed 12 and 24 h after a single application of TPA - dietary extract without TPA treatment did not induce an epidermal hyperplastic response - TPA-induced increases in skin punch wt were reduced by feeding the extract; bi-fold skin thickness was also reduced</td>
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</tr>
<tr>
<td>grape seed extract containing 89.3% proanthocyanidins</td>
<td>0, 0.25, and 0.5% in feed</td>
<td>7 male F344 rats</td>
<td>Group 1: control feed for 10 wks Group 2: control feed for 10 wks; after 1 wk, s.c. AOM 1x/wk for 2 wks Group 3: 0.25% in feed for 10 wks; after 1 wk of treated feed, s.c. AOM 1x/wk for 2 wks Group 4: 0.5% in feed for 10 wks; after 1 wk of treated feed, s.c. AOM 1x/wk for 2 wks Group 5: s.c. AOM 1x/wk for 2 wks; 4 wks later, 0.25% in feed for 4 wks Group 6: s.c. AOM 1x/wk for 2 wks; 4 wks later, 0.5% in feed for 4 wks Group 7: 0.5% in feed for 10 wks</td>
<td>intestinal AOM-induced ACF were statistically significantly decreased in groups 3-6 compared to group 2 – the inhibition was stronger in groups 3 and 4 (50-60% inhibition) than in groups 5 and 6 (34-37% inhibition); the number of ACF consisting of 1-4 or &gt;4 crypts was decreased in groups 3-6 compared to group 2; PCNA-positive cells were decreased in groups 3-6 compared to group 2, and the AOM-induced PCNA labeling index in the colonic mucosa was decreased; induction of apoptosis in groups 3-6 as evidence by a significant increase in the number of TUNEL-positive cells</td>
<td></td>
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</tbody>
</table>
**Table 11. Inhibition of Tumor Promotion**

<table>
<thead>
<tr>
<th>Test Article</th>
<th>Dose/Vehicle</th>
<th>Animals/Group</th>
<th>Procedure</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grape Seed Extract</strong></td>
<td></td>
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</tbody>
</table>
| grape seed extract containing 89.3% proanthocyanidins | 0, 0.2, and 0.5% in feed | 20 female SKH-1 hairless mice | - mice were fed treated feed for 14 days  
- starting on day 15, the mice were irradiated with 180 mJ/cm² every day for 10 days  
- 1 wk after the last UV exposure, mice were again irradiated with 180 mJ/cm² 3x/wk for 29 wks | latency period of tumors was increased by 2 wks by feeding the extract; inhibition of tumor incidence was statistically significant in the 0.5% group (35% inhibition; tumor multiplicity 46 and 65% with 0.2 and 0.5%, respectively), tumor size expressed in terms of total tumor volume per group or total tumor volume per tumor bearing mouse, and avg. tumor volume per tumor was significantly inhibited at both doses | 34 |
| | 0 and 0.5% in feed | 20 female SKH-1 hairless mice | same protocol as above performed to examine effect on malignant conversion of papillomas into carcinomas | 45% prevention by extract in terms of carcinoma incidence; prevention of UVB-induced transformation of benign papillomas to carcinomas was 65%, but when analyzed in terms of number carcinomas per carcinoma bearing mouse, there was no inhibition by the extract | 34 |
| | 0 and 0.5% in feed | 20 female SKH-1 hairless mice | - mice were fed treated feed for 14 days  
- starting on day 15, the mice were irradiated with 180 mJ/cm² every day for 10 days  
- 1 wk after the last UV exposure, both groups were treated topically with TPA (0.01 μmol/0.1 ml acetone); 3x/wk for 23 wks | latency period of tumors was increased by 3 wks by feeding the extract; a highly significant reduction in tumor incidence was observed (95%); between wks 13-15 of promotion, 10-20% of extract-fed mice developed tumors that regressed later; since these tumors were not present at the termination of the study, they were not included in tumor multiplicity and tumor multiplicity decreased by 95%; total tumor volume per group and per tumor bearing mouse was reduced | 34 |
| grape seed extract containing 89.3% proanthocyanidins | 0 and 0.5% in feed | 20 female SKH-1 hairless mice | - DMBA initiation (51.2 μg/0.01 ml acetone)  
- after 1 wk, UVB irradiation (promotion; 180 mJ/cm²); 3x/wk for 24 wks  
- treated diet was started with UVB exposure | latency period of tumors was increased by 3 wks by feeding the extract; feeding the extract resulted in a 60% reduction in the total number of tumors per group, a 74% reduction in total tumor volume per group, a 63% reduction in terms of tumor volume per tumor bearing mouse, and a 29% reduction in average tumor volume per tumor | 34 |

Abbreviations: ACF – aberrant crypt foci; AOM = azoxymethane; DMBA = dimethylbenz[a]anthracene; PCNA = proliferating cell nuclear antigen; TPA = 12-O-tetradecanoylphorbol-13-acetate
Table 12. Dermal irritation and sensitization

<table>
<thead>
<tr>
<th>Test Article</th>
<th>Concentration</th>
<th>Test Pop.</th>
<th>Procedure</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IN VITRO – IRRITATION</strong></td>
<td></td>
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<tr>
<td><em>Vitis Vinifera (Grape) Fruit Extract</em></td>
<td>3% in a sample product (extracted in water)</td>
<td>neat; test vol., 25 -125 µl</td>
<td>dermal irritation test method, standard volume-dependent dose-response study</td>
<td>predicted to be a non-irritant in human skin; human irritancy equivalent scores ranged from 0.46 to 0.61</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>product containing 10% (extracted in water)</td>
<td>neat</td>
<td></td>
<td>non-irritating/minimal</td>
<td>91</td>
</tr>
<tr>
<td><strong>Hydrolyzed Grape Skin</strong></td>
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<tr>
<td></td>
<td>hydrolyzed grape skin</td>
<td>neat</td>
<td>cultured human keratinocytes (HaCaT cells)</td>
<td>MTT cytoxicity test; 0.15 – 5 mg/ml were tested; SLS was used as a positive control</td>
<td>predicted to be non-irritating; the IC₅₀ was &gt;5 mg/ml (irritating)</td>
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<tr>
<td><strong>NON-HUMAN – IRRITATION</strong></td>
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<tr>
<td><em>Vitis Vinifera (Grape) Seed Extract</em></td>
<td>as trade name ActiVin</td>
<td>neat</td>
<td>New Zealand White rabbits; 3M/3F</td>
<td>4-h semi-occlusive application; 0.5 g of the extract moistened with 0.3 ml deionized water; applied to an intact 1 in x 1 in area of clipped skin; collars were used</td>
<td>classified as moderately irritating; all rabbits had slight to severe erythema, very slight to slight edema, and desquamation; erythema completely subsided by day 6, edema by day 8; exfoliation in one animal, eschar in 2 animals; all dermal irritation subsided by day 12</td>
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<td><strong>HUMAN – IRRITATION</strong></td>
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<tr>
<td><em>Vitis Vinifera (Grape) Seed Extract</em></td>
<td>0.15% in an after shave balm (extraction solvents were butylene glycol and water)</td>
<td>neat</td>
<td>31 male subjects</td>
<td>2-wk in-use study; product was applied at least once daily to shave skin of the face and neck</td>
<td>not an irritant; no evidence of erythema, edema, or drying</td>
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<tr>
<td><strong>IN VITRO- SENSITIZATION</strong></td>
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<tr>
<td><em>Hydrolyzed Grape Skin</em></td>
<td>hydrolyzed grape skin in ethanol</td>
<td>4 and 20 µg/ml</td>
<td>monocyte-like human cell line, THP-1 cells</td>
<td>cells were exposed for 48 h; CD80 and CD86 were used as co-stimulatory molecules; MFI was measured using a FACS; MFI of non-treated THP-1 cells was used as an internal control; nickel sulfate was used as a positive control</td>
<td>did not increase the expression of the investigated markers and did not show any stimulating potential of the immune cellular response mediated by monocyte/ macrophage</td>
</tr>
<tr>
<td><strong>HUMAN – IRRITATION AND SENSITIZATION</strong></td>
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<tr>
<td><em>Vitis Vinifera (Grape) Fruit Extract</em></td>
<td>0.0239% in a make-up primer</td>
<td>neat</td>
<td>103 subjects</td>
<td>modified HRIPT – semi-occlusive; 0.15 ml on a 20 x 20 mm pad; 9 24-h induction applications; 24-h challenge application at treated and untreated sites followed a 17 or 24-day non-treatment period</td>
<td>not an irritant or sensitizer</td>
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<td></td>
<td>blend containing 3%</td>
<td>tested at 1% aq.</td>
<td>108 subjects</td>
<td>HRIPT - semi-occlusive; 0.02-0.05 ml on a 7.5 mm paper disc; 9 24-h induction applications; challenge application at a previously untreated site after a 10-14 day non-treatment period</td>
<td>not an irritant or sensitizer</td>
</tr>
<tr>
<td></td>
<td>product containing 6%</td>
<td>10% in deionized water</td>
<td>97 subjects</td>
<td>modified HRIPT - semi-occlusive; 150 mg on a 20 x 20 mm pad; 9 24-h induction applications;24-h challenge at treated site and 48-h challenge at untreated site followed a 10-day non-treatment period</td>
<td>not an irritant or sensitizer</td>
</tr>
<tr>
<td></td>
<td>product containing 10%</td>
<td>neat</td>
<td>54 subjects</td>
<td>HRIPT – occlusive; 0.2 ml on a 20 x 20 mm Webril pad; 9 24-h induction applications; 24-h challenge at a previously untreated site after a 10-14 day non-treatment period</td>
<td>not an irritant or sensitizer</td>
</tr>
<tr>
<td><em>Vitis Vinifera (Grape) Juice</em></td>
<td>make-up primer containing 0.1%</td>
<td>neat</td>
<td>208 subjects</td>
<td>HRIPT – semi-occlusive; same induction protocol;24-h challenge application applied to a previously untreated site after a 2-wk non-treatment period</td>
<td>not an irritant or sensitizer with the exception of an occasional ± score (barely perceptible erythema), no visible reactions were noted</td>
</tr>
<tr>
<td>Test Article</td>
<td>Concentration</td>
<td>Test Pop.</td>
<td>Procedure</td>
<td>Results</td>
<td>Reference</td>
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<td><em>Vitis Vinifera (Grape) Juice Extract</em></td>
<td>hair styling product containing 0.5%</td>
<td>neat</td>
<td>100 subjects</td>
<td>modified HRIPT – occlusive; 21-day induction period, 10-24 day non-treatment period, 4-day challenge</td>
<td>not an irritant or sensitizer</td>
</tr>
<tr>
<td><em>Vitis Vinifera (Grape) Seed Extract</em></td>
<td>body lotion formulation containing 0.0002%</td>
<td>neat</td>
<td>101 subjects</td>
<td>modified HRIPT – occlusive; 21-day induction period, 10-24 day non-treatment period, 4-day challenge</td>
<td>not an irritant or sensitizer</td>
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<tr>
<td></td>
<td>hair conditioner containing 0.1%</td>
<td>10% aq. dilution</td>
<td>105 subjects</td>
<td>modified HRIPT – semi-occlusive; 0.2 ml on a 20 x 20 mm pad; 9 24-h induction applications, 24-h challenge application at treated and untreated sites followed a 10-day non-treatment period</td>
<td>not an irritant or sensitizer</td>
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<td></td>
<td>after shave balm containing 0.15% (extraction solvents were butylene glycol and water)</td>
<td>not stated; presumed neat</td>
<td>105 subjects</td>
<td>HRIPT – occlusive; 0.2 ml; air-dried at 20+ min prior to application; 9 24-h induction applications; 24-h challenge followed a 10-day non-treatment period</td>
<td>not a sensitizer; no reactions at challenge during induction, 1 subject had a minimal/doubtful response (?) at readings 2-4 and erythema (+) was observed at readings 5-8; 1 subject had a ? response at readings 1-2 and one subject had a ? response at reading 2</td>
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<td>raw material containing 1%</td>
<td>neat</td>
<td>107 subjects</td>
<td>modified HRIPT – semi-occlusive; 0.15 ml on a 20 x 20 mm pad; 9 24-h induction applications, 24-h challenge application at treated and untreated sites followed a 10-day non-treatment period</td>
<td>not an irritant or sensitizer; five grade 1 and 1 grade 2 response noted during induction; grade 1 response were noted for 3 subjects during challenge</td>
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</table>

Abbreviations: FACS = fluorescence activated cell sorter; HRIPT = human repeated insult patch test; MFI = mean fluorescence intensity; MTT = 3-(4,5-di-methyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide; SLS = sodium lauryl sulfate


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96. BioScreen Testing Services Inc. 2009. 100 Human Subject repeat insult test patch test skin irritation/sensitization valuation of Blend 3EL-New (contains 3% Vitis Vinifera (Grape) Fruit Extract (water extract)). Unpublished data submitted by the Personal Care Products Council.


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102. Product Investigations Inc. 2010. Determination of an irritating and sensitizing propensities of a hair conditioner containing 0.1% Vitis Vinifera (Grape) Seed Extract (10% dilution tested). Unpublished data submitted by Personal Care Products Council.


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<tr>
<td>VITIS VINIFERA (GRAPE) 05A - Hair Conditioner</td>
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<td>VITIS VINIFERA (GRAPE) 12D - Body and Hand (exc shave)</td>
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<td>VITIS VINIFERA (GRAPE) 12F - Moisturizing</td>
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Vitis Vinifera (Grape) Fruit Extract as Vitis Vinifera Extract

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<td>VITIS VINIFERA EXTRACT 06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)</td>
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VITIS VINIFERA (GRAPE) FRUIT POWDER 02A - Bath Oils, Tablets, and Salts

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</table>
26453: VITIS VINIFERA (GRAPE) SHOOT EXTRACT

INCI Monograph ID: 26453

Flags: ReadyToPublish, OTCDrug = True, OTCApproved = False

Definition: Vitis vinifera (Grape) Shoot Extract is the extract of the shoots of the vines of Vitis vinifera. See Reported Ingredient Functions-The Cosmetic Drug Distinction, in Regulatory and Ingredient Use Information, Volume I, Part A.

Chemical Class: C2018- Botanical Products and Botanical Derivatives

Reported Functions: F60- Antioxidant; F580- Skin Protectant

Ingredient Source: Plant

Trade Name: N99232- French Grapevine Extract (S1781- Berken)
Memorandum

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

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

DATE: December 19, 2011

SUBJECT: Information: Hydrolyzed Grape Skin

Phenbiox SRL. 2011. Technical data sheet UVIOX (Hydrolyzed Grape Skin)

Phenbiox SRL. 2011. Safety data sheet UVIOX (Hydrolyzed Grape Skin)


TECHNICAL DATA SHEET

UVIOX

Product: UVIOX is aqueous bio-liquefied Red Grape skins (INCI Name / CTFA: Hydrolyzed Grape (Vitis vinifera) Skin).

Characteristics:

➢ Aspect: aqueous solution (with some slight sedimentation)
➢ Color: ruby red
➢ Odor: characteristic, fruity
➢ pH: 2.6-3.5
➢ Dry residue: ≥ 1.5 % w/w
➢ Sugars: 2 - 6 g/Kg
➢ Phenols\(^1\): 700-1500 mg/Kg
➢ Antioxidant capacity expressed as ORAC\(^2\)/L: 3000-10000 TE
➢ Preservatives: citric acid 1.0 % w/w, sodium benzoate 0.2 % w/w, potassium sorbate 0.1 % w/w.

Recommendations:

The active principles in UVIOX are affected by pH variation. Neutral and basic pH condition can produce variation of color. The reactivity of the active principles in these conditions is far higher, which can cause a decrease in the stability of UVIOX.

Store in a cool dry place, avoid prolonged exposure to light.

Last update: 25/10/2011

Phenbiox s.r.l.

Notes:

\(^1\) Determined by the Folin-Ciocalteau method.
\(^2\) Oxygen Radical Absorbance Capacity: expressed as \(\mu\)mols of Trolox\(^\circ\) Equivalents per liter of UVIOX (TE/l).
SAFETY DATA SHEET  UVIOX

Last update: 02/03/2011

1- Chemical product and company identification

Product name: UVIOX
INCI name: hydrolyzed grape skin
CAS n°: 84929-27-1
Kind of product: bioliquefied aquose vegetal
Uses: active principle for cosmetic products
Company: Phenbiox s.r.l.
Via D' Azeglio 51, 40123 Bologna
Telefono: +39 328 6014923
Fax: +39 051 6345021

2- Composition, information on ingredients

Product name: UVIOX
Ingredients: Acqua, Vitis vinifera, citric acid, sodium benzoate, potassium sorbate.

3- Hazards identification

No known health hazards

4- First aid measures

Ingestion: considered not hazardous
Inhalation: considered not hazardous
Skin contact: considered not hazardous
Eye contact: considered not hazardous

5- Fire fighting measures

Extinguishing media: water spry, powders, carbon dioxide.

6- Accidental release measures

CLEANING TECHNIQUES

Collect with a mop and store in a bucket until disposal. Clean the contaminated zone with water.
7- Handling and storage

HANDLING

No special precautions are necessary for safe handling.

STORAGE

Store under dry conditions at room temperature in tightly closed containers, in dark place. Keep out of direct sunlight.

8- Exposure controls, personal protection

No particular protections needed.

9- Physical and chemical property

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<tr>
<th>Property</th>
<th>Value</th>
<th>Temperature or pressure</th>
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<td>pH</td>
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<td>Boiling point</td>
<td>98-102°C</td>
<td>760mmHg</td>
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<tr>
<td>Flash point</td>
<td>&gt; 100°C</td>
<td></td>
</tr>
<tr>
<td>Density</td>
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<td></td>
</tr>
<tr>
<td>Water content</td>
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</tr>
<tr>
<td>Solubility</td>
<td>Water solubility: completely soluble</td>
<td>Other solvent: soluble in alcohol and acetone</td>
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</table>

10- Stability e reactivity

CHEMICAL STABILITY

Stabile.
Condition to avoid: exposure to oxidant agent and light.

HAZARDOUS DECOMPOSITION PRODUCTS

None known.

HAZARDOUS POLYMERIZATION

None known.
11- Toxicological information

The product is not expected to be hazardous to human beings.

TOXICOLOGICAL INFORMATION

Eye irritation potential:
The product did not show an IC₅₀ higher than 5 µl/ml on fibroblasts with the NRU assay. The data is predictive of absence of irritating effects in vivo.

Skin irritation/cytotoxicity potential:
The product did not show an IC₅₀ higher than 5 µl/ml on human keratinocytes with the MTT assay. The data is predictive of a very good biocompatibility in vivo.

Pro-sensitising potential:
The product does not affect the investigated markers expression (CD80, CD86) in immunocompetent cells (monocyte cell line THP-1) and hence it does not show any stimulating potential on the immune cellular response mediated by monocyte/macrophage.

12- Ecological information

The product is not expected to be hazardous to environment.

ECOTOXICITY

No data available.

13- Disposal conditions

Product: Dispose of considering local authority regulations.
Container: Can be recycled

14- Transport information

RID/ADR
Non-hazardous for transport.

IMDG
Non-hazardous for sea transport.

IATA
Non-hazardous for air transport.

15- Regulatory information

Labelling According to Directives 67/548/EC, 1999/45/EC and CLP-Regulation EC No 1272/2008:

Symbol : N/A
R-phrase : N/A
S-phrase : N/A
This product is not considered a dangerous substance in accordance with the European Directives 88/379/EC, 67/548/EC its amendment 97/69/EC and CLP-Regulation EC No 1272/2008.

16- Additional information

The information contained herein is based on the present state of our knowledge and does not therefore guarantee certain properties. Since the conditions of handling and use are beyond our control, we make no guarantee of results, and assume no liability for damage incurred by use of this material. It is the responsibility of the user to comply with all applicable laws and regulations.
# Valutazione in vitro del potenziale di irritazione oculare

*In vitro evaluation of the eye irritation potential*

<table>
<thead>
<tr>
<th>COMMITTENTE/CUSTOMER</th>
<th>Università di Bologna - Dipartimento di chimica Industriale e dei Materiali Viale Risorgimento 4 40136 Bologna (BO) - Italia</th>
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<tr>
<td>CAMPIONE/SAMPLE</td>
<td>Hydrolyzed grape (<em>vitis vinifera</em>) fruit skin Lotto/Batch: LPUV 031-09</td>
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<td>DATA RAPPORTO/REPORT DATE</td>
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Premessa/Preliminary

Questo rapporto contiene i dati sperimentali registrati durante l'esecuzione del test di tollerabilità eseguito sul prodotto in oggetto.
I risultati del test sono presentati sotto forma di tabelle e grafici riassuntivi per agevolare l'interpretazione.
La prima parte fornisce informazioni circa il committente, il prodotto testato, il tipo di test, il laboratorio esecutore, le date di inizio e di fine studio e l'identità degli sperimentatori.
La seconda parte descrive il protocollo sperimentale.
La terza parte riporta i risultati e le conclusioni.

Nota/Note:
Il risultato dei test citati nel presente rapporto si riferisce esclusivamente al prodotto/i testato/i e alle particolari condizioni sperimentali impiegate nel test. Il presente rapporto o parti di esso possono essere ripubblicati solo con il consenso degli sperimentatori.

The test results are presented in a concise table format for easy interpretation.
The first part provides information regarding sponsor and test product identifications, assay type, entrusted laboratory, study initiation and completion dates and supervisory personnel.
The second part describes the study design, including materials and procedures.
The test results are presented in the third and last part of the report.

The results reported in the present brochure refer only to the tested sample/samples and to the particular experimental conditions hereby described. This report or parts of it can be reproduced only with the experimenters' agreement.
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1 PARTE PRIMA/PART ONE – INFORMAZIONI GENERALI/GENERAL INFORMATION

1.1 Commissitente/Customer

Università di Bologna - Dipartimento di chimica Industriale e dei Materiali
Viale Risorgimento 4
40136 Bologna (BO) - Italia

1.2 Campione Analizzato/Tested Material

<table>
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<th>Codice interno/ Internal code</th>
<th>Descrizione/Description</th>
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<td>1085/09-02</td>
<td>Liquido trasparente rosso /Clear red liquid</td>
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In aggiunta, il Sodio Lauril Solfato (SLS) è stato aggiunto nell'esperimento come controllo positivo irritante.

In addition, as an irritant positive control, Sodium Lauryl Sulfate (SLS) was included in the experimental set.

1.3 Test/Assay

➢ Citotossicità attraverso il test NRU: test di sopravvivenza cellulare con fibroblasti coltivati in monostato per la valutazione del potenziale di irritazione oculare e della biocompatibilità.

➢ Cytotoxicity by NRU test: cell survival assay using fibroblasts in monolayer cultures to assess eye irritation potential and biocompatibility.

1.4 Laboratorio incaricato/Entrusted laboratory

ABICH S.r.l.
Via 42 Martiri, 213/B – 28924
Verbania - Italy - tel +39 (0)323 586239 fax +39 (0)323 496877

1.5 Date dello studio/Study dates

Inizio/Initiation: 08/07/2009
Fine/End: 10/07/2009
1.6 Ricercatore principale/Main investigator

Dr. Aliffranchini Elena, Biotecnologa/Biotechnologist
ABICH S.r.l.

1.7 Direttore dello studio/Study director

Dr. Elena Bocchietto – Biologa specialista in
Biotecnologie / Biologist, biotechnology specialist.
- ABICH S.r.l. –
2 PARTE SECONDA/PART TWO: PROTOCOLLO SPERIMENTALE/STUDY DESIGN

2.1 Scopo del test/Aim of the test


Il saggio di citotossicità può essere eseguito al fine di valutare la potenziale irritazione oculare di un prodotto o di un ingrediente o miscela di ingredienti utilizzando colture di fibroblasti. Il test in vitro risulta essere un metodo sperimentale semplificato, ma in grado di dare molte informazioni sulle reazioni che possono verificarsi in vivo.

Test di citotossicità eseguiti su fibroblasti o cheratinociti in monostromato, sono stati ampiamente usati come indicatori predittivi del potenziale di irritazione oculare e cutanea di prodotti per uso cosmetico o biomedicale. L’obiettivo di questo test è stabilire quantitativamente gli effetti dei prodotti testati sulla vitalità cellulare attraverso il test di colorazione con NRU. Il test NRU è un metodo chimio-sensibile per la vitalità cellulare, basato sull’abilità delle cellule vive di incorporare e legare il Rosso Neutro (RN), un colorante vitale. Il RN è un debole colorante cationico che penetra la membrana cellulare per diffusione non ionica e si accumula nei lisosomi dove si lega nei siti anionic di matrice. Alterazioni della superficie cellulare o della sensibilità della membrana lisosomiale portano alla fragilità del lisosoma e ad altri cambiamenti che gradualmente diventano irreversibili. Questi cambiamenti dovuti allazione di xenobioci portano ad una diminuzione dell’”uptake” e del legame del RN. Con questo metodo è possibile distinguere tra cellule vive, danneggiate o morte.

Un limite di questa metodica è costituito dal fatto che sostanze del tutto insolubili in un medium acquoso possono non giungere a contatto con le cellule e quindi la loro potenziale tossicità può essere sottostimata. In casi in cui si sospettino condizioni di questo genere è quindi opportuno approfondire lo studio con test su epidermide ricostituita o in vivo nell’animale.

In vitro methods are an interesting alternative system to traditional in vivo tests to evaluate biological properties of biomedical and cosmetic products or raw material, according to the current European cosmetic rules that ask manufacturers to assess the product safety, without employing animals (Basic Council Directive N°76/768/ EEC of 27/07/76, EC L. 262 of 27/09/1976; VI Amendment Council Directive 76/768 EEC of the 14/06/1993 ECL.151 of the 23/06/1993). Furthermore, the use of in vitro tests, instead of in vivo models, is strongly recommended by the UNI/EN 10993 rules.

Cytotoxicity assays can be carried out in order to evaluate in vitro the potential eye irritation of a product on fibroblast cultures. The cytotoxicity assay performed in this study was designed to evaluate the eye irritation potential of the tested product using cells grown in vitro.

Cytotoxicity test performed on keratinocytes or fibroblasts in monolayer have been extensively used as indicator of the eye and skin irritation potential of a cosmetic product or of a medical device.

The objective of this assay was to assess quantitatively the effects of the test materials on cell survival through the NRU assay. A limit of this method is the fact that substances that are totally insoluble in a water-based medium may not get in contact with the cells and hence their toxicity may be underestimated. When such a case is suspected it is advised to investigate further the substance effect on tridimensional epidermis models or in vivo in the animal. The NRU assay is based on the cell ability to incorporate and bind the Neutral Red.

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REI/0444/2009/IRRO/ELB

ABICH S.r.l.
Via 42 Martiri, 213/B – 268924 – Fondotoce Verbano (Italy)
Tel: 0323 586239 / 0323 496041 - Fax: 0323 496877
www.abich.it - e.mail: info@abich.it


CIR Panel Book Page 69
(NR), a vital dye. The NR is a week cationic dye that penetrates the cell membrane through a mechanism of non ionic diffusion and that is accumulated in the lysosomes, on matrix anionic sites. Cell and lysosome membrane alterations cause lysosomes fragility and gradual irreversible changes in the cells. These changes induced by xenobiotics determinate the decrease of NR uptake and of its linkage to lysosomes. This method is able to discriminate alive, damaged or dead cells. Cells are incubated with scalar concentrations of the products and with the Neutral Red solution (NR). If the membrane is damaged, it releases the dye in the medium.

2.2 Esecuzione dei test/Assay procedures

2.2.1 Modello cellulare/ Cell model
Il modello cellulare utilizzato per il test in vitro è rappresentato da: *In vitro* test system employed consists of:

fibroblasti murini (cellule 3T3)/murine fibroblasts (3T3)

La linea cellulare deriva da fibroblasti di toppo albino ceppo Swiss stabilizzati da embrioni/Cell line derives from Swiss albino mouse fibroblasts established from albino mouse embryos.

2.2.2 Trattamento ed Esposizione/Treatment and Exposure
Le cellule sono state seminate in piastre da 96 pozzetti per 24h in MEM + 10% FBS. Quindi è stato aggiunto terreno di coltura fresco arricchito con 10% di FBS e contenente il prodotto da testare in modo da raggiungere 6 diluzioni finali comprese tra 5,00 e 0,15mg/ml. Il campione è stato sciolto in acqua alla concentrazione di 50mg/ml.

Ogni campione è stato testato in triplo. Cellule non trattate sono state utilizzate come controllo negativo, mentre come controllo positivo è stato utilizzato un tensioattivo a tossicità nota (Sodio Lauril Solfato - SLS) disolto nel terreno di coltura alle concentrazioni comprese tra 0,5mg/ml a 0,03 mg/ml.

Al termine dell’incubazione è stato quindi eseguito il test di citotossicità (NRU) per valutare la percentuale di sopravvivenza cellulare. Il test NRU valuta l'impatto tossico della sostanza in questione sulla vitalità cellulare.

Cells are seeded in 96 wells plates, for 24 h in MEM + 10% FBS. Fresh medium is added, supplemented with only 10 % FBS and with 6 scalar dilutions of the tested product ranging from 5,00 e 0,15mg/ml. The sample has been dissolved in water at 50mg/ml concentration.

For each dilution, 3 replica were performed. After 24 hour incubation, cells are tested for vitality with the citotoxicity (MTT assay). Untreated cells are used as negative control. Cells treated with a known irritating surfactant (Sodium Lauryl Sulfate – SLS) in concentration ranging from 0,5mg/ml to 0,03mg/ml were used as positive control.

The NRU assay is able to evaluate the toxic impact of the tested compound on the cells viability.
2.2.3 *Test di citotossicità NRU/ The NRU cytotoxicity assay*

Dopo l'incubazione, si sostituisce il terreno con la soluzione allo 0.33% di Rosso Neutro, le cellule vengono incubate a 37°C per 3h. Quindi vengono sottoposte a diversi lavaggi per eliminare i residui di colorante in eccesso, mentre il colorante incorporato nelle cellule viene sciolto con una soluzione solubilizzante l'NR e quantificato spettrofotometricamente a 550nm.

La piastra viene agitata su un agitatore a piastra assicurandosi che tutti i cristalli si siano sciolti ed abbiano formato una soluzione omogenea. Viene letta l'assorbanza con un colorimetro (Tecan modello Sunrise remote) equipaggiato con un lettore di piastrine sottraendo la lettura del fondino.
Il risultato è espresso come vitalità cellulare in percentuale secondo la formula:

\[
\text{% di vitalità cellulare} = \left(\frac{\text{OD}(550 \text{ nm} - 690 \text{ nm})}{\text{OD}(550 \text{ nm} - 690 \text{ nm}) \text{ controllo negativo}}\right) \times 100
\]

After incubation, the medium is replaced with 0.33% NR solution and cells are incubated for 3h at 37°C. Then cells are washed more times to eliminate exceeding dye waste, while the cells incorporate dye is dissolved with the NR Solubilization Solution and quantified spectrophotometrically at 550 nm.

The plate is shaken on a gyratory plate shaker, ensuring that all the crystals have dissolved from the cells and have formed a homogeneous solution. The absorbance is measured on a microplate reader (Tecan modello Sunrise remote), with background clearing.
The results are expressed in terms of viability:

\[
\text{% of cell viability} = \left(\frac{\text{OD}(550 \text{ nm} - 690 \text{ nm}) \text{ test product}}{\text{OD}(550 \text{ nm} - 690 \text{ nm}) \text{ negative control}}\right) \times 100
\]
2.2.4 Espressione e valutazione dei risultati/Expression and evaluation of results

I dati di citotossicità ottenuti con il test NRU sono messi in grafico contro la concentrazione del prodotto testato, creando una curva dose-risposta, che permette di determinare:

- la curva teorica di regressione
- il valore teorico di IC₅₀ (concentrazione di inibizione della crescita del 50%) ovvero la concentrazione che induce una riduzione della vitalità cellulare del 50% rispetto alle cellule non trattate

I risultati di citotossicità sono stati corretti sottraendo le letture di assorbanza dovute al mezzo diluente.

**Il valore IC₅₀ (Inhibiting Concentration 50) indica la concentrazione di prodotto necessaria per inibire la crescita cellulare del 50%. IC₅₀ è un parametro che consente di valutare il potenziale irritante di un composto**

The cytotoxicity data obtained with the NRU assay were plotted against the concentrations, which generate dose-response curves that allow to determine:

- the theoretical regression curve
- the theoretical IC₅₀ value (inhibiting concentration 50%), i.e. the concentration of test compound which causes a 50% decrease of cell survival as compared to untreated cultures

The cytotoxicity results have been corrected subtracting the cytotoxicity due to the diluent medium.

**The IC₅₀ value (Inhibiting Concentration 50) is the concentration of test compound which induces a 50% decrease of cell growth/survival. It makes it possible to evaluate the potential irritating effect of the compound**

*Per prodotti finiti, valori ≤ 1mg/ml (o 1μl/ml) possono essere considerati come irritanti. valori > 3mg/ml (o 3μl/ml) presentano un’ottima biocompatibilità. Per detergenti e prodotti da risciacquo, l’eventuale fattore di diluizione deve essere preso in considerazione. Per materie prime bisogna prendere in considerazione la concentrazione d’uso."

For finished products, values ≤ 1mg/ml (or 1μl/ml) may be considered as irritating, values> 3mg/ml (or 3μl/ml) show a very good biocompatibility. For detergents and rinsing off products, the eventual dilution factor should be taken in account. For raw materials, the use concentration should be taken in account.
3 PARTE TERZA/PART THREE - RISULTATI E CONCLUSIONI/RESULTS AND CONCLUSIONS

3.1 Risultati/Results

<table>
<thead>
<tr>
<th>SLS (controllo positivo/positive control)</th>
<th>0,50</th>
<th>0,25</th>
<th>0,13</th>
<th>0,06</th>
<th>0,03</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitalità cellulare % (rispetto al controllo negativo)/ % cell viability (vs negative control)</td>
<td>3,0</td>
<td>3,4</td>
<td>3,8</td>
<td>55,0</td>
<td>93,0</td>
</tr>
<tr>
<td>stand. dev.</td>
<td>0,3</td>
<td>0,3</td>
<td>0,7</td>
<td>5,9</td>
<td>8,2</td>
</tr>
</tbody>
</table>

IC₅₀ = 0,063mg/ml

Espressione della vitalità cellulare dopo trattamento con dosi crescenti di SLS/
Cell vitality expression after treatment with increasing amount of SLS
Curva di regressione per il calcolo dell’IC$_{50}$/Regression plot to calculate the IC$_{50}$ dose
**Hydrolyzed grape (vitis vinifera) fruit skin**
*Lotto/Batch: LPUV 031-09*
*Codice interno/internal code: 1085/09-02*

<table>
<thead>
<tr>
<th>Dose mg/ml</th>
<th>5</th>
<th>2,5</th>
<th>1,25</th>
<th>0,6</th>
<th>0,3</th>
<th>0,15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitalità cellulare % (rispetto al controllo negativo)/% cell viability (vs negative control)</td>
<td>106,5</td>
<td>101,9</td>
<td>105,3</td>
<td>112,4</td>
<td>86,9</td>
<td>79,9</td>
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<tr>
<td>stand. dev.</td>
<td>10,1</td>
<td>2,3</td>
<td>7,9</td>
<td>3,6</td>
<td>6,7</td>
<td>7,0</td>
</tr>
</tbody>
</table>

IC₅₀ > 5mg/ml

**Espressione della vitalità cellulare dopo trattamento con diverse diluizioni del prodotto testato/**
Cell vitality expression after treatment with different dilutions of the tested product
3.2 Conclusioni/Conclusions

Sulla base dei risultati sopra riportati il campione /On the bases of the results here shown, the sample:

Hydrolyzed grape (vitis vinifera) fruit skin
Lotto/Batch: LPUV 031-09

ha mostrato di possedere un’IC₅₀ maggiore di 5mg/ml su fibroblasti.
Questi dati possono essere considerati come predittivi dell’assenza di effetti irritanti per la mucosa oculare in vivo.

did show an IC₅₀ higher than 5mg/ml on fibroblasts.
These data are predictive of absence of irritating effects for the eye mucous membrane in vivo.

Data/Date: 17/07/2009

Il Direttore dello studio/ Study Director
Dr. Elena Bocchietto
4 BIBLIOGRAFIA/BIBLIOGRAPHY


Valutazione *in vitro* del potenziale di irritazione cutanea

*In vitro* evaluation of the skin irritation potential

<table>
<thead>
<tr>
<th>COMMITTENTE/CUSTOMER</th>
<th>Università di Bologna - Dipartimento di chimica industriale e dei Materiali Viale Risorgimento 4 40138 Bologna (BO) - Italia</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAMPIONE/SAMPLE</td>
<td>Hydrolyzed grape (<em>vitis vinifera</em>) fruit skin Lotto/Batch: LPUV 031-09</td>
</tr>
<tr>
<td>DATA RAPPORTO/REPORT DATE</td>
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</tr>
<tr>
<td>PROTOCOLLO N./REPORT N.</td>
<td>REL/0438/2009/IRRC/ELB</td>
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</table>

ABICH S.r.l.
Via 42 Martiri, 213/B – 28904 – Fondottoce Verbania (Italy)
Tel: 0323 585239 / 0323 496041 - Fax: 0323 496877
www.abich.it - e.mail: info@abich.it
PREMESSA/PRELIMINARY

Questo rapporto contiene i dati sperimentali registrati durante l'esecuzione del test eseguito sul prodotto in oggetto.
I risultati del test sono presentati sotto forma di tabelle e grafici riassuntivi per agevolare l'interpretazione.
La prima parte fornisce informazioni circa il committente, i prodotti testati, il tipo di test, il laboratorio esecutore, le date di inizio e fine studio e l'identità degli sperimentatori.
La seconda parte descrive il protocollo sperimentale.
La terza parte riporta i risultati e le conclusioni.

This report contains the experimental data compiled during the in vitro studies of the test product.
The test results are presented in a concise table format for easy interpretation.
The first part provides information regarding sponsor and test product identifications, assay type, entrusted laboratory, study initiation and completion dates and supervisory personnel.
The second part describes the study design, including materials and procedures.
The test results are presented in the third and last part of the report.

Nota/Note:
Il risultato dei test citati nel presente rapporto si riferisce esclusivamente al prodotto/i testato/i e alle particolari condizioni sperimentali impiegate nel test. Il presente rapporto o parti di esso possono essere riprodotti solo con il consenso degli sperimentatori.

The results reported in the present brochure refer only to the tested sample/samples and to the particular experimental conditions hereby described. This report or parts of it can be reproduced only with the experimenters' agreement.
Sommarlo/Summary

1 PARTE PRIMA/PART ONE – INFORMAZIONI GENERALI/GENERAL INFORMATION 4
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   1.3 TEST/ASSAY 4
   1.4 LABORATORIO INCARICATO/ENTRUSTED LABORATORY 4
   1.5 DATA DELLO STUDIO/STUDY DATES 4
   1.6 RICERCATORE PRINCIPALE/MAIN INVESTIGATOR 5
   1.7 DIRETTORE DELLO STUDIO/STUDY DIRECTOR 5

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   2.1 SCOPO DEL TEST/AIM OF THE TEST 6
   2.2 ESECUZIONE DEL TEST/ASSAY PROCEDURES 7
      2.2.1 Modello sperimentale/Cell model 7
      2.2.2 Trattamento ed esposizione/Treatment and exposure 7
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1 PARTE PRIMA/PART ONE – INFORMAZIONI GENERALI/GENERAL INFORMATION

1.1 Committente/Customer

Università di Bologna - Dipartimento di chimica Industriale e dei Materiali
Viale Risorgimento 4
40136 Bologna (BO) - Italia

1.2 Campione Analizzato/Tested Material

<table>
<thead>
<tr>
<th>Campione/Sample</th>
<th>Codice interno/ Internal code</th>
<th>Descrizione/Description</th>
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</thead>
<tbody>
<tr>
<td>Hydrolyzed grape (vitis vinifera) fruit skin Lotto/Batch: LPUV 031-09</td>
<td>1085/09-01</td>
<td>Liquido trasparente rosso / Clear red liquid</td>
</tr>
</tbody>
</table>

Il Sodio Lauril Solfato (SLS) è stato aggiunto nell’esperimento come controllo positivo irritante. As an irritant positive control, Sodium Lauryl Sulfate (SLS) was included in the experimental set.

1.3 Test/Assay

- Citotossicità attraverso il test MTT: test di sopravvivenza cellulare con cheratinociti umani coltivati in monostrato per la valutazione della biocompatibilità con la pelle e le mucose.
- Cytotoxicity by MTT test: cell survival assay using cultured human keratinocytes in monolayer cultures to assess biocompatibility with skin and mucosae.

1.4 Laboratorio Incaricato/Entrusted Laboratory

ABICH S.r.l.
Via 42 Martiri, 213/B – 28924
Verbania - Italy - tel +39 (0)323 586239 fax +39 (0)323 496877

1.5 Date dello Studio/Study Dates

Inizio/Initiation: 08/07/2009
Fine/End: 10/07/2009
1.6 *Ricercatore principale/Main investigator*

Dr. Alliffranchini Elena, Biotecnologa/Biotechnologist
ABICH S.r.l.

1.7 *Direttore dello studio/Study director*

Dr. Elena Bocchiello – Biologa specialista in Biotecnologie / Biologist, biotechnology specialist.
- ABICH S.r.l. –
2 PARTE SECONDA/PART TWO - PROTOCOLLO
SPERIMENTALE/STUDY DESIGN

2.1 Scopo del test/Aim of the test


Il saggio di citotossicità può essere eseguito al fine di valutare la potenziale irritazione cutanea di un prodotto finito o di un ingrediente o miscela di ingredienti utilizzando colture di cheratinociti prelevati da cute umana. Il test in vitro condotto su cellule derivate da tessuto cutaneo o epiteliale in genere risulta essere un metodo sperimentale semplificato, ma in grado di dare molte informazioni sulle reazioni che possono verificarsi in vivo. I prodotti topici cosmetici sono applicati direttamente sulla cute, spesso a contatto con le mucose, e dovrebbero quindi avere impatto tossicologico nullo o molto basso nei confronti delle cellule di derivaione cutanea e epiteliale.

I test di citotossicità eseguiti su fibroblasti e cheratinociti in monostato sono stati ampiamente usati come indicatori predittivi del potenziale di irritazione cutanee e cutanee di prodotti per uso topico, cosmetici o ingredienti. In particolare, il modello utilizzato nel presente studio è rappresentato da una linea di cheratinociti umani. L'obiettivo di questo test è stabilire quantitativamente gli effetti del prodotto testato sulla proliferazione cellulare attraverso il test di vitalità cellulare con colorazione con MTT. Questo metodo colorimetrico si basa sulla misura indiretta della vitalità cellulare attraverso la capacità di un enzima mitocondriale, la succinato deidrogenasi, di metabolizzare un substrato quale i sali di tetrazolio.

Un limite di questa metodica è costituito dal fatto che sostanze del tutto insolubili in un medium acquiso possono non giungere a contatto con le cellule e quindi la loro potenziale tossicità può essere sottostimata. In casi in cui si sospettino condizioni di questo genere è quindi opportuno approfondire lo studio con test su epidermide ricostituita o in vivo nell'animale.


Cytotoxicity assay can be carried out in order to evaluate in vitro the potential skin irritation of cosmetic ingredients or finished products on keratinocytes cultures. The in vitro test on skin-derived cells is a simplified but yet very informative model of the reactions that may occur in vivo. Cosmetic products are directly applied locally on the human skin or hair, often in contact with mucous membranes. Consequently they should exhibit no or very low toxicity to the cells that compose the skin or the epithelia.

The cytotoxicity assay performed in this study was designed to evaluate the toxic dermal irritation potential of the tested product using relevant human cells grown in vitro.

Cytotoxicity test performed on human keratinocytes or fibroblasts in monolayer have been extensively used as indicator of the eye and skin irritation potential of cosmetics and ingredients. In particular in this test the cell model utilized is a line of human keratinocytes.

A limit of this method is the fact that substances that are totally insoluble in a water-based medium may not get in contact with the cells and hence their toxicity may be underestimated. When such a case is suspected it is advised to investigate further the substance effect on tridimensional epidermis models or in vivo in the animal.

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www.abich.it - e.mail: info@abich.it
The objective of this assay was to assess quantitatively the effects of the product on cell survival through the MTT assay. This is a very simple colorimetric assay that allows to determinate the percentage of living cells within a cell culture. This assay is based on the ability of mitochondrial succinate dehydrogenase enzyme to metabolise the tetrazolium salt.

2.2 Esecuzione del test/Assay procedures

2.2.1 Modello sperimentale/Cell model

Il modello cellulare utilizzato per il test in vitro è rappresentato da: \textit{in vitro} test system employed consists of:

- cheratinociti umani stabilizzati (cellule HaCaT)/transformed human keratinocytes (HaCaT cell).

La linea cellulare deriva da pelle adulta che mantiene completa capacità di differenziazione epidermica./The cell line has been established from adult skin which maintains full epidermal differentiation capacity.

2.2.2 Trattamento ed esposizione/Treatment and exposure

Le cellule sono state seminate in piastre da 96 pozetti per 24h in DMEM + 10% FCS. Quindi è stato aggiunto terreno di coltura fresco arrostito con 10% di FCS e contenente il prodotto da testare in modo da raggiungere 6 diluzioni finali comprese tra 5 e 0,15 mg/ml. Il campione è stato sciolti in acqua alla concentrazione di 50mg/ml.

Ogni campione è stato testato in triplicato. Cellule non trattate sono state utilizzate come controllo negativo, e come controllo positivo le cellule sono state trattate con un tensioattivo a tossicità nota (Sodio Lauril Solfato - SLS) disciolto nel terreno di coltura alle concentrazioni comprese tra 0,5 mg/ml a 0,03 mg/ml. Al termine dell’incubazione è stato quindi eseguito il test di citotossicità (MTT) per valutare la percentuale di sopravvivenza cellulare. Il test MTT valuta l’impatto tossico delle sostanze in questione sulla vitalità cellulare.

Cells are seeded in 96 well plates, for 24 h in DMEM + 10% FCS. Fresh medium is added, supplemented with only 10% FCS and with 6 scalar dilutions of the tested product ranging from 5 to 0,15 mg/ml. The sample has been dissolved in water at 50mg/ml concentration.

For each dilution, 3 replica were performed. At the end of the incubation, cells are tested for vitality with the citotoxicity (MTT assay). Cells treated with a known irritating surfactant (Sodium Lauryl Sulfate – SLS) in concentration ranging from 0,5 mg/ml to 0,03 mg/ml were used as positive control. Untreated cells were used as negative control.

The MTT assay is able to evaluate the toxic impact of the tested compound on the cells viability.
2.2.3 Test di vitalità cellulare MTT/MTT cell vitality assay

Il test MTT è semplice, accurato e fornisce risultati riproducibili. Questo metodo è stato sviluppato originariamente da Mossman. Il reagente chiave è il 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide o MTT, sostanza che dà un colore giallo in soluzione acquosa. La deidrogenasi mitocondriale delle cellule vitali taglia l’anello tetrazolico, portando alla formazione di cristalli di formazano color viola porpora insolubili in acqua. I cristalli vengono sciolti con una soluzione solubilizzante l’MTT.

La soluzione viola risultante viene misurata spettrofotometricamente. Un aumento o diminuzione delle cellule vitali ha per risultato un cambiamento concomitante nella quantità di formazano che si forma e che può essere considerato come un indicatore del grado di citotossicità causato dall’esposizione alle sostanze irritanti.

Dopo 24h di trattamento, la vitalità cellulare viene valutata incubando le cellule con MTT per 3 ore (150µl per pozzetto). I cristalli di formazano precipitati vengono poi estratti utilizzando isopropanolo o DMSO (200 µl per pozzetto) e quantificati spettrofotometricamente a 550 nm.

La piastra viene agitata su un agitatore a piastra assicurandosi che tutti i cristalli si siano sciolti ed abbiano formato una soluzione omogenea. Viene letta l’assorbanza con un colorimetro (Tecan modello Sunrise remote) equipaggiato con un lettore di piastre sottraendo la lettura del fondo.

Il risultato è espresso come vitalità cellulare in percentuale secondo la formula:

% di vitalità cellulare = \[\text{OD(550 nm - 690 nm) prodotto testato/ OD(550 nm - 690 nm) controllo negativo}] \times 100

The MTT assay is simple, accurate and yields reproducible results. This method has been developed originally by Mossman. The key component is (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) or MTT. This product is of yellowish colour in solution. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, leading to the formation of purple crystals which are insoluble in aqueous solutions. The crystals are re-dissolved with the MTT Solubilization Solution and the resulting purple solution is measured spectrophotometrically. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material.

After 24h exposure of the cells to the test material, the viability is assessed by incubating the tissues for 3 hours with MTT solution (150µl per well). The precipitated formazan is then extracted using isopropanol or DMSO and quantified spectrophotometrically at 550 nm.

The plate is shaken on a gyratory plate shaker, ensuring that all the crystals have dissolved from the cells and have formed a homogeneous solution. The absorbance is measured on a microplate reader (Tecan modello Sunrise remote), with background clearing.

The results are expressed in terms of viability:

% of cell viability = \[\text{OD(550 nm - 690 nm) test product / OD(550 nm - 690 nm) negative control}] \times 100
2.2.4 Expression of results:

I dati di citotossicità ottenuti con il test MTT sono messi in grafico contro la concentrazione del prodotto testato, creando una curva dose-risposta, che permette di determinare:

- la curva teorica di regressione
- il valore teorico di IC<sub>50</sub> (concentrazione di inibizione della crescita del 50%) ovvero la concentrazione che induce una riduzione della vitalità cellulare del 50% rispetto alle cellule non trattate

I risultati di citotossicità sono stati corretti sottraendo le letture di assorbanza dovute al mezzo diluente.

**Il valore IC<sub>50</sub> (Inhibiting Concentration 50) indica la concentrazione di prodotto necessaria per inibire la crescita cellulare del 50%. IC<sub>50</sub> è un parametro che consente di valutare il potenziale irritante di un composto**

The cytotoxicity data obtained with the MTT assay were plotted against the concentrations, which generate dose-response curves that allow to determine:

- the theoretical regression curve
- the theoretical IC<sub>50</sub> value (inhibiting concentration 50%), i.e. the concentration of test compound which causes a 50% decrease of cell survival as compared to untreated cultures

The cytotoxicity results have been corrected subtracting the cytotoxicity due to the diluent medium.

**The IC<sub>50</sub> value (Inhibiting Concentration 50) is the concentration of test compound which induces a 50% decrease of cell growth/survival. It makes it possible to evaluate the potential irritating effect of the compound**

*Per prodotti finiti, valori ≤ 1mg/ml (o 1μl/ml) possono essere considerati come irritanti, valori > 3mg/ml (o 3μl/ml) presentano un’ottima biocompatibilità. Per detergenti e prodotti da risciacquo, l’eventuale fattore di diluizione deve essere preso in considerazione. Per materie prime bisogna prendere in considerazione la concentrazione d’uso.*

*For finished products, values ≤ 1mg/ml (or 1μl/ml) may be considered as irritating, values > 3mg/ml (or 3μl/ml) show a very good biocompatibility. For detergents and rinse-off products, the eventual dilution factor should be taken in account. For raw materials, the use concentration should be taken in account.*
3 PARTE TERZA/PART THREE - RISULTATI E CONCLUSIONI/RESULTS AND CONCLUSIONS

3.1 Risultati/Results

<table>
<thead>
<tr>
<th>SLS (controllo positivo/positive control)</th>
<th>0.5</th>
<th>0.25</th>
<th>0.125</th>
<th>0.06</th>
<th>0.03</th>
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<tbody>
<tr>
<td>Dose mg/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitalità cellulare % (rispetto al controllo negativo)/% cell viability (vs negative control)</td>
<td>2.2</td>
<td>2.6</td>
<td>2.7</td>
<td>85.4</td>
<td>88.2</td>
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<tr>
<td>stand. dev.</td>
<td>0.0</td>
<td>0.1</td>
<td>0.0</td>
<td>2.8</td>
<td>3.5</td>
</tr>
</tbody>
</table>

$IC_{50} = 0.083 mg/ml$

![Graph showing cell vitality expression after treatment with increasing amount of SLS](image)

Epressione della vitalità cellulare dopo trattamento con dosi crescenti di SLS/ Cell vitality expression after treatment with increasing amount of SLS
Curva di regressione per il calcolo dell'IC<sub>50</sub>/Regression plot to calculate the IC<sub>50</sub> dose
### Hydrolyzed grape (vitis vinifera) fruit skin

**Lotto/Batch:** LPUV 031-09  
**Codice interno/internal code:** 1085/09-01

<table>
<thead>
<tr>
<th>Dose mg/ml</th>
<th>5</th>
<th>2.5</th>
<th>1.25</th>
<th>0.6</th>
<th>0.3</th>
<th>0.15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitalità cellulare (% rispetto al controllo negativo)/% cell viability (vs negative control)</td>
<td>89.0</td>
<td>97.5</td>
<td>93.1</td>
<td>91.8</td>
<td>91.7</td>
<td>86.8</td>
</tr>
<tr>
<td>stand. dev.</td>
<td>2.7</td>
<td>1.1</td>
<td>3.0</td>
<td>9.5</td>
<td>3.4</td>
<td>1.1</td>
</tr>
</tbody>
</table>

IC$_{50}$ > 5mg/ml

---

**Expression della vitalità cellulare dopo trattamento con diverse diluizioni del prodotto testato**

Cell vitality expression after treatment with different dilutions of the tested product
3.2 Conclusioni/Conclusions

Sulla base dei risultati sopra riportati il prodotto /On the bases of the results here shown, the product:

**Hydrolyzed grape (vitis vinifera) fruit skin**

**Lotto/Batch: LPUV 031-09**

ha mostrato di possedere un 'IC<sub>50</sub>' maggiore di 5mg/ml su cheratinociti umani.
Questi dati possono essere considerati come predittivi di assenza di effetti irritanti in vivo.

*did show an IC<sub>50</sub> higher than 5mg/ml on human keratinocytes. These data are predictive of absence of irritating effects in vivo.*

Data/Date: 10/07/2009

Il Direttore dello studio/ Study Director
Dr. Elena Bocchietto
4 BIBLIOGRAFIA/BIBLIOGRAPHY


**ANALISI IN VITRO DEL POTENZIALE PRO-SENSIBILIZZANTE**

**IN VITRO ANALYSIS OF THE PRO-SENSITISING POTENTIAL**

<table>
<thead>
<tr>
<th>COMMITTENTE/CUSTOMER</th>
<th>Università di Bologna - Dipartimento di chimica industriale e dei Materiali Viale Risorgimento 4 40136 Bologna (BO) - Italia</th>
</tr>
</thead>
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<tr>
<td>CAMPIONE/SAMPLE</td>
<td>Hydrolyzed grape (vitis vinifera) fruit skin Lotto/Batch: LPUV 031-09</td>
</tr>
<tr>
<td>DATA RAPPORTO/REPORT DATE</td>
<td>17/07/2009</td>
</tr>
<tr>
<td>PROTOCOLLO N./REPORT N.</td>
<td>REL/0446/2009/ALTOX/ELB</td>
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</tbody>
</table>
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Premessa/Preliminary

Nella relazione vengono descritti i campioni analizzati, lo scopo dell’indagine, i metodi utilizzati e i risultati ottenuti nel corso del lavoro sperimentale. Nella prima parte sono raccolti i dati generali, nella seconda è descritto il metodo sperimentale e nella terza sono esposti i risultati e le conclusioni. I risultati del test sono forniti in forma grafica per rendere più agevole la lettura. L’interpretazione dei risultati è riassunta nella parte terminale del lavoro.

Copia del presente rapporto è conservata presso ABICH S.r.l.
I risultati grezzi relativi alle prove eseguite sono conservati presso l’Istituto Scientifico San Raffaele.

This report contains the experimental data compiled during the in vitro safety evaluation studies of the test products.
The test results are presented in a concise table format for easy interpretation.
The first part provides information regarding sponsor and test product identifications, assay type(s), entrusted laboratory, study initiation and completion dates and supervisory personnel.
The second part describes the study design, including materials and procedures.
The test results are presented in the third and last part of the report.
A copy of this report is kept on file at ABICH S.r.l.
The raw data that support the results and conclusions are kept at the San Raffaele Scientific Institute.

Nota/Note:
Il risultato dei test citati nel presente rapporto si riferisce esclusivamente al prodotto/i testato/i e alle particolari condizioni sperimentali impiegate nel test. Il presente rapporto o parti di esso possono essere riprodotti solo con il consenso degli sperimentatori.

The results reported in the present brochure refer only to the tested sample/samples and to the particular experimental conditions hereby described. This report or parts of it can be reproduced only with the experimenters’ agreement.
1 PARTE PRIMA/FIRST PART – Dati generali/General facts

1.1 Committente/Customer

Università di Bologna - Dipartimento di chimica Industriale e dei Materiali
Viale Risorgimento 4
40136 Bologna (BO) - Italia

1.2 Campione testato/Test sample

Hydrolyzed grape (vitis vinifera) fruit skin; Lotto/Batch: LPUV 031-09, codice interno/internal code 085/09-03

1.3 Data di ricevimento del campione/Sample receipt date

30/06/2009

1.4 Test eseguiti/Executed tests

➤ Valutazione preliminare della vitalità cellulare/citotossicità su monconi umani tramite metodica MTT
➤ Valutazione dello stimolo pro-sensibilizzante su monconi umani tramite FACS.
➤ Preliminary evaluation of cytoxicity on human monocytes through MTT assay
➤ Evaluation of pro-sensitising activity on human monocytes through FACS analysis

1.5 Laboratorio incaricato/Entrusted laboratory

Istituto Scientifico Ospedale San Raffaele, via Olgettina 60 Milano, Laboratorio di immunologia molecolare e cellulare San Raffaele Scientific Institute, via Olgettina 60, Milano – Italy, Cell and molecular Immunology Laboratory

1.6 Data di fine del test/Test ending date

16/07/2009

1.7 Sperimentatore/Experimenter

Dr. Samuele Burastero, Medico chirurgo, Specialista in Allergologia e Immunologia Clinica/MD, Allergology and Clinical Immunology Specialist, Ricercatore presso l'Istituto Scientifico Ospedale San Raffaele/Researcher at Scientific Institute San Raffaele Hospital

1.8 Supervisore scientifico/Scientific monitor

Dr. Elena Bocchietto, Biologa specialista in Biotecnologie/Biotechnology specialist ABICH S.r.l. - Via 42 Martiri- 28924 -Verbania -Italy tel +39 (0)323 586239
2 PARTE SECONDA/SECOND PART - Descrizione dello studio/Study description

2.1 Obiettivo/Aim

Scopo del test è valutare l'assenza di effetti pro-sensibilizzanti da parte di prodotti finiti o materie prime per uso cosmetico o biomedicale. Nella definizione di un quadro di tollerabilità cutanea, è importante considerare oltre al potenziale di irritazione anche il potenziale sensibilizzante dei prodotti e degli ingredienti per uso topico ai fini della sicurezza d'impiego.

Nel test effettuato si è ritenuto opportuno utilizzare un modello cellulare per valutare la reattività immunologica di tipiche cellule immunitarie (monociti in questo caso) esposte a contatto prolungato (48h) con il prodotto in analisi, a due diverse concentrazioni. Occorre premettre che i dati tossicologici forniti dai test su modelli cellulari non possono essere considerati integralmente sostitutivi dei test in vivo, ma senz'altro l'ampia letteratura scientifica pubblicata oggi consente di ritenersi affidabili e utilmente informativi circa le caratteristiche biologiche delle sostanze testate. Dal momento inoltre che la VI e VII modifica alla Dir. 76/768 CE prevedono l'abbandono dell'utilizzo di animali per testare prodotti finiti e ingredienti di cosmetici e considerato che il test di sensibilizzazione su volontari umani è eticamente di difficile applicazione, è d'obbligo la ricerca di modelli predittivi in vitro, che consentano di effettuare una quanto più ampia possibile valutazione della sicurezza d'impiego dei prodotti finiti, così come richiesto dalla legislazione(1-11). Analogamente, anche per i dispositivi medici le norme UNI/EN 10993 prevedono, dove possibile, l'impiego di test alternativi per limitare l'uso di animali (12).

Il test è stato condotto su una linea cellulare di monociti denominata THP-1, poiché tale tipo cellulare è fortemente coinvolto nelle risposte immunitarie della cute, organo bersaglio tipico dei prodotti topici. Su queste cellule si è valutata la modulazione dell'espressione di due molecole costimolatorie, CD80 (B7.1) e CD86 (B7.2) utilizzando come controllo positivo una tipica sostanza sensibilizzante da contatto, il Nichel Solfato. Il Nichel è capace di suscitare in vivo reazioni immunitarie di tipo allergizzante (sensibilizzazione da contatto) ed è stato largamente utilizzato in vitro per studiare la modulazione della risposta immune.

Il riconoscimento dell'antigene da parte del TCR (T Cell Receptor) dei linfociti T (segnale 1) non è funzionale alla maturazione di una risposta immune efficiente se non avviene a livello della membrana di una cellula presentante l'antigene stesso e in grado di fornire un ulteriore segnale (segnale 2 o costimolo) qualitativamente fondamentale per la definizione del tipo di risposta (umorale, cellulare, etc.). Sia B7.1 che B7.2 (collettivamente: B7) sono glicoproteine di membrana presenti sulla superficie di numerose cellule che presentano l'antigene (cellule dendritiche, cellule di Langerhans, monociti/macrocopi, linee cellulari diverse tra cui cheratinociti) e agiscono come costimoli. Infatti, entrambe le molecole sono ligandi di una glicoproteina denominata CD28, presente sulla membrana del linfocito T. L'insnesco del sistema ligando/recettore CD28/B7 previene l'apoptosi (morte cellulare programmatata) delle cellule T e coopera nel sostenere la loro proliferazione e differenziazione. Nelle prime fasi della risposta immune “fisiologica” B7.2 è espresso costitutivamente e modula sia le risposte Th1 che Th2. Con il progredire della risposta immune, anche B7.1 viene up-regolata e incrementa l'intensità del segnale costimolatorio, con espansione delle cellule T e produzione di varie citochine. B7.1, inoltre, è preferenzialmente up-regolato durante la fase acuta delle risposte autoimmuni (13-25).
L'aumento dell'espressione di queste molecole costimolatorie sul monocito è quindi segno di attivazione di una risposta immunitaria in seguito ad esposizione ad un antigeone potenzialmente allergizzante. Funzionalmente, infatti, l'espressione di molecole costimolatorie su questo tipo cellulare corrisponde all'acquisizione di competenza per la presentazione dell'antigeone nella tipica sede (la cute nel nostro caso) in cui in vivo si manifesta sensibilizzazione da contatto.

The aim of the test is to evaluate that the tested product or raw material does not cause pro-sensitising effects on the involved cell model. For every product intended to come into direct and prolonged contact with the skin, it is important to consider, beside the irritating potential, even the sensitising potential in order to predict the general safety of the finished formula to avoid risks for the consumers.

In the test we used a blood-derived cell model (monocytes/macrophages) and we exposed them for prolonged time (48h) with two different concentrations of the tested sample. It is necessary to premise that toxicological data from cell models cannot totally replace in vivo tests, but a wide scientific literature is available to support their reliability. Furthermore, the VI\textsuperscript{th} and VII\textsuperscript{th} amendment at the EC Dir. 76/768 plans the abandon of animal testing for cosmetic products, and as sensitisation testing on human volunteers is ethically problematic, the development of alternative in vitro method to predict skin sensitisation is a forced route to evaluate the safety of employ of cosmetics (1-11). Furthermore, the use of in vitro tests, instead of in vivo models, is strongly recommended by the UNI/EN 10993 rules (12).

In the present study, we used a monocytes cell line, named THP-1, as prototypic blood-derived immunologically active cell. On these cells we checked out the expression of two costimulatory molecules, CD80 (B7.1) and CD86 (B7.2), using as a positive control Nickel sulphate, a well known contact sensitising agents, both in in vitro and in vivo models.

When the lymphocytes T TCR recognize the antigen (signal 1) on the Antigen-Presenting Cell (APC), additional molecules (called co-stimulatory) on the APC's membrane are necessary to obtain a complete functional immune response (signal 2).

The signal 2 is very important to define the kind of immune response that is going to be activated (umoral, cellular, etc.)

The costimulatory molecules CD80 and CD86 (also called B7.1 and B7.2) are necessary to obtain an efficient antigen presentation by the T cell receptor (TCR) and hence to obtain a correct immune response. Both these molecules are membrane glycoprotein expressed on the surface of different antigen-presenting cells (dendritic cells, Langerhans cells, monocytes/macrophages, keratinocytes) and they recognise a further molecule, a glycoprotein called CD28 on the T lymphocyte membrane.

The switching on of the ligand/receptor system CD28/B7 avoids the T cell apoptosis and sustains their proliferation and differentiation and the production of many cytokines.

In the first phase of the physiological immune response, B7.2 is expressed as default and it modulates both the Th1 and Th2 responses. As the immune response goes on, also B7.1 is up-regulated and the costimulatory signal increase, as the T cells and the production of different cytokines. B7.1 is also preferentially up-regulated during the acute phase of auto-immune response (13-25).

The increasing level of expression of CD80 and CD86 on monocytes is a signal of activation of the immune response derived from the exposition to a potentially sensitising contact antigen.
2.2 Descrizione dei campioni/Samples description

<table>
<thead>
<tr>
<th>Nome/Name</th>
<th>Descrizione/Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolyzed grape (Vitis vinifera) fruit skin Lotto/Batch: LPUV 031-09 Codice interno/Internal code: 1085/09-03</td>
<td>Liquido trasparente rosso /Clear red liquid</td>
</tr>
<tr>
<td>Nickel solfato/Nickel sulfate</td>
<td>In cristalli discolto in PBS, controllo positivo sensibilizzante/Crystals dissolved in PBS, positive control as a sensitizer</td>
</tr>
</tbody>
</table>

2.3 Preparazione del campione/Sample preparation

Il campione è stato discolto in etanolo e quindi diluito direttamente nel medium di coltura delle cellule a diverse diluzioni e sottoposto a test preliminare di citotoxicità su cellule THP-1 per valutare a quale concentrazione la sostanza poteva essere impiegata in vitro senza causare mortalità cellulare, evento che provoca alterazione dei risultati. Come controllo negativo è stato considerato il terreno di crescita delle cellule lasciato nelle stesse condizioni sperimentali.

The sample was dissolved in ethanol and then diluted in the cell culture medium at different concentrations. It underwent a preliminary cytotoxicity screening on the cells to decide the best concentration to test it without cytotoxic effects on the cells, in order to avoid false results. Cell medium exposed to the same experimental conditions were used as a negative control.

2.4 Modello sperimentale/Cell model

È stata utilizzata una linea monocitoide umana, denominata THP-1. Le cellule sono coltivate in RPMI contenente 10% FCS e 2 mM di glutamina.

The test is carried out on a monocyte-like human line called THP-1. Cells are kept in RPMI containing 10% FCS and 2 mM glutamine.

2.5 Esecuzione del test preliminare MTT/MTT preliminary assay execution

Il test MTT è semplice, accurato e fornisce risultati riproducibili. Questo metodo è stato sviluppato originariamente da Mossman (27). Il reagente chiave è il 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide o MTT; sostanza che dà un colore giallo in soluzione acquosa. La deidrogenasi mitocondriale delle cellule vitali taglia l'anello tetrazolico, portando alla formazione di cristalli di formazano color viola porpora insolubili in acqua. I cristalli vengono sciolti in isopropanolo acidificato e la soluzione viola risultante viene misurata allo spettrofotometro.

Un aumento o diminuzione delle cellule vitali ha per risultato un cambiamento concomitante nella quantità di formazano che si forma e che può essere considerato come un indicatore del grado di citotoxicità causato dall'esposizione alle sostanze testate.

Il terreno per MTT viene preparato come descritto (27). Dopo il trattamento, le cellule vengono lavate con PBS e la soluzione MTT viene aggiunta ad ogni pozzetto, con successiva incubazione a 37°C. Alla fine del periodo di incubazione, il terreno-MTT viene rimosso e in ogni pozzetto viene aggiunta la soluzione solubilizzante MTT (27). La piastra viene agitata su un agitatore a piastra per 20-30 minuti,
assicurandosi che tutti i cristalli si siano sciolti ed abbiano formato una soluzione omogenea. 
Viene letta l'assorbanza e sottratta il fondo come descritto nella metodica. 
Il risultato è espresso come:

\[
\text{% sopravvivenza} = \frac{\text{OD cellule trattate}}{\text{OD cellule non trattate}} \times 100
\]

The MTT assay is simple, accurate and yields reproducible results. This method has been developed originally by Mossman (27). The key component is (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) or MTT. This product is of yellowish colour in solution. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, leading to the formation of purple crystals which are insoluble in aqueous solutions. The crystals are re-dissolved in acidified isopropanol and the resulting purple solution is measured spectrophotometrically. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material.

MTT-medium is prepared as described (27). After exposure of the cells to the test material, the cells are washed with PBS and exposed to the MTT-medium at 37°C. At the end of the incubation period, the MTT-medium is removed and the cells receive the MTT solubilization solution. The plate is shaken on a rotatory plate shaker for 20-30 minutes, ensuring that all the crystals have dissolved from the cells and have formed a homogeneous solution. The absorbance is measured as described with background elimination. The results are expressed in terms of viability:

\[
\text{% Viability} = \frac{\text{OD treated cultures}}{\text{OD untreated control cultures}} \times 100
\]
2.6 Trattamento ed esposizione/Treatment and exposure

A seguito dei risultati del test preliminare, il campione è stato impiegato a due diverse diluzioni finali sulle cellule THP-1, diluendolo nel medium di coltura delle cellule in modo da ottenere le concentrazioni finali desiderate a contatto con le cellule. Le cellule sono state esposte per 48 h a 37°C con il 5% di CO₂.

Following the results of the preliminary assay, the sample was diluted in the cell medium at the two different dilutions in order to obtain the desired final concentrations in contact with the THP-1 cells in vitro. The exposure has been carried out for 48 h at 37°C with 5% CO₂.

2.7 Esecuzione del test di sensibilizzazione/Sensitisation assay execution

Dopo l’esposizione le cellule sono state esaminate con colorazione di Tripan Blue e osservazione microscopica in camera conta-globuli per la vitalità, raccolte, lavate in un tampone isotonic (PBS) e marcate con un anticorpo fluoresceinato diretto contro B7.1 o B7.2. Dopo ulteriori lavaggi per asportare l’anticorpo in eccesso, le cellule sono state immesse in un citofluorimetro di flusso (FACS, Fluorescence Activated Cell Sorter, Becton Dickinson, Mountain View, CA) per la valutazione della MFI (Mean Fluorescence Intensity), proporzionale al numero di molecole marcate per cellula, e quindi rappresentativo del livello di espressione delle molecole costimolatorie da noi indagate.

Si sono inoltre rilevati parametri qualitativi morfologici (alterazione del volume delle cellule, modificazioni delle granulazioni cellulari) legati a necrosi cellulare e apoptosi, eventi strettamente connessi al processo di elicitation allergica.

Come controllo (fluorescenza di base) è stata valutata la MFI sia delle cellule THP-1 non trattate in alcuna maniera (tranne i lavaggi in PBS) sia delle cellule THP-1 fatte reagire con un anticorpo monoclonale fluoresceinato (come quelli anti-B7.1 e anti-B7.2) ma di specificità irrilevante (isotype-matched control).

After the incubation with the tested substance and the controls, cells are collected, checked under the microscope for their vitality by staining with Tripan Blue dye and counting in a cell counter chamber, washed in PBS and then marked with a fluoresceinated anti-B7.1 or B7.2 antibody. After washing, to eliminate the excess antibody, the MFI (Mean Fluorescence Intensity) linked to the cells was evaluated by means of a flux cytofluorimeter (FACS, Fluorescence Activated Cell Sorter, Becton Dickinson, Mountain View, CA). This value is proportional to the expression of costimulatory molecules.

The MFI of the non-treated THP-1 cells and of cells after reaction with a monoclonal isotype-matched antibody was used as an internal control (basal fluorescence).
3 PARTE TERZA/THIRD PART - Risultati e conclusioni/Results and conclusions

3.1 Risultati/Results

In tabella 1 sono riportati i risultati dell’analisi della linea di monociti THP-1 per espressione di molecole costimolatorie al citofluorimetro di flusso dopo 48h di reazione con il campione alle due concentrazioni testate e con i controlli, corretti per il controllo negativo.

In table 1 are reported the results expressed as co-stimulatory molecules expression after the exposition of the monocyte cell line THP-1 for 48 h to the investigated and control substances, subtracted of the negative controls (non treated cells basal values).

<table>
<thead>
<tr>
<th>Campioni/Samples</th>
<th>CD80 (MFI*)</th>
<th>CD86 (MFI*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nickel Sulfate 2C µg/ml</td>
<td>48,85</td>
<td>74,66</td>
</tr>
<tr>
<td>Nickel Sulfate 1C µg/ml</td>
<td>23,78</td>
<td>16,33</td>
</tr>
<tr>
<td>Nickel Sulfate 4 µg/ml</td>
<td>0,51</td>
<td>2,44</td>
</tr>
<tr>
<td>Hydrolyzed grape (vitis vinifera) fruit skin 20 µl/ml</td>
<td>-0,04</td>
<td>-0,4</td>
</tr>
<tr>
<td>Hydrolyzed grape (vitis vinifera) fruit skin 4 µl/ml</td>
<td>-0,12</td>
<td>-0,33</td>
</tr>
</tbody>
</table>

* MFI = Mean Fluorescence Intensity - è la media geometrica dell’intensità di fluorescenza delle cellule decorate con l’anticorpo fluoresceinato ed è proporzionale al n. di molecole decorate per cellula.

* MFI = Mean Fluorescence Intensity – it is the geometric average of the fluorescence intensity of the cells decorated with the fluoresceinated antibody and it is proportional to the number of stained molecules per cell.
In figura 1 sono riportati in grafico i valori di espressione di CD80 e CD86 riscontrati per il campione analizzato ed i relativi controlli, sottratti del controllo negativo, ossia del valore del campione trattato con un anticorpo fluoresceinato di specificità irrelevante.

In figure 1 are plotted the values of expression of CD80 and CD86 found for the tested sample and its relative controls, subtracted of the negative control (value of the sample treated with a fluoresceinated antibody of irrelevant specificity).

Figura 1/Figure 1

Osservando il comportamento del Nichel, tipica sostanza allergizzante, si può rilevare come questo sia caratterizzato da a) incremento elevato di entrambi i marcatori; b) correlazione diretta dell'incremento dell'intensità della risposta con la concentrazione; c) effetto rilevabile anche a dosi molto basse.

La dose testata di 4 μg/ml di Nichel Solfato (NiSO₄·6H₂O) corrisponde a circa 1 ppm di Nichel, valore che si colloca intorno alla dose soglia allergizzante nei soggetti già sensibilizzati e su cute irritata (28, 29). Nell'esperimento si è verificato come questa concentrazione sia in grado di causare un aumento rilevabile di CD 80, rispetto ai controlli non trattati (dati non mostrati).

La concentrazione in grado di causare una reazione allergica nella maggior parte dei soggetti sensibili si colloca tuttavia intorno a valori ben più alti, oltre le 100 ppm di Nichel Solfato-6H₂O, a contatto con la cute integra e sana.

AZIENDA CERTIFICATA
UNI EN ISO 9001:2000
Certificato N. 501004992
Alle concentrazioni saggiate, il campione analizzato non ha evidenziato alcuna modulazione dei marcatori indagati.

Il campione analizzato non ha mostrato effetti citotossici sulle cellule utilizzate per il saggio (IC$_{50}$= 100 µl/ml).

Alle diluzioni utilizzate non si sono riscontrati effetti apoptotici sulle cellule in esame.

Showing the behaviour of Nickel, a prototypic sensitising substance, we can see how this is characterised by a) high increase of both the markers; b) direct correlation between concentration and intensity of the response; c) relevant effects even at very low doses.

The tested dose of 4 µg/ml of Nickel Sulphate (NiSO$_4$.6H$_2$O) corresponds to more or less 1 ppm of Nickel, dosage that is around the minimal sensitising threshold in already sensitised individuals with irritated skin (28, 29). From above reported data, we can notice how a so low dose is already able to cause a detectable increase of CD80 respect to untreated cells(data not shown). The concentration that is able to cause an allergic reaction in most of the sensitive subjects is anyway around higher value, over the 100 ppm of Nickel Sulphate.6H$_2$O in contact with safe and intact skin .

The sample did not show any increase in the expression of both the investigated markers.

The sample did not show cytotoxic effects on the cells used for the test (IC$_{50}$= 100 µl/ml).

We did not observed any signal related to apoptosis on the treated cells.
3.2 Conclusioni/Conclusions

Nel test sopra riportato il campione / In the above experimental conditions, the sample:

**Hydrolyzed grape (vitis vinifera) fruit skin**

*Lotto/Batch: LPUV 031-09*

non aumenta in vitro l'espressione di nessuno dei marcatori indagati nei monociti umani, mostrando quindi di non possedere un potenziale stimolatorio del sistema immunitario mediato dal monocito/macrophago.

does not affect in this in vitro model the investigated markers expression in immunocompetent cells and hence it does not show any stimulating potential on the immune cellular response mediated by monocyte/macrophage.

Data/Date: 17/07/2009

Il Responsabile dello studio/ Study Monitor

Dr. Elena Bocchietto
4 Bibliografia/Bibliography


19) Burastero SE, Magnani Z, Confetti C, Balbo P, Oddera S, RossI GA. CD80 Molecule expression by alveolar macrophages (AM) is enhanced in allergic asthma and it is involved in allergen presentation to allergen-specific T-cells. Eur Respir J 1998;12 (suppl. 28):159s.

21) Schweitzer AN, Sharpe AH. Studies using antigen-presenting cells lacking expression of both B7-1 (CD80) and B7-2 (CD86) show distinct requirements for B7 molecules during priming versus restimulation of Th2 but not Th1 cytokine production. J Immunol 1998;161:2762-71.


Memorandum

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

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

DATE: June 14, 2012

SUBJECT: Updated Concentration of Use by FDA Product Category: Grape-Derived Ingredients
## Concentration of Use by FDA Product Category*

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Product Category</th>
<th>Maximum Concentration of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Bubble baths</td>
<td>0.002%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Other bath preparations</td>
<td>0.003%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Eye lotion</td>
<td>0.02-0.09%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Other eye makeup preparations</td>
<td>0.0002%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Colognes and toilet waters</td>
<td>0.0002%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Powders (dusting and talcum)</td>
<td>0.0002%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Other fragrance preparations</td>
<td>0.0002%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Hair conditioners</td>
<td>0.0002-0.1%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Hair sprays</td>
<td>0.00002%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>pump sprays</td>
<td></td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Shampoos (noncoloring)</td>
<td>0.00008-0.1%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Tonics, dressings and other hair grooming aids</td>
<td>0.0002-0.005%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Foundations</td>
<td>0.001-0.005%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Lipstick</td>
<td>0.0002%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Other makeup preparations</td>
<td>0.004%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Nail polish and enamel removers</td>
<td>0.001%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Bath soaps and detergents</td>
<td>0.0002-0.02%</td>
</tr>
</tbody>
</table>

* Vitis Vinifera (Grape) Leaf Water, Vitis Vinifera (Grape) Leaf Wax, Vitis Vinifera (Grape) Root Extract, Vitis Vinifera (Grape) Seed, Vitis Vinifera (Grape) Seed Powder, Vitis Vinifera (Grape) Skin Extract, Vitis Vinifera (Grape) Skin Powder, Vitis Vinifera (Grape) Vine Extract, Vitis Vinifera (Grape) Vine Sap, Hydrolyzed Grape Fruit, Hydrolyzed Grape Skin.
<table>
<thead>
<tr>
<th>Vitis Vinifera (Grape) Seed Extract</th>
<th>Other personal cleanliness products</th>
<th>0.0005%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Aftershave lotions</td>
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</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Skin cleansing (cold creams, cleansing lotions, liquids and pads)</td>
<td>0.0002-0.001%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Face and neck creams, lotions and powders</td>
<td>0.001-0.1%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Body and hand creams, lotions and powders</td>
<td>0.0003-0.01%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Foot powders and sprays</td>
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</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Moisturizing creams, lotions and powders</td>
<td>0.0001-0.02%</td>
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<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Night creams, lotions and powders</td>
<td>0.0002-0.03%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Paste masks and mud packs</td>
<td>0.0002%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Skin fresheners</td>
<td>0.0002%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Other skin care preparations</td>
<td>0.0003-0.02%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Sun tan gels, creams and liquids not spray</td>
<td>0.001%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed Extract</td>
<td>Indoor tanning preparations</td>
<td>0.001%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape)</td>
<td>Bath soaps and detergents</td>
<td>0.1%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Bud Extract</td>
<td>Shampoos (noncoloring)</td>
<td>0.08%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Baby lotions, oils and creams</td>
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<td>Bubble baths</td>
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<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Other bath preparations</td>
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</tr>
<tr>
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<td>Eye liner</td>
<td>0.002%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Eye shadow</td>
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<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Eye lotion</td>
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<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Other eye makeup preparations</td>
<td>0.002%</td>
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<tr>
<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Colognes and toilet waters</td>
<td>0.05%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Powders (dusting and talcum)</td>
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<td>Ingredient</td>
<td>Product Type</td>
<td>Concentration</td>
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<tr>
<td>----------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
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<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Other fragrance preparations (not spray)</td>
<td>0.002%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Hair conditioners</td>
<td>0.002-0.3%</td>
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<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Tonics, dressings and other hair grooming aids</td>
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</tr>
<tr>
<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Other hair preparations (noncoloring)</td>
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<td>Hair dyes and colors (all types requiring caution statement and patch test)</td>
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<tr>
<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Other hair coloring preparations</td>
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<tr>
<td>Vitis Vinifera (Grape) Fruit Extract</td>
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<td>Lipstick</td>
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<tr>
<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Makeup fixatives</td>
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<tr>
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<td>Other makeup preparations</td>
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<td>Bath soaps and detergents</td>
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<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Other personal cleanliness products</td>
<td>0.02-0.002%</td>
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<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Skin cleansing (cold creams, cleansing lotions, liquids and pads)</td>
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</tr>
<tr>
<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Face and neck creams, lotions and powders</td>
<td>0.00005-0.7%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Body and hand creams, lotions and powders</td>
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</tr>
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<td>Vitis Vinifera (Grape) Fruit Extract</td>
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</tr>
<tr>
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<td>Night creams, lotions and powders</td>
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</tr>
<tr>
<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Paste masks and mud packs</td>
<td>0.05-0.8%</td>
</tr>
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<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Skin fresheners</td>
<td>0.006%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Other skin care preparations</td>
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<tr>
<td>Vitis Vinifera (Grape) Fruit Extract</td>
<td>Suntan gels, creams and liquids not spray</td>
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<td>Indoor tanning preparations</td>
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<tr>
<td>Vitis Vinifera (Grape) Fruit Water</td>
<td>Face powders</td>
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<td>Foundations</td>
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<td>Other makeup preparations</td>
<td>0.1%</td>
</tr>
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<td>Paste masks and mud packs</td>
<td>2%</td>
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<td>Perfumes</td>
<td>3%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Leaf Extract</td>
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<td>Vitis Vinifera (Grape) Leaf Extract</td>
<td>Face and neck creams, lotions and powders</td>
<td>0.04%</td>
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<tr>
<td>Vitis Vinifera (Grape) Leaf Extract</td>
<td>Other skin care preparations</td>
<td>0.01%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed</td>
<td>Skin cleansing (cold creams, cleansing, lotions, liquids and pads)</td>
<td>0.08%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed</td>
<td>Body and hand creams, lotions and powders</td>
<td>0.05%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Seed</td>
<td>Other skin care preparations</td>
<td>0.05%</td>
</tr>
<tr>
<td>Vitis Vinifera (Grape) Vine Extract</td>
<td>Face and neck creams, lotions and powders</td>
<td>0.004%</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.

Information collected in 2012; Table prepared April 2, 2012
Updated June 14, 2012 (added Hydrolyzed Grape Fruit and Hydrolyzed Grape Skin to the title as these ingredients were included in the title of the survey, but no uses were reported)
Memorandum

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

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

DATE:          August 1, 2012

SUBJECT:       Concentration of Use by FDA Product Category: Vitis Vinifera (Grape) Shoot Extract
### Concentration of Use by FDA Product Category - *Vitis Vinifera* (Grape) Shoot Extract

<table>
<thead>
<tr>
<th>Product Category</th>
<th>Maximum Concentration of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powders (dusting and talcum)</td>
<td>0.00005%</td>
</tr>
<tr>
<td>Bath soaps and detergents</td>
<td>0.003%</td>
</tr>
</tbody>
</table>

Information collected in 2012  
Table prepared July 31, 2012, 2012
Memorandum

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

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

DATE:     May 9, 2012

SUBJECT:  Studies of Products Containing Vitis Vinifera (Grape) Seed Extract

The extraction solvents used to prepare the Vitis Vinifera (Grape) Seed Extract contained in the aftershave lotion tested in the attached studies were Butylene Glycol and Water.

Institute for In Vitro Sciences. 2006. Bovine corneal opacity and permeability assay with optional histology (aftershave lotion containing 0.15% Vitis Vinifera (Grape) Seed Extract). Study Number: 06AF67.350049.

Clinical Research Laboratories, Inc. 2006. An in-use safety evaluation to determine the dermal irritation potential of a cosmetic product or toiletry (aftershave lotion containing 0.15% Vitis Vinifera (Grape) Seed Extract). CRL Study Number: CRL66106.

TKL Research. 2006. Summary report: Repeated insult patch test of an aftershave lotion containing 0.15% Vitis Vinifera (Grape) Seed Extract. TKL Study No.: DS103906-5.
FINAL REPORT

Study Title

BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY WITH
OPTIONAL HISTOLOGY

Test Article

Authors
Janet W. Luczak, M.G.A.
Nicole Barnes, B.S.

Study Completion Date
August 18, 2006

Performing Laboratory
Institute for In Vitro Sciences, Inc.
21 Firstfield Road, Suite 220
Gaithersburg, MD 20878

Study Number
06AF67.350049

Laboratory Project Number
4536
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<th>Page</th>
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<tr>
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<td>TEST ARTICLE RECEIPT</td>
<td>4</td>
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<tr>
<td>BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY WITH OPTIONAL HISTOLOGY</td>
<td></td>
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<td>RESULTS AND DISCUSSION</td>
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<td>APPENDIX A</td>
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<td>SP350049 (PROTOCOL)</td>
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<tr>
<td>PROTOCOL ATTACHMENT 1</td>
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<td>APPENDIX B</td>
<td>B1-B3</td>
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</table>
SIGNATURE PAGE

BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY WITH
OPTIONAL HISTOLOGY

Initiation Date: July 25, 2006

Completion Date: August 18, 2006

Sponsor:

Sponsor's Representative:

Testing Facility: Institute for In Vitro Sciences, Inc.
21 Firstfield Road, Suite 220
Gaithersburg, MD 20878

Archive Location: Institute for In Vitro Sciences, Inc.
Gaithersburg, MD 20878

Study Director:

Laboratory Supervisor:

Gregory O. Moyer, M.B.A.

Project 4536, Final Report

3

CIR Panel Book Page 117
## TEST ARTICLE RECEIPT

| IIVS Test Article Number | Sponsor's Designation | Physical Description | Receipt Date | Storage Conditions*
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<th></th>
<th></th>
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<th></th>
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<tbody>
<tr>
<td>06AF67</td>
<td></td>
<td>white semi-viscous liquid</td>
<td>7/21/06</td>
<td>room temperature</td>
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</table>

* - Protected from exposure to light
BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY WITH OPTIONAL HISTOLOGY
INTRODUCTION

The Bovine Corneal Opacity and Permeability Assay (BCOP) was used to assess the potential ocular irritancy of the test article to isolated bovine corneas. Bovine corneas, obtained as a by-product from freshly slaughtered animals, were mounted in special holders and exposed to the test article. An *in vitro* score was determined for the test article based on the induction of opacity and permeability (to fluorescein) in the isolated bovine corneas.

The purpose of this study was to evaluate the potential ocular irritancy of the test article, supplied by ..., as measured by changes in opacity and permeability (to fluorescein) in isolated bovine corneas. The laboratory phase of this study was conducted on July 25, 2006 at the Institute for In Vitro Sciences, Inc. Five corneas were treated with the test article. Based on changes in corneal opacity and permeability (relative to the control corneas), an *in vitro* score was determined.
MATERIALS AND METHODS

Bovine Eyes

Bovine eyes were obtained from a local abattoir as a by-product from freshly slaughtered animals (J.W. TREUTH & SONS, Inc., Baltimore, MD). The eyes were excised and then placed in Hanks' Balanced Salt Solution, containing Penicillin/Streptomycin (HBSS), and transported to the laboratory on ice packs. Immediately upon receipt of the eyes into the laboratory, preparation of the corneas was initiated.

Preparation of Corneas

The eyes were grossly examined for damage and those exhibiting defects were discarded. The tissue surrounding the eyeball was carefully pulled away and the cornea was excised such that a 2 to 3 mm rim of sclera was present around the cornea. The isolated corneas were then stored in a petri dish containing HBSS until they were mounted in a corneal holder. The corneas were mounted in the holders with the endothelial side against the O-ring of the posterior chamber. The anterior chamber was then positioned on top of the cornea and the screws were tightened. Starting with the posterior chamber, the two chambers were then filled with Minimum Essential Medium (EMEM) without phenol red, containing 1% fetal bovine serum and 2mM L-glutamine (Complete MEM). Each corneal holder was uniquely identified with a number written in permanent marker, on both the anterior and posterior chambers. The corneal holders were incubated at 32 ± 1°C for a minimum of 1 hour.

Controls

The positive control used in this study was ethanol (Parnaco). The negative control used in this study was sterile, deionized water (Quality Biological).

Test Article Preparation

As instructed by the Sponsor, the test article was administered to the test system without dilution.

Test Article pH Determination

The pH of the test article was determined using pH paper (EMD Chemicals Inc.). Initially, the test article was added to 0-14 pH paper with 1.0 pH unit increments to approximate a narrow pH range. Next, the test article was added to 0-6 and 5-10 pH paper with 0.5 pH unit increments, to obtain a more accurate pH value. The pH value obtained from the narrower range pH paper is presented in Table 1.

Bovine Corneal Opacity and Permeability Assay

After a minimum of 1 hour of incubation, the corneas were removed from the incubator. The medium was removed from both chambers and replaced with fresh Complete MEM. The initial opacity was determined for each cornea using a Spectro Designs OP-KIT opacimeter. Three corneas, whose initial opacity readings were close to the median opacity for all the
corneas, were selected as the negative control corneas. The treatment of each cornea was identified with the test article number written in permanent marker on colored tape, affixed to each holder. The medium was then removed from the anterior chamber and replaced with the test article, positive control, or negative control.

**Method for Testing Liquid or Surfactant Materials**

The liquid test article, was tested neat. An aliquot of 750 μL of the test article, positive control, or negative control was introduced into the anterior chamber while slightly rotating the holder to ensure uniform distribution over the cornea. Due to its viscous nature, the test article, , was administered directly onto the exposed cornea using a positive displacement pipet. Each treated cornea was completely covered with the test article. Five corneas were incubated in the presence of the test article at 32 ± 1°C for 10 minutes. Three corneas were incubated in the presence of the negative control at 32 ± 1°C for 10 minutes. Three corneas were incubated in the presence of the positive control at 32 ± 1°C for 10 minutes. After the 10-minute exposure time, the control or test article treatments were removed. The epithelial side of the cornea was washed at least three times with Complete MEM (containing phenol red).

For corneas directly exposed to the test article (without anterior chamber window), the test article was removed from the treated corneas by rinsing the exposed epithelium of the corneas (special care was taken not to spray the corneas directly) with Complete MEM. The chamber windows were returned to the chamber when most or all of the test article had been removed. The rinsing process continued in the same manner as the positive and negative control corneas. The corneas were then given a final rinse with Complete MEM (without phenol red). The anterior chamber was refilled with fresh Complete MEM (without phenol red) and an opacity measurement was performed. The corneas were returned to the incubator for approximately 2 hours after which a final measure of opacity was obtained.

After the final opacity measurement was performed, the medium was removed from both chambers of the holder. The posterior chamber was filled with fresh Complete MEM and 1 mL of a 4 mg/mL fluorescein solution was added to the anterior chamber. The corneas were then incubated in a horizontal position (anterior side up) for approximately 90 minutes at 32 ± 1°C. At the end of the 90-minute incubation period, the medium was removed from the posterior chamber and placed into tubes numbered corresponding to chamber number. Aliquots of 360 μL from the numbered tubes were placed into their designated wells on a 96-well plate. The optical density at 490 nm (OD<sub>490</sub>) was determined using a Molecular Devices Vmax kinetic microplate reader. If the OD<sub>490</sub> value of a control or test article sample was 1.500 or above, a 1:5 dilution of the sample was prepared in Complete MEM (to bring the OD<sub>490</sub> value within the linear range of the platereader). A 360 μL sample of each 1:5 dilution was transferred to its specified well on the 96-well plate. The plate was read again and the final reading was saved to a designated print file.

**Fixation of Corneas**

After the medium was removed for the permeability determination, each cornea was carefully separated from its corneal holder and transferred to an individual prelabeled tissue cassette containing a biopsy sponge. The endothelial surface of each cornea was placed on the sponge to protect it. The cassettes were placed in 10% neutral buffered formalin to fix the corneal tissue for at least 24 hours. The fixed corneas will be stored up to one year.
**Histological Evaluation**

As instructed by the Sponsor, a histological evaluation was not performed.

**Presentation of Data**

Opacity Measurement: The change in opacity for each cornea (including the negative control corneas) was calculated by subtracting the initial opacity reading from the final opacity reading. These values were then corrected by subtracting from each the average change in opacity observed for the negative control corneas. The mean opacity value of each treatment group was calculated by averaging the corrected opacity values of each cornea for that treatment condition.

Permeability Measurement: The mean OD$_{490}$ for the blank wells was calculated. The mean blank OD$_{490}$ was then subtracted from the raw OD$_{490}$ of each well (corrected OD$_{490}$). Any dilutions that were made to bring the OD$_{490}$ readings into the linear range of the platteread (OD$_{490}$ should be less than 1.500), had each diluted OD$_{490}$ reading multiplied by the dilution factor. The final corrected OD$_{490}$ of the test article and the positive control was then calculated by subtracting the average corrected OD$_{490}$ of the negative control corneas from the corrected OD$_{490}$ value of each treated cornea:

Final Corrected OD$_{490}$ = (raw OD$_{490}$ – mean blank OD$_{490}$) – average corrected negative control OD$_{490}$

The mean OD$_{490}$ value of each treatment group was calculated by averaging the final corrected OD$_{490}$ values of the treated corneas for that treatment condition.

The following formula was used to determine the *in vitro* score:

$$In \ Vitro \ Score = Mean \ Opacity \ Value + (15 \times Mean \ OD_{490} \ Value)$$

**Criteria for Determination of a Valid Test**

The BCOP assay was accepted when the positive control (ethanol) caused an *in vitro* score that fell within two standard deviations of the historical mean.
RESULTS AND DISCUSSION

Bovine Corneal Opacity and Permeability Assay

Table 1 summarizes the opacity, permeability, and \textit{in vitro} score for the test article. Table 2 summarizes the opacity, permeability and \textit{in vitro} score for the positive control. Since the results of the positive control fell within two standard deviations of the historical mean (within a range of 39.4 to 64.2), the assay was considered valid. The opacity and permeability data for the individual corneas may be found in Appendix B.

The following classification system was established by Sina et al.\textsuperscript{1} based on studies with a wide range of test materials. While this classification system provides a good initial guide to interpretation of these \textit{in vitro} data, these specific ranges may not be applicable to all classes of materials.

\textit{In Vitro} Score:

\begin{itemize}
  \item from 0 to 25 = mild irritant
  \item from 25.1 to 55 = moderate irritant
  \item from 55.1 and above = severe irritant
\end{itemize}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
Assay Date & IVS Test Article Number & Sponsor’s Designation & Conc. & Exposure Time & Mean Opacity Value & Mean OD\textsubscript{490} Value & \textit{In Vitro} Score & pH \\
\hline
7/25/06 & 06AF67 & Neat & 10 minutes & 0.9 & 0.004 & 1.0 & 6.0 \\
\hline
\end{tabular}
\caption{BCOP Results of the Test Article}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Assay Date & Positive Control & Exposure Time & Mean Opacity Value & Mean OD\textsubscript{490} Value & \textit{In Vitro} Score \\
\hline
7/25/06 & Ethanol & 10 minutes & 26.3 & 1.126 & 43.2 \\
\hline
\end{tabular}
\caption{BCOP Results of the Positive Control}
\end{table}

Final Report

An In-Use Safety Evaluation to Determine the Dermal Irritation Potential of a Cosmetic Product or Toiletry

CLIENT:

ATTENTION:

TEST MATERIAL: After Shave Balm

CRL STUDY NUMBER: CRL66106

AUTHORIZED SIGNATURES:

Bruce E. Kanengiser, M.D.
President/Medical Director

Michael J. Muscattello, Ph.D.
Executive Vice President/COO

George J. Neuman, M.D.
Diplomate American Board of Dermatology

REPORT DATE: July 11, 2006
Good Clinical Practice
Quality Assurance Audit Statement

Clinical Study Number: CRL66106
Start Date: June 15, 2006
Completion Date: June 30, 2006

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

[Signature]
Signature of QA Auditor

[Date]
July 11, 2006
Date
FINAL REPORT

An In-Use Safety Evaluation to Determine the Dermal Irritation Potential of a Cosmetic Product or Toiletry

PURPOSE

The purpose of this study was to evaluate the dermal irritation potential of a cosmetic product following a 2-week use period.

INVESTIGATOR

George Neumaier, M.D.
Diplomate American Board of Dermatology

Clinical Research Laboratories, Inc.
371 Hoes Lane
Piscataway, New Jersey 08854
732-981-1616

SPONSOR

TEST MATERIAL

The following test material was provided by Research Laboratories, Inc. on June 9, 2006: and was received by Clinical After Shave Balm

STUDY DATES

This study was initiated on June 15, 2006 and was completed on June 30, 2006.
STUDY POPULATION

A total of 32 male subjects, ranging in age from 18 to 69 years old and in generally good health, were selected for the study (Panelist Demographics – Appendix I). Each subject was assigned a permanent CRL Identification Number. All panelists signed an Informed Consent Form in conformance with 21 CFR Part 50: “Protection of Human Subjects” and a HIPAA Authorization Form in conformance with 45 CFR Parts 160 and 164. All subjects completed a Panelist Profile/Medical History Form provided by Clinical Research Laboratories, Inc. prior to study enrollment. Subjects who met the following criteria were impaneled for this study.

Subject Inclusion Criteria:

1. Subject is male between 18 and 70 years of age;
2. Subject is free from any dermal disorders which may affect test results;
3. Subject has signed an Informed Consent Form in compliance with 21 CFR Part 50: “Protection of Human Subjects”;
4. Subject has completed a HIPAA Authorization Form in conformance with 45 CFR Parts 160 and 164;
5. Subject is dependable and able to follow directions;
6. Subject is in generally good health and has a current Panelist Profile/Medical History Form on file;
7. Subject agrees not to introduce any new cosmetic or personal care products, other than the assigned test material, during the course of the study.

Subject Exclusion Criteria:

1. Subject has received treatment with sympathomimetics, antihistamines, vasoconstrictors, non-steroidal anti-inflammatory agents, and/or systemic or topical corticosteroids within one week prior to initiation of the study;
2. Subject has known allergies to cosmetics or toiletries;
3. Subject has participated in a dermal study within one week of study initiation;
4. Subject has participated in an investigational systemic drug study within two weeks of study initiation;
5. Subject has a history of acute or chronic dermatologic, medical, and/or physical conditions which would preclude application of the test material and/or could influence the outcome of the study.
STUDY EVALUATIONS

Safety Assessments – Dermal Evaluations

The face and neck of each subject were examined for signs of irritation including erythema, edema, and dryness. Any observed irritation was graded and the results recorded on the dermal examination score sheet using the following scoring scales:

<table>
<thead>
<tr>
<th>Erythema</th>
<th>Edema</th>
<th>Dryness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = None</td>
<td>1 = None</td>
<td>1 = None</td>
</tr>
<tr>
<td>2 = Mild</td>
<td>2 = Mild</td>
<td>2 = Mild</td>
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<tr>
<td>3 = Moderate</td>
<td>3 = Moderate</td>
<td>3 = Moderate</td>
</tr>
<tr>
<td>4 = Severe</td>
<td>4 = Severe</td>
<td>4 = Severe</td>
</tr>
</tbody>
</table>

Efficacy Assessments – Questionnaires

An assessment of the effects of the test material was determined by questioning the treated subject with regard to the efficacy of the product following use. Questionnaires answered by subjects were tallied to determine the consensus opinion of the panel.

STUDY EXECUTION

Informed Consent

At the baseline/screening visit, the study procedures were explained to all subjects intending to participate. All subjects were completely informed about the pertinent details and purpose of the study, according to the Informed Consent guidelines. A written Informed Consent was read, understood, and signed by each subject. Each subject was given a copy of the signed Informed Consent Form.

Subject Identification

All subjects were initially identified by a permanent CRL identification number. Once the subject met qualification criteria, a study subject number was assigned at the Baseline Visit. This permanent subject number was assigned in sequence as subjects were enrolled in the study.

Product Use

Each subject was given a copy of the Sponsor’s use directions and panelist instructions (Appendix III). Each subject was instructed to use the test material once daily for two weeks. Other than the assigned test material, no new toiletries or personal care products were to be introduced by the panelists during the study.
STUDY EXECUTION (Continued)

Baseline Visit

All subjects reported to CRL for the Baseline Visit. The face and neck of each subject were examined and evaluated for evidence of irritation (erythema, edema and dryness) as described in the Study Evaluations section of this report. After acceptance onto the study, subjects were assigned sequential subject numbers in the order of qualification and were issued the identically numbered test product. Subjects were given verbal and written instructions outlining study requirements and restrictions and a Daily Diary to note each use of the assigned product.

Final Visit

After the approximately two-week use period, each subject was given a dermal examination as described in the Study Evaluations section of this report. The Daily Diaries were reviewed and collected along with test products at the conclusion of the study. A computerized questionnaire regarding product performance was completed at the final visit.

STATISTICAL ANALYSIS

Questionnaire responses, for which response category comparisons are informative, were analyzed by Z-tests. Z-tests were used to determine statistically significant differences in the proportions of subjects responding positively or negatively to each question offering a range of responses. The proportions of subjects choosing the central (neutral) responses were split equally and added to the response proportion of the top and bottom choices. The split proportions were compared by calculation of a Z-Score to determine statistically significant differences. Statistical significance exists for Z-scores greater than or equal to the absolute value of 1.96 at the 95% confidence level.

TEST RESULTS

A total of 31 subjects completed the study. One subject (#32) discontinued study participation for reasons unrelated to test material use. Dermal examination results appear in Table I. A summary and statistical analysis of questionnaire responses appears in Table II.

Daily Diaries

Comments recorded on the Daily Diary that were related to reactions or symptoms perceived during use of the test material included reports from three subjects. Subject #10 reported razor burn on one day of the study. Subjects #20 and #25 reported slight burning following application. All three subjects completed the study with no modification to the schedule of application and no dermal irritation of the face or neck was observed at the final visit. Individual comments recorded on the Daily Diary appear in Appendix IV.
CONCLUSION

The dermal evaluation of After Shave Balm over a 2-week use period revealed no evidence of erythema, edema, or dryness of the face or neck. In this limited test population, the test material identified as After Shave Balm does not demonstrate a clinically significant potential for eliciting dermal irritation.

Subject questionnaire responses indicated that the following qualities or test material effects were perceived with statistical significance in the majority of the test population:

- Liked the appearance of the product.
- Liked the scent of the product.
- The product was gentle to skin.
- The product was easy to apply.
- The product absorbed easily into skin.
- The product was soothing to skin.
- The product made skin feel cool.
- The product helped to moisturize skin.
- The product helped to condition skin.
- The product helped soften skin.
- The product comforted skin after shaving.
- The product was calming to skin.
- The product left a pleasant scent on skin

RETENTION

Test materials and all original forms of this study will be retained by Clinical Research Laboratories, Inc. as specified in CRL Standard Operating Procedure 30.6C, unless designated otherwise by the Sponsor.
Table I

Dermal Examination Results

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<th>Subject Number</th>
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<th>Final</th>
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Dermal Scoring Scale: (Erythema, Edema, Dryness)
1 = None
2 = Mild
3 = Moderate
4 = Severe

Discontinued Study Participation
Table 1
(Continued)

Dermal Examination Results

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</table>

Dermal Scoring Scale: (Erythema, Edema, Dryness)
1 = None
2 = Mild
3 = Moderate
4 = Severe

Discontinued Study Participation
Appendix III

Use Instructions

DIRECTIONS FOR USE:

1. Use this product once daily for a period of 2 weeks.

2. Apply the product at least once daily on clean shaven skin, apply a small amount to face and the neck. Avoid the eye area and contact with eyes.

3. You are not to introduce any new shave balms or personal care products during this study.

4. Avoid direct contact with eyes.
**SUMMARY REPORT**

TKL Study No.: DS103906-5  
**Date of Report:** July 12, 2006

**Study Title:** Repeated Insult Patch Test

**Study Sponsor:**

**TKL Protocol No.:** TKL-1000  
**Sponsor Reference No.:**

**Study Objective:** To assess the sensitization potential of topically applied study material.

**Study Design:** Standard RIPT methodology with nine 24-hour induction applications and a single 24-hour challenge application following a 10-15 day rest period.

**Principal Investigator:** Jonathan S. Dosik, MD – Dermatologist  
**Manager, Dermatologic Safety Testing:** Kathleen Georgeian

**Study Center:** TKL Research, Inc.  
4 Forest Avenue  
Paramus, NJ 07652  
**Study Site:** Paramus, NJ

**Study Dates:**  
**Date Study Initiated:** May 8, 2006  
**Date Study Completed:** June 15, 2006

**Study Material:**

<table>
<thead>
<tr>
<th>SPL Code and Category</th>
<th>Amount Applied</th>
<th>Patch Condition</th>
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</thead>
<tbody>
<tr>
<td>After Shave Balm</td>
<td>0.2 mL</td>
<td>Occlusive</td>
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</table>

**Special Instructions:** Air dried at least 20 minutes prior to patch application.

**Number of Subjects:**  
Enrolled: 117  
Completed: 105  
Discontinued: 12  
Lost to follow-up: 5  
Voluntary withdrawal: 4  
Protocol violation: 3
SUMMARY REPORT (Continued)

TKL Study No.: DS103906-5

Listing of Attached Tables:

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Summary Tables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendix</td>
<td>Data Listings</td>
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</table>

SUMMARY AND CONCLUSION:

There were no adverse events reported.

A summary of response data is provided in Table 3, Appendix I. Individual dermatological response grades are provided in Data Listing 3, Appendix II.

Under the conditions employed in this study, there was no evidence of sensitization to SPL Code.

SIGNATURES:

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.

Jonathan S. Dosik, MD
Dermatologist/Principal Investigator

Kathleen Georgian
Clinical Research Coordinator and Manager, Dermatologic Safety Testing

This report has been reviewed by the TKL Corporate Quality Assurance Department and the report accurately reflects the raw data for this study.

Quality Assurance

Date

[em.j]/Dipt/MCI DS103906-5R
TABLE 1: SUMMARY OF SUBJECT ENROLLMENT AND DISPOSITION

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<tr>
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<th>n (%)</th>
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<tr>
<td>Subjects enrolled</td>
<td>117</td>
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<tr>
<td>Subjects completed induction</td>
<td>106 (90.6)</td>
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<tr>
<td>Subjects completed all phases</td>
<td>105 (89.7)</td>
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<tr>
<td>Total subjects discontinued</td>
<td>12 (10.3)</td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>5 (4.3)</td>
</tr>
<tr>
<td>Voluntary withdrawal</td>
<td>4 (3.4)</td>
</tr>
<tr>
<td>Protocol violation</td>
<td>3 (2.6)</td>
</tr>
</tbody>
</table>

Note: All percentages are relative to total subjects enrolled

See Data Listing 1 for further detail

Program: DISPSWY.SAS/USES: FINAL/29JUN06:16:01:39
TABLE 2: SUMMARY OF SUBJECT DEMOGRAPHICS
ALL ENROLLED SUBJECTS

<table>
<thead>
<tr>
<th>Age</th>
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<tr>
<td>n (%) 18 to 44</td>
<td>47 (40.2)</td>
</tr>
<tr>
<td>n (%) 45 to 84</td>
<td>57 (48.7)</td>
</tr>
<tr>
<td>n (%) 65 and up</td>
<td>13 (11.1)</td>
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</table>

<table>
<thead>
<tr>
<th>Mean (SD)</th>
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<tr>
<td>46.7 (14.3)</td>
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<table>
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<th>Median</th>
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<th>Range</th>
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<td>18.6 to 70.7</td>
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<table>
<thead>
<tr>
<th>Gender</th>
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<tr>
<td>n (%) Male</td>
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<tr>
<td>n (%) Female</td>
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<table>
<thead>
<tr>
<th>Race</th>
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<tr>
<td>n (%) Asian</td>
<td>3 (2.6)</td>
</tr>
<tr>
<td>n (%) Black</td>
<td>1' (9.4)</td>
</tr>
<tr>
<td>n (%) Caucasian</td>
<td>8' (69.2)</td>
</tr>
<tr>
<td>n (%) Hispanic</td>
<td>2' (17.9)</td>
</tr>
<tr>
<td>n (%) Other</td>
<td>' (0.9)</td>
</tr>
</tbody>
</table>

See Data Listing 2 for further detail

Program: DEMOSMY.SAS/USES: DEMOGS/29JUN06:16:01:41
TABLE 3: SUMMARY OF DERMATOLOGIC RESPONSE GRADES
NUMBER OF SUBJECTS BY PRODUCT

<table>
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<td></td>
<td>-</td>
<td>107</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>?</td>
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<td>3</td>
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<tr>
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<td>+</td>
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<td>Total evaluable</td>
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<td>Number discontinued</td>
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MAXIMUM ELICITED RESPONSE DURING INDUCTION
ALL SUBJECTS COMPLETING INDUCTION (N=105)

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<td>103 (97.2%)</td>
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<tr>
<td>?</td>
<td>2 (1.9%)</td>
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<tr>
<td>+</td>
<td>1 (0.9%)</td>
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</table>

(*) when required

Key to Symbols:
- = No reaction
? = Minimal or doubtful response, slightly different from surrounding normal skin
+ = Definite erythema, no edema
+++ = Definite erythema, definite edema and vesiculation
D = Damage to epidermis: oozing, crusting and/or superficial erosions
p = Papular response >50%

Program: SUMMARY.SAS/USES: RESPONSE, PROD LIST, FINAL/29JUN05:16:01:50
APPENDIX II

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</table>

Key: Last Reading # (I=Induction Phase, C=Challenge Phase)
    Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal
    V=Protocol violation, AE=Adverse event, O=Other)

Program: DISPLIST.SAS/USES: DEMOGRS, RESPONSE, FINAL/29JUN06:16:01:23
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Key: Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal)
V=Protocol violation, AE=Adverse event, O=Other)

Program: DISPLIST.SAS/USES: DEMOGS, RESPONSE, FINAL/29JUN06:15:01:23
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Key: Last Reading #: (I=Induction Phase, C=Challenge Phase)
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**TKL STUDY NO. DS103906**

**DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES BY PRODUCT AND SUBJECT**

**PRODUCT**

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Key to Symbols:

- = No reaction

? = Minimal or doubtful response, slightly different from surrounding normal skin

+ = Definite erythema, no edema

++ = Definite erythema, definite edema

+++ = Definite erythema, definite edema and vesiculation

N9G = No ninth grading

NA = Not applied

NP = Not patched due to reaction achieved

X = Reading not performed due to missed visit or subject discontinuation

D = Damage to epidermis: oozing, crusting and/or superficial erosions

p = Papular response >50%

MU = Make-up reading for missed induction visit

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PROD LIST/29/JUN05:16:01:25
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TKL STUDY NO. DS103906
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

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Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/29JUN06:16:01:25
Memorandum

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

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

DATE: June 1, 2012


Memo - The date on the memo should be the date the memo was written, rather than the date of the meeting. Knowing the date the memo was written is helpful when checking to be sure all information provided by industry is in the report. Information provided after the memo was written would not be expected to be in the report, while information provided before the memo was written should be in the report.

p.1 - It may be helpful to state in the Introduction (or somewhere else in this report) that although these ingredients may be used as cosmetic colors somewhere in the world, in the United States, grape-derived ingredients are not approved color additives for use in cosmetic products.

p.3 - Please note that grape skin extract is only approved for coloring beverages.

p.4 - In the Cosmetic Use section, it may be helpful to state that although some drug functions are reported for some grape-derived ingredients, none of the ingredients have approved drug uses in the United States.

p.7 - In the description of the results of the skin lightening study (reference 28), were the markers significantly decreased compared to controls (referring to the last sentence of the description of this study)?

p.8 - Something is missing from the following “in several clinical chemistry were observed...”

p.8 - In the last paragraph of this page, it is not necessary to describe a rabbit study as “non-human”. Please change “Vitis Vinifera (Cucumber) Juice” to “Vitis Vinifera (Grape) Juice”.

p.17-18 - Please cite IARC conclusions (caffeic acid, ferulic acid, tannins) to IARC not a CIR report.

p.21-22, Table 7 - Please include a key for the abbreviations included in this table.

p.23, Table 8 - The source of the extract used in the first study (reference 83) is not clear. It is presented under the heading “Grape”, which has been used to represent the fruit in other tables, but in the test article column it says “(plant)”. If this is a whole plant extract, perhaps the heading needs to be revised.

p.23, Table 8 - In the Results column for reference 34, please change “those that weren’t” to “untreated controls”
Memorandum

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

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

DATE: July 3, 2012

SUBJECT: Comments on the Tentative Report on Grape-Derived Ingredients

Please see the attached completed monograph for Vitis Vinifera (Grape) Shoot Extract.

p.7, 9 - Please revise (occurs on both p. 7 and 9) "...in a chromosomal aberration, and the mixed..."
   (the word "assay" needs to be added after the word aberration)

p.7 - In the first paragraph of the Carcinogenicity section, please delete "of" in the following: "to
determine whether dietary [of] grape seed extract alone had any effect on skin tumor
formation."

p.9, third paragraph of the Summary - The information in the following sentences does not appear to be
presented elsewhere in the report (not found in either the text or tables). This information
should either be presented elsewhere in the report, or deleted from the Summary. "In an in vitro
study using reconstructed three-dimensional skin equivalent model, grape seed extract
stimulated keratinocyte proliferation, fibrillin-1, elastin, and collagen type-1. Grape seed
extract inhibited mushroom tyrosinase activity, and it inhibited melanogenesis in cultured B16
melanoma cells."

p.10, first complete paragraph - "Cucumber" needs to be changed to "Grape"

p.19, Table 6 - If available, please provide some indication of the dose of Leucocyanidin that resulted
   in effects. If no further details are available, it would be helpful to state: "Without stating any
details, a review stated that ..."

p.19, Table 6 - In the D,L-citronellol paragraph, please change "inhalation NOAEL" to "inhalation
   NOAEC" as mg/m³ is a concentration.

p.25, Table 10, Procedure column, grape seed extract - correct "2ks" to "2 wks"
26453: VITIS VINIFERA (GRAPE) SHOOT EXTRACT

INCI Monograph ID: 26453

Flags: ReadyToPublish, OTCDrug = True, OTCApproved = False

Definition: Vitis Vinifera (Grape) Shoot Extract is the extract of the shoots of the vines of Vitis vinifera. See Reported Ingredient Functions-The Cosmetic Drug Distinction, in Regulatory and Ingredient Use Information, Volume I, Part A.

Chemical Class: C2018- Botanical Products and Botanical Derivatives

Reported Functions: F60- Antioxidant; F580- Skin Protectant

Ingredient Source: Plant

Trade Name:
N99232- French Grapevine Extract
(S1781- Berkem)