Safety Assessment of *Ginkgo biloba*-Derived Ingredients as Used in Cosmetics

Status: Draft Tentative Report for Panel Review
Release Date: February 9, 2018
Panel Meeting Date: March 5-6, 2018
Memorandum

To: CIR Expert Panel Members and Liaisons
From: Christina L. Burnett, Senior Scientific Writer/Analyst
Date: February 9, 2018
Subject: Draft Tentative Safety Assessment on Ginkgo biloba-Derived Ingredients

Enclosed is the Draft Tentative Report of the Safety Assessment of Ginkgo biloba-Derived Ingredients as Used in Cosmetics. (It is identified as ginkgo032018rep in the pdf document).

In December 2017, the Panel issued an Insufficient Data Announcement for these 10 ingredients. The Panel’s data needs were:

- Method of manufacturing for each of these Ginkgo biloba-derived cosmetic ingredients
- Composition and impurities data for each of these Ginkgo biloba-derived cosmetic ingredients
- 28-Day dermal toxicity data
- Dermal irritation and sensitization data at leave-on use concentrations (i.e., up to 1% Ginkgo Biloba Leaf Extract)
- Ocular irritation data, if available
- Genotoxicity data
- Developmental and reproductive toxicity data
- Data on the absorption spectra or phototoxicity of these cosmetic ingredients

Since the December Panel meeting, CIR has received the following requested data, which have been incorporated into the report and have been designated with [brackets] in the text or highlighting in the tables (ginkgo032018 data1 through ginkgo032018data5).

- HRIPT on a lotion containing 0.2% Ginkgo Biloba Leaf Extract
- Certificate of analysis for a Ginkgo Biloba Leaf Extract
- Composition, method of manufacturing, and toxicity data on Ginkgo Biloba Meristem Cell
- Absorption spectra for of a Ginkgo Biloba Leaf Extract
- Summaries of HRIPTs, phototoxicity/photoallergy, and in vitro ocular tests on Ginkgo Biloba Leaf Extract

Additional data from the published literature has also been incorporated in the report and designated appropriately.

Comments provided by the Council prior to the December meeting on the draft report have been addressed (ginkgo03218pcpc). Additionally, comments provided by the American Herbal Products Association (AHPA) have been addressed (ginkgo032018ahpacomments). The AHPA also provided their comments on the 2-year gavage studies performed by the NTP (ginkgo032018ahpa_ntp).

Based on the proceedings and comments from the December meeting, we have started a draft Discussion with some points for the Panel to consider, including the findings on the genotoxicity and carcinogenicity studies by the NTP, sensitization concerns, and the outstanding data needs.

The Panel should carefully consider and discuss the data and the draft Abstract and Discussion presented in this report and issue a Tentative Report with a safe, safe with qualifications, insufficient data, or split conclusion.
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY  
Ginkgo biloba-derived ingredients

MEETING  
March 2018

<table>
<thead>
<tr>
<th>Public Comment</th>
<th>CIR</th>
<th>Expert Panel</th>
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**Ginkgo biloba-Derived Ingredients History**

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

**December 2017** - The Panel issued an Insufficient Data Announcement for these 10 ingredients. The Panel’s data needs were:

- Method of manufacturing for each of these Ginkgo biloba-derived *cosmetic ingredients*
- Composition and impurities data for each of these Ginkgo biloba-derived *cosmetic ingredients*
- 28-Day dermal toxicity data
- Dermal irritation and sensitization data at leave-on use concentrations
- Ocular irritation data, if available
- Genotoxicity data
- Developmental and reproductive toxicity data
- Data on the absorption spectra or phototoxicity of these *cosmetic ingredients*
### Ginkgo biloba-Derived Ingredients Data Profile – March 2018 – Writer, Christina Burnett

<table>
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<tr>
<th>Ingredient</th>
<th>In-Use</th>
<th>Physical/Chemical Properties</th>
<th>Method of Manufacturing</th>
<th>Composition/Impurities</th>
<th>UV Absorption</th>
<th>Acute Toxicity</th>
<th>Repeated Dose Toxicity</th>
<th>Genotoxicity</th>
<th>Reproductive and Developmental Toxicity</th>
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<th>Toxicokinetics</th>
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“X” indicates that data were available in the category for that ingredient.
## Ginkgo-Derived Ingredients

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<th>TOXNET</th>
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### Botanical and/or Fragrance Websites (if applicable)

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<th>GRIN</th>
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NA = Not applicable

Distributed for comment only -- do not cite or quote
Search Strategy

SciFinder

Search for CAS # and INCI names yielded 14 returns (8 for “Ginkgo Biloba Leaf”, 6 for CAS #), reference search was for “adverse effect, including toxicity” (some hits were repeated under the terpenoids CAS #).

Ginkgo Biloba Leaf = 0 hits
107438-79-9 = 3 hits, 2 relevant
15291-75-5 = 14 hits, 5 relevant
15291-76-6 = 4 hits, 3 relevant
15291-77-7 = 123 hits, 10 relevant
33570-04-6 = 23 hits, 15 relevant
90045-36-6 = 1 hit, 1 relevant

PubMed Search: ((((((((((((((ginkgo biloba leaf extract) OR ginkgo biflavones) OR ginkgo biloba leaf) OR ginkgo biloba leaf powder) OR ginkgo biloba leaf water) OR ginkgo biloba leaf cell extract) OR ginkgo biloba meristem cell) OR ginkgo biloba nut extract) OR ginkgo biloba root extract) OR ginkgo leaf terpinoids) OR 90045-36-6) OR 107438-79-9) OR 15291-75-5) OR 15291-76-6) OR 15291-77-7) OR 33570-04-6 AND (tox[sb]) = 605 hits; 53 useful

Search updated January 2018 – 7 more references were found relevant and ordered.

Search Engines

- Toxnet [https://toxnet.nlm.nih.gov/] (includes Toxline; HSDB; ChemIDPlus; DART; IRIS; CCRIS; CPDB; GENE-TOX)
- SciFinder [https://scifinder.cas.org/scifinder]

Pertinent Websites

- wINCI - [http://webdictionary.personalcarecouncil.org]
- FDA databases [http://www.ecfr.gov/cgi-bin/ECFR?page=browse]
- FDA search databases: [http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm]
- GRAS listing: [http://www.fda.gov/Food/IngredientsPackagingLabeling/gras/default.htm]
- SCOGS database: [http://www.fda.gov/Food/IngredientsPackagingLabeling/gras/scogs/ucm2006852.htm]
- Indirect Food Additives: [http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives]
- Drug Approvals and Database: [http://www.fda.gov/Drugs/InformationOnDrugs/default.htm]
- [http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf]
- FDA Orange Book: [https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm]
- OTC ingredient list: [https://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf]
- (inactive ingredients approved for drugs: [http://www.accessdata.fda.gov/scripts/cder/iig/]
- HPVIS (EPA High-Production Volume Info Systems) - [https://ofmext.epa.gov/HPVIS/HPVISlogan]
- NIOSH (National Institute for Occupational Safety and Health) - [http://www.cdc.gov/niosh/]
- NTIS (National Technical Information Service) - [http://www.ntis.gov/]
- NTP (National Toxicology Program) - [http://ntp.niehs.nih.gov/]
- Office of Dietary Supplements [https://ods.od.nih.gov/]

LINKS
- FEMA (Flavor & Extract Manufacturers Association) - [http://www.femaflavor.org/search/apachesolr_search/](http://www.femaflavor.org/search/apachesolr_search/)
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - [http://www.ecetoc.org](http://www.ecetoc.org)
- [www.google.com](http://www.google.com) - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

**Botanical Websites, if applicable**
- GRIN (U.S. National Plant Germplasm System) - [https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx](https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx)
- National Agricultural Library NAL Catalog (AGRICOLA) - [https://agricola.nal.usda.gov/](https://agricola.nal.usda.gov/)

**Fragrance Websites, if applicable**
- Research Institute for Fragrance Materials (RIFM)

**Note:** ChemPortal can be used to search several of the above databases simultaneously - [http://www.echemportal.org/echemportal/index?pageID=0&request_locale=en](http://www.echemportal.org/echemportal/index?pageID=0&request_locale=en)
Dr. Belsito’s Team

DR. BELSITO: So then Ginkgo biloba. So this is the first time that we're looking at this report. Ten ingredients derived from Ginkgo biloba. There were no publicly available toxicity data available on any of the cosmetic products or any of the ingredients specifically and all of the end points were taken from the literature that variably described extracts of Ginkgo biloba leaves or some of non-cosmetic ingredients and source.

So with that as background, where are we here? I thought that we needed sensitization and irritation data for all of it depending on what we learn from composition and manufacturing. The ginkgolic acid is related to urushiol. And wave two we did get HRIPT but it was at 1 percent, the maximum leave on is 1 percent. There was no irritation at 100 percent in 20 individuals, I thought that was good. The composition, we need it all except for the leaf extracts.

Manufacturing, again the same we just need the leaf extracts. Ocular irritation would be nice if it were available since it's used around the eyes. I guess phototoxicity we may need depending upon the chemical composition of the non-leaf parts, we don't need it for the leaf parts because I think they're - you know what we really need is manufacturing and composition to be able to go ahead and decide.

DR. LIEBLER: Right. I think not surprisingly the leaf extract derived material is why we used and that’s what drives the whole report. On the other hand, we’ve got all these other ingredients that are not leaf derived, not leaf terpenoids. Leaf terpenoids by the way is not well defined even though it's from the leaf.

Depending on what it is and how concentrated these things are that could be problem. Meristem, of course the usual meristem, rather old for us. And all the leaf derived ingredients I think the information we have on leaf extract is probably fine. But everything else remains - except for sensitization.

I have a note that if you're not happy with sensitization then we're not happy with it so, but as far as composition and method of manufacture and components of concern I think we've got enough data on the leaf extracts to represent all the leaf stuff.

DR. BELSITO: I'll assume you’re talking about -

DR. LIEBLER: Leaf powder -

DR. BELSITO: (inaudible) leaf powder, leaf water, you're happy with all of those?

DR. LIEBLER: yeah, I think those would be represented by leaf.

DR. BELSITO: Okay.

DR. LIEBLER: Okay. but that won't cover the root, the nut, the leaf terpenoids, and the meristem cell.

DR. BELSITO: There was a question back there?

DR. ZIMMERMANN: Yes, Merle Zimmermann from the American Herbal Product Association. We have done some studying in particular on some of the different leaf extracts and in the draft here it looks like GBE is being used to refer to sort of a generally leaf extracts that are few in the draft that have somewhat different characteristics than the description upfront I'm not sure (inaudible)

DR. LIEBLER: You have new data that we don't have already?
DR. ZIMMERMANN: Well, I have some data from the office and some comments of the NTP’s particular analysis of the material. It's a little different than the material that's manufacturing here in the draft.

DR. LIEBLER: Okay, was that material available to us?

DR. ZIMMERMANN: Yeah, I've got a copy available that I can -

DR. LIEBLER: Okay, but it wasn't something that you’d seen before, Christina?

DR. ZIMMERMANN: These were in the comments of the draft of the NTP article I'm not sure it was previously distributed.

DR. HELDRETH: Yeah, we've seen lots of different compositions when we're out there looking for it but as Christina stated in the report and Don reiterated, none of them seem to have the necessary association what the cosmetic ingredients might end up being. So that's why we put a plethora of different compositions in there because none of them seem to be more important than the other in terms of cosmetic ingredient composition.

DR. LIEBLER: But I think even if we don't know what you have in the green folder represents the cosmetic ingredient, if it represents data on ginkgo leaf extracts that we didn't already have, it would be useful to have.

DR. BELSITO: So you have data on composition but is it composition on Ginkgo biloba leaf that is used in a cosmetic or it's just a composition without known use?

MR. ZIMMERMANN: The compositions that are described here in the methods of manufacturing are a little different than the compositions of the particular concentrated extract that the NTP study was done with. It's included in the text of the NTP study but it's kind of buried in there. And I have it pulled out here if you'd like it.

DR. ANSELL: The point being that we think that some of that data cited as it related to NTP is not relevant to the commercial cosmetic material and we have a composition description as it relates to the NTP standardized materials which cite how they differ from the cosmetic grade and I think that's the point the gentleman's making.

DR. ZIMMERMANN: Yes.

DR. HELDRETH: Yeah as far as we could see when we were looking at it we're seeing, yes, there's a different between whatever NTP looked at and the "standardized" version but that really doesn't tell us anything about the cosmetic version, neither one of them are the cosmetic version. And we really have no information telling us how the cosmetic version is different.

DR. LIEBLER: But we have information in our report already about extracts that are not necessarily the cosmetic ingredient and then we have other information about other extracts that are also not necessary the cosmetic ingredient but why do we not want to even look at that? That doesn't make any sense to me.

MS. BURNETT: The data is in there. I think the issue is, I did not separate the constituent profile of the NTP study from that of the standardized extract. I think that's the issue. The data's there, it's just I'm showing a range and I think I need to separate the NTP -

DR. LIEBLER: So it's incorporated in to the data that we've already seen?

MS. BURNETT: Yes, it's there. It's all there. It's showing - instead of separating out the two different things, I just lumped them together and the issue is that I lumped them together.
DR. ANSELL: yes, I think we just jumped two or three steps ahead of where we were conversationally. I think there is significant data on the use of equivalent materials, as it relates to the leaf and all the leaf extracts, we agree that some of the discussions about some of the other sections, other preparations.

There's less data we think that the topical data is in fact needed but as it relates to the interpretation specifically of the NTP data we think that that needs to be highlighted. That the material used there is not reflected of the commercial grade used.

MS. BURNETT: I do want to note that there is no different constituent. It's just that different concentration of use. Well, not concentrations of use but different concentration profile so it's not like one thing has ginkgolic acid and one doesn't. It just has it at different -

DR. ANSELL: Right and we will provide that data so that the panel can decide how relevant the standardized material is to the interpretation of cosmetic materials.

DR. LIEBLER: So we're still lacking in data on the Ginkgo leaf extract that's actually supplied as a cosmetic ingredient?

MS. BURNETT: We received something in wave two. The extraction method is different from the standardized and from the NTP. The standardized is extracted with acetone and what we received was extracted with ethanol and or ethanol butylene glycol, I think

DR. BERGFELD: Glycol.

DR. LIEBLER: So the method could be different. There could be variations in the method even among cosmetic ingredient producers. The composition of those extracts, if I had to bet a beer, it would be similar.

MS. BURNETT: Just different percentages of each little constituent.

DR. LIEBLER: Yeah, because those are very similar extraction methods basically.

DR. BELSITO: But you're right. We have no data specific to the material that is used in a cosmetic.

MS. BURNETT: Except for the wave two, what we received is the only thing that we have.

DR. HELDRETH: And that's why we didn't callout the differences between the standardized versus the NTP. It didn't seem super relevant to the end result here.

DR. BELSITO: And wave two is just one manufacturer's way of doing this?

MS. BURNETT: Correct.

DR. BERGFELD: Well, sometimes we've only had one manufacturer's way.

DR. BELSITO: No I understand. Paul, just a question to you. I think it's not relevant given these concentrations but your assessment of the effects of the DART study

DR. SNYDER: I didn’t alert to anything there.

DR. BELSITO: Okay, go ahead.
DR. SNYDER: Related to the concentration of use, we're going to make a request of sensitization at the highest concentration of use, we don't have 2017 concentration of use data, we have 2014, we have categories for 2017- are we going to get new data for 2017 so we don't get caught up in getting a higher concentration use again?

MS. BURNETT: I do not know if council will resurvey. It took a while to write the report, I had other Citrus things going on. I finally got around to it this year. I can ask Carol-

DR. SNYDER: Caveat to, I just want to makes sure that we're aware, we don't have most current concentration of use. The most current is 1 percent is based on 2014 when our other survey data is from 2017.

MS. BURNETT: I can ask Carol if she's thinking of resurveying.

DR. BELSITO: I think probably -

MS. BURNETT: I doubt it, I doubt it.

DR. BELSITO: These botanicals are just going crazy and the changes that have occurred in their use in the past 3 years has just been amazing. I mean, you read the levels on shampoos, conditioners, makeup, everything I mean it's one plant product after the other after the other. I mean, I think that that is an insufficiency as well. That she needs to go out and resurvey industry and find out where they're using it and what the concentration ranges are.

DR. ANSELL: And while that's certainly true but for Ginkgo which is going to rely to great extent as it's use as a dietary supplement, I mean the concentrations were just simply going to swamp any cosmetic application. I think that would be more relevant for materials which are more esoteric but at any given time we have to do a survey and it's a snapshot in time.

DR. BELSITO: I'm not talking about toxicity issues, I'm talking about - I mean systemic toxicity issues, I'm talking about skin sensitization because basically what we've said it that we're okay on all the leaf derived ingredients except for sensitization at the highest concentration of use. If we don't know what that concentration of use is then, you know, I guess we can say in the discussion that it's our assumption that that concentration of use has not changed from 2014, but that seems pretty lame when this publication will come out in 2018 or 2019.

To say that the panel that's looking at ingredients for the cosmetic ingredient review doesn't know having the updated dates for 4 years in a cosmetic ingredient that they are reviewing.

DR. ANSELL: But that's always true in these reports that they're snapshots in time and that the panels opinion is based on, is predicated on the use concentrations and applications within the report.

DR. BELSITO: I agree, and it's always a snapshot but the snapshot is usually taken just as the report is coming out. Where we have 2017 data and safe as used, now we're saying safe as used we don't know how it - this report will have a publication date of 2018 and we don't know how it's used in 2018.

DR. ANSELL: When we do the systematic reviews at 15 years, clearly we need to redo it but we would be chasing our tails here if at every stage we have to go back and resurvey whether there's been a change between the SLR and the final report. Many of these take several years to lock down and so it's not a trivial exercise. I think if there were a specific reason that the use concentrations and the data available were very tight that it might be required but in this case, I just don't see it.

DR. SNYDER: So I had a note, coming at that point of discussion at a later botanical, that we use a terminology that this safety assessment is based upon information sourced from published and nonpublished. For botanicals I really don't care about the source of the information but we really need to say it's based upon the data presented in this report. It goes to what Jay is saying because the for the botanicals we make a lot of assumptions based upon
composition data, extraction methods that we have, and it's all based upon the data that we have. It's not based upon unknown data. It's based upon the data that we have on this report and so I think that statement needs to be changed, that it's based up on the data in this report because that does reflect what we actually looked at.

Because we don't know what other extraction methods or compositions, from different country sourced botanical, et cetera, et cetera. So I think that's important for us to have that in the botanical reports in particular.

DR. HELDRETH: And that's something that we do include in the conclusion. I mean our conclusions in those cases where it's safe, typically read safe as used in present practices of use and concentration described in this report. So I think that -

DR. SNYDER: But we don't use that language in the beginning when we talk about the safety assessment is based on information sourced from published, nonpublished and so to me it's based upon information in this report.

DR. HELDRETH: So you'd like to see that pre-iterated earlier in report.

DR. SNYDER: Yes, exactly.

DR. BERGFELD: Just to follow that through, Bart. Are you adding that last little phrase to all the ingredients? I don't think so.

DR. SNYDER: No, just particularly to the botanicals.

DR. BERGFELD: I know, but it does apply to all, what's in this report.

DR. SNYDER: Yeah, I mean at some instances it does but -

DR. BERGFELD: It's not always there.

DR. HELDRETH: No, I mean our conclusions were different in the past but that is our standard -

DR. BERGFELD: That's your standard now? That's what I'm asking.

DR. HELDRETH: Yes.

DR. BELSITO: Okay, so here's my assessment of where we're at, let me see if I summarize it correctly. This is currently insufficient. For all of the leaf products, it's insufficient for sensitization at the highest concentration of use. For all the nonleaf products, it's insufficient for method of manufacture, composition except for the Ginkgo biflavones and the Ginkgo leaf terpenoids, Ginkgo leaf terpenoids has the word leaf in it, but I don't think that's comparable to the leaf extracts. There's something else that's done to that material to produce terpenoids and we would need to know about that. So we don't know that and I would say the same for biflavones even though it doesn't have the leaf. So the leaf things except for the leaf terpenoids I think are okay.

DR. BELSITO: And then of course, the pesticide and respiratory boilerplate would need to be brought into discussion for these.

DR. BERGFELD: So you're sending this out, that insufficient data announcement, that's your movement now?

DR. BELSITO: Mhmm.
Dr. Marks’ Team

DR. EISENMANN: Before we begin, I'd like to introduce Dr. Merle Zimmermann from the American Herbal Products --

DR. ZIMMERMANN: Hi.


DR. MARKS: Hey, welcome.

DR. ZIMMERMANN: Thank you.

DR. EISENMANN: So he might have some comments on ginkgo.

DR. MARKS: Do you want to come up here to the table --

DR. ZIMMERMANN: Yeah, (inaudible).

DR. MARKS: That way, you'll be a microphone. You'll have to come up. It's -- this is the first -- oh, you're welcome. This is the first time that we're looking at these ginkgo-derived ingredients. So I'm going to pull that up. So it's a draft report for 10 ingredients. We're -- Ron, Ron, and Tom do like the extract, leaf, meristem, nut, roots - so we get some different parts of the plant with ginkgo. Do you like them?

Are all the ingredients, okay. I can't imagine how we can eliminate any. At least, at this point is -- are there that should be eliminated? Hearing no response, I assume that means no.

DR. SLAGA: Right.

DR. HILL: Are the ginkgo bioflaviones a botanical, or are they sufficiently processed that we can consider them to be --

DR. HELDRETH: It's a mixture.

DR. HILL: (Inaudible)

DR. HELDRETH: It's not (inaudible).

MS. BURNETT: And we don't have the data on this.

DR. MARKS: So needs? Ron, Ron and Tom?

DR. HILL: What do I have?

MS. BURNETT: (Inaudible)

DR. MARKS: I can see --

DR. SHANK: I'm going to get in trouble.

DR. MARKS: No, I --

DR. HILL: And go for it.

DR. MARKS: -- it's -- I really --
DR. SHANK: (Inaudible)

DR. MARKS: -- I really like that I sit across from you all because you can read mine on herbal and vice versa. And then Dr. Shank has a lot of non-verbal communication.

MS. BURNETT: Yes.

DR. MARKS: So who wants to go first?

DR. SHANKS: It's old age. It's my --

DR. MARKS: Nah.

DR. SHANKS: Well, from the toxicology point of view, there are a lot of red flags. One, I think we may have trouble with is that the -- there are several genotoxicity studies with mixed results. Some positive, some negative. So we say, well, better to have carcinogenicity data than we do. And in the NTP study, ginkgo biloba -- I forget what exactly the form was -- yielded liver carcinomas in bone marrow lymphomas.

So the IARC has taken a position that ginkgo leaf extract is classified as a possible human carcinogen and actually cautions that it may be unsafe for use in cosmetics. This is not withstanding the argument that low dose exposures from cosmetic use versus a high dose exposures in animal tests have to be weighed. But you add to it that there is a study on a mouse lymph node assay which showed a positive lymph -- called popliteal reaction which would support the bone marrow carcinogenicity.

But we have a long history of human use of ginkgo leaf preparations in folk medicine which have not shown any serious effects. So this is -- I think, this is going to be a hard one. I also found it hard to know all these ginkgo leaf preparation used in cosmetics. Is everybody using the same thing? Are all the preparations the same? What actually chemically is going into the cosmetics? Getting down to sensitization. Wave two has HRIPT for the leaf extract of at a tenth of a percent with -- apparently, it used as high 1 percent.

DR. MARKS: I --

DR. SHANK: That's --

DR. MARKS: -- sorry.

DR. SHANK: -- 10 times higher.

DR. MARKS: Mm-hmm.

DR. SHANK: There's no other information on ginkgo ingredients. So we need method of preparation, composition, impurities, 28-day dermal tox, genotox, DART, irritation and sensitization on the others besides the ginkgo leaf extract that was studied by NTP.

DR. MARKS: Repeat that --

DR. SHANK: Is that enough?

DR. MARKS: -- method of manufacturer?

DR. SHANK: Oh. Method of manufacture, composition, impurities, 28-day tox, genotox, developmental-reproductive tox, irritation, sensitization.
MS. BURNETT: And this is for the non-leaf extract?

DR. SHANK: Yes.

MS. BURNETT: So none of the other leaves -- leaves ingredient -- ingredients. Sounds like the leaf --

DR. SHANK: Okay. Let me get my ginkgo stuff.

MS. BURNETT: Sorry.

DR. SHANK: So the NTP study was done on the leaf extract.

MS. BURNETT: Right.

DR. EISENMANN: Merle, you can fall in.

DR. ZIMMERMANN: If could comment. Merle Zimmermann from the American Herbal Products Association. The particular leaf extract that was used at the NTP study had some really distinct differences from the ones described in the manufacturing section as far as the components were concerned. That supplier had some patents and stuff for a super-concentrated ginseng extract product which the patents seemed to have things in common with what was reported by NTP as characteristics of the material they used in their study. So --

DR. SHANK: So does the NTP material have any relevance to cosmetics?

DR. ZIMMERMANN: Umm --

DR. SHANK: Because this -- the prior IARC actually indicated I would not use for cosmetics.

DR. ZIMMERMANN: Want to know how many folks have sourced their extracts from Shanghai and Shingling (phonetic) because that's the one supplier that NTP tested. And it's -- as far as the American Herbal Products Association is aware, that material never really been used in the United States for the traditional dietary supplement kinds of uses that some of the other ingredients that are more described by the methods of manufacturing and the compositional description up here in front matter correspond with --

DR. SHANK: Okay.

DR. ZIMMERMANN: -- and having study on their own.

DR. SHANK: Very important --

DR. ZIMMERMANN: Yeah.

DR. SHANK: -- information. Thank you.

DR. ZIMMERMANN: Thank you.

DR. EISENMANN: I have a quick matter just to tell you all. So far, we've gotten one response from a cosmetic company on what the composition is and it's in wave two. And this shows what the NTP versus what is the Schwabe --

DR. SHANK: Oh.
DR. EISENMANN: -- the one that sold most, that also has -- so it kind -- so it has similar components, but the concentration that cosmetic is -- ingredient is much, much lower. So we got something like 25 -- 1 percent of the -- none of the first class. But (inaudible) --

DR. ZIMMERMANN: That's (inaudible) --

DR. EISENMANN: Right.

DR. ZIMMERMANN: Those are flavonoid replacements (phonetic)

DR. EISENMANN: Right.

DR. EISENMANN: So in other words, they don't -- for dietary supplements, I think they concentrated the extract. Whereas, I'm not sure they're concentrating it for cosmetic and things like -- well, I do want to go out more, and push more for more composition data from the cosmetic ingredient suppliers. But --

DR. HELDRETH: So this --

DR. SHANK: Can --

DR. HELDRETH: -- highlights the differences between this "standardized" version and the NTP studied one. But nothing's being said about how either one of those relate to the cosmetic ingredient.

DR. EISENMANN: Other than that one supplier now. Then --

DR. HELDRETH: Well, yeah.

DR. SHANK: So --

DR. HELDRETH: The debate about the difference between NTP and the standardized version seems irrelevant --

MS. BURNETT: Yeah. I just --

DR. HELDRETH: -- to the cosmetic (inaudible).

MS. BURNETT: -- when I made the table, I just put them all together. Because I also had found an article that evaluated different brands of herbal supplements -- herbal ginkgo supplements and even though they're supposed to have a standardized composition, they were all over the map. So I kind of felt in terms of cosmetic use not separating them out. It didn't really have a relevance to separate them out for our terms; for our purposes.

DR. HELDRETH: Yeah.

DR. EISENMANN: Well, I thought it did have relevance in that that's should give the composition of what was tested.

DR. SHANK: Yes, that's important.

DR. EISENMANN: So I mean, having the general is important, but also you would probably need the composition of what was tested in the report. Because of the most of the studies I, if I'm not mistaken, most of the studies are either on NTP material called the NTP studies, or the -- predominantly the EGB --

DR. ZIMMERMAN: Well --
DR. EISENMANN: -- as I recall.

DR. ZIMMERMAN: Yeah, there's characterized ingredient I think is -- sort of, falls on the German pharmacopeia.

MS. BURNETT: But they all have the same standard constituents, correct? It's just different --

DR. ZIMMERMAN: Well --

MS. BURNETT: -- ranges of composition.

DR. ZIMMERMAN: -- for example, the German government doesn't allow five parts per million of the -- I think they're allergenic ginkgolic --

DR. SHANK: Ginkgolic --

DR. ZIMMERMAN: -- (inaudible) acids.

DR. SHANK: Yeah.

DR. ZIMMERMAN: But the material that the NTP used had made 10.5 parts per million which is more than double what --

DR. EISENMANN: And if it was --

DR. ZIMMERMAN: -- the standard of the --

DR. EISENMANN: -- one supplier --

DR. ZIMMERMAN: -- normal -- that one supplier, yeah.

DR. EISENMANN: And then, we've got data from one supplier that's (inaudible). One, because then I can just use less than.1 percent of that.

DR. ZIMMERMAN: Yeah.

DR. EISENMANN: (Inaudible)

DR. HILL: Well, it's actually less than 21 ppm--

MS. BURNETT: Oh, yeah. PPM.

DR. HILL: -- on this table you gave them.

MS. BURNETT: Yeah. That's -- and I'm sorry.

DR. HILL: And that's -- you know, what, what's going on here in this cosmetic ingredient that's listed here is that they do an ethanol water extraction. Don't know whether -- how long and what temperature and evaporated the dryness and take it back up at 50 percent; butylene glycol. So that they had a.51 percent flavonoid glycosides; .16 percent triterpene lactones and less than.1 ppm of the ginkgolic acids, which is just a general thing when you say extract, it could be anything from very concentrated to very dilute because it's not sold as in so many milligrams in a solid is, you know, dissolution that can be all over the map. And then if you give the extraction, then you would super critical fluid from carbon dioxide, for example, you get something different. And then if you do the extraction that you -- what -- solvent matters greatly in terms of what you get which is -- I don't know how you deal with that. Something we've talked about this --
DR. EISENMANN: I don't think they're doing --

DR. HILL: Well, they're not.

DR. EISENMANN: -- CO2.

DR. HILL: I'm just -- you know, my point is -- and you just say extract of --

DR. EISENMANN: Right.

DR. HILL: -- this. I mean, I honestly think there should be some means of figuring out what rough concentration of something you got in there, which we don't and we aren't. It complicates matters greatly.

DR. HELDRETH: All right. So we -- if we work somewhat around the fact that maybe somebody else makes it differently. As long as we have at least one example of how it can be made because our conclusion says, "As displayed in this book." And so then the conclusion hints to that and we don't have to worry if somebody else is doing it differently, they -- this conclusion doesn't really apply to their product. But that -- it -- except for what we've got there in wave 2, showing the composition for a specific cosmetic ingredient. The other data that we have in the report, I don't know what that's really applying to.

DR. SHANK: Yes.

DR. HELDRETH: I mean, most of the time it just says, "leaf", or it just says, "pulp" or something that's not necessarily a cosmetic ingredient composition.

DR. HILL: And one comment I was going to make in the tail end of that was, is that if I take this NTP material and dilute it down by a factor of 70 or something like that, I will get pretty close to what this other one is. So -- including the ppm that we just talked about.

DR. MARKS: So let me summarize, I think.

DR. SHANK: Good luck.

DR. MARKS: I think the bottom line's going to be an insufficient data announcement tomorrow this second again, but ultimately, we're going to get a formulate to be non-sensitizing, so I should think we should take that in consideration about the sensitization needs; although I agree we need them. That Ron Shank, if I heard you correctly -- and Tom, help me, how we sort this out. The leaf extract gives mixed results with the genotoxicity. IARC said it's a possible carcinogen. Folk medicine not an issue. So is the leaf extract that we need more from a carcinogenic point of view?

DR. SHANK: Well, I mean it -- I mean, the statements from NTP that it's -- have no neoplastic lesions, right?

DR. MARKS: Mm-hmm, okay.

DR. SHANK: You know, I think it's logically --

DR. SLAGA: (inaudible)

DR. HILL: Yeah, I didn't see it yet because --

DR. EISENMANN: Because they discussed the (inaudible) -- a little bit.

DR. SHANK: Isn't that true? I believe I read that.
MS. BURNETT: (Inaudible)

DR. SHANK: I don't know. Let me find it again.

DR. MARKS: Yeah, because that's why we're dealing with --

DR. SLAGA: I mean, there was some --

DR. MARKS: -- (inaudible). So it's this

(inaudible) --

DR. SLAGA Inflammation and (inaudible) --

DR. MARKS: Yeah. One of the ones --

MS. BURNETT: There was --

DR. MARKS: (Inaudible)

MS. BURNETT: -- there's this --

DR. EISENMANN: (Inaudible)

MS. BURNETT: There's a second study below the NTP study. It's just, like three sentences without further detail. That one says no neoplastic or pre-neoplastic effects were observed in a dietary study of the ginkgo

DR. SLAGA: And it's significant. I mean, that's where the (inaudible) --

DR. SHANK: That was an NTP study.

MS. BURNETT: Yeah, that's below the NTP study.

DR. SLAGA: Genotoxicity was in vitro, but in (inaudible) there was none. So -- but that happens a lot of times. So I don't know if I put a lot of weight on that.

DR. HILL: So the short answer is, no, I have not read this paper and (inaudible) --

DR. MARKS: So our -- (inaudible)

DR. EISENMANN: (Inaudible)

(crosstalk)

DR. MARKS: The leaf extract, Tom, you feel is okay?

DR. SLAGA: Yeah, I think it's okay.

DR. MARKS: Okay. And am I correct, Ron Shank, you said that IARC said it's a possible carcinogen?

DR. SHANK: No, NTP.

DR. MARKS: Oh, NTP --

DR. SHANK: Said --
DR. MARKS: (Inaudible)

MS. BURNETT: Well, IARC also --

DR. SLAGA: IARC said the same. Not, not NTP.

DR. MARKS: Yeah, okay. That's --

DR. SLAGA: But they say that about a lot of the compounds. So I -- you know, it's really hard to document saying this. I'd go on the NTP data, you know. And granted - -

DR. MARKS: And the NTP was --

DR. SLAGA: -- they have large number of animals.

DR. MARKS: Right.

DR. SLAGA: Males and females of rats and mice, and --

DR. MARKS: NTP was okay.

DR. EISENMANN: But it's from material that's not being used even as a dietary supplement. And this maybe goes into a little bit about mechanistically about why and also the doses that were used in the NTP are much, much higher than --

DR. SLAGA: Yeah.

DR. EISENMANN: -- as used as a dietary supplement. Although you might have more that of mechanistic or --

DR. SLAGA: And you know, it's not just NTP with genotoxicity, invitro, in vivo. The -- like, the tumors. But they do short term, you know, 28-day; they do a whole series before they even do the long-term dose finding. They -- I'd put more weight on that, than on this similar discovery.

DR. MARKS: So you would you then -- to address Ron Shank's concerns, would you say just handle this in the discussion?

DR. SLAGA: Yeah.

DR. MARKS: And obviously not -- we don't need more -- this isn't an insufficient data. Obviously, we got enough data it sounds like. Now, Ron Shank, you mentioned other extracts. I am specific because it's just a root and nut are the other extracts, right?

MS. BURNETT: Right. And then --

DR. MARKS: That we --

MS. BURNETT: -- other leaf components, like the water, the powder.

DR. MARKS: Yeah. I think if we had the leaf extract, that should cover the --

MS. BURNETT: Okay.

DR. MARKS: -- other leaf components.
DR. SHANK: On this, yeah.

DR. MARKS: So the root and nut extracts, we need method of manufacturer, composition, impurities, 28-day, geno development and repro. What did use as that acronym? DARTH?

MS. BURNETT: DART.

DR. MARKS: DART. D-A-R-T development and reproductive toxicity. I like it. And was there -- am I summarizing that correctly? What the needs were for the root and nut extracts?

DR. HILL: Did we include something about composition already?

DR. MARKS: Yes.


MS. BURNETT: Right.

DR. MARKS: Yep.

DR. SHANK: Composition.

DR. MARKS: Impurities.

DR. SHANK: Impurities. Then you have 28-day --

DR. MARKS: Yeah.


DR. MARKS: Mm-hmm.

DR. SHANK: Developmental. You know, reproductive-tox, irritation, sensitization.

DR. MARKS: Yeah and I have that. So from the irritation, I agree with Ron Shank with what you mentioned. We need the sensitization on the leaf extract at 1 percent. We only have it -- it's okay at 0.1 percent. But it's -- despite the fact we're going to say formulate to be non-sensitizing, I'd still like to see that. And then the nut and root extract that leave on concentration, I'd like to see the sensitivity data, but we don't know what the concentration of use is. So should -- we probably need the concentration of use of the nut and root extract on the leave ons. Am I correct on that, Christina? That we don't have that? And there are case reports of allergic contact dermatitis at a clinical alerts ginkgo is a sensitizer.

MS. BURNETT: We have -- no, we -- there are uses reported from the extract, but we do not have --

DR. MARKS: Oh.

MS. BURNETT: -- the concentration of use.

DR. MARKS: Yeah, so I want the leave on concentration. I want the sensitization data on leave on concentration. Obviously, we don't have that at this point. Does that sort of summarize insufficient data announcement and those needs?

DR. SHANK: Yeah.
DR. MARKS: Then we'll go from there.

DR. SHANK: Well --

DR. MARKS: Oh, more?

DR. SHANK: Let's go back and talk about this carcinogenicity presents.

DR. MARKS: And that's the leaf extract that you started with, with a mixed geno?

DR. SHANK: Yes.

DR. MARKS: Okay.

DR. SHANK: And on Page 17. Third -- under the cancer study, the third paragraph at the end of that study above "other relevant studies." It says, "the study concluded that ginkgo biloba extract caused cancers of the thyroid in male and female rats, and male mice; and cancers of the liver in male and female mice." In a different study, they didn't get neoplastic changes. "And the International Agency for Research of Cancer has determined that ginkgo biloba extract is possibly carcinogenic to humans, based on the inadequate human geno (inaudible) carcinogenicity data and sufficient evidence in experimental animals." How do we handle that position -- because you're either going to have to say that really is faulty, or not pertinent because it was the wrong extract. But if you accept that data, then why ask really the other? Because you're going to have to say unsafe.

DR. ZIMMERMANN: Well, Merle here from the American Herbal Products Association. When we had -- were looking at the information moving around at these studies. The NTP material from the Shanghai Shingling factory had these observations from the citations marked 32 in the summary, but it's kind of labelled exactly the same way throughout; that it is a -- well, it is a ginseng leaf extract that Shanghai Shingling sells.

DR. SHANK: Mm-hmm.

DR. ZIMMERMANN: But the other ones -- you can see where the -- they have different results. Were from a different study with, I believe, of material that might reflect better what's used in the market for humans. And then, I believe, IARC is kind of -- their hands are tied when NTP releases a paper that they have to say something to report on the NTP material, I think. When the opals (inaudible) look into it -- the processes.

DR. MARKS: Tom, do you want to say anything there in terms of --

DR. SLAGA: Well, if -- can we handle that in the discussion?

DR. EISENMANN: For this paper that I suggested goes into some of the mechanisms, they say for the conclusion about this NTP study, "the underlying mechanisms behind thyroid and liver tumor inductions seen in NTP studies is an increased activity paddock drug metabolism enzymes. This increase was ultimately accelerated self-proliferation from common neoplasm." So the NTP study also did use very high doses --

DR. SHANK: Right.

DR. EISENMANN: -- compared to even oral -- compared to human oral doses. So cosmetic dose would also be much lower.

DR. SHANK: Mm-hmm.

DR. EISENMANN: And the -- at least, according to the studies reviewed in this paper, it has a much greater tendency to result in enzyme induction in mice and rats, relatively (inaudible).
DR. SLAGA: Can we handle that in the discussion?

DR. EISENMANN: I think so. And I don't think it'd be necessary to go through every single study. And this --

DR. SHANK: Right.

DR. EISENMANN: -- review has already done a lot of it.

DR. SHANK: Right.

DR. EISENMANN: And I think this review is available on the internet for free. So I think it'd be easily cited, rather than having to go through each and every study. But like, I said (inaudible) --

DR. MARKS: Well, we're going to see it again. Does that sound moving forward handle it in the discussion and see how well that shakes out. And then, we'll be revisiting it, I'm sure. Does that sound good? We'll definitely handle it in the discussion.

DR. SHANK: Yeah. Absolutely because on the face of it, I would think consumer groups might have concerns.

DR. MARKS: Mm-hmm.

DR. SHANK: And then I did not read that paper on the immunotoxicity assay that was on crude ginkgo biloba extracts, plural. So apparently, they looked at several and it's immunotoxic in causing proliferation of the lymphocytes and what else?

DR. EISENMANN: Let's see, this one, it says, it has ginkgolic acid at 5-1/2 percent and 24.6 percent. Which is -- I mean, even the NTP which had a high level of this, but had it at 10-something ppm. So that study definitely had a high --

DR. SHANK: Fruit study.

DR. EISENMANN: -- high level of the --

DR. SHANK: The mouse lymphoma?

DR. EISENMANN: Yes.

DR. SHANK: Can it -- can (inaudible)?

DR. EISENMANN: 24.6 percent. And the NTP has it at 10-something ppm.

DR. SHANK: There is --

DR. EISENMANN: 10.5 ppm.

DR. SHANK: -- then there's something wrong with the data. I mean, everything that came in from toxic to (inaudible).

DR. HILL: That can't quite be right, I don't think.

DR. SHANK: There's something wrong in the immunotoxicity lymph node assay. Apparently, they incubated or used the concentrations in terms of 10 percent of extract. Well, you know, and that's not -- I'm surprised that it ever got published.
MS. BURNETT: Well --

DR. HILL: Yeah.

MS. BURNETT: -- it's not the -- wasn't the extract. It was -- sorry, I just lost it. Where did it go? Found it. The ginkgolic acid was 5.5 percent and 24.6 percent. That was the chemical analysis of the crude extract.

DR. SHANK: Oh, okay. But that's not what they used in the assay; can't be.

MS. BURNETT: No. I'm not sure what the concentration was. It's saying it was just a crude ethanolic aqueous ginkgo biloba leaf extract, or a hexane fraction of the crude extract, or a positive ginkgo. But --

DR. SHANK: But they don't say the concentration?

MS. BURNETT: No. Just that it's a crude extract.

DR. SHANK: I'll have to --

MS. BURNETT: I can --

DR. SHANK: -- try to get that paper. There's something wrong there.

MS. BURNETT: I can --

DR. MARKS: Okay.

MS. BURNETT: -- get it to you.

DR. SHANK: Yeah.

DR. MARKS: So formulate our insufficient data announcement. The needs are (1) clarify the leaf extract carcinogenicity issue; (2) root and nut extracts -- a lot of things basically all method of manufacturer, composition, impurities, 28-day tox and DART.

DR. HILL: I am --

DR. MARKS: And then, (3) sensitization on a leaf extract at 1 percent, and a nut and root extract at leave on concentration, which we can't say the percents we don't know. And do you think that's where we'll land tomorrow, and we'll see what the Belsito team thinks? Sound good?

DR. SHANK: Mm-hmm.

DR. MARKS: Excellent discussion thank you. Ron Hill, you're fine with that?

DR. HILL: Mm-hmm.
**Full Panel Meeting**

DR. BERGFELD: The last ingredient that we have this morning is Dr. Belsito and ginkgo.

DR. BELSITO: So this is the first time we're looking at this botanical family of 10 ingredients of ginkgo biloba. And it was interesting because there's really essentially no data we can actually say is the ginkgo biloba that is used in cosmetics. So we are going much by ginkgo biloba extract. Having looked at all this data we felt that it was insufficient for a number of reasons. We needed method of manufacturing and impurities for all except for the ginkgo leaf biloba extract. We needed irritation and sensitization for all of the ingredients or a representative ingredient. We needed -- well, yea, we needed irritation and sensitization for all depending upon what we learn about the composition and the manufacturing. Particularly, since ginkgolic acid is related to urushiol. We did receive an HRIPT at 1 in wave 2 but the maximum leave on is 1 percent. We needed ocular irritation if available since it's used around the eye area and we needed either an absorption spectrum or a photo toxicity photo sensitization if it does absorb.

DR. BERGFELD: Anything to be added to that or commented upon? Dr. Marks?

DR. MARKS: Our team seconds the insufficient data announcement. Thank you, Dan. Our team seconds --

DR. BERGFELD: Are you adding anything to it though?

DR. MARKS: Well, that would be in a discussion. I first want to second the motion. And then we have largely the same needs -- first of all, we had pretty robust discussion about the leaf extract carcinogenicity. There was mixed results looking at the genotox. IARC says possible carcinogen. The NTP looked okay. Our cosmetic dose is low. It's used in folk medicine. It's not carcinogenic. We thought a robust discussion as to why it's safe in cosmetics is important.

We have the same needs - root and nut extracts, method of manufacture, composition and impurity, 28 day tox, the DART. And then we had the same needs on sensitization although we felt in all, definitely we thought all the extracts would represent the group of 10. Maybe that won't be as we move forward. But we thought if we had the leaf extract that 1 percent as you said, Don, for sensitization and also the nut and root extract on leave on concentrations and we don't know those leave on concentrations that would be also a need. I think if you combine what we both said were the needs it is going to be pretty robust.

DR. BERGFELD: And lengthy. Ron Hill?

DR. HILL: I'd like to include the extract -- they excluded it from the method of manufacture. I'd like to include it because clearly there's no one extract. There are multiple possible extractions, solvents and then it is brought up again. If look at the NTP I think it was the NTP material, well that we weren't sure about that because that was the Japanese source. If you look at the one extract where they did the total extract it appears that they extracted with multiple solvents of different kinds and then combined everything and then we got a composition from that well and good. Then if you look at other test data it was something like that we don't know for sure if the ginkgolic acids had been removed at any level and then brought back up in 50/50 water butylene glycol. Are all the ones that are using cosmetic ingredients brought up in 50/50 butylene glycol and then really at what composition of dissolved solvents, so. For solvents because that is the deal with the NTP extract is the concentrations were a lot higher, but if you dilute that by a factor of 200 you get down to the composition we were looking at for the extract that's in here. And then it's the percentage of that extract that is reported when they do the study. So dilute by a factor of 200 and then that extract is present at 5 percent or whatever it is. So there are some ambiguities here in meaning in terms of how much substance is actually in. If we at least get some information about the method of manufacture of the extracts and use that as representative of what is on the market and review based on that we have a little more to go on.

DR. BERGFELD: You want to comment on that, Don?

DR. BELSITO: Yea, we're insufficient. All the data we can get the better.

DR. LIEBLER: Yea and one of the points I think I didn't hear, Don said it I know we talked about yesterday the idea the leaf extract could represent many of the leaf ingredients. But I did have a concern about the leaf terpenoids
because that could be substantially similar to the leaf extract. Might just call it leaf terpenoids even though they prepare it in much the same way, but unless we know how that is made I don't think the leaf extract at this point necessarily covers for that for composition, method of manufacture, etc.

DR. HILL: And more to the point if that was included at X concentration of which data we don't have it could be a lot higher exposure.

DR. LIEBLER: Yea, right it could be much more concentrated with respect to certain molecules that are in the others, but less concentrated.

DR. BERGFELD: All right, Monice, do you have the list? Good.

MS. FIUME: Christina do you have all the names?

DR. BERGFELD: Ron, Shank?

DR. SHANK: I think this is where we should discuss how we are going to handle the NTP carcinogenicity study and the International Agency for Research in Cancer's conclusion that this is a possible human carcinogen and that it is an animal carcinogen. If we take that as the conclusion then there is no need to ask for any more data because it would be unsafe. So at this time I think we need to discuss how are we going to handle the carcinogenicity data in the discussion. Because apparently, everybody feels that is not going to be the main criterion upon which we judge this ingredient.

DR. BERGFELD: Tom, you have a comment? You have a suggestion?

DR. SLAGA: Well, I put more weight on the NTP because of the extensive work that goes into testing a compound or extract they use large a lot more animals than most scientists do. They do a lot of pre-- testing before they do short-term toxicity and so sometimes you know, and they use a very high concentration so I really thought that it was emphasized that it was -- didn't produce tumors.

DR. BERGFELD: Don?

DR. BELSITO: Yea, I mean I asked Paul about this yesterday in our team discussion. I mean those effects were seen at 100 mg per kg and we are talking about a product that's highest leave on is 1 percent in a cosmetic product. And, I mean, if Paul actually didn’t feel we needed to discuss it and gave not much merit to these high levels, but I will let him address that.

DR. SNYDER: Yes. I don't need to reiterate, but that was my feelings. Mono cell leukemia is a specific tumor. It has no relationship to humans. So some of the findings like the thyroid also are not very predictive for humans. And so I felt that even knowing that the highest dose 1000 there was some of those effects. Many of those were not
relevant to humans. There were no effects at 200 for the females and 100 for the males and so those levels were very comfortable with the data. The IARC a little less. I didn't go and look at the report because I put most of my emphasis on the NTP study because I thought it was a much more robust study.

DR. BERGFELD: Do you think a short sentence in the discussion could clarify that because you've already mentioned in the text of the document you've got it cited?

DR. SLAGA: Yea, we could handle that a discussion about the -- it's not relevant to humans.

DR. BERGFELD: Nothing large.

DR. SNYDER: Well, it's a slippery slope because you have to -- sort of just talk about everything in a large paragraph and it almost brings too much attention to it. But we can come up with something.

DR. SHANK: Okay, but you would put it in the discussion.

DR. SNYDER: Yes.

DR. SHANK: Okay.

DR. BERGFELD: Okay.

DR. LIEBLER: I don't know if this is -- how useful this might be but if you say that the some of the carcinogenicity in the animal models is animal specific and then you also rely on the sort of argument its a very high dose and then on the other hand you've got this history of intake of ginkgo extracts for a long time. Might it be helpful to get a little bit of information on what is typical for ginkgo extract, ginkgo bilobo extracts that are taken as herbal medicinals or dietary supplements? If there are a big disparity between that exposure level, which is apparently not associated with toxicities or cancers. And the high dose for the animals that might help to tamper that dosing argument a little bit more.

MS. BURNETT: Dr. Liebler, I didn't look at that in terms of anecdotal relationships. Ginkgo extracts are used for different maladies and the dose is kind of all over the place. On the back of a supplement bottle it recommends, I believe, 120 mg a day.

DR. LIEBLER: I wonder if you just took the higher dose at range of what's used in supplements is that -- question I would have is there a big disparity between that and the dose that produced cancers in animals?

DR. ANSELL: We think there's also the NTP sample was not particularly representative of materials that are available commercially. That there as what is given to us yesterday from the herbal supplement folks the NTP material was significantly different than the materials that are items of commerce.

DR. SHANK: That would be in the discussion.

DR. BERGFELD: And we have a reference for that?

DR. ANSELL: I believe so.

DR. BERGFELD: We need to put that in the document.

DR. BELSITO: I think, Christina, didn't you say that information was already there, but you just put it into board categories or concentrations?

MS. BURNETT: I put it in a broad range just because at the time that I'm writing the report I don't know the extraction method for the cosmetics, so I didn't see the need to separate out the different constituent levels to what was used when I was gathering the composition data. And then I had found a study that compared different
supplement brands and they found a huge disparity between brands, so when comparing those I didn't know how to interpret that data and just thought it was best to show it as a range.

DR. ANSELL: We will send the comments again.

MS. BURNETT: I have them. Thank you.

DR. BERGFELD: Ron Hill?

DR. HILL: I mean, again the table that you showed us if I take the NTP sample and just dilute it by a factor of 100 to 200 the composition is pretty consistent with what you had for the extract. I don't know if it was representative in terms of the constituents. The question is the concentration which was very much higher in their deal. And the gavage where they were giving high doses for 104 weeks I believe it is five times a week I believe is the important study at a level because again, I say if you are giving something at low level in a diet or even consuming human supplements you are subject to a first pass metabolism that wouldn't happen in the skin, but what we depend on is the normal penetration rates are typically much smaller and so we really don't have any information about these constituents that I know of in terms of normal penetration. That's the fly in the ointment as well. And we're not talking about mouthwash in general here. It might be. I didn't look, but I think we are mostly talking about skin exposure at low concentrations in the formulations. That's the margin right there is the low concentrations in those cosmetic formulations.

DR. BERGFELD: I think, information which has been listed in both the needs assessment by the team leaders and also in the discussion that we need to put together and make an announcement, an insufficient data announcement. I'd like to call the question if you're all agreeable. All those in favor of an insufficient data announcement. Thank you.

(MOTION PASSED UNANIMOUSLY)

DR. BERGFELD: I'd like to also open up to the panel if you have any other thoughts before this goes out to the public about the requests that you send them in to Bart or Monice.
DRAFT ABSTRACT

The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) assessed the safety of 10 Ginkgo biloba-derived ingredients, which are most frequently reported to function in cosmetics as skin conditioning agents or antioxidants. The Panel reviewed the available data to determine the safety of these ingredients. Because final product formulations may contain multiple botanicals, each containing similar constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. Industry should use good manufacturing practices to limit impurities that could be present in botanical ingredients. The Panel concluded that …[to be determined].

INTRODUCTION

Most of the Ginkgo biloba-derived ingredients detailed in this safety assessment are reported to function as skin conditioning agents, while some are reported to function as antioxidants in cosmetics, according to the web-based International Cosmetic Ingredient Dictionary and Handbook (wINCI; Dictionary; see Table 1).1 Ginkgo Leaf Terpenoids is reported to function as an antiacne agent, an antifungal agent, and an external analgesic. These functions are not considered a cosmetic function in the United States and, therefore, do not fall under the purview of CIR. This assessment of the safety of the following 10 Ginkgo biloba-derived ingredients is based on the data contained in this report:

- Ginkgo Biloba Leaf Extract
- Ginkgo Biloba Leaf Water
- Ginkgo Biloba Cell Extract
- Ginkgo Biloba Root Extract
- Ginkgo Biloba Leaf Powder
- Ginkgo Biloba Nut Extract
- Ginkgo Biloba Meristem Cell
- Ginkgo Biloba Leaf Terpenoids

Ginkgo biloba leaves and nuts (also called seeds) have been used as a source of traditional Chinese medicines.2 More recently, extracts of the leaves of Ginkgo biloba have been used as herbal medicines or dietary supplements in the treatment of heart disease, eye ailments, tinnitus, cerebral and peripheral vascular insufficiency, injuries involving brain trauma, dementias, short-term memory improvement, cognitive disorders secondary to depression, vertigo, and various other cognitive disorders.2,3 Investigations into the efficacy of the leaf extract for these uses are numerous and are mainly based on oral administration of supplements. However, there are no publically available toxicity data that corresponds to specific use of these ingredients as cosmetics. The focus of this safety assessment will be on data relevant to the use of Ginkgo biloba-derived ingredients in cosmetics, with specific focus on dermal application when available.

Because often in the published literature the information provided is not sufficient to determine how well the tested substance represents the cosmetic ingredient, the taxonomic name is used unless it is clear that the test substance is similar to a cosmetic ingredient. However, in the case of data on the extract of Ginkgo biloba leaves, the abbreviation GBE will be used, unless the data specifically are related to the cosmetic use of Ginkgo Biloba Leaf Extract.

Botanicals, such as Ginkgo biloba-derived ingredients, may contain hundreds of constituents, some of which may have the potential to cause toxic effects. In this assessment, CIR is reviewing the potential toxicity of each of the Ginkgo biloba-derived ingredient as a whole, complex mixture. CIR is not reviewing the potential toxicity of the individual constituents, except wherein such constituents are also ingredients under review.

This safety assessment includes relevant published and unpublished data for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world’s literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that CIR typically evaluates, is provided on the CIR website (http://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; http://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

CHEMISTRY

Definition and Plant Identification

The definitions and functions of the Ginkgo biloba-derived ingredients included in this report are provided in Table 1. The raw materials for the ingredients in this report are obtained from the deciduous tree, Ginkgo biloba, which has fan-shaped leaves that turn golden yellow in autumn and which can grow to 40 m (~131 ft) tall.2 The female trees bear offensive-smelling, inedible fruit that contain a single thin-shelled semi-edible nut. Ginkgo trees are planted widely as ornamental trees via cultivation. Few naturally-occurring specimens grow in Zhejiang province China. Trees grown commercially for the leaves are found in China, France, and in the United States.

Methods of Manufacturing

Ginkgo Biloba Leaf Extract

A general description of manufacturing for “medicinal” GBE reported that the leaves of the Ginkgo biloba tree are harvested either mechanically or by hand from plantations or in the wild.3 The leaves are then dried and pressed into balls.
dry extract from the dried leaf of *Ginkgo biloba* can be manufactured using acetone/water and subsequent purification steps without addition of concentrates or isolated ingredients.

GBEs may be full extracts or standardized extracts. Full extracts are prepared with alcohol and contain all constituents soluble in alcohol. Standardized extracts (one of which is referred to as EGB 761 in published literature) are more common, especially in herbal supplements, and are prepared in manufacturer-dependent multi-step processes (Scheme 1). These processes may include additional steps in which some compounds, such as flavonoids and lactones, are enriched while others, such as ginkgolic acids, are removed.

**Scheme 1.** General manufacturing process of a standardized *Ginkgo biloba* leaf extract

A manufacturer has reported that one Ginkgo Biloba Leaf Extract product is produced through extraction with an ethanol-water solution, while another product is produced through extraction with an ethanol-water solution before being evaporated and resolved in 50% butylene glycol.

**Ginkgo Biloba Meristem Cell**

Ginkgo Biloba Meristem Cell is produced by sterilizing cambium-containing tissue from the *Ginkgo biloba* plant, isolating the cambial meristem cells from the tissue, and then culturing the cells for proliferation. The cultured cambial meristem cells are then subjected to specific culture conditions (details not provided) in order to produce various secondary metabolites. Finally the cultured cambial meristem cells are harvested with a filter-press.

**Composition/Impurities**

**Ginkgo Biloba Leaf Extract**

Table 2 summarizes the composition ranges of the major constituents of various extracts (standardized and non-standardized) of *Ginkgo biloba* leaves.

The target levels of the major constituents of the standardized GBE EGB 761 are reported to be: not less than 6% total terpene trilactone content, not less than 24% total flavonol glycosides, and not more than 5 ppm (0.0005%) ginkgolic acids. This extract is reported to be a brown powder with characteristic smell containing not more than 20 ppm heavy metals and not more than 2 ppm arsenic. The standardized extract used in National Toxicology Program (NTP) studies is reported to contain 15.4% terpene trilactones, 31.2% flavonol glycosides, and 10.45 ppm (0.001%) ginkgolic acids.

According to an analysis of crude extracts of *Ginkgo biloba* leaves, there are seasonal differences in the levels of certain constituents, with concentrations of flavonol glycosides higher in the spring than in the autumn (136.3 mg/100 g versus 46.0 mg/100g) and biflavones higher in the autumn than in the spring (194.8 mg/100 g versus 44.28 mg/100 g).

General *Ginkgo biloba* composition was reported in the *Physician’s Desk Reference for Herbal Medicines* to be the following: flavonoids (0.5% to 1.8%) including monosides, biosides and triosides of quercetin, isorhamnetins, 3-O-methylmyristicins, and kaempferol (may be esterified with p-coumaric acid); biflavonoids (0.4% to 1.9%) including...
amentoflavone, bilobetin, 5-methoxybilobetin, ginkgetin, and isoginkgetin; proanthocyanidins (8% to 12%); trilactonic diterpenes (0.06% to 0.23%) including ginkgolide A, B, and C; and trilactonic sesquiterpene bilobalide (0.04% to 0.2%).

The **United States Pharmacopeia** states that “ginkgo” consists of the dried leaf of Ginkgo biloba Linne (Fam. Ginkgoaceae). It contains not less than 0.5% of flavonoids, calculated as flavonol glycosides, with a mean molecular mass of 756.7; and not less than 0.1% of terpene lactones, calculated as the sum of bilobalide, ginkgolide A, ginkgolide B, and ginkgolide C, both on the dried basis. This reference also states that “powdered ginkgo extract” is prepared from dried and comminuted leaves of Ginkgo extracted with an acetone-water mixture or other suitable solvents. It contains not less than 22.0% and not more than 27.0% of flavonoids, calculated as flavonol glycosides, with a mean molecular mass of 756.7; and not less than 5.4% and not more than 12.0% of terpene lactones, consisting of between 2.6% and 5.8% of bilobalide and between 2.8% and 6.2% of ginkgolide A, ginkgolide B, and ginkgolide C.

The **British Pharmacopoeia** states that “ginkgo leaf” content should be not less than 0.5% of flavonoids, calculated as flavone glycosides (dried drug).

An extraction with 60% w/w ethanol of dried green Ginkgo biloba leaves yielded an extract comprised of 3.4% flavone glycosides, 0.7% terpene lactones, and 5.5% ginkgolic acids. Further fractionation by liquid-liquid partition between water and heptane yielded a fraction containing 0.3% flavone glycosides, 0.1% terpene lactones, and 24.6% ginkgolic acids.

For use as an herbal medicine in Germany, GBE must be extracted with acetone/water and contain 22%-27% flavone glycosides (queretin and kaempferol) with a molar mass of 756.7 (queretin glycoside) and 740.7 (kaempferol glycoside); 5%-7% terpene lactones of which 2.8%-3.4% consists of ginkgolides A, B, and C and 2.6%-3.2% bilobalide; and less than 5 ppm (0.0005%) ginkgolic acids.

Ginkgolic acid is a salicylic acid derivative with a C15 side chain that is related to the pentadecylcatechols (i.e. urushiol) found in poison ivy. One analysis found crude aqueous extracts of Ginkgo biloba leaf contained up to a total of 30 ppm urushiols, while the process described in Scheme 1 (i.e., production of a particular standardized GBE) removed long chain alklyphenols to below detection levels. Other extraction processes have been seen to result in a specific standardized extract material containing 10.45 ppm (0.001%) urushiols.

A manufacturer has reported that a Ginkgo Biloba Leaf Extract produced with butylene glycol contains 0.51% flavonol glycosides, 0.16% terpene lactones (0.08% bilobalide, 0.04% ginkgolide A, 0.02% ginkgolide B, and 0.02% ginkgolide C), 0.21% quercetin, and less than 0.1 ppm ginkgolic acid.

A certificate of analysis on a Ginkgo Biloba Leaf Extract (solvent not specified) described the sample as a light tan powder that contained 25.3% ginkgo flavonol, 6.4% ginkgolides (bilobalide, ginkgolide A, ginkgolide B, ginkgolide C), 2.3 ppm ginkgolic acid, 0.1 mg free quercetin, 0.2 mg free kaempferol, and less than 20 ppm heavy metals.

**Ginkgo Biloba Meristem Cell**

A supplier has reported that Ginkgo Biloba Meristem Cell is distinctly different from general GBEs, with major constituents being catechin, gallocatechin, epigallocatechin, and bilobalide.

**UV Absorption**

In a spectral analysis provided by a supplier of a Ginkgo Biloba Leaf Extract (ethanol: water:butylene glycol extract), no maximum UV absorption peaks were observed in the 280 to 450 nm range.

**USE**

**Cosmetic**

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

According to 2017 VCRP survey data, Ginkgo Biloba Leaf Extract has the most reported uses in cosmetic products, with a total of 726; the majority of the uses are in leave-on eye makeup preparations and skin care products (Table 3). Two other *Ginkgo*-derived ingredients are reported to be in use, with 24 or fewer uses reported in the VCRP. However, the results of the concentration of use survey on these 10 ingredients conducted in 2014 by the Council indicate use for only Ginkgo Biloba Leaf Extract, at a maximum of 1%, as reported in face and neck skin preparations. A concentration of use survey has not yet been completed for Ginkgo Biloba Leaf Cell Extract. Ingredients with no reported uses in the VCRP or by the Council are listed in Table 4.

Ginkgo Biloba Leaf Extract may be used in products that can be incidentally ingested or come into contact with mucous membranes; for example, use is reported in a lipstick at up to 0.2%. Additionally, Ginkgo Biloba Leaf Extract
GBE is used extensively as an herbal supplement for anti-inflammatory, cognitive-promoting, antioxidant, and vascular effects at daily doses of 120 to 240 mg. In Germany, GBE is an approved herbal medicine for use for treatment of memory deficits, dementia, and other organic brain syndromes when extracted with acetone/water. It is not approved when extracted with other solvents due to lack of supporting safety data.

GBE as a herbal supplement may interact with pharmaceutical drugs and act as or enhance anticoagulants, anti-inflammatory agents, antihypertensives, and/or anesthetics which may lead to hemorrhage, apraxia, hematoma, hyphema, permanent neurological deficit, and death. The Physician's Desk Reference for Herbal Medicines reports major drug interaction risks with anticoagulants, nonsteroidal anti-inflammatory drugs (NSAIDs), and trazodone and moderate drug interaction risks with low molecular weight heparins and thrombolytic agents. GBE may also interact with anticonvulsants, bupirone, insulin, monoamine oxidase (MAO) inhibitors, nicardipine, nifedipine, omeprazole, papaverine, St. John’s wort, selective serotonin reuptake inhibitors, and thiazide diuretics.

The nuts of Ginkgo biloba are a delicacy in Japan and China, but must be removed completely from the pulp, boiled or roasted, and eaten sparingly (limit 8 - 10 per day). In traditional Chinese medicine, the nut is dried and used to treat such ailments as asthma, cough, bronchitis, scabies, and sores.

TOXICOKINETICS

In general, toxicokinetics data are not expected to be found on botanical ingredient because each botanical ingredient is a complex mixture of hundreds of constituents. However, there have been many pharmacokinetics studies on GBEs, specifically on some of the key constituents, which indicate GBE may be well absorbed after oral administration.

Dermal Penetration

The ability of the GBE constituent, quercetin, to penetrate the skin while in a cosmetic formulation was studied in vitro with human dermatomed skin. The absorption, distribution, and elimination of a radiolabeled GBE were studied in male and female Sprague-Dawley rats. The rats received a single oral suspended dose (20 μCi; 380 mg/kg) of a radiolabeled GBE. The test
material was obtained from *Ginkgo biloba* grown under a supply of $[^{14}\text{C}]-\text{acetate}$. Analysis showed that the flavonol glycosides and proanthocyanidins bore the radiolabel; no radioactivity was detected in the terpenes or the main sugars after the hydrolysis of glycosides. The pharmacokinetic results, based on blood specific activity data versus time course, were characteristic of a two-compartment model with an apparent first order phase and a half-life of approximately 4.5 h. Expired $[^{14}\text{C}]-\text{CO}_2$ represented 16% of the administered dose 3 h post-treatment. After 72 h, 38% of the radioactivity was excreted via exhalation, while 21% was determined to be excreted in the urine and 29% was excreted in the feces. The researchers of this study concluded that at least 60% of the radiolabeled GBE was absorbed. The site of absorption was likely the upper gastrointestinal tract.

**Human**

The bioavailability and pharmacokinetics of *Ginkgo biloba* L. in a human plasma study was investigated using 3 different preparations. The preparations were a tincture of fresh *Ginkgo biloba* leaves (extracted with 65% v/v ethanol; 1 ml contains 920 mg *Ginkgo biloba* leaves as active ingredient), *Ginkgo biloba* fresh plant extract tablets (extracted with 67% v/v ethanol; one 250 mg tablet contains 90 mg fresh plant extract), and *Ginkgo biloba* extract EGB 761 tablets (extracted with 60% m/m acetone; one tablet contains 40 mg purified dried extract). The study was performed on 24 healthy volunteers (6 males and 18 females): each volunteer received a single oral dose of the maximum registered daily dosage of either the tincture (90 drops or 2.73 ml), the fresh plant extract (4 tablets), or EGB 761 (3 tablets) with 100 ml. Prior to dosing, each preparation was analyzed for concentrations of bilobalide (646.93 µg, 1974.96 µg, and 3672.39 µg for the tincture, fresh plant extract, and EGB 761, respectively), ginkgolide A (298.14 µg, 881.52 µg, and 1571.37 µg for the tincture, fresh plant extract, and EGB 761, respectively), and ginkgolide B (147.45 µg, 524.56 µg, and 836.46 µg for the tincture, fresh plant extract, and EGB 761, respectively) prior to the plasma study with liquid chromatography-mass spectrometry (LC-MS).

Blood samples (36 ml) were taken 30 min prior to administration and 15, 30, 45, 60, and 360 min after administration. The samples were centrifuged to separate the plasma and plasma was analyzed by LC-MS. The resulting maximum concentrations (median) of bilobalide, ginkgolide A and ginkgolide B in plasma after administration of the maximum daily dose of the different *Ginkgo biloba* products were as follows: 3.53, 3.62, and 1.38 ng/ml, respectively, after administration of the tincture; 11.68, 7.36, and 4.18 ng/mL, respectively, after administration of the fresh plant extract tablets; and 26.85, 16.44, 9.99 ng/mL, respectively, after administration of EGB 761 tablets. The authors of study concluded that ginkgolide A and B and bilobalide are bioavailable after oral dosing of 3 different *Ginkgo biloba* preparations.

**TOXICOLOGICAL STUDIES**

**Acute Toxicity Studies**

*Ginkgo Biloba Leaf Extract*

**Oral**

The LD$_{50}$ of a standardized GBE administered orally to mice was reported to be 7730 mg/kg.

**Intravenous**

The LD$_{50}$ after intravenous administration of a standardized GBE was 1100 mg/kg for both rats and mice.

*Ginkgo Biloba Meristem Cell*

**Oral**

In a toxicity test to determine lethal dose, a single oral dose of 0 or 2000 mg/kg Ginkgo Biloba Meristem Cell was administered to 5 male and female Sprague-Dawley rats in each group (written as provided, no further details). After a 14 day observation period, the animals were killed and underwent necropsy. No unscheduled deaths or treatment-related effects were observed during the observation period or at necropsy. The lethal dose for Ginkgo Biloba Meristem Cell was greater than 2000 mg/kg in this rat study.

In a single dose oral volume increase toxicity test, 2 male and female Beagle dogs (written as provided, no further details) received Ginkgo Biloba Meristem Cell at 250, 500, and 1000 mg/kg, respectively, for 4 days. No unscheduled deaths were observed. All animals vomited after receiving 500 and 1000 mg/kg of the test material. Only 1 animal vomited after receiving the 250 mg/kg dose, but the effects were determined to be too slight a symptom to confirm treatment-related effects. No adverse effects were observed in body weights or at necropsy. The maximum tolerated dose for Ginkgo Biloba Meristem Cell was determined to be greater than 1000 mg/kg in this dog study.

**Short-Term Studies**

*Ginkgo Biloba Leaf Extract*

**Oral**

The results of a combined liver comet assay (see Genotoxicity section) using male and female C3H-derived constitutive androstane receptor knockout (CARKO) and wild-type mice found no abnormal clinical signs and no treatment-
related effects on body weight following oral exposure of up to 2000 mg/kg body weight/day of a GBE for 3 days in either mouse genotype. Relative liver weights were significantly increased in male and female wild-type mice at all doses of a GBE in a dose-dependent manner. The liver weights in the CARKO mice were similar to the negative control group. The wild-type mice in all GBE-treated groups had dose-dependent slight-to-moderate hepatocellular hypertrophy in the centrilobular area: this effect was only observed in a single CARKO mouse in the highest dose group. No histopathological findings suggesting cytotoxicity in the liver was observed in any GBE-treated groups.

**Ginkgo Biloba Meristem Cell**

*Oral*

In a dose-range finding study for a 13-week oral repeated dose toxicity test (see below), groups of male and female Sprague-Dawley rats received 500, 1000, or 2000 mg/kg Ginkgo Biloba Meristem Cell for 4 weeks (number of rats/group and route of administration not described). No unscheduled deaths or clinical signs of toxicity were observed during the treatment period. Additionally, no treatment-related changes in body weight gains, feed intake, hematological/biochemical measurements, or organ weights were observed. No adverse effects were noted at necropsy in any dose group.

**Ginkgo Biloba Leaf Extract**

*Oral*

The toxicity of a particular GBE was investigated in a 3-month mouse study performed by the NTP. Groups of 10 male and 10 female B6C3F1/N mice received 0, 125, 250, 500, 1000, or 2000 mg/kg body weight of the GBE in corn oil via gavage, 5 days per week for 14 weeks. Control groups received corn oil (5 ml/kg). Clinical findings and body weights were recorded initially, then weekly, and at the end of the study. Blood was collected at the end of the study from all animals for hematological analyses. Sperm motility and vaginal cytology evaluations were made on the mice in the 0, 500, 1000, and 2000 mg/kg dose groups. At the end of the study period, tissues from over 40 sites were examined for every animal, including ovaries and uteri in females and prostate gland and testes with epididymis and seminal vesicles in males.

One female mouse in the 1000 mg/kg group died of a dosing accident during week 11. Mean body weights of 2000 mg/kg females were significantly less than those of the vehicle control group. Ruffled fur was observed in two 1000 mg/kg males between weeks 7 and 8 and all 2000 mg/kg males between weeks 5 and 9. No treatment-related differences were observed in sperm parameters in males administered 500, 1000, or 2000 mg/kg or in the estrous cycle of females administered 500 or 1000 mg/kg when compared to controls. Female mice in the 2000 mg/kg group had a significantly higher probability of extended estrus than did the vehicle control females. Liver weights of males of the 250 mg/kg or greater dose groups and females of all dose groups were significantly greater than those of the vehicle control groups. Kidney weights of males of the 2000 mg/kg group were significantly less than those of the vehicle control group. Incidences of hepatocytic hypertrophy were significantly increased in males and females dosed with 250 mg/kg or greater. Significantly increased incidences of focal hepatocytic necrosis occurred in males of the 1000 and 2000 mg/kg dose groups. The incidences of hyaline droplet accumulation in the respiratory epithelium of the nose were significantly increased in males of the 500 mg/kg and females of the 1000 and 2000 mg/kg dose groups. In the olfactory epithelium of the nose, the incidences of hyaline droplet accumulation were significantly increased in the 125 (female only), 500, and 1000 mg/kg groups. Incidences of atrophy of the olfactory epithelium were significantly increased in the 1000 mg/kg groups. The incidences of pigment accumulation in macrophages in the olfactory epithelium were significantly increased in males in the 500 mg/kg or greater groups and in females in the 1000 and 2000 mg/kg dose groups.

The NTP also performed a 3-month study of the same GBE used above in rats. Groups of 10 male and 10 female F344/N rats received 0, 62.5, 125, 250, 500, or 1000 mg/kg body weight of the GBE in corn oil via gavage, 5 days per week for 14 weeks. Additional groups of 10 male and 10 female rats received the same doses for a clinical pathology study, 5 days per week for 23 days. Control groups received corn oil (2.5 ml/kg). The same methods that were followed in the mouse study described above were used in the main study animals, while animals in the clinical pathology study had blood samples collected on days 4 and 23.

All rats survived to the end of the study. Mean body weights of all dosed groups were similar to those of the vehicle control groups. No treatment-related clinical findings were observed. Liver weights of all dosed groups of males and females were significantly greater than those of the vehicle control groups. Incidences of hepatocyte hypertrophy in all dosed groups of males and in 500 and 1000 mg/kg females were significantly greater than those in the vehicle control groups; there was a dose-related increase in severity of this lesion in males. “Hepatocyte fatty change” occurred in all dosed males. The incidences of thyroid gland follicular cell hypertrophy were significantly increased in 500 and 1000 mg/kg males and in 1000 mg/kg females. The incidences of pigmentations in the olfactory epithelium of the nose were significantly increased in 500 and 1000 mg/kg males and in females administered 125 mg/kg or greater.
The reproductive and developmental toxicity of a standardized GBE was studied in mice. In one study, groups of 25 mated female CD-1 mice received 0, 100, 350, or 1225 mg/kg/day GBE in tap water via gavage (20 ml/kg) on days 6 through 15 of gestation.47 The dams were observed daily for clinical signs of toxicity. Feed and water consumption were monitored during the study. Body weight was measured daily. On day 17 of gestation, the dams were killed and the ovaries, uteri, and the fetuses were removed. The internal organs and the placentae of the dams were examined macroscopically. The fetuses were examined for several parameters, including external and internal damages (malformations), sex, viability, and weight. The skeletal systems and soft tissues of the fetuses were also examined.

No clinical signs of toxicity were observed in the dams and there were no unscheduled deaths. No treatment related effects were observed in body weight gains or feed and water consumption. There were no pathological findings observed during necropsy. No embryotoxic effects were observed during external and internal examinations of the fetuses nor were any observed in skeletal or soft tissues. There were no increased incidences of malformation, variations, or retardations. The authors concluded the no-observed-effect-level (NOEL) was greater than 1225 mg/kg/day for both the dams and the fetuses in this study of a standardized GBE.47

Another study examined the effects of oral administration of a standardized GBE in saline on the mouse reproductive and developmental toxicity.48 Female Swiss albino mice received 0, 3.7, 7.4, or 14.8 mg/kg body weight/day for 28 days prior to mating, from day 1 to day 7 of gestation, or from day 10 to day 18 of gestation. There were 10 animals per dose group to study the anti-implantation and abortifacient activities for this GBE, while there were 10 mice per dose group to study the reproductive cycle and 20 mice per dose group to study the developmental cycle (12 test groups total). Blood hormones were measured in the pre-mating group on day 28. Vaginal smears were performed daily. The mice were observed daily for clinical signs of toxicity and premature deaths. Body weights were recorded weekly. On day 20 of gestation, the remaining mice were killed and their kidneys, liver, brain, placenta, spleen and ovaries were removed and weighed. The ovaries were prepared for histological examinations, and then ovarian follicles were counted. Maternal toxicity, estrous cycle, reproductive hormones, ovarian follicle counts, resorption index, implantation index, fetal viability and fetuses, and placenta mean weights were evaluated.

No symptoms of clinical toxicity such as depressed activities, respiratory distress, salivation, tremor, fasciculation, dull eyes, diarrhea, or change in fur appearance were observed in the dams during treatment, and there were no unscheduled deaths. Statistically significant decreases in body weight gains were observed in the 14.8 mg/kg/day dose group when compared to the controls. There were no treatment-related differences in the relative weights of the liver, kidney, brain, spleen, ovary, and placenta, but there was a significant dose-dependent decrease in the relative weight of the gravid uterus in the 14.8 mg/kg/day dose group for 28 days when compared to controls. Ovarian follicle counts, resorption index, implantation index, and fetal viability were significantly reduced in 14.8 mg/kg/day dose group. Treatment with 14.8 mg/kg bw/day of this particular GBE induced disruption of estrous cycle and caused maternal toxicity, in addition to fetal toxicity. No adverse effects were observed in the 3.7 or 7.4 mg/kg bodyweight/day dose groups. The authors concluded that 14.8 mg/kg body weight/day of this GBE produced adverse effects on the estrous cycle, fertility, abortifacient, reproductive performance, and hormone levels of female mice and may cause adverse effects on ovarian function as an antifertility agent.48

The effects of an aqueous GBE on embryo-fetal development were investigated in pregnant Wistar rats.49 Groups of 17 rats received 0, 3.5, 7, or 14 mg/kg/day of the test material during the tubal transit and implantation period of pregnancy. The dams were then killed on the 15th day of pregnancy. The following parameters were evaluated during the study: clinical
symptoms of maternal toxicity; maternal body weight; feed and water intake; maternal liver, kidney, and ovary weights; number of corpora lutea; implants per group ratio; pre- and post-implantation loss per group ratio; live fetuses mean; dead fetuses percentage; fetus and placenta weight per offspring ratio; and fetal external malformation. No significant adverse effects were observed for any of the parameters in the dams or the embryos. The authors of this study concluded that the studied GBE did not produce adverse effects in maternal or embryonic rats.

**GENOTOXICITY**

**In Vitro**

**Ginkgo Biloba Leaf Extract**

The NTP tested a specific GBE at up to 10,000 µg/plate was mutagenic in an Ames test using *Salmonella typhimurium* strains TA98 and TA100 and *Escherichia coli* strain WP2 uvrA/pKM101, with and without metabolic activation. The genotoxicity of the same GBE and eight of its constituents (quercetin; quercetin-3-β-D-glucoside; kaempferol; isorhamnetin; ginkgolide A; ginkgolide B; ginkgolide C; and bilobalide) were evaluated in mouse L5178Y cells using a lymphoma assay and a Comet assay. The GBE (0.2-1.2 mg/ml) and the eight constituents were tested in a dimethyl sulfoxide (DMSO) solution. A dose-dependent increase in mutant frequency was observed in the studied GBE, quercetin (10-100 µM), quercetin-3-β-D-glucoside (200-1000 µM), and kaempferol (10-200 µM) without metabolic activation. DNA double-strand breaks were also observed in dose-dependent increases in the studied GBE, quercetin, and kaempferol. Negative results were observed in the other constituents. A Western blot analysis confirmed that GBE, quercetin, and kaempferol activated the DNA damage signaling pathway. Additionally, GBE produced reactive oxygen species and decreased glutathione levels in L5178Y cells. An analysis of loss of heterozygosity in *Tk* mutants indicated that GBE, quercetin, and kaempferol resulted in extensive chromosomal damage. The authors concluded that the studied GBE, quercetin, and kaempferol are mutagenic in mouse L5178Y cells.

**Gingko Biloba Meristem Cell**

Ginkgo Biloba Meristem Cell at up to 5000 µg/plate was not mutagenic in an Ames test in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 or in *E. coli* strain WP2 uvrA/pKM101, with and without metabolic activation. Ginkgo Biloba Meristem Cell did not induce chromosomal aberrations in Chinese hamster lung cultured cells, with and without metabolic activation. The cells were treated with 210.0 µg/ml without metabolic activation (short-time treatment), 333.6 µg/ml with metabolic activation (short-time treatment), and 202.2 µg/ml without metabolic activation (24 h continuous treatment). Short-time treatment was not defined.

**In Vivo**

**Ginkgo Biloba Leaf Extract**

In a micronucleus test in male and female B6C3F1/N mice performed by the NTP, no increase in the frequency of micronucleated erythrocytes was observed in peripheral blood of male mice administered 125 to 2000 mg/kg/day of a GBE orally for 3 months. Female mice that received the same doses had results that were deemed equivocal based on a significant trend test and due to no individual dose group being significantly elevated over the vehicle control group. A significant (P < 0.001) dose-related decreased in the percentage of circulating polychromatic erythrocytes (PCEs) was observed in male mice, which may indicate the studied GBE induced bone marrow toxicity. In the female mice, a significant (P = 0.001) decrease in the percentage of circulating PCEs was also observed, but the response was not as correlated with dose as it was in the males.

In a reporter gene mutation assay using male B6C3F1 gpt delta mice, oral dosing of the GBE used in the NTP studies at up to 2000 mg/kg body weight/day (in corn oil) for 90 days did not produce remarkable increases in gpt or Spi mutation frequencies in DNA extracted from the liver. No treatment-related clinical signs or deaths were observed during the treatment period. Relative liver weights were significantly increased in the 2000 mg/kg group. Hepatocellular hypertrophy in the centrilobular area and slight focal necrosis were observed in the 2000 mg/kg group.

This assay was performed in conjunction with a combined liver comet assay and bone marrow micronucleus assay using male and female CARKO and wild-type mice. The short-term toxicity effects were described in the Toxicological Studies section. In the micronucleus study, no significant alterations in the percentages of PCEs were observed in females of either genotype; however, a significant decrease in the percentage of PCEs were observed in both genotypes in males, indicating the studied GBE induced bone marrow toxicity in male mice. In the comet assay, there was no significant difference in the percent tail DNA in any of the GBE-treated groups in either mouse genotype. Heavily damaged cells called “hedgehogs” indicating cytotoxic effects were not detected in any animals. The researchers performing these 3 assays concluded that the studied GBE is not genotoxic.


**Ginkgo Biloba Meristem Cell**

In a micronucleus test, no increase in the frequency of micronucleated polychromatophilic erythrocytes in bone marrow was observed in male mice administered 500 to 2000 mg/kg/day Ginkgo Biloba Meristem Cell.\(^{45}\) There was no significant difference in the ratio of polychromatophilic erythrocytes in total red blood cells when compared to the negative control. The positive control yielded expected results. No further details were provided.

**CARCINOGENICITY**

**Oral**

The carcogenic potential of a GBE administered orally was studied by the NTP in male and female rats and mice.\(^8\) In the study on mice, groups of 50 male and 50 female B6C3F1/N mice received 200, 600, or 2000 mg/kg of this GBE in corn oil 5 day per week for 104 weeks via gavage. In the study on rats, groups of 50 F344/N male and 50 female rats received 100, 300, or 1000 mg/kg body weight of this GBE for 104 (males) or 105 (females) weeks via gavage. Control groups received corn oil (5 ml/kg in mice and 2.5 ml/kg in rats). In rats involved in what was deemed a “special study,” groups of 10 male and female rats received the same doses as in the main study; blood was collected from these rats on day 22 and at week 14 for thyroid hormone analyses and other analyses of the liver and thyroid gland. All animals were observed twice daily. Body weights were evaluated at study beginning and ending and at different intervals during the course of the study. At the end of the study period, tissues from over 40 sites were examined for every animal, including ovaries and uteri in females and prostate gland and testes with epididymis and seminal vesicles in males.

In mice, mortality was significantly higher in the 600 and 2000 mg/kg males than in the vehicle controls, with the most frequent cause of death being liver tumors. Survival in the 600 mg/kg females was significantly greater than that of the vehicle controls. Mean body weights in the mid- and high-dose group male mice were less than (10% or more) those of the vehicle controls after weeks 85 and 77, respectively. The mean body weights of the high-dose females were generally less than the vehicle controls between weeks 17 and 69 and after week 93.

In rats, mortality in the 1000 mg/kg males was significantly higher than that of the vehicle controls, with the most frequent cause of death being mononuclear cell leukemia. The survival of the treated female rats was comparable to the vehicle control. In week 14, all dose group males and females of the 1000 mg/kg group in the special study had increased levels of thyroid stimulating hormone compared to the vehicle controls; the increase was dose-related in the male rats. Mean body weights in the mid- and high-dose male and female rats were less than (10% or more) those of the vehicle controls after weeks 93 and 89, respectively.

Lesions in the liver, thyroid gland, and nose were observed in all the studied GBE dose groups in mice and rats. These lesions included hypertrophy in the liver and thyroid gland in rats and mice, liver hyperplasia in male and female rats, and hyperplasia and atrophy of the epithelium in the nose of male and female rats. Inflammation, hyperplasia, hyperkeratosis, and ulcers were also observed in the forestomach of male and female mice. Additionally, increased incidences of cancers of the thyroid gland were observed in male and female rats and male mice and of liver cancers in male and female mice. The study concluded that the studied GBE caused cancers of the thyroid gland in male and female rats and male mice, and cancers of the liver in male and female mice.\(^8\)

In dietary carcinogenicity studies of a standardized GBE in mice (at up to 200 mg/kg/day) or rats (at up to 100 mg/kg/day), no neoplastic or pre-neoplastic effects were observed.\(^{51}\) The rodents received the test material for up to 85 weeks. No changes in body weight gain were reported. No further details are available. The International Agency for Research on Cancer (IARC) has determined that GBEs are possibly carcinogenic to humans (group 2B) based on inadequate human carcinogenicity evidence and sufficient evidence in experimental animals.\(^{52}\) The animal data used to reach this determination were from the NTP studies that are described above that used a specific GBE. IARC reviewed the findings of a few randomized and case-control epidemiological studies researching the potential effects of the use of GBE dietary supplements in elderly patients and ovarian cancer patients. IARC suggested that the mechanisms for carcinogenicity associated with GBEs may be genotoxicity and/or topoisomerase inhibition that could be related to the constituents quercetin, kaempferol, and/or rutin.

**OTHER RELEVANT STUDIES**

**Immunotoxicity**

In a popliteal lymph node assay (PLNA), the sensitization potential of a GBE was evaluated.\(^{12}\) Groups of male C57BL/6 mice received subplantar injections of 10 \(\mu\)l DMSO (induction) followed by another injection of DMSO (negative control group), a crude ethanolic-aqueous GBE, heptane fraction of the crude GBE, or diphenylhydantoin (positive control group) at doses of 2 mg each. The negative control yielded small enlargement of the lymph nodes, while the crude ethanolic-aqueous GBE resulted in statistically significant lymphoproliferative reaction (LPR) in the ipsilateral popliteal lymph node. A massive lymph node hyperplasia that was almost comparable to the positive control was observed in the heptane solution fraction of the crude GBE. Chemical analyses of the crude extract and the heptane fraction found ginkgolic acid at 5.5% and 24.6%, respectively, which were theorized to be responsible for the LPR observed in this study.
DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Human

No irritation was observed in a 24-h human patch test of a Ginkgo Biloba Leaf Extract (100%; ethanol:water:butylene glycol extract) in 20 subjects. No further details were provided.

Sensitization

Animal

The sensitizing potential of ginkgolic acid and a GBE was studied in 10 female albino guinea pigs using a modified Freund’s complete adjuvant (FCA) technique. The pure ginkgolic acid was extracted from Ginkgo biloba fruit and the GBE was a prepared through water:acetone extraction and contained 24% flavone glycosides and ~1000 ppm (~0.1%) ginkgolic acid. The animals received intradermal injections (up to 0.15 ml) of an emulsion containing 4 ml physiological saline, 4 ml FCA, 15 mg of the pure ginkgolic acid, and 30 mg ginkgolic acid-containing leaf extract on to the clipped and shaved shoulder area on days 1, 5, and 9 of the study. After an 11 day rest period, the animals were challenged with 0.1% and 1% ginkgolic acid and 10% GBE in acetone on the clipped and shaved right flank. All animals exhibited sensitization to pure ginkgolic acid, while none were sensitized to the GBE that contained 1000 ppm ginkgolic acid.

Human

Human dermal sensitization studies are summarized in Table 5. No dermal irritation or sensitization was observed in human repeat insult patch tests (HRIP Ts) of products containing up to 0.2% Ginkgo Biloba Leaf Extract.

Cross-Reactivity

Guinea pig sensitization studies of crude Ginkgo biloba fruit extract, the main aromatic components of the fruit, and urushiol found no cross-reactions among the compounds. It was also determined that ginkgolic acid was the main allergen in Ginkgo biloba.

Phototoxicity/Photosensitization

No phototoxicity or photosensitization was reported to a lip product containing 0.0072% Ginkgo Biloba Leaf Extract in a study of 29 subjects. The test material was applied neat under semi-occlusive patches. No further details were provided.

OCULAR IRRITATION STUDIES

In an EpiOcular in vitro assay of an eye product containing 0.013% Gingko Biloba Leaf Extract, it was predicted that the test substance had no potential for eye irritation. No further details were provided.

CLINICAL STUDIES

Case Studies

The fruit pulp of the Ginkgo biloba tree has been reported to cause contact dermatitis, with several cases reported after patients handled the fruit pulp during extraction of the edible nut center. Symptoms include intense itching, edema, papules, and pustules that usually resolve in 7-10 days. A 66-year-old woman presented with progressive erythematous eruption over the face, neck, trunk, and extremities that started approximately one week after the patient had ingested two 60 mg doses of a GBE supplement. No other new medications or changes in behavior were reported. A physical examination, complete blood cell count, and chemistry panel were unremarkable. The authors of the report did not disclose if patch or skin prick tests were performed. A 45-year-old man developed acute generalized exanthematous pustulosis on his limbs and face 48 h after starting an oral GBE treatment for tinnitus. The patient had not previously taken any GBEs before and was not taking any other medication. The patient had no history of adverse drug reactions or psoriasis. The rash cleared within 10 days of stopping the GBE treatment. The patient refused a follow-up cutaneous patch test.

In anecdotal accounts from Chinese medicine, consumption of fresh Ginkgo biloba nuts may cause stomachache, nausea, diarrhea, convulsions, weak pulse, restlessness, difficulty breathing, and shock. Death has been reported in children following consumption of fresh nuts.

Other Clinical Reports

No adverse effects were reported in clinical studies of a cosmetic formulation containing 1.5% GBE and other antioxidants in 45 volunteers (twice daily for 90 days) and of a cosmetic formulation containing 0.30% GBE in 20 volunteers (twice daily for 28 days).
Numerous studies have investigated the efficacy and safety of GBEs in humans in the treatment of various afflictions. In a cross-matching review of much of this published toxicological and clinical data on GBEs (mainly the herbal supplement EGb761), the authors of the review evaluated the findings of 75 clinical studies with a total of 7115 patients treated orally with GBEs and found no specific or serious undesired reactions to GBEs.\(^ {31} \) Any adverse events observed frequently occurred at the same frequency as placebo treatments. Based on cross-matching data on the historic use by humans, large intake, toxicological and clinical studies, the authors concluded that GBEs are well tolerated and safe.

**SUMMARY**

According to the *Dictionary*, most of the *Ginkgo biloba*-derived ingredients detailed in this safety assessment are reported to function as skin conditioning agents, while some are reported to function as antioxidants in cosmetics. Investigations into the efficacy of the leaf extract for these uses are numerous and are mainly based on oral administration of supplements. There are no publicly available toxicity data that corresponds to specific use of these ingredients as cosmetics. This safety assessment focuses on data relevant to the use of *Ginkgo biloba*-derived ingredients in cosmetics, with specific attention on dermal application when available.

According to 2017 VCRP survey data, *Ginkgo Biloba Leaf Extract* has the most reported uses in cosmetic products, with a total of 726; the majority of the uses are in leave-on eye makeup preparations and skin care products. Two other *Ginkgo*-derived ingredients are reported to be in use, with 24 or less uses reported in the VCRP. The results of the concentration of use survey on these 10 ingredients conducted in 2014 by the Council indicate use for only *Ginkgo Biloba Leaf Extract*, which is reported to be used at a maximum of 1%, as reported in face and neck skin preparations.

GBEs are used extensively as an herbal supplement for anti-inflammatory, cognitive-promoting, antioxidant, and vascular effects and is an approved herbal medicine in Germany for use for treatment of memory deficits, dementia, and other organic brain syndromes when extracted with acetone/water. GBEs may interact with pharmaceutical drugs. Nuts from *Ginkgo biloba* are consumed as a delicacy in Japan and China and are used in traditional Chinese medicine. Anecdotal accounts report that consumption of the nuts may have acute adverse effects.

In general, toxicokinetics data are not expected to be found on botanical ingredient because each botanical ingredient is a complex mixture of hundreds of constituents. However, there have been many pharmacokinetics studies on GBEs, specifically on some of the key constituents, which indicate GBEs may be well absorbed after oral administration. The GBE constituent, quercetin, was found to penetrate human dermatomed skin. In an oral ADME study in rats, at least 60% of a radiolabeled GBE (flavonol glycosides and proanthocyanidins) were absorbed, with the main site of absorption likely in the upper gastrointestinal tract. Radioactivity was measured in exhalation and elimination products. In a human plasma study ginkgolide A, ginkgolide B, and bilobalide were found to be bioavailable after single oral dosing of 3 different *Ginkgo biloba* preparations.

The LD\(_{50}\) of a particular standardized GBE administered orally to mice was reported to be 7730 mg/kg, and the LD\(_{50}\) after intravenous administration of another standardized GBE was 1100 mg/kg for both rats and mice. The lethal dose for *Ginkgo Biloba Meristem Cell* was greater than 2000 mg/kg in rats and the maximum tolerated dose for this ingredient was greater than 1000 mg/kg in dogs.

In 3-month studies by the NTP of a specific GBE at up to 2000 mg/kg/day, increased liver weights, decreased kidney weights, increased incidences of hepatic cytotoxicity and focal hepatic necrosis, and increased incidences hyaline droplet accumulation, atrophy and pigment accumulation in macrophages in the olfactory epithelium were observed in mice. In a similar NTP study of the same GBE test material in rats, increased liver weights, increased incidences of hepatocyte hypertrophy, increased incidences of thyroid gland follicular cell hypertrophy, and increased incidences of pigmentation in the olfactory epithelium of the nose were observed. There was no evidence of organ damage or impairment of hepatic or renal function when a specific GBE was administered orally over 27 weeks to rats and mice at doses ranging from 100 to 1600 mg/kg. In a 4-week oral repeated dose study, no adverse effects were observed in rats that received up to 2000 mg/kg *Ginkgo Biloba Meristem Cell*. In the follow-up 13-week oral study, the NOAEL in rats for *Ginkgo Biloba Meristem Cell* was greater than 1000 mg/kg.

In an oral DART study of a standardized GBE in mice, the NOEL for dams and fetuses was greater than 1225 mg/kg/day. No clinical signs of toxicity were observed in the dams and no embryotoxic effects were observed in the fetuses. In another oral DART study in mice, a standardized GBE at 14.8 mg/kg/day produced adverse effects on the estrous cycle, fertility, abortifacient, reproductive performance, and hormone level in female mice and may cause adverse effects on ovarian function as an antifertility agent. No adverse effects in maternal or embryonic rats were observed in an embryo-fetal development study in rats at doses up to 14 mg/kg/day of an aqueous GBE.

The GBE specific to NTP studies was mutagenic in an Ames test at up to 10,000 µg/plate, and the same GBE (0.2 - 1.2 mg/ml) was mutagenic in mouse L5178Y cells. In a mouse micronucleus test of the same GBE at up to 2000 mg/kg/day, no increase in the frequency of micronucleated erythrocytes was observed in male mice, but the results were deemed equivocal in female mice. The same GBE at up to 2000 mg/kg/day was not genotoxic in a reporter gene mutation assay, a combined liver comet assay, and bone marrow micronucleus assay in mice. *Ginkgo Biloba Meristem Cell* was not mutagenic in an Ames test at up to 5000 µg/plate, nor did it induce chromosomal aberrations in Chinese hamster lung cultured cells.
with and without metabolic activation. Ginkgo Biloba Meristem Cell did not increase the frequency of micronucleated erythrocytes in male mice at up to 2000 mg/kg/day.

In oral carcinogenicity studies of rats and mice conducted by the NTP, lesions in the liver, thyroid gland and nose were observed in all GBE dose groups (200 - 2000 mg/kg/day, by gavage). Lesions included hypertrophy in the liver and thyroid gland in rats and mice, liver hyperplasia in male and female rats, and hyperplasia and atrophy of the epithelium in the nose of male and female rats. Inflammation, hyperplasia, hyperkeratosis, and ulcer were also observed in the forestomach of male and female mice. Additionally, increased incidences of cancers of the thyroid gland were observed in male and female rats and male mice, as were liver cancers in male and female mice. In dietary carcinogenicity studies of another standardized GBE in mice (at up to 200 mg/kg/day) or rats (at up to 100 mg/kg/day) for up to 85 weeks, no neoplastic or pre-neoplastic effects were observed. IARC has determined that GBEs are possibly carcinogenic to humans (group 2B).

In a PLNA validation study, a GBE exposure yielded statistically significant lymphoproliferative reactions in the ipsilateral popliteal lymph node, which may have been caused by ginkgolic acid.

No irritation was observed in a 24-h human patch test of Ginkgo Biloba Leaf Extract (100%; ethanol:water:butylene glycol extract).

In a guinea pig study, sensitization was observed to ginkgolic acid at concentrations of 0.1% and 1%, but no sensitization was observed to a GBE that contained ~1000 ppm (~0.1%) ginkgolic acid. No dermal sensitization was reported in HRIPTs of products containing 0.2% Ginkgo Biloba Leaf Extract.

Guinea pig sensitization studies of crude Ginkgo biloba fruit extract, the main aromatic components of the fruit, and urushiol found no cross-reactions among the compounds. It was also determined that ginkgolic acid was the main allergen in Ginkgo biloba.

No phototoxicity or photosensitization was reported to a lip product containing 0.0072% Ginkgo Biloba Leaf Extract.

No ocular irritation was predicted in an in vitro assay using an eye product containing 0.013% Ginkgo Biloba Leaf Extract.

Reports of contact dermatitis have been reported following exposure to the fruit pulp of Ginkgo biloba. Patients have reported erythematous reactions and generalized exanthematous pustulosis following ingestion of certain GBE supplements. No adverse effects were reported in clinical studies of cosmetic formulations containing up to 1.5% GBEs. In anecdotal accounts from Chinese medicine, consumption of fresh Ginkgo biloba nuts may cause stomachache, nausea, diarrhea, convulsions, weak pulse, restlessness, difficulty breathing, and shock. Death has been reported in children following consumption of fresh nuts. A cross-matching review of multiple clinical studies found no specific or serious undesired reactions to GBEs.

**DRAFT DISCUSSION**

The ingredients in this report are each a mixture of botanical constituents derived from the plant Ginkgo biloba. Because final product formulations may contain multiple botanicals, each possibly containing similar constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. For Ginkgo biloba-derived ingredients, the Panel was concerned about the presence of ginkgolic acid, quercetin, and kaempferol in cosmetics. Dermal sensitization has been associated with ginkgolic acid, and positive results have been observed in genotoxicity assays with quercetin and kaempferol. [The Panel should expand discussion on the quercetin and kaempferol findings here.] When formulating products, manufacturers should avoid reaching levels of plant constituents that may cause sensitization or other adverse health effects.

The Panel also expressed concern about pesticide residues, heavy metals, and other plant species that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit impurities.

Ginkgo Biloba Leaf Extract was reported to be used in spray and powder products that could possibly be inhaled, such as pump spray suntan products at a maximum concentration of 0.05%, and face powders at a maximum concentration of 0.05%. There were no inhalation toxicity data available. Although the Panel noted that droplets/particles from spray and loose-powder cosmetic products would not be respirable to any appreciable amount, the potential for inhalation toxicity is not limited to respirable droplets/particles deposited in the lungs. In principle, inhaled droplets/particles deposited in the nasopharyngeal and thoracic regions of the respiratory tract may cause toxic effects depending on their chemical and other properties. However, 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 is available at http://www.cir-safety.org/cir-findings.

Additionally, the Panel may like to address the following:

- The findings of the genotoxicity studies and carcinogenicity studies described in this safety assessment. Do the findings of the dermal penetration study with quercetin coupled with the low use concentration address any concerns the Panel has with Ginkgo Biloba Leaf Extract and other Ginkgo biloba-derived ingredients?
• Sensitization data on products containing Ginkgo Biloba Leaf Extract at up to 0.2% were negative. Ginkgo Biloba Leaf Extract is used at up to 1% in leave-on products. Are these data sufficient to address concerns, or does the Panel want to impose a limit on use since the data are not at use concentration? How does the Panel want to address ginkgolic acid?
• The importance of knowing the composition of a botanical ingredient as used in cosmetics, especially since different toxicological results have been observed with extracts that have varying constituent levels.
• In December 2017, the Panel issued an Insufficient Data Announcement with the following needs:
  • Method of manufacturing for each of these Ginkgo biloba-derived cosmetic ingredients
  • Composition and impurities data for each of these Ginkgo biloba-derived cosmetic ingredients
  • 28-Day dermal toxicity data
  • Dermal irritation and sensitization data at leave-on use concentrations
  • Ocular irritation data, if available
  • Genotoxicity data
  • Developmental and reproductive toxicity data
  • Data on the absorption spectra or phototoxicity of these cosmetic ingredients

Have the data needs been met for any or all of the Ginkgo biloba-derived ingredients described in this safety assessment. If the answer is no, the Panel should list the data that is still needed and provide the appropriate safety conclusion.

**CONCLUSION**

To be determined…
### TABLES

**Table 1. Definitions, Structures, and functions of the ingredients in this safety assessment.**

<table>
<thead>
<tr>
<th>Ingredient/CAS No.</th>
<th>Definition &amp; Structure</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ginkgo Biloba Leaf Extract 90045-36-6</td>
<td>Ginkgo biloba leaf extract is the extract of the leaf of <em>Ginkgo biloba</em>.</td>
<td>skin-conditioning agent – misc.</td>
</tr>
<tr>
<td>Ginkgo Bilavones 90045-36-6</td>
<td>Ginkgo bilavones is a mixture of bilavones derived from the leaves of <em>Ginkgo biloba</em>. It consists predominantly of sciadopitysin, bilobetin, ginkgetin, and isoginkgetin.</td>
<td>antioxidant</td>
</tr>
<tr>
<td>Ginkgo Biloba Leaf 90045-36-6</td>
<td>Ginkgo biloba leaf is the leaf of <em>Ginkgo biloba</em>.</td>
<td>skin-conditioning agent – misc.</td>
</tr>
<tr>
<td>Ginkgo Biloba Leaf Cell Extract 90045-36-6</td>
<td>Ginkgo biloba leaf cell extract is the extract of a culture of the leaf cells of <em>Ginkgo biloba</em>.</td>
<td>flavoring agents; skin protectant</td>
</tr>
<tr>
<td>Ginkgo Biloba Leaf Powder 90045-36-6</td>
<td>Ginkgo biloba leaf powder is the powder obtained from the dried, ground leaves of <em>Ginkgo biloba</em>.</td>
<td>skin-conditioning agent – misc.</td>
</tr>
<tr>
<td>Ginkgo Biloba Leaf Water 90045-36-6</td>
<td>Ginkgo biloba leaf water is the aqueous solution of the steam distillate obtained from the leaves of <em>Ginkgo biloba</em>.</td>
<td>fragrance ingredient; skin-conditioning agent – misc.</td>
</tr>
<tr>
<td>Ginkgo Biloba Meristem Cell 90045-36-6</td>
<td>Ginkgo biloba meristem cell are the cultured meristem cells isolated from <em>Ginkgo biloba</em>.</td>
<td>antimicrobial agent; antioxidant; skin-conditioning agent – misc.</td>
</tr>
<tr>
<td>Ginkgo Biloba Nut Extract 90045-36-6</td>
<td>Ginkgo biloba nut extract is the extract of the seeds of <em>Ginkgo biloba</em>.</td>
<td>cosmetic astringent; hair conditioning agent; nail conditioning agent; skin-conditioning agent – misc.</td>
</tr>
<tr>
<td>Ginkgo Biloba Root Extract 90045-36-6</td>
<td>Ginkgo biloba root extract is the extract of the roots of <em>Ginkgo biloba</em>.</td>
<td>skin-conditioning agent – misc.</td>
</tr>
<tr>
<td>Ginkgo Leaf Terpenoids 107438-79-9, 15291-75-5, 15291-76-6, 15291-77-7, 33570-04-6</td>
<td>Ginkgo leaf terpenoids is a mixture of terpenoids isolated from the leaves of <em>Ginkgo biloba</em> consisting chiefly of ginkgolide A, ginkgolide B, ginkgolide C, ginkgolide J, and bilobalide.</td>
<td>antiacne agent; antifungal agent; antimicrobial agent; antioxidant; external analgesics; hair conditioning agent</td>
</tr>
</tbody>
</table>

---

**Table 2. Major constituents of GBEs (%).**

<table>
<thead>
<tr>
<th>Class</th>
<th>Identified</th>
<th>Standardized Extract (Egb 761) Specification</th>
<th>Range* (%)</th>
<th>NTP Study Extract*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Terpene trilactones</strong></td>
<td>Total 6</td>
<td>0.07-14.23</td>
<td>15.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bilobalide</td>
<td>0.03-8.64</td>
<td>6.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ginkgolide A</td>
<td>0.01-2.90</td>
<td>3.74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ginkgolide B</td>
<td>&lt; 0.005-1.75</td>
<td>1.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ginkgolide C</td>
<td>&lt; 0.005-1.75</td>
<td>3.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ginkgolide J</td>
<td>0.03-0.78</td>
<td>Not measured</td>
<td></td>
</tr>
<tr>
<td><strong>Flavonol glycosides</strong></td>
<td>Total 24</td>
<td>0.18-35.54</td>
<td>31.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>&lt; 0.01-8.34</td>
<td>16.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kaempferol</td>
<td>0.02-5.57</td>
<td>12.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isorhamnetin</td>
<td>0.04-1.13</td>
<td>2.37</td>
<td></td>
</tr>
<tr>
<td><strong>Alkylphenols</strong></td>
<td>Ginkgolic acids, cardanol</td>
<td>0.0005</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

*Adapted from the NTP 2013 report.

*Constituent ranges are not specific to the cosmetic ingredient Ginkgo Biloba Leaf Extract but to constituent ranges of standardized and non-standardized GBEs found in the published literature.
Table 3. Frequency (2017) and concentration of use (2014) according to duration and type of exposure for *Ginkgo biloba*-derived ingredients\textsuperscript{19,20}

<table>
<thead>
<tr>
<th>Exposure Type</th>
<th>Duration of Use</th>
<th># of Uses</th>
<th>Max Conc of Use (%)</th>
<th># of Uses</th>
<th>Max Conc of Use (%)</th>
<th># of Uses</th>
<th>Max Conc of Use (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ginkgo Biloba Leaf Powder</td>
<td>Ginkgo Biloba Leaf Extract*</td>
<td>Ginkgo Biloba Nut Extract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Totals</td>
<td>NR</td>
<td>726</td>
<td>0.00002-1</td>
<td>24</td>
<td>NR</td>
</tr>
<tr>
<td>Eye Area</td>
<td>Leave-On</td>
<td>3</td>
<td>NR</td>
<td>637</td>
<td>0.000002-1</td>
<td>14</td>
<td>NR</td>
</tr>
<tr>
<td>Incidental Inhalation-Spray</td>
<td>1\textsuperscript{a}; 1\textsuperscript{b}</td>
<td>NR</td>
<td>7; 165\textsuperscript{a}; 101\textsuperscript{b}</td>
<td>0.05; 0.0005-0.0041\textsuperscript{a}</td>
<td>2\textsuperscript{a}; 6\textsuperscript{b}</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Dermal Contact</td>
<td>2</td>
<td>NR</td>
<td>664</td>
<td>0.00001-0.01</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Dermal Contact</td>
<td>Deodorant (underarm)</td>
<td>2</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Dermal Contact</td>
<td>Hair - Non-Coloring</td>
<td>2</td>
<td>NR</td>
<td>48</td>
<td>0.00005-0.001</td>
<td>1</td>
<td>NR</td>
</tr>
<tr>
<td>Dermal Contact</td>
<td>Hair - Coloring</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Dermal Contact</td>
<td>Nail</td>
<td>NR</td>
<td>NR</td>
<td>5</td>
<td>0.000002-0.24</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Dermal Contact</td>
<td>Mucous Membrane</td>
<td>NR</td>
<td>NR</td>
<td>21</td>
<td>0.00002-0.2</td>
<td>1</td>
<td>NR</td>
</tr>
<tr>
<td>Dermal Contact</td>
<td>Baby Products</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>0.005</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

NR = Not reported.
\* Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
\* Combined with the generic entry “Ginkgo Extract” in the VCRP database, which is not an INCI name.
\* It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.
\* Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.
\* It is possible these products may be powders, but it is not specified whether the reported uses are powders.

Table 4. Ingredients not reported in use\textsuperscript{19,20}

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Ginkgo Biflavones</th>
<th>Ginkgo Biloba Extract</th>
<th>Ginkgo Biloba Leaf Water</th>
<th>Ginkgo Biloba Meristem Cell</th>
<th>Ginkgo Biloba Root Extract</th>
<th>Ginkgo Leaf Terpenoids</th>
<th>Ginkgo Biloba Leaf Cell Extract*</th>
</tr>
</thead>
</table>

* concentration of use data have not yet been received

Table 5. Human dermal sensitization studies on Ginkgo Biloba Leaf Extract

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Number of Subjects</th>
<th>Method</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0085% in a cream</td>
<td>48</td>
<td>HRIPT, tested neat under occlusive patch</td>
<td>No dermal irritation or sensitization</td>
<td>56</td>
</tr>
<tr>
<td>0.0072% in a lip product</td>
<td>109</td>
<td>HRIPT, tested neat under occlusive patch</td>
<td>No dermal irritation or sensitization</td>
<td>56</td>
</tr>
<tr>
<td>0.1% in a leave-on product</td>
<td>201</td>
<td>HRIPT, 4 cm\textsuperscript{2} semi-occlusive patches; dose density = 0.05 mg/cm\textsuperscript{2}</td>
<td>No sensitization</td>
<td>55</td>
</tr>
<tr>
<td>0.2% in a lotion</td>
<td>208</td>
<td>HRIPT, 0.2 ml applied with a 2 cm\textsuperscript{2} Webril pad and semi-occluded</td>
<td>No sensitization</td>
<td>55</td>
</tr>
</tbody>
</table>
REFERENCES


<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
<th>Ingredient</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>03D</td>
<td>Eye Lotion</td>
<td>GINKGO BILOBA (GINKGO) LEAF POWDER</td>
<td>1</td>
</tr>
<tr>
<td>05C</td>
<td>Hair Straighteners</td>
<td>GINKGO BILOBA (GINKGO) LEAF POWDER</td>
<td>1</td>
</tr>
<tr>
<td>05G</td>
<td>Tonics, Dressings, and Other Hair Grooming Aids</td>
<td>GINKGO BILOBA (GINKGO) LEAF POWDER</td>
<td>1</td>
</tr>
<tr>
<td>12D</td>
<td>Body and Hand (exc hair)</td>
<td>GINKGO BILOBA (GINKGO) LEAF POWDER</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Eye Lotion</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>2</td>
</tr>
<tr>
<td>03C</td>
<td>Eye Shadow</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>172</td>
</tr>
<tr>
<td>03D</td>
<td>Eye Lotion</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>24</td>
</tr>
<tr>
<td>03F</td>
<td>Mascara</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>4</td>
</tr>
<tr>
<td>03G</td>
<td>Other Eye Makeup Preparations</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>15</td>
</tr>
<tr>
<td>04E</td>
<td>Other Fragrance Preparation</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>3</td>
</tr>
<tr>
<td>05A</td>
<td>Hair Conditioner</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>12</td>
</tr>
<tr>
<td>05B</td>
<td>Hair Spray (aerosol fixatives)</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>2</td>
</tr>
<tr>
<td>05F</td>
<td>Shampoos (non-coloring)</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>18</td>
</tr>
<tr>
<td>05G</td>
<td>Tonics, Dressings, and Other Hair Grooming Aids</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>3</td>
</tr>
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<td>05I</td>
<td>Other Hair Preparations</td>
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<td>9</td>
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<tr>
<td>07A</td>
<td>Blushers (all types)</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>21</td>
</tr>
<tr>
<td>07B</td>
<td>Face Powders</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>46</td>
</tr>
<tr>
<td>07C</td>
<td>Foundations</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>13</td>
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<tr>
<td>07E</td>
<td>Lipstick</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>5</td>
</tr>
<tr>
<td>07G</td>
<td>Rouges</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>1</td>
</tr>
<tr>
<td>07H</td>
<td>Makeup Fixatives</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>1</td>
</tr>
<tr>
<td>07I</td>
<td>Other Makeup Preparations</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>11</td>
</tr>
<tr>
<td>08B</td>
<td>Cuticle Softeners</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>1</td>
</tr>
<tr>
<td>08E</td>
<td>Nail Polish and Enamel</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>2</td>
</tr>
<tr>
<td>08G</td>
<td>Other Manicuring Preparations</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>2</td>
</tr>
<tr>
<td>10A</td>
<td>Bath Soaps and Detergents</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>4</td>
</tr>
<tr>
<td>10E</td>
<td>Other Personal Cleanliness Products</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
<td>10</td>
</tr>
<tr>
<td>11E</td>
<td>Shaving Cream</td>
<td>GINKGO BILOBA LEAF EXTRACT</td>
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Memorandum

TO: Bart Heldreth, Ph.D.
    Executive Director - Cosmetic Ingredient Review (CIR)

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

DATE: December 5, 2017

SUBJECT: Ginkgo Biloba Leaf Extract

TKL Research. 2003. Repeated insult patch test study (lotion containing 0.2% Ginkgo Biloba Leaf Extract).
REPEATED INSULT PATCH STUDY

Lotion containing 0.2% Ginkgo Biloba Leaf Extract

TKL STUDY NO. DS104903/105003-1

CONDUCTED FOR:

DATE OF ISSUE:

October 1, 2003
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APPENDICES

I SUMMARY TABLES
II DATA LISTINGS
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IV INFORMED CONSENT DOCUMENT
SIGNATURES

Kathleen Georgian, Clinical Research Coordinator and Manager, Dermatologic Safety Testing

Date: 9/26/03

Jonathan S. Dosik, MD
Principal Investigator

Date: 9/30/03

STATEMENT OF QUALITY ASSURANCE

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

Clinical research studies are performed by TKL in accordance with all applicable federal regulations and proposed guidelines for Good Clinical Practices, which include:

21 CFR Part 312, Investigational New Drug Application
21 CFR Part 50, Protection of Human Subjects
21 CFR Part 56, Institutional Review Boards

Quality Assurance

Date: 10/1/03
TITLE OF STUDY
Repeated Insult Patch Study

SPONSOR

STUDY MATERIAL

DATE STUDY INITIATED
July 21, 2003

DATE STUDY COMPLETED
September 5, 2003

DATE OF ISSUE
October 1, 2003
INVESTIGATIVE PERSONNEL

Jonathan S. Dosik, MD
Principal Investigator

Robert C. Reardon, PhD
Director of Operations

Kathleen Georgian
Clinical Research Coordinator
and Manager, Dermatologic Safety Testing

Silvia Guadalupe
Assistant Clinical Research Coordinator

Holanda Ayon
Supervisor, Dermatologic Safety

Joanne Mruczek, RN
Senior Clinical Assistant

CLINICAL SITES

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578 Driggs Avenue
Brooklyn, NY 11211

TKL RESEARCH, INC.
1099 Wall Street West
Lyndhurst, NJ 07071
SUMMARY

One study material, Sample No. [redacted] was evaluated neat to determine its ability to sensitize the skin of normal volunteer subjects using a semi-occlusive repeated insult patch study. Two hundred eight subjects completed the study.

Under the conditions employed in this study, there was no evidence of sensitization to Sample No. [redacted].
1.0 OBJECTIVE

The objective of this study was to determine the ability of the study material to cause sensitization by repeated applications to the skin of humans under controlled patch study conditions.

2.0 RATIONALE

Substances that come into contact with human skin need to be evaluated for their propensity to irritate and/or sensitize. Once an appropriate pre-clinical safety evaluation has been performed, a reproducible, standardized, quantitative patch evaluation procedure must be used to demonstrate that a particular material can be applied safely to human skin without significant risk of adverse reactions. The method herein employed is generally accepted for such a purpose.

Repeated insult patch evaluation is a modified predictive patch study that can detect weak sensitizers that require multiple applications to induce a cell-mediated (Type IV) immune response sufficient to cause an allergic reaction. Irritant reactions may also be detected using this evaluation method, although this is not the primary purpose of this procedure. Results are interpreted according to interpretive criteria based upon published works, as well as the clinical experience of TKL Research, Inc. These interpretive criteria are periodically reviewed and amended as new information becomes available.

3.0 STUDY DESIGN

3.1 STUDY POPULATION

A sufficient number of subjects were enrolled to provide 200 completed subjects.

3.1.1 Inclusion Criteria

Individuals eligible for inclusion in the study were those who:

1. were males or females, 18 to 70 years of age, in general good health;

2. were free of any systemic or dermatologic disorder which, in the opinion of the investigative personnel, would have interfered with the study results or increased the risk of adverse events;

3. were of any skin type or race, providing the skin pigmentation would allow discernment of erythema;

4. had completed a patch study Medical Screening form as well as a Medical/Personal History form; and

5. had read, understood, and signed an informed consent agreement.
3.1.2 Exclusion Criteria

Individuals excluded from participation in the study were those who:

1. had any visible skin disease at the study site which, in the opinion of the investigative personnel, would have interfered with the evaluation;

2. were receiving systemic or topical drugs or medication which, in the opinion of the investigative personnel, would have interfered with the study results;

3. had psoriasis and/or active atopic dermatitis/eczema;

4. were females who were pregnant, planning to become pregnant during the study, or breast-feeding;

5. had a known sensitivity to cosmetics, skin care products, or topical drugs as related to the material being evaluated; and/or

6. were participating in another study or had been recruited to participate in another study concurrently.

3.1.3 Informed Consent

A properly executed informed consent document in compliance with FDA regulations (21 CFR Part 50) was obtained from each subject prior to entering the study. The signed informed consent document is maintained in the study file. In addition, the subject was provided with a copy of the informed consent document (see Appendix IV).

3.2 Description of Study

3.2.1 Outline of Study Procedures

Subjects participated in the study over a 6-week period involving 3 phases: (1) Induction, (2) Rest, and (3) Challenge. Prior to study entry, the subjects were screened to assure that they met the inclusion/exclusion criteria. Informed consent was obtained. Each subject was provided with a schedule of the study activities. All subjects were told to avoid wetting the patches and were asked not to engage in activities that caused excessive perspiration. They were instructed to notify the staff if they experienced any discomfort beyond mild itching or observed any adverse changes at the patch sites, while on the study or within 2 weeks of completing the study.

The Induction Phase consisted of 9 consecutive applications of the study material and subsequent evaluations of the patch sites. Prior to application of the patches, the sites were outlined with a skin marker, e.g. gentian violet. The subjects were required to remove the patches approximately 24 hours
after application. They returned to the facility at 48-hour intervals to have the sites evaluated and identical patches applied to the same sites. Patches applied on Friday were removed by subjects after 24 hours. The sites were evaluated on the following Monday, ie, 72 hours after patch application. *

Following the ninth evaluation, the subjects were dismissed for a rest period of approximately 10-15 days.

Subjects who were absent once during the induction phase received a make-up (MU) patch at the last induction visit. The MU applications were graded 48 hours later at the MU visit, or were recorded as N9G (no ninth grading).

The Challenge Phase was initiated during the sixth week of the study. Identical patches were applied to sites previously unexposed to the study material. The patches were removed by subjects after 24 hours and the sites graded after additional 24-hour and 48-hour periods (ie, 48 and 72 hours after application). Rechallenge was performed whenever there was evidence of possible sensitization.

To be considered a completed case, a subject must have had 9 applications and no fewer than 8 subsequent readings during induction, and a single application and 2 readings during challenge. Only completed cases were used to assess sensitization.

3.2.2 Definitions Used for Grading Responses

The symbols found in the scoring scales below were used to express the response observed at the time of examination:

- = 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

SPECIAL NOTATIONS

E = Marked/severe erythema
S = Spreading of reaction beyond patch site (ie, reaction where material did not contact skin)
p = Papular response > 50%
pv = Papulovesicular response > 50%
D = Damage to epidermis: oozing, crusting and/or superficial erosions
I = Itching

* A Monday or Friday holiday could result in evaluation at 96 hours after patch application.
X = Subject absent
PD = Patch dislodged
NA = Not applied
NP = Not patched (due to reaction achieved)
N9G = No ninth grading

3.2.3 Evaluation of Responses

All responses were graded by a trained dermatologic evaluator meeting TKL's strict certification requirements to standardize the assignment of response grades.

4.0 NATURE OF STUDY MATERIAL

4.1 Study Material Specifications

Identification : [Redacted] Lotion
Amount Applied : 0.2 mL
Special Instructions : Volatilized for at least 30 minutes prior to patch application.

4.2 Storage, Handling, and Documentation of Study Material

Receipt of the material used in this study was documented in a general logbook, which serves as a permanent record of the receipt, storage, and disposition of all study material received by TKL. On the basis of information provided by the sponsor, the study material was considered reasonably safe for evaluation on human subjects. A sample of the study material was reserved and will be stored for a period of 6 months. At the conclusion of the clinical study, the remaining study material was discarded or returned to the sponsor and the disposition documented in the logbook. All information regarding the receipt, storage, and disposition of the study material was also recorded on a Clinical Material Record form (see Appendix III), which is incorporated in this study report. All study material is kept in a locked product storage room accessible to clinical staff members only.

4.3 Application of Study Material

Study material was applied to the patch as instructed. The patch was applied to the infrascapular area of the back, either to the right or left of the midline, or to the upper arm.

4.4 Description of Patch Conditions

Material evaluated under occlusive patch conditions was applied to a 2-cm x 2-cm Webril pad attached to a non-porous, plastic film adhesive bandage (3M medical tape). The patch was secured with hypoallergenic tape (Micropore), as needed.
Material evaluated under semi-occlusive patch conditions was applied to a 2-cm x 2-cm Webril pad. The pad was affixed to the skin with hypoallergenic tape (Micropore).

5.0 INTERPRETATION

Sensitization is characterized by an acute allergic contact dermatitis. Typical sensitization reactions begin with an immunologic response in the dermis resulting in erythema, edema formation, and secondary epidermal damage (vesiculation), sometimes extending beyond the patch site and often accompanied by itching. Sensitization reactions tend to be delayed. The reaction typically becomes evident between 24 and 48 hours, peaks at 48-72 hours and subsequently subsides. The reaction is often greater at 72 hours than at 48 hours. The severity of the reaction is generally greater during the challenge phase of a Repeated Insult Patch Test (RIPT) than that seen during induction.

Irritant reactions are characterized as a non-immunologic, localized, superficial, exudative, inflammatory response of the skin due to an externally applied material. The typical initial reaction does not develop much edema or vesiculation but results in scaling, drying, cracking, oozing, crusting, and erosions. The reaction is usually sharply delineated, not spreading beyond the patch site. Irritant reactions are typically evident by 24 hours and diminish over the next 48-72 hours. Removal of the offending agent results in gradual improvement of the epidermal damage. The reaction seen at 72 hours is, therefore, less severe than that seen at 48 hours. Finally, the severity of the reaction experienced in the challenge phase is generally similar to that seen during induction.

If the results of the study indicate the likelihood of sensitization, the recommended practice is to rechallenge the subjects who have demonstrated sensitization-like reactions to confirm that these reactions are, indeed, associated with the product. Our preferred rechallenge procedure involves the application of the product to naïve sites, under both occlusive and semi-occlusive patch conditions. Use of the semi-occlusive patch condition helps to differentiate irritant and sensitization reactions. Generally speaking, if a product is a sensitizer it will produce a similar reaction under both occlusion and semi-occlusion. Whereas, if the product has caused an irritant reaction, the reactions will be less pronounced under the semi-occlusive condition.

6.0 DOCUMENTATION AND RETENTION OF DATA

The case report forms (CRFs) are designed to identify each subject by subject number and initials, and to record demographics, examination results, adverse events, and end of study status. Originals or copies of all CRFs, source documents, IRB documents (if required), correspondence, study reports, and all source data will be kept on hard-copy file for a minimum of 5 years from completion of the study. Storage is maintained either at a TKL facility in a secured room accessible only to TKL employees, or at an offsite location which provides a secure environment with burglar/fire alarm systems, camera detection and controlled temperature and humidity. Documentation will be available for the sponsor's review on the premises of TKL.
7.0 RESULTS AND DISCUSSION

One study material, Sample No. [redacted], was evaluated neat to determine its ability to sensitize the skin of normal volunteer subjects using a semi-occlusive repeated insult patch study. Two hundred nineteen subjects between the ages of 18 and 69 were enrolled and 208 subjects completed the study (see Tables 1 and 2 in Appendix I and Data Listings 1 and 2 in Appendix II).

The following table summarizes subject enrollment and disposition.

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Source: Table 1, Appendix I

There were no adverse events.

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

8.0 CONCLUSION

Under the conditions employed in this study, there was no evidence of sensitization to Sample No. [redacted]
9.0 REFERENCES


Marzulli FN, Maibach HI. Effects of vehicles and elicitation concentration in contact dermatitis testing I: experimental contact sensitization in humans. Contact Dermatitis 1976; 2:325.


Kligman AM, Epstein W. Updating the maximization test for identifying contact allergens. Contact Dermatitis 1975; 1:231-239.

Memorandum

TO: Bart Heldreth, Ph.D.
    Executive Director - Cosmetic Ingredient Review (CIR)

FROM: Carol Eisenmann, Ph.D.
       Personal Care Products Council

DATE: December 11, 2017

SUBJECT: Ginkgo Biloba Leaf Extract

Certificate of Analysis

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SpecPure™ GBE

(Ginkgo Biloba Extract)

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Q.C. Manager: Heyi

Inspector: 02
Memorandum

TO: Bart Heldreth, Ph.D.
    Executive Director - Cosmetic Ingredient Review (CIR)

FROM: Carol Eisenmann, Ph.D.
       Personal Care Products Council

DATE: January 2, 2018

SUBJECT: Ginkgo Biloba Meristem Cell

The supplier stated the following: “When we compared the chemical production pattern of Ginkgo Biloba Meristem Cell with general Ginkgo Biloba Extract (GBE), there was a distinct difference in these two ingredients in terms of chemical compositions. Normally the constituents of GBE are bilobalide, ginkgolide A, B, C, etc., but major compounds produced in Ginkgo Biloba Meristem Cell are catechin and gallocatechin and bilobalide; ginkgolide A, B, C, etc. were not detected. Thus, we can say Ginkgo Biloba Meristem Cell is distinct from GBE.” A chromatogram is attached.


GINKGO BILOBA MERISTEM CELL
METHOD OF MANUFACTURING

1. Sterilization of plant tissue
   - Plant tissue containing cambium is taken from Ginkgo Biloba plant and is sterilized

2. Isolation of cambial meristem cells
   - Cambial meristem cells are obtained from the cambium of the sterilized plant tissue of Ginkgo Biloba

3. Cambial meristem cell culture
   - Isolated cells are placed on the media and are cultured for proliferation in petri-dish, flasks, bioreactor then scaled up to tanks

4. Secondary metabolites production in cultured cambial meristem cells through specific culture condition
   - Specific culture condition is given to cultured cambial meristem cells to produce various secondary metabolites

5. Cell separation
   - Cultured cambial meristem cells are harvested with filter-press
GINKGO BILoba MERistem CELL
TOXICITY TESTING

1. SINGLE DOSE ORAL TOXICITY TEST ON RAT

This test was conducted to evaluate toxicity and determine lethal dose of the test sample, *ginkgo biloba* meristem cell, via conducting a single dose oral administration on 6-week old male and female Sprague-Dawley rats.

0 (control group, water for injection) and 2,000 mg/kg of the test sample was orally administered to 5 male and female rats in each group. 14 days after administration, general symptom and weight were studied. At the end of the observation period, euthanasia was administered for autopsy.

No death was observed in the 2,000 mg/kg administration group. Also, no treatment-effect of the test sample was observed concerning general symptom, weight, and autopsy.

As a result of conducting a single dose oral administration of *ginkgo biloba* meristem cell, it is determined that estimated lethal dose would surpass 2,000mg/kg for both male and female group.

2. SINGLE DOSE ORAL VOLUME INCREASE TOXICITY TEST ON BEAGLE (DOG BREED)

This test was conducted to evaluate both qualitative and quantitative toxic response per single oral administration of the test sample, *ginkgo biloba* meristem cell, to 2 male and female beagle dog breeds in a volume increase of 500→250→1,000 mg/kg for 4 days.

No death was observed during the test period, but all animals in male and female groups vomited after being administered with 500 and 1,000 mg/kg of the test sample. After the 250 mg/kg administration, 1 animal vomited the test sample, but it was too slight a symptom to confirm the treatment-effect of the test sample. Apart from this, no change was observed in weight and autopsy after the administration of test sample.

As a result, vomiting was observed after administering 500 and 1,000 mg/kg of the test sample to male and female groups under this test condition. But treatment-effect of the test sample was not confirmed in the 250 mg/kg administration. Thus it is determined that the estimated maximum tolerated dose (MTD) would surpass 1,000 mg/kg.
3. REVERSE MUTATION TEST

The histidine requisite salmonella (TA98, TA100, TA1535 and TA1537) and the tryptophan requisite colon bacterium (WP2uvrA(pKM101)) were used to examine the mutagenicity of test sample (ginkgo biloba meristem cell) with and without metabolic activation.

As a result of conducting a dosage-range finding study, growth/development arrest due to test sample confirmed negative in all strain capacities, irrespective of metabolic activation. Sedimentation of the test sample was observed in volumes over 128 µg/plate, irrespective of metabolic activation. Accordingly, 5000 µg/plate was determined as the maximum volume for this test and 5 capacity levels, including maximum capacity, were established for treatment group. Also, negative and positive controls were established.

As a result of this study, the number of reverse mutation colony in the treatment group did not show volume increase with or without metabolic activation and it did not show more than a two fold increase of the negative control group. Growth/development arrest due to test sample was not observed in any strain capacity. However, sedimentation due to test sample was observed in capacity levels over 313 µg/plate. On the other hand, the number increase of reverse mutation colony was confirmed in each positive control strain compared to that of negative control.

As a result, the mutagenicity of test sample, ginkgo biloba meristem cell, confirmed negative under this test condition.

4. MICRONUCLEI TEST ON MOUSE

This test was conducted to study the influence of ginkgo biloba meristem cell on the micronuclei induction of marrow cell in mouse.

In order to determine maximum capacity and processing time of the test sample, a test for capacity-determination and for test sample processing time was conducted. As a result of conducting the capacity determination study, no general symptom or death due to the test sample was observed in any male mouse. To determine processing time of the test sample, 2,000 mg/kg of test sample was administered to male mice to create bone marrow aspiration per 24, 48, and 72 hours. No frequency increase of micronuclei was observed in all time periods, thus 24 hours—as this time period is commonly used—was determined as general time for myelocyte collection. In this experiment, bone marrow aspiration was created 24 hours after administering 500, 1,000, and 2,000 mg/kg of test sample to negative and positive control groups.

As a result, in all treatment groups, no significant statistical increase of micronuclei frequency in polychromatophilic erythrocytes was observed in all capacity levels compared to negative control. Also, no treatment group showed a significant difference in the ratio of polychromatophilic erythrocytes in total red blood cells compared to negative control.
On the other hand, a notable increase in the frequency of micronuclei in polychromatic erythrocytes was observed in positive control compared to negative control, but no significant difference in the ratio of polychromatic erythrocytes in total red blood cells was observed compared to negative control.

The above results indicate that *ginkgo biloba* meristem cell does not induce micronuclei of myelocytes in mouse under given test condition.

5. CHROMOSOME ABERRATION STUDY USING MAMMalian CULTURED CELL

In order to examine chromosomal aberrations of *ginkgo biloba* meristem cell, a chromosome aberration test was conducted using Chinese Hamster Lung (CHL/1U) cultured cells.

The cell growth inhibition test confirmed cell toxicity in short-time treatment with and without metabolic activation, and in 24-hour continuous treatment without metabolic activation. Thus IC₅₀ value for the short-time treatment without metabolic activation was 210.0 μg/mL, 333.6 μg/mL for the short-time treatment with metabolic activation, and 202.2 μg/mL for the 24-hour continuous treatment without metabolic activation.

Accordingly, the maximum capacity determined for short-time treatment without metabolic activation and for 24-hour continuous treatment without metabolic activation was 210.0 μg/mL, and 340 μg/mL for short-time treatment with metabolic activation. Thus 4 capacity levels were established for treatment group. Also, negative and positive controls were set up.

After producing sample slides, 200 metaphase cells were observed in maximum capacities of both short-time treatment and continuous treatment. Therefore, 3 slides per capacity level (including maximum capacity) were studied for both short-time treatment and continuous treatment.

As a result, sedimentation of test sample was confirmed for all capacities in short-time treatment with and without metabolic activation and in 24-hour continuous treatment without metabolic activation. Also, the frequency of cells with structural and/or numerical aberration was less than 5% in short-time treatment with and without metabolic activation and in 24-hour continuous treatment without metabolic activation. Thus no chromosomal aberration was confirmed nor significant statistical increase observed.

On the other hand, a notable statistical increase in the frequency of cells with structural aberration was observed in positive control compared to negative control.

The results acquired from the given test conditions indicate that *ginkgo biloba* meristem cell do not induce chromosomal aberration in the short-time treatment with and without metabolic activation and in the 24-hour continuous treatment without metabolic activation concerning Chinese Hamster Lung (CHL/1U) cultured cells.
6. 4-WEEK REPEATED DOSE ORAL TOXICITY TEST ON RAT

This test was conducted to evaluate toxicity after 4-week repeated dose oral administration of *ginkgo biloba* meristem cell in 3 capacities—500, 1,000, and 2,000 mg/kg—to male and female Sprague-Dawley rats and use this data as a foundation of determining capacity for 13-week repeated oral toxicity test. The control group was injected with diluting agent.

No death or abnormal symptom was observed in all male and female treatment groups.

In male and female treatment groups, no change in weight and/or feed intake was observed. Also, no treatment-effect was observed in hematological examination/biochemical examination of blood or in organ weight. After performing autopsy, no treatment-effect was observed in male and female treatment group. And histopathological examination demonstrated no test substance-effect in the 2,000 mg/kg administration group.

After conducting a 4-week repeated dose oral administration of *ginkgo biloba* meristem cell to determine capacity, no treatment-effect was observed in male and female treatment groups. Accordingly, considering the total amount of administration in this 13-week repeated dose toxicity test, the administration amount for high-dose group was determined 1,000 mg/kg, and 250 mg/kg for low-dose group. The total amount of administration was determined 20mL, which ought to be divided in two parts for daily administration.

7. 13-WEEK REPEATED DOSE ORAL TOXICITY TEST ON RAT

This test was conducted to assess safety concerning repeated dose administration of *ginkgo biloba* meristem cell. 6-week old male and female Sprague-Dawley rats were administered with test substance for 13 weeks, 10 rats per each capacity of 250, 500, and 1,000 mg/kg.

During the observational period, various tests were conducted for general symptom, weight and feed intake, ophthalmology, and urinalysis. After performing autopsy, hematological examination/biochemical examination of blood, organ weight measurement, microscopy, and histopathology were conducted.

No death or abnormal symptom was observed in all male and female treatment groups. Also, no change due to treatment was observed in all male and female treatment groups concerning weight, feed intake, ophthalmology, urinalysis, hematological examination/biochemical examination of blood, and autopsy. As a result of conducting histopathological study, no treatment-effect was observed in the male and female 1,000 mg/kg administration group.

Following the result of 13-week repeated dose oral administration of *ginkgo biloba* meristem cell, NOAEL for both male and female is expected to exceed 1,000 mg/kg.
Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review (CIR)

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: January 16, 2018

SUBJECT: Ginkgo Biloba Leaf Extract

Anonymous. 2018. Absorption spectra of Ginkgo Biloba Leaf Extract (Ginkgo Extract BG (ethanol-water extract evaporated and resolved in 50% Butylene Glycol) as described in Council memo 5 provided November 13, 2017).
Absorption spectra of Ginkgo Biloba Leaf Extract (Ginkgo Extract BG (ethanol-water extract evaporated and resolved in 50% Butylene Glycol) as described in Council memo 5 provided November 13, 2017)

Detected UV peak

The maximum UV absorption peak was not observed in the range of 280 nm to 450 nm in Ginkgo Extract BG.
Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review (CIR)

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: January 23, 2018

SUBJECT: Ginkgo Biloba Leaf Extract

### Summaries of Studies on Products Containing Ginkgo Biloba Leaf Extract

<table>
<thead>
<tr>
<th>Study Type</th>
<th>Test Sample</th>
<th>Test Condition</th>
<th>Test Dates</th>
<th>Completed Subjects #</th>
<th>Conclusion</th>
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<td>HRIPT</td>
<td>cream containing 0.0085% of the extract</td>
<td>neat, occlusive patch</td>
<td>6/10-7/18/2002</td>
<td>48</td>
<td>no dermal irritation, or dermal sensitization potential</td>
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<td>Phototox and photoallergy</td>
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<td>neat, semi-occlusive patch</td>
<td>7/26-9/3/2010</td>
<td>29</td>
<td>no phototox, or photoallergy potential</td>
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<tr>
<td>EpiOcular</td>
<td>eye product containing 0.0013% of the extract</td>
<td>neat</td>
<td>8/15/2012</td>
<td>not applicable</td>
<td>ET&lt;sub&gt;30&lt;/sub&gt; &gt; 24 hours, no eye irritation potential</td>
</tr>
</tbody>
</table>
Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review (CIR)

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

DATE: November 28, 2017

SUBJECT: Draft Tentative Report: Safety Assessment of Ginkgo biloba-Derived Ingredients as Used in Cosmetics (draft prepared for the December 4-5, 2017 CIR Expert Panel Meeting)

Key Issues
Although there is a section on pharmacology, the focus of the Heinonen and Gaus (2015) review is safety. This review cites many primary references. Rather than reviewing many references in the CIR report, some key conclusions of the review should be mentioned in the CIR report and the reference should be provided so that the reader of the CIR report can go to the review for more details (especially about the safety conclusions for the numerous clinical studies).

As most of the studies in the CIR report are either on the standardized leaf extract EGB 761, or the extract tested by the NTP. The composition of these two extracts should be clearly stated in the CIR report. The following statement in the memo, Introduction and the Summary is not correct for the majority of the studies currently in the report: “...the test article is a vaguely and variably described extract of Ginkgo biloba leaves...”

Statements from the American Herbal Products Association (AHPA) regarding how well the extract tested by NTP represents the extract on the market should be added to the CIR report.

The Introduction states: “CIR is not reviewing the potential toxicity of the individual constituents, except wherein such constituents are also ingredients under review.” One INCI name Ginkgo Biflavones is defined as “a mixture of biflavones derived from the leaves of Ginkgo biloba. It consists predominantly of sciaqopitysin, bilobetin, ginkgetin, and isoginkgetin.” The search strategy does not indicate that these named components were used as search terms.

Additional Considerations
Search strategy - The meaning of the checks, “X”, “NA” in the search strategy table are not clear.
Non-Cosmetic - To provide perspective to cosmetic use, the typical oral dose of ginkgo leaf extract should be stated.

ADME, Summary - What compounds were labeled in the rat oral ADME study (references 32, 34)? The introduction of the NTP bioassay says that it was mainly flavonol glycosides.

Acute, Chronic - It is not appropriate to cite the acute studies and the 27 week rat study to NTP (reference 32). NTP cites these studies to: Salvador, R.L. (1995). Herbal medicine - ginkgo. *Can. Pharmacists J.* 52, 39-41. This reference appears to be a review. What was the extract used in these studies? Citing these studies to NTP may lead the reader to think that the extract used in the NTP studies was also used in these studies.

Carcinogenicity, Summary - The basis for the IARC conclusion should be stated. The human data concerning carcinogenicity should be mentioned in the CIR report.

Other Clinical Reports - What was the duration of the clinical study of the anti-aging formulation containing 1.5% ginkgo leaf extract?

Table 2 - As the German specifications indicate that ginkgolic acids must be <5 ppm this table does not appear to represent the full concentration range of constituents. It should be made clear that the units for 10.45 ginkgolic acids is ppm not %.
AHPA comments on Cosmetic Ingredient Review Draft Safety Assessment of *Ginkgo biloba*-Derived Ingredients as Used in Cosmetics

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Comments on Composition and Impurities ....................................................................................... 3
Comments on Main body ..................................................................................................................... 4
Comments on Summary ..................................................................................................................... 5
Conclusion ............................................................................................................................................ 6
Introduction and summary
AHPA appreciates your work and the opportunity to participate at the meeting this week and comment on the draft Safety Assessment of Ginkgo biloba-Derived Ingredients as Used in Cosmetics.

To improve the precision of the draft, AHPA suggests that the early sections in the Chemistry segment of the draft (Methods of Manufacturing and Composition/Impurities) be adjusted to reflect the use of the term GBE as used in the remainder of the report, where it refers to a wide variety of distinct Ginkgo biloba leaf extracts. Minor changes are suggested for the Main body and the closing Summary to clarify when single, or multiple distinct GBEs are being discussed.

AHPA proposes these revisions:

Comments on Methods of Manufacturing
Under Methods of Manufacturing AHPA suggests revising the second paragraph and caption for Scheme 1:

GE may be full extracts or standardized extracts. Full extracts are prepared with alcohol and contain all constituents soluble in alcohol. Standardized extracts (referred to as EGB 761 in published literature) are more common, especially in herbal supplements, and are prepared in a manufacturer-dependent multi-step processes in which some compounds, such as flavonoids and lactones, are enriched while others, such as ginkgolic acids, are removed (Scheme 1). Standardized extracts are reported to contain 6% terpene triactones, 24% flavonol glycosides, and less than 5 ppm ginkgolic acids.

Scheme 1. General manufacturing process of a standardized Ginkgo biloba

AHPA proposes removing the text mentioning EGB 761 (a specific standardized Ginkgo biloba extract marketed in the U.S. exclusively as a dietary supplement ingredient) from this paragraph and adjusting the narrow description of a standardized extract to be wider so that it can cover all ingredient discussed in the draft report, which contains references to a variety of standardized GBE extracts that are different from the specific material used as an example in this Methods paragraph.

AHPA also recommends removing the text discussing the composition of a standardized extract from this paragraph as the specific GBE used in reference 32, the National Toxicology Program (NTP) study, does not meet the definition provided here. The test material used from Shanghai Xing Ling Science and Technology Pharmaceutical Company (Shanghai, China) (Shanghai Xing Ling) was reported to contain 15.4% terpene bi- and triactones, 31% flavonol glycosides, and over 10 ppm ginkgolic acids, which is quite different from this description (and even other specific leaf extracts that
follow the established pharmacopeial standards for *Ginkgo biloba* leaf extract material, see below). AHPA conducted a survey of its members and was not able to identify a case where the specific material studied by NTP was in use in the U.S. dietary supplement industry.

To illustrate the difference between the NTP's Shanghai Xing Ling leaf extract and established pharmacopoeial standards currently mentioned in Composition/Impurities, comparison of the composition of the NTP test article and the international standards may be helpful. This table summarizes differences between this NTP test article and these established standards and AHPA encourages you to use the information as needed:

<table>
<thead>
<tr>
<th></th>
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<tr>
<td>Health Canada¹</td>
<td>22-27%</td>
<td>-</td>
<td>-</td>
<td>5-7%</td>
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<tr>
<td>German Commission E²</td>
<td>22-27%</td>
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<td>American Herbal Pharmacopoeia³</td>
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<td>-</td>
<td>5-7%</td>
<td>-</td>
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<td>European Pharmacopoeia⁴</td>
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<td>6.94%</td>
<td>8.42%</td>
<td>15.4%</td>
<td>10.45 ±2.40</td>
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</tbody>
</table>


**Comments on Composition and Impurities**

AHPA suggests revision to the fifth paragraph of Composition/Impurities:

Ginkgolic acid is a salicylic acid derivative with a C₁₅ side chain that is related to the pentadecylcatechols (i.e. urushiol) found in poison ivy.⁸ One analysis found crude aqueous extracts of GBE contained up to a total of 30 ppm urushiois while the process described in Scheme 1 to produce a particular standardized GBE removed long chain alkylphenols to below detection levels.⁵ Other extraction processes not in use in the US dietary supplement market have been seen to
result in a specific standardized extract material containing 10.45 ppm urushiols.\textsuperscript{32}

Comments on Main body
With the above changes, the use of the term GBE in the main body of the review is nearly consistent. AHPA suggests references to specific GBEs being studied be made slightly clearer by using language such as “a GBE” or “a particular standardized leaf extract of GBE” or “the studied GBE” or something similar in cases where research focused on a specific test material and a plural such as “specific GBEs” when more than one is being discussed.

For example, under the Toxicological Studies section language like the following might better represent the variety of materials under research:

**Acute Toxicity Studies**

*Animal Oral*

The LD\textsubscript{50} of a specific standardized leaf extract of GBE administered orally to mice was reported to be 7.73 g/kg.\textsuperscript{32}

It is essential to note that this specific ingredient used in the NTP study, an extract from Shanghai Xing Ling, did not meet established pharmacopoeial standards for the levels of constituents in Ginkgo biloba leaf extracts, and as a result it is important to distinguish this ingredient and the conclusions drawn from its use from other distinct ingredients that do meet established international standards. With respect to this particular reference from the National Toxicology Program and its use within the CIR draft, AHPA provided comments on the draft of the NTP report (attached) which may be relevant when discussing the specific ingredient studied therein in the context of the safety of other distinct Ginkgo biloba extracts.

AHPA proposes that throughout the sections referring to the research on this Shanghai Xing Ling material, it be made abundantly clear that the results apply to “a specific GBE” with language like “the test material studied” or “a particular GBE” to ensure clarity for the reader.

From AHPA’s review of the International Agency for Research on Cancer (IARC), while the discussion covered a variety of Ginkgo biloba extracts, conclusions depended directly on the unique results of NTP’s study of its particular test material from Shanghai Xing Ling. AHPA suggests revision of the last paragraph in the Carcinogenicity, Oral section as follows to make more evident the source of the information used in IARC’s decision-making process:

The International Agency for Research on Cancer (IARC), based on the National Toxicology Program’s research on a specific GBE, has determined that GBE is
possibly carcinogenic to humans (group 2B) based on inadequate human carcinogenicity evidence and sufficient evidence in experimental animals.

Comments on Summary
AHPA suggests that the plural be used in referring to extracts except in cases where a specific extract is being considered, and in these use text such as “a particular GBE” or language to that effect. This would retain the tone of the text with respect to the variety of GBE ingredients that exist without implying that GBEs with different compositional characteristics and manufacturing standards are equivalent to each other.

Here is a proposed revision of the Summary section starting with paragraph 3 of the draft, making GBE plural in cases where the text refers to many distinct extracts and adding notation when a particular one is under consideration:

**GBEs** is a re used extensively as an herbal supplement for anti-inflammatory, cognitive-promoting, antioxidant, and vascular effects and is an approved herbal medicine in Germany for use for treatment of memory deficits, dementia, and other organic brain syndromes when extracted with acetone/water. **GBEs** may interact with pharmaceutical drugs. Nuts from *Ginkgo biloba* are consumed as a delicacy in Japan and China and are used in traditional Chinese medicine. Anecdotal accounts report that consumption of the nuts may have acute adverse effects. In general, toxicokinetics data are not expected to be found on botanical ingredient because each botanical ingredient is a complex mixture of hundreds of constituents. However, there have been many pharmacokinetics studies on **GBEs**, specifically on some of the key constituents, which indicate **GBEs** may be well absorbed after oral administration. The GBE constituent, quercetin, was found to penetrate human dermatomed skin. In an oral ADME study in rats, at least 60% of a radiolabeled GBE were absorbed, with the main site of absorption likely in the upper gastrointestinal tract. Radioactivity was measured in exhalation and elimination products. The LD50 of a particular standardized GBE administered orally to mice was reported to be 7.73 g/kg, and the LD90 after intravenous administration of this specific standardized GBE was 1.1 g/kg for both rats and mice. In 3-month studies of a specific GBE at up to 2000 mg/kg/day, increased liver weights, decreased kidney weights, increased incidences of hepatocytic hypertrophy and focal hepatocytic necrosis, and increased incidences hyaline droplet accumulation, atrophy and pigment accumulation in macrophages in the olfactory epithelium were observed in mice. In a similar study of the same GBE test material in rats, increased liver weights, increased incidences of hepatocytic hypertrophy, increased incidences of thyroid gland follicular cell hypertrophy, and increased incidences of pigmentation in the olfactory epithelium of the nose were observed. There was no evidence of organ damage or impairment of hepatic or renal function when a specific GBE was administered orally over 27 weeks to rats and mice at doses ranging from 100 to 1600 mg/kg. In an oral DART study of a standardized GBE in mice, the NOEL for dams and fetuses was greater than 1225 mg/kg/day. No clinical signs of toxicity were observed in the dams and no embryotoxic effects were observed in the fetuses. In another oral DART study in mice, a standardized GBE at 14.8 mg/kg/day produced adverse effects on the estrous cycle, fertility, abortifacient, reproductive performance, and hormone level in female mice and may cause adverse effects on ovarian function as an antifertility agent.

**A GBE** at up to 10,000 μg/plate was mutagenic in an Ames test. **A GBE** (0.2 - 1.2 mg/ml) was mutagenic in mouse L5178Y cells. In a mouse micronucleus test of **a GBE** up to 2000 mg/kg/day, no increase in the frequency of micronucleated erythrocytes was observed in male mice, but the results were deemed equivocal in female mice. **A GBE** at up to 2000 mg/kg/day was not genotoxic.
in a reporter gene mutation assay, a combined liver comet assay, and bone marrow micronucleus assay in mice.

In carcinogenicity studies of rats and mice conducted by the NTP, lesions in the liver, thyroid gland and nose were observed in all GBE dose groups (200 - 2000 mg/kg/day). Lesions included hypertrophy in the liver and thyroid gland in rats and mice, liver hyperplasia in male and female rats, and hyperplasia and atrophy of the epithelium in the nose of male and female rats. Inflammation, hyperplasia, hyperkeratosis, and ulcer were also observed in the forestomach of male and female mice. Additionally, increased incidences of cancers of the thyroid gland were observed in male and female rats and male mice, as were liver cancers in male and female mice. IARC has determined that GBEs are possibly carcinogenic to humans (group 2B).

In a PLNA validation study, a GBE exposure yielded statistically significant lymphoproliferative reactions in the ipsilateral popliteal lymph node, which was may have been caused by ginkgolic acid.

Reports of contact dermatitis have been reported following exposure to the fruit pulp of Ginkgo biloba. Patients have reported erythematous reactions and generalized exanthematous pustulosis following ingestion of certain GBE supplements.

No adverse effects were reported in clinical studies of cosmetic formulations containing up to 1.5% GBEs. In anecdotal accounts from Chinese medicine, consumption of fresh Ginkgo biloba nuts may cause stomachache, nausea, diarrhea, convulsions, weak pulse, restlessness, difficulty breathing, and shock. Death has been reported in children following consumption of fresh nuts. No dermal or ocular irritation and no dermal sensitization studies were found in the published literature.

Conclusion
The above suggested changes should improve the ability of the review to most effectively communicate the state of safety research on Ginkgo biloba across the variety of forms and ingredients studied. AHPA is glad for the opportunity to participate in the Cosmetic Ingredient Review development process for these Ginkgo biloba herbal ingredients and looks forward to continuing to share information and expertise.
Draft NTP TR 578: Analysis of the specific Ginkgo biloba extract used in 2-year gavage studies
Prepared by the American Herbal Products Association (AHPA)
January 31, 2012

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Re: the NTP's particular GBE is a bit different than those described in the front matter of the GBE draft
Introduction and summary
The “Peer Review Draft” of NTP TR 578 (hereinafter Draft TR 578) identifies the test article that was used in the 2-year rodent gavage studies that are the subject of Draft TR 578 as “Ginkgo biloba extract from leaves.” Draft TR 578 further reports that two lots of the material were obtained from a company identified as Shanghai Xing Ling Science and Technology Pharmaceutical Company, Ltd. (hereinafter Shanghai Xing Ling); that just one of these lots, identified as Lot 020703 was used as the test article in the 2-year studies; that analyses of the test article were conducted by an analytical lab for identity, purity, stability, and moisture; and that confirming analysis for identity of the test article was conducted by another analytical lab.

This introduction provides a summary of a review undertaken by staff of the American Herbal Products Association (AHPA) in communication with several marketers of extracts of Ginkgo biloba leaf. Background and substantiating information for this summary are provided elsewhere in this AHPA review.

The test article is not similar to commonly marketed Ginkgo biloba leaf extracts. Draft TR 578 provides quantititative data from the above described analyses of the test article. Draft TR 578 also states that the test article is “similar to” a ginkgo leaf extract marketed by Dr. Willmar Schwabe GmbH & Co. as EGB 761® and that the levels of certain constituents of the test article “have a similar ratio” of these constituents as is found in EGB 761®. Draft TR 578 also states that the levels of these constituents of the test article “reflect concentrations measured in commercially available [ginkgo] products” in the U.S.

In fact, the test article used in the described 2-year gavage studies is dissimilar to EGB 761®. NTP’s analysis of the test article measured flavonol glycosides at 31.2%, terpene lactones at 15.4%, 2 and ginkgolic acids at 10.45 ±2.40 ppm. 3, 4 By comparison, EGB 761® is standardized to contain 24% flavonol glycosides and 6% terpene lactones and is manufactured to ensure that ginkgolic acids are present at no more than 5 ppm. It is thus quite clear that the test article is dissimilar to EGB 761® with regard to the levels of these compounds present.

And Draft TR 578 provides no quantitative information on other constituents or classes of compounds that may or may not be present in the test article other than the 46.6% represented by flavonol glycosides and terpene lactones. There

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1 The common name for Ginkgo biloba is ginkgo and the two terms are used interchangeably throughout this document.
2 AHPA notes that the described quantitative assay of the terpene lactones bilobalide and ginkgolides were conducted after strong acidic hydrolysis. This is unusual as terpene lactones may be cleaved under such conditions. The chromatogram of the sample (page 1-6) shows strong baseline noise as high as the signals of ginkgolide B and C. It is questionable how an extract quantitation was feasible under such conditions and there is some possibility that measurement of these compounds has been understated.
3 This mean with stated standard deviation represents three separate results for the analysis of ginkgolic acid in the test article. The three data points were reported by the contracted analytical lab to be 8.975, 9.152, and 13.223 ppm.
4 Draft TR 578 states on page 1-3, “HPLC/MS analyses for the presence of ginkgolic acids I, II, and III ... resulted in no observable peaks of ginkgolic acids ....” This is apparently an error and should be corrected.
is thus no data presented on which to base a conclusion that this other 46.6% of the test article is “similar to” the other 70% of EGB 761®.

In addition, it is not accurate to identify the levels of flavonol glycosides and terpene lactones present in the test article as “reflect[ing] concentrations measured in commercially available [ginkgo] products” in the U.S. As Draft TR 578 points out in its Table 1, there is significant variation in ginkgo leaf extracts in the U.S. marketplace. The test article is just one of many of such variations, and compared to analysis of 50 other ginkgo leaf extract products identified in published studies, contains the highest level of terpene lactones (over 35% higher than the next highest sample) and the fourth highest level of flavonol glycosides. Draft TR 578 should refrain from making any statements that implies that the specific and unique Ginkgo biloba leaf extract used as the test article is in any manner representative of other ginkgo leaf extracts.

Any eventual final version of NTP TR 578 should therefore be revised, both in the title of the final draft and throughout the text, to clearly state that these 2-year gavage studies were conducted with a test article that is specific and unique, and that is dissimilar to commercially marketed ginkgo leaf extracts. Any eventual final version of NTP TR 578 should also state that the conclusions drawn from these 2-year gavage studies have not been shown to be relevant to any other ginkgo leaf extract.

There is no market data to support that the test article is sold in the U.S. Draft TR 578 also states that NTP had been informed that the Ginkgo biloba leaf extract produced by Shanghai Xing Ling was “widely distributed in commerce.” Draft TR 578 lists “personal communication” as the citation for this information but provides no other details as to the nature of this personal communication.

AHPA was not aware at the time that Draft TR 578 was issued in December 2011 of the presence in the U.S. marketplace of Shanghai Xing Ling as a marketer of a ginkgo leaf extract or any other herbal extract. AHPA has since found that this company holds four U.S. patents on a proprietary ginkgo leaf extract that is significantly dissimilar to generally and commercially available ginkgo leaf extracts in the U.S.⁵, and that the company intended about a decade ago to seek approval of this proprietary extract as a drug in the U.S.⁶ AHPA contacted Shanghai Xing Ling through a Chinese speaking representative of one AHPA member; this representative was informed that Shanghai Xing Ling does not sell or market its own products that contain its proprietary ginkgo leaf extract in the United States. In addition, AHPA has never found this proprietary ginkgo leaf extract offered for sale in the U.S. as an ingredient for use in ginkgo products and is not aware of any ingredient supplier who offers for sale any Ginkgo biloba leaf extract that is standardized to contain more than 24% flavonol glycosides and 6% terpene lactones. Any eventual final version of NTP TR 578 should

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⁵ U.S. Patents 06030621, 06187314, 06475534, and 06632460.
therefore be revised to remove any statements to the effect that the test material is widely distributed in commerce, and in fact should state that the ingredient is apparently not in commerce in the United States.

**Significant revisions should be made to Draft TR 578.** An addendum to this report provides specific suggestions for revisions to the title and text of Draft TR 578 to reflect the following facts with regard to the *Ginkgo biloba* leaf extract that served as the test article in the 2-year gavage studies that are its subject:

- That the test article is a unique and specific extract of *Ginkgo biloba* leaf.
- That the test article is dissimilar to EGB 761®; in fact, it is AHPA’s view that NTP TR 578 should limit its mention of this specific brand to the single report of NTP’s original intention to use this brand in these 2-year studies, and perhaps to its description in Table 1 therein.
- That the test article is dissimilar to most other commonly available *Ginkgo biloba* leaf extracts available in the U.S. market.
- That the test article is not known to be sold in the United States.
- That the conclusions presented in Draft TR 578 are not applicable to other *Ginkgo biloba* leaf extracts.

**Consideration should be given to other factors.** Consideration should be given to other possible explanations for and issues related to the study results reported in Draft TR 578 even as such results apply to the specific *Ginkgo biloba* leaf extract that served as the test article. These include the following at a minimum; each of these are discussed below, except that the first listed topic is discussed in a review of Draft TR 578 prepared for AHPA by Intertek Cantox and dated January 25, 2012.

- Absence of any information about analysis of the test article for residues of heavy metals, mycotoxins, microbiology, polyaromatic hydrocarbons, or pesticides that may have been present.
- The very high level of ginkgolic acids in the test article (10.45 ±2.40 ppm compared to an established standard of 5 ppm).
- Selection of corn oil as the vehicle for gavage administration.
- Various nomenclatural issues, including the use of a CAS number and botanical names to describe the specific tested ginkgo leaf extract.
- Absence of sufficient information to describe how the specific test article was manufactured.

**Identity of the specific *Ginkgo biloba* leaf extract test article**
The abstract of draft TR 578 provided the results of analysis of the test article provided by Shanghai Xing Ling. The test article is characterized as containing 31.2% flavonol glycosides, 15.4% terpene lactones, and 10.45 ±2.40 ppm ginkgolic acid.

Shanghai Xing Ling has intentionally developed a unique *Ginkgo biloba* leaf extract that is intended to be dissimilar to other ginkgo leaf extracts. This extract or an apparently similar *Ginkgo biloba* leaf extract manufactured by Shanghai
Xing Ling has been described “as a new multicomponent drug” in the scientific literature.\(^7\)\(^8\) The company holds four U.S. patents, dated between 2000 and 2003, for a ginkgo leaf extract.\(^9\) The most recent of these patents\(^{10}\) includes several statements that are relevant to any evaluation as to whether ginkgo leaf extracts provided by this supplier are similar or dissimilar to other ginkgo leaf extracts sold in the U.S. For example, this patent states (emphasis added throughout):

- That one object of the company’s invention of a specific and proprietary ginkgo leaf extract is “to provide a Ginkgo biloba extract with a highly concentrated effective content, that include 44 to 78% flavonoids [later described as having a content of “about 20% to about 75% flavonol glycosides], 2.5 to 10% ginkgolides and 2.5 to 10% bilobalide.” Note that the described extract could consist, at the high end of each of the stated ranges, of as much as 95% of a combination of flavonol glycosides and terpene lactones (i.e., the ginkgolides and bilobalide), leaving only 5% for those constituents that make up 70% of the Schwabe ginkgo leaf extract, EGB 761\(^\circ\).

- States, “Until now it has not been possible to prepare such highly concentrated extracts from Ginkgo biloba leaves.”

- Identifies an “advantage of a Ginkgo biloba extract with highly concentrated effective content” as “the reduced daily dosage and smaller size of the pharmaceutical prepared from it.”

- Claims another “advantage of a Ginkgo biloba extract with highly concentrated effective content” to be “further removal of inactive substances,” apparently meaning removal of any constituents other than the flavonoids or terpene lactones.

- Notes that the patent “relates generally to compositions extracted from Ginkgo biloba leaves and particularly to a different composition comprising new active components and combinations.”

In summary, Shanghai Xing Ling clearly produces a different, unique, and proprietary Ginkgo biloba leaf extract. The ginkgo leaf extract described in its U.S. patents is novel and “until now ... has not been possible” to produce; contains “more highly concentrated” levels of flavonol glycosides than other such extracts; seeks “further removal” of any other constituents naturally found in ginkgo leaf; is of a “different composition” than other ginkgo leaf extracts; and allows for a “reduced daily dosage.”

This patent also states that Shanghai Xing Ling’s proprietary ginkgo leaf extract is manufactured to contain “about 0.1 ppm to 5 ppm ginkgolic acids,” though at

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\(^9\) U.S. Patents 06030621, 06187314, 06475534, and 06632460.

other times the patent identifies the intended level of ginkgolic acids to be “about 0.1 ppm to 0.5 ppm.”

AHPA cannot say with any certainty whether the Ginkgo biloba leaf extract provided by Shanghai Xing Ling to NTP as the test article for the 2-year gavage studies that are the subject of Draft TR 578 is or is not the same article as is described in the company’s U.S. patents. AHPA notes however that the test article’s analysis conforms to the patent with regard to the levels of flavonol glycosides (present at 31.2%, within the patents’ range of 20-75%); bilobalide (6.94%, in the patents’ range of 2.5-10%); and ginkgolides (8.42%, again in the stated 2.5-10% range of the patented ingredient). In fact the only measured parameter described in the patent that is not met in the test article is the level of ginkgolic acids, which was recorded as present at 10.45 ±2.40 ppm, and so considerably higher than described in the patent.

Of additional interest is that Shanghai Xing Ling reportedly signed an agreement with a contract research organization to conduct clinical trials on its patented ginkgo leaf extract with a goal of obtaining FDA drug approval for the treatment of stable angina.11

It is apparent that the Ginkgo biloba leaf extract used as the test article in NTP’s 2-year gavage studies is certainly a different ingredient than EGb 761®. In addition, it cannot be characterized as representative of any other ginkgo leaf extract. It is also probable that the manufacturer intended, through its proprietary manufacturing process, to create a unique ingredient that is unlike other Ginkgo biloba leaf extracts. The results of these 2-year studies should therefore not be assumed to be readily extrapolated to any other ginkgo leaf extract and any revision to Draft TR 578 should refrain from making any statements that expressly or implicitly associate the test article with EGb 761® or any other Ginkgo biloba leaf extract.

Ginkgo biloba leaf extracts in the U.S. market
Extracts of ginkgo leaf are broadly sold in the U.S. as dietary supplements. While products that contain EGb 761® are present in the U.S. market, as Draft TR 578 notes (citing Kressmann, 2002), there is great variety among the numerous ginkgo leaf extracts in the U.S. marketplace.

It is absolutely certain that the test article was not EGb 761® and its chemical profile is significantly different than that of this branded Schwabe product. Draft TR 578 should be revised to remove any statement to the effect that the test article is in any way similar to EGb 761®. Except for the fact that both are derived from the leaf of the Ginkgo biloba tree, there is nothing to associate these two dissimilar ingredients.

It is also clear that the specific ginkgo leaf extract used as the test article cannot be represented as the same as any other marketed ginkgo leaf extract. The

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Kressmann (2002) article cited in Draft TR 578 shows a very broad range in the levels of flavonol glycosides (from 23.88 ±0.21 to 35.54 ±1.03%), terpene lactones (from 3.87 ±1.09 to 11.31 ±0.17%), and ginkgolic acids (from <500 ppm (the study’s limit of quantification) to 89576 ±2297 ppm) in 26 analyzed Ginkgo biloba leaf extract products marketed in the U.S. Only three of these tested products were reported to contain more than 31.2% flavonol glycosides, the amount found in the test article; none contained as high a level of terpene lactones as the 15.4% reportedly in the test article. A similar product review conducted at about the same time measured a range of from 0.4 to 26.2% flavonol glycosides and 0.6 to 8.2% terpene lactones in 14 ginkgo products in tablet or capsule forms. A more recent analysis of 10 U.S. marketed products containing ginkgo leaf extracts standardized to 24% flavonol glycosides and 6% terpene lactones found three of them to contain less than the amount claimed but none were reported to contain an excess of either. Thus the test article contains a higher level of flavonol glycosides than all but three of the 50 products tested in these three studies and more terpene lactones than any other of these tested products. These facts, combined with the analytical data that shows that the amount of ginkgolic acids in the test article exceeds the limits established by regulatory and pharmacopeial standards by more than 100%, must be seen as contradicting any representation of the test article as “reflect[ing] concentrations [of contained constituents] measured in commercially available [ginkgo] products” in the U.S.

Regulatory and pharmacopeial standards for Ginkgo biloba leaf extract
Every dietary supplement that consists of or contains a Ginkgo biloba leaf extract that is marketed in the United States is required to meet all claims made on the product’s label, including any claims for the ingredient’s identity and for the level of contained constituents, such as flavonol glycosides and terpene lactones. But the U.S. law does not require every ginkgo leaf extract to be...

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12 Kressmann S, Müller WE, and Blume HH. Pharmaceutical quality of different Ginkgo biloba brands. 2002. J Pharm Pharmacol 54:661-669. The authors analyzed 27 products, one of which consisted only of ginkgo leaf (not an extract) and is not considered here.
15 21 CFR 111.75(c).
16 21 CFR 101.36(1) and 101.9(g)(3) and (g)(4). These Federal regulations require any "added" constituent to be present in an amount that is "at least equal to the value" declared on labeling (referred to as "the 100 percent standard"). FDA has communicated its position that a substance is considered an added substance "if the manufacturer manipulates its level .... For example, in a standardized herbal extract, because the manufacturer controls the amount of the standardized substance in the extract, that substance (the dietary ingredient) is an added dietary ingredient and is subject to the 100 percent standard." (Letter from FDA (DE Baker, Associate Commissioner for Regulatory Affairs) to Capsugel (RJ Dennin); October 19, 1999).
m to any specific standard, although any product that claims sentenced standard must, in fact, comply with that standard.\textsuperscript{17} however, exist in some other countries and in extant references. The following table provides some examples of such int for comparison the analytical data on the test article ear gavage studies.

\textbf{for Levels of Constituents in Ginkgo biloba Extracts}

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<tr>
<td>Health Canada\textsuperscript{18}</td>
<td>22-27%</td>
<td>-</td>
<td>-</td>
<td>5-7%</td>
<td>-</td>
</tr>
<tr>
<td>German Commission E\textsuperscript{19}</td>
<td>22-27%</td>
<td>2.6-3.2%</td>
<td>2.8-3.4%</td>
<td>5-7%</td>
<td>&lt;5 ppm</td>
</tr>
<tr>
<td>American Herbal Pharmacopoeia\textsuperscript{20}</td>
<td>22-27%</td>
<td>-</td>
<td>-</td>
<td>5.7%</td>
<td>-</td>
</tr>
<tr>
<td>European Pharmacopoeia\textsuperscript{21}</td>
<td>22.0-27.0%</td>
<td>2.6-3.2%</td>
<td>2.8-3.4%</td>
<td>5.4-6.6%</td>
<td>&lt;5 ppm</td>
</tr>
<tr>
<td>US Pharmacopoeia\textsuperscript{22}</td>
<td>22.0-27.0%</td>
<td>2.6-5.8%</td>
<td>2.8-6.2%</td>
<td>5.4-12.0%</td>
<td>&lt;5 ppm</td>
</tr>
<tr>
<td>Test article</td>
<td>31.2%</td>
<td>6.94%</td>
<td>8.42%</td>
<td>15.4%</td>
<td>10.45 ±2.40</td>
</tr>
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</table>

As is obvious from Table A, the NTP test article fails to meet any of the listed standards because the level of each of the described and quantified constituents is higher in the test article than is accepted by any of the standards.

It must therefore be concluded that the 2-year gavage studies reported in Draft TR 578 have relevance only to the test article itself, as provided by Shanghai Xing Ling, and that these studies provide no information that has been shown to be relevant to any \textit{Ginkgo biloba} leaf extract that conforms to one or another of these standards.

\textbf{Use of corn oil as the vehicle for the test article raises questions}

Draft TR 578 reports that corn oil was used as the vehicle for gavage administration of the test article, a detail that raises significant concerns.

\textsuperscript{17} 21 U.S.C. 343(s)(2)(D).
\textsuperscript{21} European Pharmacopoeia 7.0. Monograph: Ginkgo Dry Extract, Refined and Quantified. 2010. Strasbourg, France: European Directorate for the Quality of Medicines & Healthcare.
Corn oil is commonly used as a vehicle for gavage administration of pure chemicals. No analysis has been done, however, to determine whether it is an appropriate vehicle for a “complex mixture of chemical constituents,” as Draft TR 578 accurately describes Ginkgo biloba leaf and as is also accurate for the tested ginkgo leaf extract. There is little or no information, and no analytical data, to indicate that the test article was distributed in the corn oil vehicle in a manner that ensured absorption of the entire test article. For example, no analysis was done to determine whether some compounds within the specific Ginkgo biloba leaf extract used as the test article may have been enriched in this vehicle or whether other compounds, including potentially protective compounds, were present in a form in the corn oil vehicle that would allow for absorption.

Corn oil has frequently been used in toxicity studies as the dosing vehicle for lipophilic chemicals such as halogenated hydrocarbons. But ginkgo leaf extracts are usually produced by extraction with aqueous alcohols or acetone, and generally include lipophilic constituents, such as ginkgolic acids, as well as compounds that are water soluble and would not be dissolved in oil. It is therefore possible that the actual material fed to the test animals – that is, the mixture of corn oil and the specific Ginkgo biloba leaf extract used in these studies – was not precisely representative of the extract itself. Absent analysis of this extract/oil mixture there can be no certainty of the identity of the material actually consumed in these studies.

There is also concern related to the known potential for corn oil itself to have a toxicological effect on test animals. NTP produced a Technical Report in 1992 to evaluate the comparative toxicology of corn oil, safflower oil, and tricaprylin for use as a vehicle for gavage in studies in male F344/N rats.23 One of the conclusions in this report stated, “the use of corn oil as a gavage vehicle may have a confounding effect on the interpretation of chemical-induced proliferative lesions of the exocrine pancreas and mononuclear cell leukemia in male F344/N rats.” An increased rate of mononuclear cell leukemia was observed in the male rats in the 2-year gavage studies of the tested ginkgo extract, yet there is no discussion in Draft TR 578 of the noted “confounding effect” of the vehicle on this finding of the study.

This concern is not entirely conceptual as there is some evidence that corn oil as a vehicle for gavage can have an effect on the toxicology of a studied material. For example, administration of chloroform by corn oil gavage for 90 days resulted in significantly greater hepatotoxicity in male and female B6C3F1 mice than with aqueous administration.24 It has also been suggested that oral consumption of corn oil enhances the toxicity and carcinogenicity of volatile organic compounds in rodents, and that this effect could be “due to induction of metabolizing

enzymes, thus increasing the generation of reactive intermediates." In addition, in a study that examined the effects of various levels of corn oil and lard fed during the initiation stage of azoxymethane-induced hepatocarcinogenesis in male Fischer 344 rats, an enhancing effect on hepatocarcinogenesis was observed with a corn oil diet compared with a lard diet.

In presenting this issue AHPA is not suggesting that all of the effects observed in the 2-year gavage studies were caused by the corn oil used as the gavage vehicle. Rather this information suggests that the conclusions presented in Draft TR 578 must be seen as inconclusive until the well established adverse effects of corn oil itself are addressed, and analysis is conducted to demonstrate that corn oil is a valid vehicle for administration of the complex mixture represented by the test article.

**Potential safety concerns related to ginkgolic acids**

As noted in Table A, it is a common practice to limit the level of ginkgolic acids in ginkgo leaf extracts to 5 ppm, even though both beneficial and harmful properties have been reported to be associated with these alkylphenol compounds. Reported negative associations have included "contact allergenic, cytotoxic, embryotoxic, immunotoxic, mutagenic and slight neurotoxic" properties, though it is also reported that "there is no conclusive evidence that oral consumption of Ginkgo leaves or full extracts containing as much as 22,000 ppm (2.2%) of ginkgolic acids leads to allergic reactions or other serious side effects."

Nonetheless, the fact that the test article was measured to contain 10.45 ±2.40 ppm ginkgolic acid should be considered as a possible explanation for at least some of the results observed in the NTP studies.

**Use of a CAS number to describe the test article is inaccurate**

Draft TR 578 includes in its description of the identity of the test article a specific CAS number, number 90045-36-6. CAS No. 90045-36-6 is defined as:

"Extractives and their physically modified derivatives such as tinctures, concretes, absolutes, essential oils, oleoresins, terpenes, terpene-free fractions, distillates, residues, etc., obtained from *Ginkgo biloba*, Ginkgoaceae."

This definition is so broad as to include virtually any extractive or derivative obtained from *Ginkgo biloba* leaf, stem, root, bark, fruit or seed, regardless of significant variations in the chemical composition among all of these possible substances. To represent the test article used in the 2-year gavage studies by such a broad term is inaccurate, and could have the effect of implying that the results of these studies are relevant to any and all extractives and derivatives.

25 Ibid.
28 Ibid.
obtained from any part of the ginkgo tree. This term should be removed from any future revisions to Draft TR 578.

Scientific and common names of the *Ginkgo biloba* tree are not synonyms of *Ginkgo biloba* extract
Draft TR 578 presents *Ginkgo biloba* as the "botanical name" of the test article, and also lists several of the common name synonyms for the ginkgo tree as synonyms for the ginkgo extract used in the study. Use of these names for a *Ginkgo biloba* extract is not strictly accurate and AHPA recommends that these names be removed.

**Study reproducibility is compromised**
The National Center for Complementary and Alternative Medicine (NCCAM) at NIH has recognized the importance of proper characterization of study materials in order to fund replicable research on natural products, including complex botanical products. For animal studies that employ complex botanical products, such as extracts made from the leaves of *Ginkgo biloba*, NCCAM's Policy on Natural Product Integrity requires, among other things, information relevant to the standardization process. That information should include a description of the manufacturing process with details of the extraction such as solvent(s), ratio of plant to solvent, extraction time and temperature, and data on batch-to-batch reproducibility.\(^{29}\)

Without this information it is not possible to reproduce the research on another batch of the specific *Ginkgo biloba* leaf extract from Shanghai Xing Ling, much less to apply the research to any dissimilar ginkgo extract such as EGB 761\(^{29}\) or any other ginkgo leaf extract.

Addendum
In order to make the above comments most useful and to best clarify AHPA's intended meanings herein, in several places within these comments AHPA has suggested that revisions be made Draft TR 578. These recommendations are clarified below in the form of proposed textual revisions. With each proposal, AHPA identifies language recommended for deletion with strikethrough text, and language recommended for addition in bold underline font.

This addendum should not, however, be viewed as an exhaustive list of changes that would need to be made to Draft TR 578 in order to take into account the comments submitted in this review and AHPA requests that complete review and revision of Draft TR 578 be undertaken in order to ensure that any final Technical Report on these 2-year studies is completely accurate and does not in any manner imply that these studies are relevant to any Ginkgo biloba leaf extract other than the specific test article.

Page P1:

NTP TECHNICAL REPORT
ON THE
TOXICOLOGY AND CARCINOGENESIS
STUDIES OF A SPECIFIC
GINKGO BILoba LEAF EXTRACT

(CAS NO.: 90045-36-6)

Page P3:

NTP TECHNICAL REPORT
ON THE
TOXICOLOGY AND CARCINOGENESIS
STUDIES OF A SPECIFIC
GINKGO BILoba LEAF EXTRACT

(CAS NO.: 90045-36-6)

Header on pages 3, 5, 6, 8, 9, 10, and wherever the term "Ginkgo biloba Extract" appears throughout the document:

A Specific Ginkgo biloba Leaf Extract, NTP TR 578

Pages 7 and 19:

A SPECIFIC GINKGO BILoba LEAF EXTRACT
CAS-No.: 90045-36-6
**Synonyms:** Ginkgo, Ginkgo biloba, fossil tree, maidenhair tree, Japanese silver apricot, baiguo, bai guo ye, kow tree, yinheung (yin heung)

**Botanical name:** Ginkgo biloba

The Ginkgo biloba extract used in the current studies was procured from a supplier known to provide material to United States companies and contained 31.2% flavonol glycosides, 15.4% terpene lactones (6.94% bilobalide, 3.74% ginkgolide A, 1.62% ginkgolide B, 3.06% ginkgolide C), and 10.45 ppm ginkgolic acid.

**Page 12:**

Conclusions

Under the conditions of these 2-year gavage studies, there was some evidence of carcinogenic activity* of a specific Ginkgo biloba leaf extract in male F344/N rats based on increased incidences of thyroid gland follicular cell adenoma. The increased incidences of mononuclear cell leukemia and hepatocellular adenoma may have been related to Ginkgo biloba extract administration. There was some evidence of carcinogenic activity of the specific Ginkgo biloba leaf extract in female F344/N rats based on increased incidences of thyroid gland follicular cell neoplasms. Increased occurrence of respiratory epithelium adenomas in the nose may have been related to Ginkgo biloba extract administration. There was clear evidence of carcinogenic activity of the specific Ginkgo biloba leaf extract in male B6C3F1/N mice based on increased incidences of hepatocellular carcinoma and hepatoblastoma. The increased incidences of thyroid gland follicular cell adenoma were also related to the specific Ginkgo biloba leaf extract administration. There was clear evidence of carcinogenic activity of the specific Ginkgo biloba leaf extract in female B6C3F1/N mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma.

Administration of the specific Ginkgo biloba leaf extract resulted in increased incidences of nonneoplastic lesions in the liver, thyroid gland, and nose of male and female rats and mice and the forestomach of male and female mice. Increased severity of nephropathy in male rats was also due to administration of the specific Ginkgo biloba leaf extract.

Because the specific Ginkgo biloba leaf extract used in these studies may or may not be similar to other Ginkgo biloba leaf extracts sold in the U.S. or in other countries the conclusions given here should not be extrapolated to any other Ginkgo biloba leaf extract.

**Page 20:**

The main constituents of Ginkgo biloba leaves and their concentrations in standardized Ginkgo biloba extract (EGb 761®) and other commercially available preparations are shown in Table 1. The extract used in this study was not characterized to this extent and had significant chemical differences with respect to all quantified constituents including flavonol glycosides, terpene lactones, and ginkgolic acids.

**Table 1**

<table>
<thead>
<tr>
<th>Constituents of Ginkgo biloba</th>
<th>Identified Chemical Constituents</th>
<th>Target Specification in EGb 761®</th>
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In 1965, the German physician-pharmacist Dr. Willmar Schwabe III developed Ginkgo biloba leaf extracts (De Feucus, 2003). The final product, a standardized Ginkgo biloba extract (EGb 761®), has been subsequently marketed by Dr. Willmar Schwabe Pharmaceuticals under the trade names Ginkgold® (Nature’s Way™), Kaver®, Rōkan®, Tanakan®, and Tebonin®. EGB 761® is a quantified refined extract, standardized to containing 24% flavonoid glycosides (primarily derivatives of quercetin, kaempferol, and isorhamnetin), 6% terpene lactones [3.1% ginkgolides (A, B, C, J) and 2.9% bilobalide], various organic acids (5% to 10%), and other constituents (Table 1). Many Ginkgo biloba components are biologically active, and it is believed that the action of multiple constituents contributes to the medicinal properties of the plant leaf extract. However, the standardization of EGB 761® and other Ginkgo biloba extracts is based on their flavonoid and terpene trilactone content (Figure 1), as these compounds are thought to be primarily responsible for the pharmacological activity associated with Ginkgo biloba extract.

In the United States, herbal formulations sold as dietary supplements such as Ginkgo biloba extract are regulated under the Dietary Supplement Health and Education Act of 1994 (DSHEA). As such, they are not subject to the same standards of pre-market testing as drugs intended to treat, cure, prevent, diagnose, or mitigate disease. In contrast, in Germany and France Ginkgo biloba dried leaf extract is regulated as a prescription drug and therefore, requires registration and adherence to specified content standards. For Ginkgo biloba dried leaf extracts, these are 22.0% to 27.0% flavonoid glycosides, 5% to 7% terpene lactones (2.82.6% to 3.43.2% ginkgolides A, B, C, and 2.9% to 3.2% bilobalide), and not more than 5 ppm ginkgolic acids, due to their cytotoxic and allergenic potential (Kressmann et al., 2002). In the United States, a wide range of component concentrations is observed in available Ginkgo biloba products (Table 1).
(Kressmann et al., 2002). However, analyses by independent investigators showed variation even in the composition of the standardized extracts (Woerdenbag and van Beek, 1997).

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The test article selection was based on availability of bulk product and market share of the manufacturer at the study initiation.

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MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

A Specific Ginkgo biloba Leaf Extract

The original intent was to use standardized extract EGB 761®, manufactured by Wilhelm Schwabe, due to its use in many human studies. However, this material was not available to the NTP because unformulated EGB 761® was exclusively sold to pharmaceutical companies at the time of procurement for the NTP studies. Through industry contacts, the NTP learned that Shanghai Xing Ling Science and Technology Pharmaceutical Company (Shanghai, China) produced an extract purported to be similar to the Schwabe extract that was said to be widely distributed in commerce (personal communication). NTP does not however know whether this ingredient is now or has ever been sold or offered for sale in the United States. A specific Ginkgo biloba extract made from leaves was nonetheless obtained from Shanghai Xing Ling Science and Technology Pharmaceutical Company, Ltd., in two lots (020703 and GBE-50-001003). Lot 020703 was used during the 3-month and 2-year studies. Lot GBE-50-001003 was used only for methods development. Identity, purity, stability, and moisture analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO); in addition, the study laboratory at Battelle Columbus Operations (Columbus, OH) confirmed the identity of the test article by infrared spectroscopy (Appendix I). Reports on analyses performed in support of the Ginkgo biloba extract studies are on file at the National Institute of Environmental Health Sciences.

Page 36:

Quantitation assays of α-glycosides in the hydrolyzed extracts using HPLC/UV indicated that the test material contained 16.71% quercetin glycosides, 12.20% kaempferol glycosides, and 2.37% isorhamnetin glycosides.
Page 101:

DISCUSSION AND CONCLUSIONS

Ginkgo biloba extract is a popular herbal supplement used to improve brain function. As with many natural products, there is significant variability in the contents of Ginkgo biloba extract available in the marketplace (Kressmann et al., 2002; Agnolet et al., 2010; Gawron-Czella et al., 2010; Chandra et al., 2011). In a 2002 study analyzing Ginkgo biloba extract constituents from products available in the United States, Kressmann et al. (2002) found a range of concentrations for flavonol glycosides (24% to 36%), terpene lactones (4% to 11%), and ginkgolic acids (less than 500 ppm to 90,000 ppm). The Ginkgo biloba extract used in the present studies contained 31.2% flavonol glycosides, 15.4% terpene lactones (6.94% bilobalide, 3.74% ginkgolide A, 1.62% ginkgolide B, 3.06% ginkgolide C), and 10.45 ppm ginkgolic acid. These values do not reflect concentrations measured in the most common commercially available products in the United States and have a similar ratio of active ingredients to all exceed the specifications for the standardized Ginkgo biloba leaf extract known as (EGb 761®).

Page 105-106:

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was some evidence of carcinogenic activity* of a specific Ginkgo biloba leaf extract in male F344/N rats based on increased incidences of thyroid gland follicular cell adenoma. The increased incidences of mononuclear cell leukemia and hepatocellular adenoma may have been related to Ginkgo biloba leaf extract administration. There was some evidence of carcinogenic activity of the specific Ginkgo biloba leaf extract in female F344/N rats based on increased incidences of thyroid gland follicular cell neoplasms. Increased occurrence of respiratory epithelium adenomas in the nose may have been related to Ginkgo biloba leaf extract administration. There was clear evidence of carcinogenic activity of the specific Ginkgo biloba leaf extract in male B6C3F1/N mice based on increased incidences of hepatocellular carcinoma and hepatoblastoma. The increased incidences of thyroid gland follicular cell adenoma were also related to the specific Ginkgo biloba leaf extract administration. There was clear evidence of carcinogenic activity of the specific Ginkgo biloba leaf extract in female B6C3F1/N mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma.

Administration of the specific Ginkgo biloba leaf extract resulted in increased incidences of nonneoplastic lesions in the liver, thyroid gland, and nose of male and female rats and mice and the forestomach of male and female mice. Increased severity of nephropathy in male rats was also due to administration of the specific Ginkgo biloba leaf extract.

Because the specific Ginkgo biloba leaf extract used in these studies may or may not be similar to other Ginkgo biloba leaf extracts sold in the U.S. or in other countries the conclusions given here should not be extrapolated to any other Ginkgo biloba leaf extract.

Page 1-2:
CHEMICAL CHARACTERIZATION
AND DOSE FORMULATION STUDIES
PROCUREMENT AND CHARACTERIZATION
*Ginkgo biloba* Leaf Extract

Although the original study planned to use standardized extract EGB 761®, manufactured by Wilhelm Schwabe, this material was not available to the NTP because at the time of procurement for the NTP studies, this standardized extract was sold unformulated only to Pharma. Through industry contacts, the NTP learned that Shanghai Xing Ling Science and Technology Pharmaceutical Company (Shanghai, China) produced an extract purported to be similar to the Schwabe extract and that was said to be widely distributed in commerce. NTP does not however know whether this ingredient is now or has ever been sold or offered for sale in the United States. A *Ginkgo biloba* leaf extract was nonetheless obtained from Shanghai Xing Ling Science and Technology Pharmaceutical Company, Ltd. in two lots (020703 and GBE-50-001003). Lot 020703 was used during the 3-month and 2-year studies. Identity, purity, stability, and moisture analyses were conducted by the analytical chemistry laboratory at Midwest Research Institute (Kansas City, MO); in addition, the study laboratory at Battelle Columbus Operations (Columbus, OH) confirmed the identity of the test article versus a frozen reference of the same lot, shipped separately, by infrared spectroscopy. Reports on analyses performed in support of the *Ginkgo biloba* extract studies are on file at the National Institute of Environmental Health Sciences.

... For these assays, methanol:water (50:50) extracts of the specific *Ginkgo biloba* powdered leaf extract were partitioned with dichloromethane and dried over anhydrous sodium sulfate. The residue was reconstituted with methanol and analyzed using total ion current and single ion response mode following the methodology of Ndjoko et al. (2000) and Li et al. (2002). Further information on these methods can be found in Gray et al. (2005, 2007).

Page 1-3:

Quantitation assays of α-glycosides in the hydrolyzed extracts using HPLC/UV indicated that the test material contained 16.71% quercetin glycosides, 12.20% kaempferol glycosides, and 2.37% isorhamnetin glycosides. ... HPLC/MS analyses for the presence of ginkgolic acids I, II, and III using standards from ChromaDex, Inc. (Irvine, CA), and for colchicine using the colchicine standard from Sigma-Aldrich, resulted in no observable peaks of ginkgolic acids or colchicine in the test material.
Safety Assessment of *Ginkgo biloba*-Derived Ingredients as Used in Cosmetics

Status: Draft Report for Panel Review
Release Date: November 10, 2017
Panel Meeting Date: December 4-5, 2017

The 2017 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A. Hill, Ph.D. James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer.
INTRODUCTION

Most of the *Ginkgo biloba*-derived ingredients detailed in this safety assessment are reported to function as skin conditioning agents, while some are reported to function as antioxidants in cosmetics, according to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (v1NCl; Dictionary; see Table 1). This report assesses the safety of the following 10 *Ginkgo biloba*-derived ingredients:

- Ginkgo Biloba Leaf Extract
- Ginkgo Biloba Leaf
- Ginkgo Biloba Leaf Cell Extract
- Ginkgo Biloba Leaf Powder
- Ginkgo Biloba Flavones
- Ginkgo Biloba Meristem Cell
- Ginkgo Biloba Nut Extract
- Ginkgo Biloba Root Extract
- Ginkgo Leaf Terpenoids

*Ginkgo biloba* leaves and nuts (also called seeds) have been used as a source of traditional Chinese medicines. More recently, extracts of the leaves of *Ginkgo biloba* have been used as herbal medicines or dietary supplements in the treatment of heart disease, eye ailments, tinnitus, cerebral and peripheral vascular insufficiency, injuries involving brain trauma, dementias, short-term memory improvement, cognitive disorders secondary to depression, vertigo, and various cognitive disorders. Investigations into the efficacy of the leaf extract for these uses are numerous and are mainly based on oral administration. There are no publically available toxicity data that corresponds to any one of these cosmetic ingredients, specifically. For all of the endpoint results summarized in this report, the test article is a vaguely and variably described extract of *Ginkgo biloba* leaves, or some other non-cosmetic-ingredient source, such as "fruit pulp." The focus of this safety assessment will be on data relevant to the use of *Ginkgo biloba*-derived ingredients in cosmetics, with specific focus on dermal application when available.

Often in the published literature, the information provided is not sufficient to determine how well the tested substance represents the cosmetic ingredient; therefore, the taxonomic name is used unless it is clear that the test substance is similar to a cosmetic ingredient. However, in the case of data on the extract of *Ginkgo biloba* leaves, the abbreviation GBE will be used, unless the data specifically are related to the cosmetic use of Ginkgo Biloba Leaf Extract.

Botanicals, such as *Ginkgo biloba*-derived ingredients, may contain hundreds of constituents, some of which may have the potential to cause toxic effects. In this assessment, CIR is reviewing the potential toxicity of each of the *Ginkgo biloba*-derived ingredient as a whole, complex mixture. CIR is not reviewing the potential toxicity of the individual constituents, except wherein such constituents are also ingredients under review.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world’s literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that CIR typically evaluates, is provided on the CIR website (http://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; http://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

CHEMISTRY

Definition and Plant Identification

The definitions and functions of the *Ginkgo biloba*-derived ingredients included in this report are provided in Table 1. The raw materials for the ingredients in this report are obtained from the deciduous tree, *Ginkgo biloba*, which has fan-shaped leaves that turn golden yellow in autumn and which can grow to 40 m (~131 ft) tall. The female trees bear offensive-smelling, inedible fruit that contain a single thin-shelled semi-edible nut. *Ginkgo* trees are planted widely as ornamental trees via cultivation. Few naturally-occurring specimens grow in Zhejiang province China. Trees grown commercially for the leaves are found in China, France, and in the United States.

Methods of Manufacturing

A general description of manufacturing for "medicinal" GBE reported that the leaves of the *Ginkgo biloba* tree are harvested either mechanically or by hand from plantations or in the wild. The leaves are then dried and pressed into balls. A dry extract from the dried leaf of *Ginkgo biloba* can be manufactured using acetone/water and subsequent purification steps without addition of concentrates or isolated ingredients.

GBE may be full extracts or standardized extracts. Full extracts are prepared with alcohol and contain all constituents soluble in alcohol. Standardized extracts (preferred to as GBT-761 in published literature) are more common, especially in herbal supplements, and are prepared in a manufacturer-dependent multi-step process in which some compounds, such as flavonoids and lactones, are enriched while others, such as ginkgolic acids, are removed (Scheme 1).

Standardized extracts are reported to contain 6% terpene trilactones, 24% flavonol glycosides, and less than 5 ppm ginkgolic acids.

**NOTE** NTP GBEE: 15.4% 31.2% 10.45 ± 2.40 ppm from Shanghai Xing Ling
Scheme 1. General manufacturing process of standardized Ginkgo biloba extract.

**Composition/Impurities**

Table 2 summarizes the composition ranges of the major constituents of various extracts (standardized and non-standardized) of Ginkgo biloba leaves.

General Ginkgo biloba composition was reported in the *Physician’s Desk Reference for Herbal Medicines* to be the following flavonoids (0.5% to 1.8%) including monosides, bisides and trisides of quercetin, isorhamnetins, 3-O-methylhyrocorisins, and kaempferol (may be ester with p-coumaric acid); biflavonoids (0.4% to 1.9%) including amentoflavone, bilobetin, 5-methoxybilobetin, ginkgetin, and isoginkgetin; proanthocyanidins (8% to 12%); triacontane diterpenes (0.06% to 0.23%) including ginkgolide A, B, and C; and triacontane sesquiterpene bilabiolids (0.04% to 0.2%).

An extraction of 60% w/v ethanol of dried green Ginkgo biloba leaves yielded an extract comprised of 3.4% flavone glycosides, 0.7% terpene lactones, and 5.5% ginkgolic acids. Further fractionation by liquid-liquid partition between water and heptane yielded a fraction comprised of 0.3% flavone glycosides, 0.1% terpene lactones, and 24.6% ginkgolic acids.

For use as an herbal medicine in Germany, GBE must be extracted with acetone/water and contain 22%-27% flavone glycosides (quercetin and kaempferol) with a molar mass of 756.7 (quercetin glycoside) and 740.7 (kaempferol glycoside), 5%-7% terpene lactones of which 2.8%-3.4% consists of ginkgolides A, B, and C and 2.6%-3.2% bilobalide, and less than 5 ppm ginkgolic acids. An example of standardized GBE specifications is the following: brown powder with characteristic smell containing not more than 20 ppm heavy metals; not more than 2 ppm arsenic; not more than 5 ppm ginkgolic acid; not less than 24.0% total flavonoid content; and not less than 6.0% total terpene triacontane content.

Ginkgolic acid is a salicylic acid derivative with a C15 side chain that is related to the pentadecycyclotechols (i.e. urushiol) found in poison ivy. One analysis found crude aqueous extracts of GBE contained up to a total of 30 ppm urushiol while the process to produce standardized GBE removed long chain alkylphenols to below detection levels.

**USE**

**Cosmetic**

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

According to 2017 VCRP survey data, Ginkgo Biloba Leaf Extract has the most reported uses in cosmetic products, with a total of 726; the majority of the uses are in leave-on eye makeup preparations and skin care products (Table 3).
other Ginkgo-derived ingredients are reported to be in use, with 24 or less uses reported in the VCRP. A concentration of use survey on these 10 ingredients conducted in 2014 by the Council indicate use for one Leaf Extract, which is reported to be used at a maximum of 1%, as reported in face and neck skin preparations. A concentration of use survey has not yet been completed for Ginkgo Biloba Leaf Cell Extract. Ingredients with uses in the VCRP or by the Council are listed in Table 4.

In some cases, reports of uses were received from the VCRP, but no concentration of use data were provided. For example, Ginkgo Biloba Nut Extract is reported to be used in 24 formulations, but no use concentration data were provided.

Some of these ingredients may be used in products that can be incidentally ingested or come into contact with mucous membranes; for example, Ginkgo Biloba Leaf Extract is used in lipstick at up to 0.2%. Additionally, some of these ingredients are used in formulations that are used near the eyes; for example, Ginkgo Biloba Leaf Extract is used in eye shadows and eye lotions at up to 0.01%. Moreover, some of these ingredients were reported to be used in sprayed products that could possibly be inhaled. For example, Ginkgo Biloba Leaf Extract was reported to be used in pump spray suntan products at a maximum concentration of 0.05%. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 μm, with propellant sprays yielding a greater fraction of droplets/particles below 10 μm compared with pump spray. Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable extent. Ginkgo Biloba Leaf Extract is also used in powders, and these products could possibly be inhaled; for example, it is used in face powders at a maximum concentration of 0.05%. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

The Ginkgo biloba-derived ingredients described in this report are not restricted from use in any way under the rules governing cosmetic products in the European Union.

Non-Cosmetic

Ginkgo biloba is used extensively as an herbal supplement for anti-inflammatory, cognitive-promoting, antioxidant, and vascular effects. In Germany, GBE is an approved herbal medicine for use for treatment of memory deficits, dementia, and other organic brain syndromes when extracted with acetone/water. It is not approved when extracted with other solvents due to lack of supporting safety data.

Standardized GBE and/or constituents of the extract, such as bilobalide, kaempferol and ginkgetin, have also been studied for potential neuroprotective effects against Alzheimer’s disease, anti-inflammatory and analgesic effects on post-surgical incisions and diseases such as osteoarthritis and atopic dermatitis, protective effects (antioxidant) against radiation and chemotherapy-induced toxicity, anticancer effects, and therapy for uveitis. GBE as an herbal supplement may interact with pharmaceutical drugs and act as an enhancer of anticoagulants, anti-inflammatory agents, antihypertensives, and/or anesthetics which may lead to hemorrhage, apraxia, hematoma, hyperemia, permanent neurological deficit, and death. The Physician’s Desk Reference for Herbal Medicines reports major drug interaction risks with anticoagulants, nonsteroidal anti-inflammatory drugs (NSAIDs), and trazodone and moderate drug interaction risks with low molecular weight heparins and thrombolytic agents. GBE may also interact with anticonvulsants, buspirone, insulin, monoamine oxidase (MAO) inhibitors, nitrindipine, nifedipine, omeprazole, papaverine, St. John’s wort, selective serotonin reuptake inhibitors, and thiazide diuretics.

The United States Pharmacopeia states that "ginkgo" consists of the dried leaf of Ginkgo biloba Linne (Fam. Ginkgoaet). It contains not less than 0.5% of flavonoids, calculated as flavonol glycosides, with a mean molecular mass of 756.7; and not less than 0.1% of terpene lactones, calculated as the sum of bilobalide, ginkgolide A, ginkgolide B, and ginkgolide C, both on the dry basis. This reference also states that "powdered ginkgo extract" is prepared from dried and comminuted leaves of Ginkgo extracted with an acetone-water mixture or other suitable solvents. It contains not less than 22.0% and not more than 27.0% of flavonoids, calculated as flavonol glycosides, with a mean molecular mass of 756.7; and not less than 5.4% and not more than 12.0% of terpene lactones, consisting of between 2.6% and 5.8% of bilobalide and between 2.8% and 6.2% of ginkgolide A, ginkgolide B, and ginkgolide C.

The British Pharmacopoeia states that "ginkgo leaf" content should be not less than 0.5% of flavonoids, calculated as flavone glycosides (dried drug). The nuts of Ginkgo biloba are a delicacy in Japan and China, but must be removed completely from the pulp, boiled or roasted, and eaten sparingly (limit 5 - 10 per day). In traditional Chinese medicine, the nut is dried and used to treat such ailments as asthma, cough, bronchitis, scabies, and sores.

TOXICOKINETICS

In general, toxicokinetics data are not expected to be found on botanical ingredient because each botanical ingredient is a complex mixture of hundreds of constituents. However, there have been many pharmacokinetics studies on GBE, specifically on some of the key constituents, which indicate GBE may be well absorbed after oral administration.
Dermal Penetration

Quercetin, to penetrate the skin while in a cosmetic formulation was studied in a cosmetic formulation used in the study was an emulsion containing triaureth-4
ate/VP copolymer and emollients, slescorum gum, humectants, preservatives and with 6.0% (w/w) titrated Ginkgo biloba glycolic leaf extract. An analysis of the
12% quercetin. The test formulation (10 mg/cm²) was applied to the skin
of human volunteers for 24 h. Samples of the receptor fluid (citrate buffer with 0.5% d 24 h exposures and quantified with high performance liquid chromatography.
the stratum corneum was removed by tape
surface of the exposed skin. Quercetin in the dermis and the receptor fluid was below
in skin. Approximately 40% quercetin was measured in the washing solution.

Absorption, Distribution, Metabolism, and Excretion (ADME)

The absorption, distribution, and elimination of radiolabeled GBE were studied in male and female Sprague-Dawley rats. The rats received a single oral suspended dose (20 μCi; 380 mg/kg) of the radiolabeled GBE. The test material was obtained from Ginkgo biloba grown under a supply of [14C]-acetate. The pharmacokinetic results, based on blood specific activity data versus time course, were characteristic of a two-compartment model with an apparent first order phase and a half-life of approximately 4.5 h. Expired [14C]-CO₂ represented 16% of the administered dose 3 h post-treatment. After 72 h, 38% of the radioactivity was excreted via exhalation, while 21% was determined to be excreted in the urine and 29% was excreted in the feces. The researchers of this study concluded that at least 60% of the radiolabeled GBE was absorbed. The site of absorption was likely the upper gastrointestinal tract.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Animal

Oral

The LD₅₀ of standardized GBE administered orally to mice was reported to be 7.73 g/kg.³²

Intravenous

The LD₅₀ after intravenous administration of standardized GBE was 1.1 g/kg for both rats and mice.³²

Short-Term Studies

Oral

The results of a combined liver comet assay (see Genotoxicity section) using male and female C3H-derived constitutive androstane receptor knockout (CARKO) and wild-type mice found no abnormal clinical signs and no treatment-related effects on body weight following oral exposure of up to 2000 mg/kg body weight/day GBE for 3 days in either mouse genotype.³⁵ Relative liver weights were significantly increased in male and female wild-type mice at all doses of GBE in a dose-dependent manner. The liver weights in the CARKO mice were similar to the negative control group. The wild-type mice in all GBE-treated groups had dose-dependent slight-to-moderate hepatocellular hypertrophy in the centrilobular area: this effect was only observed in a single mouse in the highest dose group. No histopathological findings suggesting cytotoxicity in the liver was observed in any GBE-treated groups.

Subchronic Toxicity Studies

Oral

The toxicity of GBE was investigated in a 3-month mouse study performed by the National Toxicology Program (NTP).³² Groups of 10 male and 10 female B6C3F1/N mice received 0, 125, 250, 500, 1000, or 2000 mg/kg body weight GBE in corn oil via gavage, 5 days per week for 14 weeks. Control groups received corn oil (5 ml/kg). Clinical findings and body weights were recorded initially, then weekly, and at the end of the study. Blood was collected at the end of the study from all animals for hematology analyses. Sperm motility and vaginal cytology evaluations were made on the mice in the 0, 500, 1000, and 2000 mg/kg dose groups. At the end of the study period, tissues from over 40 sites were examined for every animal, including ovaries and uterus in females and prostate gland and testes with epididymis and seminal vesicles in males.

One female mouse in the 1000 mg/kg group died of a dosing accident during week 11. Mean body weights of 2000 mg/kg females were significantly less than those of the vehicle control group. Ruffled fur was observed in two 1000 mg/kg males between weeks 7 and 8 and all 2000 mg/kg males between weeks 5 and 9. No treatment-related differences were observed in sperm parameters in males administered 500, 1000, or 2000 mg/kg or in the estrous cycle of females.
administered 500 or 1000 mg/kg when compared to controls. Female mice in the 2000 mg/kg group had a significantly higher probability of extended estrous than did the vehicle control females. Liver weights of males of the 250 mg/kg or greater dose groups and females of all dose groups were significantly greater than those of the vehicle control groups. Kidney weights of males of the 2000 mg/kg group were significantly less than those of the vehicle control group. Incidences of hepaticocyte hypertrophy were significantly increased in males and females dosed with 250 mg/kg or greater. Significantly increased incidences of focal hepaticocyte necrosis occurred in males of the 1000 and 2000 mg/kg dose groups. The incidences of hyaline droplet accumulation in the respiratory epithelium of the nose were significantly increased in males of the 500 mg/kg and females of the 1000 and 2000 mg/kg dose groups. In the olfactory epithelium of the nose, the incidences of hyaline droplet accumulation were significantly increased in the 125 (female only), 500, and 1000 mg/kg groups. Incidences of atrophy of the olfactory epithelium were significantly increased in the 1000 mg/kg groups. The incidences of pigment accumulation in macrophages in the olfactory epithelium were significantly increased in males in the 500 mg/kg or greater groups and in females in the 1000 and 2000 mg/kg dose groups.32

The NTP also performed a 3-month study of GBE in rats.32 Groups of 10 male and 10 female F344/N rats received 0, 62.5, 125, 250, 500, or 1000 mg/kg body weight GBE in corn oil via gavage, 5 days per week for 14 weeks. Additional groups of 10 male and 10 female rats received the same doses for a clinical pathology study, 5 days per week for 23 days. Control groups received corn oil (2.5 ml/kg). The same methods that were followed in the mouse study described above were used in the main study animals, while animals in the clinical pathology study had blood samples collected on days 4 and 23.

All rats survived to the end of the study. Mean body weights of all dosed groups were similar to those of the vehicle control groups. No treatment-related clinical findings were observed. Liver weights of all dosed groups of males and females were significantly greater than those of the vehicle control groups. Incidences of hepaticocyte hypertrophy in all dosed groups of males and in 500 and 1000 mg/kg females were significantly greater than those in the vehicle control groups; there was a dose-related increase in severity of this lesion in males. Hepaticocyte fatty change occurred in all dosed males. The incidences of thyroid gland follicular cell hypertrophy were significantly increased in 500 and 1000 mg/kg males and in 1000 mg/kg females. The incidences of pigmentation in the olfactory epithelium of the nose were significantly increased in 500 and 1000 mg/kg males and in females administered 125 mg/kg or greater.32

**Chronic Toxicity Studies**

**Oral**

There was no evidence of organ damage or impairment of hepatic or renal function when GBE was administered orally over 27 weeks to rats and mice at doses ranging from 100 to 1600 mg/kg.32

The results of the NTP chronic toxicity bioassays are summarized in the Carcinogenicity section below.

**DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES**

**Oral**

The reproductive and developmental toxicity of standardized GBE was studied in mice. In one study, groups of 25 mated female CD-1 mice received 0, 100, 350, or 1225 mg/kg/day GBE in tap water via gavage (20 ml/kg) on days 6 through 15 of gestation.36 The dams were observed daily for clinical signs of toxicity. Feed and water consumption were monitored during the study. Body weight was measured daily. On day 17 of gestation, the dams were killed and the ovaries, uterus, and the fetuses were removed. The internal organs and the placenta of the dams were examined macroscopically. The fetuses were examined for several parameters, including external and internal damages (malformations), sex, viability, and weight. The skeletal systems and soft tissues of the fetuses were also examined.

No clinical signs of toxicity were observed in the dams and there were no unscheduled deaths. No treatment-related effects were observed in body weight gains or feed and water consumption. There were no pathological findings observed during necropsy. No embryotoxic effects were observed during external and internal examinations of the fetuses nor were any observed in skeletal or soft tissues. There were no increased incidences of malformation, variations, or retardations. The authors concluded the no-observed-effect-level (NOEL) was greater than 1225 mg/kg/day for both the dams and the fetuses in this study of standardized GBE.36

Another study examined the effects of oral administration of standardized GBE in saline on the mouse reproductive and developmental toxicity.37 Female Swiss albino mice received 0, 3.7, 7.4, or 14.8 mg/kg body weight/day for 28 days prior to mating, from day 1 to day 7 of gestation, or from day 10 to day 18 of gestation. There were 10 animals per dose group to study the anti-implantation and abortifacient activities for GBE, while there were 10 mice per dose group to study the reproductive cycle and 20 mice per dose group to study the developmental cycle (12 test groups total). Blood hormones were measured in the pre-mating group on day 28. Vaginal smears were performed daily. The mice were observed daily for clinical signs of toxicity and premature deaths. Body weights were recorded weekly. On day 20 of gestation, the remaining mice were killed and their kidneys, liver, brain, placenta, spleen and ovaries were removed and weighed. The ovaries were prepared for histological examinations, and then ovarian follicles were counted. Maternal toxicity, estrous cycle, reproductive...
s, ovarian follicle counts, resorption index, implantation index, fetal viability and fetuses, and placenta mean weights.

No clinical signs of toxicity were observed in the dams during treatment and there were no unscheduled deaths.

Statistically significant decreases in body weight gains were observed in the 14.8 mg/kg/day dose group when compared to the controls. There were no treatment-related changes in the relative weights of the liver, kidney, brain, spleen, ovary, and placenta, but there was a significant dose-dependent decrease in the relative weight of the gravid uterus in the 14.8 mg/kg/day dose group for 28 days when compared to controls. Ovarian follicle counts, resorption index, implantation index, fetal viability were significantly reduced in 14.8 mg/kg/day dose group. Treatment with 14.8 mg/kg bw/day GBE induced disruption of estrous cycle and caused maternal toxicity, in addition to fetal toxicity. No adverse effects were observed in the 3.7 or 7.4 mg/kg bodyweight/day dose groups. The authors concluded that 14.8 mg/kg body weight/day GBE produced adverse effects on the estrous cycle, fertility, abortifacient, reproductive performance, and hormone levels of female mice and may cause adverse effects on ovarian function as an antifertility agent.

**GENOTOXICITY**

**In Vitro**

GBE at up to 10,000 μg/plate was mutagenic in an Ames test using *Salmonella typhimurium* strains TA98 and TA100 and in *Escherichia coli* strain WP2 uvrA/pKM101, with and without metabolic activation.

The genotoxicity of GBE and eight of its constituents (quercetin; quercetin-3-β-D-glucoside; kaempferol; isorhamnetin; ginkgolide A; ginkgolide B; ginkgolide C; and bilobalide) were evaluated in mouse L5178Y cells using a lymphoma assay and a Comet assay. The GBE (0.2-1.2 mg/ml) and the eight constituents were tested in a DMSO solution. A dose-dependent increase in mutant frequency was observed in GBE, quercetin (10-100 μM), quercetin-3-β-D-glucoside (200-1000 μM), and kaempferol (10-200 μM) without metabolic activation. DNA double-strand breaks were also observed in dose-dependent increases in GBE, quercetin, and kaempferol. Negative results were observed in the other constituents. A Western blot analysis confirmed that GBE, quercetin, and kaempferol activated the DNA damage signaling pathway. Additionally, GBE produced reactive oxygen species and decreased glutathione levels in L5178Y cells. Loss of heterozygosity analysis of mutants indicated that GBE, quercetin, and kaempferol resulted in extensive chromosomal damage. The authors concluded that GBE, quercetin, and kaempferol are mutagenic in mouse L5178Y cells.

**In Vivo**

In a micronucleus test in male and female B6C3F1/N mice, no increase in the frequency of micronucleated erythrocytes was observed in peripheral blood of male mice administered 125 to 2000 mg/kg/day GBE orally for 3 months. Female mice that received the same doses had results that were deemed equivocal based on a significant trend test and due to no individual dose group being significantly elevated over the vehicle control group. A significant (P = 0.001) dose-related decrease in the percentage of circulating polychromatic erythrocytes (PCEs) was observed in male mice, which may indicate GBE induced bone marrow toxicity. In the female mice, a significant (P = 0.001) decrease in the percentage of circulating PCEs was also observed, but the response was not as correlated with dose as it was in the males.

In a reporter gene mutation assay using male B6C3F1 gpt delta mice, oral dosing of GBE in corn oil at up to 2000 mg/kg body weight/day for 90 days did not produce remarkable increases in gpt or Spi mutation frequencies in DNA extracted from the liver. No treatment-related clinical signs or deaths were observed during the treatment period. Relative liver weights were significantly increased in the 2000 mg/kg group. Hepatocellular hypertrophy in the centrilobular area and slight focal necrosis were observed in the 2000 mg/kg group.

This assay was performed in conjunction with a combined liver comet assay and bone marrow micronucleus assay using male and female CARKO and wild-type mice. The short-term toxicity effects were described in the Toxicological Studies section. In the micronucleus study, no significant alterations in the percentages of PCEs were observed in females of either genotype; however, a significant decrease in the percentage of PCEs were observed in both genotypes in males, indicating GBE induced bone marrow toxicity in male mice. In the comet assay, there was no significant difference in the percent tail DNA in any of the GBE-treated groups in either mouse genotype. Heavily damaged cells called “hedgehogs” indicating cytotoxic effects were not detected in any animals. The researchers performing these 3 assays concluded that GBE is not genotoxic.

**CARCINOGENICITY**

**Oral**

The carcinogenic potential of GBE administered orally was studied by the NTP in male and female rats and mice. In the study on mice, groups of 50 male and 50 female B6C3F1/N mice received 200, 600, or 2000 mg/kg GBE in corn oil 5 day per week for 104 weeks via gavage. In the study on rats, groups of 50 F344/N male and 50 female rats received 100, 300, or 1000 mg/kg body weight GBE for 104 (males) or 105 (females) weeks via gavage. Control groups received corn oil (5 ml/kg in mice and 2.5 ml/kg in rats). In rats involved in what was deemed a “special study,” groups of 10 male and female rats received the same doses as in the main study; blood was collected from these rats on day 22 and at week 14 for thyroid
hormone analyses and other analyses of the liver and thyroid gland. All animals were observed twice daily. Body weights were evaluated at study beginning and ending at different intervals during the course of the study. At the end of the study period, tissues from over 40 sites were examined for every animal, including ovaries and uteri in females and prostate gland and testes with epididymis and seminal vesicles in males.

In mice, mortality was significantly higher in the 600 and 2000 mg/kg males than in the vehicle controls, with the most frequent cause of death being liver tumors. Survival in the 600 mg/kg females was significantly greater than that of the vehicle controls. Mean body weights in the mid- and high-dose group male mice were less than those of the vehicle controls after weeks 85 and 77, respectively. The mean body weights of the high-dose females were generally less than the vehicle controls between weeks 17 and 69 and after week 93.

In rats, mortality in the 1000 mg/kg males was significantly higher than that of the vehicle controls, with the most frequent cause of death being mononuclear cell leukemia. The survival of the treated female rats was comparable to the vehicle control. In week 14, all dose group males and females of the 1000 mg/kg group in the special study had increased levels of thyroid stimulating hormone compared to the vehicle controls; the increase was dose-related in the male rats. Mean body weights in the mid- and high-dose male and female rats were less than the vehicle controls after weeks 93 and 89, respectively.

Lesions in the liver, thyroid gland, and nose were observed in all GBE dose groups in mice and rats. These lesions included hypertrophy in the liver and thyroid gland in rats and mice, liver hyperplasia in male and female rats, and hyperplasia and atrophy of the epithelium in the nose of male and female rats. Inflammation, hyperplasia, hyperkeratosis, and ulcers were also observed in the forestomach of male and female mice. Additionally, increased incidences of cancers of the thyroid gland were observed in male and female rats and male mice and of liver cancers in male and female mice. The study concluded that GBE caused cancers of the thyroid gland in male and female rats and male mice, and cancers of the liver in male and female mice.

No neoplastic or preneoplastic effects were observed in dietary carcinogenicity studies of standardized GBE in mice (at up to 200 mg/kg/day) or rats (at up to 100 mg/kg/day). The rodents received the test material for up to 85 weeks. No changes in body weight gain were reported. No further details are available.

The International Agency for Research on Cancer (IARC) has determined that GBE is possibly carcinogenic to humans (group 2B) based on inadequate human carcinogenicity evidence and sufficient evidence in experimental animals.

OTHER RELEVANT STUDIES

Immunotoxicity

In a popliteal lymph node assay (PLNA), the sensitization potential of GBE was evaluated. Groups of male C57BL/6 mice received subplantar injections of 10 μl DMSO (induction) followed by another injection of DMSO (negative control group), crude ethanolic-aqueous GBE, heptane fraction of the crude GBE, or diphenylhydantoin (positive control group) at doses of 2 mg each. The negative control yielded small enlargement of the lymph nodes, while the crude ethanolic-aqueous GBE resulted in statistically significant lymphoproliferative reaction (LPR) in the ipsilateral popliteal lymph node. A massive lymph node hyperplasia that was almost comparable to the positive control was observed in the heptane solution fraction of the crude GBE. Chemical analyses of the crude extract and the heptane fraction found ginkgolic acid at 5.5% and 24.6%, respectively, which were theorized to be responsible for the LPR observed in this study.

DERMAL IRRITATION AND SENSITIZATION STUDIES

No dermal irritation or sensitization studies were found in the published literature.

OCULAR IRRITATION STUDIES

No ocular irritation studies were found in the published literature.

CLINICAL STUDIES

Case Studies

The fruit pulp of the Ginkgo biloba tree has been reported to cause contact dermatitis, with several cases reported after patients handled the fruit pulp during extraction of the edible nut center. Symptoms include intense itching, edema, papules, and pustules that usually resolve in 7-10 days.

A 66-year-old woman presented with progressive erythematous eruption over the face, neck, trunk, and extremities that started approximately one week after the patient had ingested two 60 mg doses of a GBE supplement. No other new medications or changes in behavior were reported. A physical examination, complete blood cell count, and chemistry panel were unremarkable. The authors of the report did not disclose if patch or skin prick tests were performed.

A 45-year-old man developed acute generalized exanthematous pustulosis on his limbs and face 48 h after starting oral GBE treatment for tinnitus. The patient had not previously taken GBE before and was not taking any other medication. The patient had no history of adverse drug reactions or psoriasis. The rash cleared within 10 days of stopping GBE treatment. The patient refused a follow-up cutaneous patch test.
In anecdotal accounts from Chinese medicine, consumption of fresh *Ginkgo biloba* nuts may cause stomachache, nausea, diarrhea, convulsions, weak pulse, restlessness, difficulty breathing, and shock. Death has been reported in children following consumption of fresh nuts.

**Other Clinical Reports**

No adverse effects were reported in clinical studies of an anti-aging cosmetic formulation containing 1.5% GBE and other antioxidants in 45 volunteers and of an anti-wrinkle cosmetic formulation containing 0.30% GBE in 20 volunteers.

**SUMMARY**

According to the *Dictionary*, most of the *Ginkgo biloba*-derived ingredients detailed in this safety assessment are reported to function as skin conditioning agents, while some are reported to function as antioxidants in cosmetics. Investigations into the efficacy of the leaf extract for these uses are numerous and are mainly based on oral administration. There are no publicly available toxicity data that corresponds to any one of these ingredients specifically. For all of the endpoint results summarized in this report, the test extract is a vaguely and variably described extract of *Ginkgo biloba* leaves, or some other non-cosmetic-ingredient source such as “fruit pulp.” The focus of this safety assessment has been on data relevant to the use of *Ginkgo biloba*-derived ingredients in cosmetics, with specific focus on dermal application when available.

According to 2017 VCRP survey data, Ginkgo Biloba Leaf Extract has the most reported uses in cosmetic products, with a total of 726; the majority of the uses are in leave-on eye makeup preparations and skin care products. Two other *Ginkgo*-derived ingredients are reported to be in use, with 24 or less uses reported in the VCRP. The results of the concentration of use survey on these 10 ingredients conducted in 2014 by the Council indicate use for only Ginkgo Biloba Leaf Extract, which is reported to be used at a maximum of 1%, as reported in face and neck skin preparations.

GBE is extensively used as an herbal supplement for anti-inflammatory, cognitive-promoting, antioxidant, and vascular effects and is an approved herbal medicine in Germany for use for treatment of memory deficits, dementia, and other organic brain syndromes when extracted with acetone/water. GBE may interact with pharmaceutical drugs. Nuts from *Ginkgo biloba* are consumed as a delicacy in Japan and China and are used in traditional Chinese medicine. Anecdotal accounts report that consumption of the nuts may have acute adverse effects.

In general, toxicokinetics data are not expected to be found on botanical ingredients because each botanical ingredient is a complex mixture of hundreds of constituents. However, there have been many pharmacokinetics studies on GBE, specifically on some of the key constituents, which indicate GBE may be well absorbed after oral administration. The GBE constituent, quercetin, was found to penetrate human dermatomed skin. In an oral ADME study in rats, at least 60% of radio-labeled GBE absorbed, with the main site of absorption likely in the upper gastrointestinal tract. Radioactivity was measured in exhalation and elimination products.

The LD₅₀ of standardized GBE administered orally to mice was reported to be 7.73 mg/kg, and the LD₅₀ after intravenous administration of standardized GBE was 1.1 g/kg for both rats and mice.

In 3-month studies of GBE at up to 2000 mg/kg/day, increased liver weights, decreased kidney weights, increased incidences of hepatic cystic hypertrophy and focal hepatic necrosis, and increased incidences hyaline droplet accumulation, atrophy and pigment accumulation in macrophages in the olfactory epithelium were observed in mice. In a similar study of GBE in rats, increased liver weights, increased incidences of hepatic cystic hypertrophy, increased incidences of thyroid gland follicular cell hypertrophy, and increased incidences of pigmentation in the olfactory epithelium of the nose were observed. There was no evidence of organ damage or impairment of hepatic or renal function when GBE was administered orally over 27 weeks to rats and mice at doses ranging from 100 to 1600 mg/kg.

In an oral DART study of standardized GBE in mice, the NOEL for dams and fetuses was greater than 1225 mg/kg/day. No clinical signs of toxicity were observed in the dams and no embryo-toxic effects were observed in the fetuses. In another oral DART study in mice, standardized GBE at 14.8 mg/kg/day produced adverse effects on the estrous cycle, fertility, abortifacient, reproductive performance, and hormone levels in female mice and may cause adverse effects on ovarian function as an anti-fertility agent.

GBE at up to 10.000 μg/plate was mutagenic in an Ames test. GBE (0.2 - 1.2 mg/ml) was mutagenic in mouse L5178Y cells. In a mouse micronucleus test of GBE up to 2000 mg/kg/day, no increase in the frequency of micronucleated erythrocytes was observed in male mice, but the results were deemed equivocal in female mice. GBE at up to 2000 mg/kg/day was not genotoxic in a reporter gene mutation assay, a combined liver comet assay, and bone marrow micronucleus assay in mice.

In carcinogenicity studies of rats and mice conducted by the NTP, lesions in the liver, thyroid gland and nose were observed in all GBE dose groups (200 - 2000 mg/kg/day). Lesions included hypertrophy in the liver and thyroid gland in rats and mice, liver hyperplasia in male and female rats, and hyperplasia and atrophy of the epithelium in the nose of male and female rats. Inflammation, hyperplasia, hyperkeratosis, and ulcer were also observed in theforestomach of male and female mice. Additionally, increased incidences of cancers of the thyroid gland were observed in male and female rats and male mice, as were liver cancers in male and female mice. IARC has determined that GBE is possibly carcinogenic to humans (group 2B).
In a PLNA validation study, GBE exposure yielded statistically significant lymphoproliferative reactions in the ipsilateral popliteal lymph node, which was may have been caused by ginkgolic acid.

Reports of contact dermatitis have been reported following exposure to the fruit pulp of Ginkgo biloba. Patients have reported erythematous reactions and generalized exanthematous pustulosis following ingestion of GBE supplements. No adverse effects were reported in clinical studies of cosmetic formulations containing up to 1.5% GBE. In anecdotal accounts from Chinese medicine, consumption of fresh Ginkgo biloba nuts may cause stomachache, nausea, diarrhea, convulsions, weak pulse, restlessness, difficulty breathing, and shock. Death has been reported in children following consumption of fresh nuts.

No dermal or ocular irritation and no dermal sensitization studies were found in the published literature.

**DISCUSSION**

To be determined...

**CONCLUSION**

To be determined...
Draft NTP TR 578: Analysis of the specific Ginkgo biloba extract used in 2-year gavage studies
Prepared by the American Herbal Products Association (AHPA)
January 31, 2012

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Introduction and summary
The “Peer Review Draft” of NTP TR 578 (hereinafter Draft TR 578) identifies the test article that was used in the 2-year rodent gavage studies that are the subject of Draft TR 578 as “Ginkgo biloba extract from leaves.” Draft TR 578 further reports that two lots of the material were obtained from a company identified as Shanghai Xing Ling Science and Technology Pharmaceutical Company, Ltd. (hereinafter Shanghai Xing Ling); that just one of these lots, identified as Lot 020703 was used as the test article in the 2-year studies; that analyses of the test article were conducted by an analytical lab for identity, purity, stability, and moisture; and that confirming analysis for identity of the test article was conducted by another analytical lab.

This introduction provides a summary of a review undertaken by staff of the American Herbal Products Association (AHPA) in communication with several marketers of extracts of Ginkgo biloba leaf. Background and substantiating information for this summary are provided elsewhere in this AHPA review.

The test article is not similar to commonly marketed Ginkgo biloba leaf extracts. Draft TR 578 provides quantitative data from the above described analyses of the test article. Draft TR 578 also states that the test article is “similar to” a ginkgo¹ leaf extract marketed by Dr. Willmar Schwabe GmbH & Co. as EGB 761® and that the levels of certain constituents of the test article “have a similar ratio” of these constituents as is found in EGB 761®. Draft TR 578 also states that the levels of these constituents of the test article “reflect concentrations measured in commercially available [ginkgo] products” in the U.S.

In fact, the test article used in the described 2-year gavage studies is dissimilar to EGB 761®. NTP’s analysis of the test article measured flavonol glycosides at 31.2%, terpene lactones at 15.4%,² and ginkgolic acids at 10.45 ±2.40 ppm.³ ⁴ By comparison, EGB 761® is standardized to contain 24% flavonol glycosides and 6% terpene lactones and is manufactured to ensure that ginkgolic acids are present at no more than 5 ppm. It is thus quite clear that the test article is dissimilar to EGB 761® with regard to the levels of these compounds present. And Draft TR 578 provides no quantitative information on other constituents or classes of compounds that may or may not be present in the test article other than the 46.6% represented by flavonol glycosides and terpene lactones. There

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¹ The common name for Ginkgo biloba is ginkgo and the two terms are used interchangeably throughout this document.
² AHPA notes that the described quantitative assay of the terpene lactones bilobalide and ginkgolides were conducted after strong acidic hydrolysis. This is unusual as terpene lactones may be cleaved under such conditions. The chromatogram of the sample (page 1-6) shows strong baseline noise as high as the signals of ginkgolide B and C. It is questionable how an extract quantitation was feasible under such conditions and there is some possibility that measurement of these compounds has been understated.
³ This mean with stated standard deviation represents three separate results for the analysis of ginkgolic acid in the test article. The three data points were reported by the contracted analytical lab to be 8.975, 9.152, and 13.223 ppm.
⁴ Draft TR 578 states on page 1-3, “HPLC/MS analyses for the presence of ginkgolic acids I, II, and III ... resulted in no observable peaks of ginkgolic acids ....” This is apparently an error and should be corrected.
is thus no data presented on which to base a conclusion that this other 46.6% of the test article is "similar to" the other 70% of EGB 761®.

In addition, it is not accurate to identify the levels of flavonol glycosides and terpene lactones present in the test article as "reflect[ing] concentrations measured in commercially available [ginkgo] products" in the U.S. As Draft TR 578 points out in its Table 1, there is significant variation in ginkgo leaf extracts in the U.S. marketplace. The test article is just one of many of such variations, and compared to analysis of 50 other ginkgo leaf extract products identified in published studies, contains the highest level of terpene lactones (over 35% higher than the next highest sample) and the fourth highest level of flavonol glycosides. Draft TR 578 should refrain from making any statements that implies that the specific and unique Ginkgo biloba leaf extract used as the test article is in any manner representative of other ginkgo leaf extracts.

Any eventual final version of NTP TR 578 should therefore be revised, both in the title of the final draft and throughout the text, to clearly state that these 2-year gavage studies were conducted with a test article that is specific and unique, and that is dissimilar to commercially marketed ginkgo leaf extracts. Any eventual final version of NTP TR 578 should also state that the conclusions drawn from these 2-year gavage studies have not been shown to be relevant to any other ginkgo leaf extract.

There is no market data to support that the test article is sold in the U.S. Draft TR 578 also states that NTP had been informed that the Ginkgo biloba leaf extract produced by Shanghai Xing Ling was "widely distributed in commerce." Draft TR 578 lists "personal communication" as the citation for this information but provides no other details as to the nature of this personal communication.

AHPA was not aware at the time that Draft TR 578 was issued in December 2011 of the presence in the U.S. marketplace of Shanghai Xing Ling as a marketer of a ginkgo leaf extract or any other herbal extract. AHPA has since found that this company holds four U.S. patents on a proprietary ginkgo leaf extract that is significantly dissimilar to generally and commercially available ginkgo leaf extracts in the U.S.⁵, and that the company intended about a decade ago to seek approval of this proprietary extract as a drug in the U.S.⁶ AHPA contacted Shanghai Xing Ling through a Chinese speaking representative of one AHPA member; this representative was informed that Shanghai Xing Ling does not sell or market its own products that contain its proprietary ginkgo leaf extract in the United States. In addition, AHPA has never found this proprietary ginkgo leaf extract offered for sale in the U.S. as an ingredient for use in ginkgo products and is not aware of any ingredient supplier who offers for sale any Ginkgo biloba leaf extract that is standardized to contain more than 24% flavonol glycosides and 6% terpene lactones. Any eventual final version of NTP TR 578 should

⁵ U.S. Patents 6030621, 60187314, 05475534, and 06632460.
therefore be revised to remove any statements to the effect that the test material is widely distributed in commerce, and in fact should state that the ingredient is apparently not in commerce in the United States.

**Significant revisions should be made to Draft TR 578.** An addendum to this report provides specific suggestions for revisions to the title and text of Draft TR 578 to reflect the following facts with regard to the *Ginkgo biloba* leaf extract that served as the test article in the 2-year gavage studies that are its subject:

- That the test article is a unique and specific extract of *Ginkgo biloba* leaf.
- That the test article is dissimilar to EGB 761®, in fact, it is AHPA’s view that NTP TR 578 should limit its mention of this specific brand to the single report of NTP’s original intention to use this brand in these 2-year studies, and perhaps to its description in Table 1 therein.
- That the test article is dissimilar to most other commonly available *Ginkgo biloba* leaf extracts available in the U.S. market.
- That the test article is not known to be sold in the United States.
- That the conclusions presented in Draft TR 578 are not applicable to other *Ginkgo biloba* leaf extracts.

**Consideration should be given to other factors.** Consideration should be given to other possible explanations for and issues related to the study results reported in Draft TR 578 even as such results apply to the specific *Ginkgo biloba* leaf extract that served as the test article. These include the following at a minimum; each of these are discussed below, except that the first listed topic is discussed in a review of Draft TR 578 prepared for AHPA by Intertek Cantox and dated January 25, 2012.

- Absence of any information about analysis of the test article for residues of heavy metals, mycotoxins, microbiology, polyaromatic hydrocarbons, or pesticides that may have been present.
- The very high level of ginkgolic acids in the test article (10.45 ±2.40 ppm compared to an established standard of 5 ppm).
- Selection of corn oil as the vehicle for gavage administration.
- Various nomenclatural issues, including the use of a CAS number and botanical names to describe the specific tested ginkgo leaf extract.
- Absence of sufficient information to describe how the specific test article was manufactured.

**Identity of the specific *Ginkgo biloba* leaf extract test article**

The abstract of draft TR 578 provided the results of analysis of the test article provided by Shanghai Xing Ling. The test article is characterized as containing 31.2% flavonol glycosides, 15.4% terpene lactones, and 10.45 ±2.40 ppm ginkgolic acid.

Shanghai Xing Ling has intentionally developed a unique *Ginkgo biloba* leaf extract that is intended to be dissimilar to other ginkgo leaf extracts. This extract or an apparently similar *Ginkgo biloba* leaf extract manufactured by Shanghai
Xing Ling has been described "as a new multicomponent drug" in the scientific literature.\textsuperscript{7,8} The company holds four U.S. patents, dated between 2000 and 2003, for a ginkgo leaf extract.\textsuperscript{9} The most recent of these patents\textsuperscript{10} includes several statements that are relevant to any evaluation as to whether ginkgo leaf extracts provided by this supplier are similar or dissimilar to other ginkgo leaf extracts sold in the U.S. For example, this patent states (\textit{emphasis added} throughout):

- That one object of the company's invention of a specific and proprietary ginkgo leaf extract is "to provide a Ginkgo biloba extract with a \textit{highly concentrated effective content}, that include 44 to 78% flavonoids [later described as having a content of "about 20% to about 75% flavonol glycosides], 2.5 to 10% ginkgolides and 2.5 to 10% bilobalide." Note that the described extract could consist, at the high end of each of the stated ranges, of as much as 95% of a combination of flavonol glycosides and terpene lactones (i.e., the ginkgolides and bilobalide), leaving only 5% for those constituents that make up 70% of the Schwabe ginkgo leaf extract, EGB\textsubscript{761}.\textsuperscript{6}

- States, "\textit{Until now it has not been possible} to prepare such highly concentrated extracts from Ginkgo biloba leaves."

- Identifies an "advantage of a Ginkgo biloba extract with highly concentrated effective content" as "the \textit{reduced daily dosage} and smaller size of the pharmaceutical prepared from it."

- Claims another "advantage of a Ginkgo biloba extract with highly concentrated effective content" to be "\textit{further removal of inactive substances,}" apparently meaning removal of any constituents other than the flavonoids or terpene lactones.

- Notes that the patent "relates generally to compositions extracted from Ginkgo biloba leaves and particularly to a \textit{different composition comprising new active components and combinations}.

In summary, Shanghai Xing Ling clearly produces a different, unique, and proprietary \textit{Ginkgo biloba} leaf extract. The ginkgo leaf extract described in its U.S. patents is novel and "until now ... has not been possible" to produce; contains "more highly concentrated" levels of flavonol glycosides than other such extracts; seeks "further removal" of any other constituents naturally found in ginkgo leaf; is of a "different composition" than other ginkgo leaf extracts; and allows for a "reduced daily dosage."

This patent also states that Shanghai Xing Ling's proprietary ginkgo leaf extract is manufactured to contain "about 0.1 ppm to 5 ppm ginkgolic acids," though at


\textsuperscript{9} U.S. Patents 60630621, 06187314, 06475534, and 06632460.

other times the patent identifies the intended level of ginkgolic acids to be “about 0.1 ppm to 0.5 ppm.”

AHPA cannot say with any certainty whether the *Ginkgo biloba* leaf extract provided by Shanghai Xing Ling to NTP as the test article for the 2-year gavage studies that are the subject of Draft TR 578 is or is not the same article as is described in the company’s U.S. patents. AHPA notes however that the test article’s analysis conforms to the patent with regard to the levels of flavonol glycosides (present at 31.2%, within the patents’ range of 20-75%); bilobalide (6.94%, in the patents’ range of 2.5-10%); and ginkgolides (8.42%, again in the stated 2.5-10% range of the patented ingredient). In fact the only measured parameter described in the patent that is not met in the test article is the level of ginkgolic acids, which was recorded as present at 10.45 ±2.40 ppm, and so considerably higher than described in the patent.

Of additional interest is that Shanghai Xing Ling reportedly signed an agreement with a contract research organization to conduct clinical trials on its patented ginkgo leaf extract with a goal of obtaining FDA drug approval for the treatment of stable angina.\(^{11}\)

It is apparent that the *Ginkgo biloba* leaf extract used as the test article in NTP’s 2-year gavage studies is certainly a different ingredient than EGb 761\(^{\circledast}\). In addition, it cannot be characterized as representative of any other ginkgo leaf extract. It is also probable that the manufacturer intended, through its proprietary manufacturing process, to create a unique ingredient that is unlike other *Ginkgo biloba* leaf extracts. The results of these 2-year studies should therefore not be assumed to be readily extrapolated to any other ginkgo leaf extract and any revision to Draft TR 578 should refrain from making any statements that expressly or implicitly associate the test article with EGb 761\(^{\circledast}\) or any other *Ginkgo biloba* leaf extract.

**Ginkgo biloba** leaf extracts in the U.S. market

Extracts of ginkgo leaf are broadly sold in the U.S. as dietary supplements. While products that contain EGb 761\(^{\circledast}\) are present in the U.S. market, as Draft TR 578 notes (citing Kressmann, 2002), there is great variety among the numerous ginkgo leaf extracts in the U.S. marketplace.

It is absolutely certain that the test article was not EGb 761\(^{\circledast}\) and its chemical profile is significantly different than that of this branded Schwabe product. Draft TR 578 should be revised to remove any statement to the effect that the test article is in any way similar to EGb 761\(^{\circledast}\). Except for the fact that both are derived from the leaf of the *Ginkgo biloba* tree, there is nothing to associate these two dissimilar ingredients.

It is also clear that the specific ginkgo leaf extract used as the test article cannot be represented as the same as any other marketed ginkgo leaf extract. The

Kressmann (2002) article cited in Draft TR 578 shows a very broad range in the levels of flavonol glycosides (from 23.88 ±0.21 to 35.54 ±1.03%), terpene lactones (from 3.87 ±1.09 to 11.31 ±0.17%), and ginkgolic acids (from <500 ppm (the study's limit of quantification) to 89576 ±2297 ppm) in 26 analyzed Ginkgo biloba leaf extract products marketed in the U.S. Only three of these tested products were reported to contain more than 31.2% flavonol glycosides, the amount found in the test article; none contained as high a level of terpene lactones as the 15.4% reported in the test article. A similar product review conducted at about the same time measured a range of from 0.4 to 26.2% flavonol glycosides and 0.6 to 8.2% terpene lactones in 14 ginkgo products in tablet or capsule forms. A more recent analysis of 10 U.S. marketed products containing ginkgo leaf extracts standardized to 24% flavonol glycosides and 6% terpene lactones found three of them to contain less than the amount claimed but none were reported to contain an excess of either. Thus the test article contains a higher level of flavonol glycosides than all but three of the 50 products tested in these three studies and more terpene lactones than any other of these tested products. These facts, combined with the analytical data that shows that the amount of ginkgolic acids in the test article exceeds the limits established by regulatory and pharmacopoeial standards by more than 100%, must be seen as contradicting any representation of the test article as "reflect[ing] concentrations [of contained constituents] measured in commercially available [ginkgo] products" in the U.S.

Regulatory and pharmacopoeial standards for Ginkgo biloba leaf extract
Every dietary supplement that consists of or contains a Ginkgo biloba leaf extract that is marketed in the United States is required to meet all claims made on the product's label, including any claims for the ingredient's identity and for the level of contained constituents, such as flavonol glycosides and terpene lactones. But the U.S. law does not require every ginkgo leaf extract to be

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12 Kressmann S, Müller WE, and Blume HH. Pharmaceutical quality of different Ginkgo biloba brands. 2002. J Pharm Pharmacol 54:661-669. The authors analyzed 27 products, one of which consisted only of ginkgo leaf (not an extract) and is not considered here.


15 21 CFR 111.75(c).

16 21 CFR 101.36(f)(1) and 101.9(g)(3) and (g)(4). These Federal regulations require any "added" constituent to be present in an amount that is "at least equal to the value" declared on labeling (referred to as "the 100 percent standard"). FDA has communicated its position that a substance is considered an added substance "if the manufacturer manipulates its level .... For example, in a standardized herbal extract, because the manufacturer controls the amount of the standardized substance in the extract, that substance (the dietary ingredient) is an added dietary ingredient and is subject to the 100 percent standard."(Letter from FDA (DE Baker, Associate Commissioner for Regulatory Affairs) to Capsugel (RJ Dennin); October 19, 1999).
identical or to conform to any specific standard, although any product that claims
to comply with an identified standard must, in fact, comply with that standard.17

Such standards do, however, exist in some other countries and in extant
pharmacopoeial references. The following table provides some examples of such
standards and presents for comparison the analytical data on the test article
used in the NTP 2-year gavage studies.

Table A. Standards for Levels of Constituents in Ginkgo biloba Extracts

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Health Canada18</td>
<td>22-27%</td>
<td>-</td>
<td>-</td>
<td>5-7%</td>
<td>-</td>
</tr>
<tr>
<td>German Commission E19</td>
<td>22-27%</td>
<td>2.6-3.2%</td>
<td>2.8-3.4%</td>
<td>5-7%</td>
<td>&lt;5 ppm</td>
</tr>
</tbody>
</table>
| American Herbal
Pharmacopoeia20        | 22-27%                 | -            | -             | 5-7%                        | -                |
| European Pharmacopoeia21| 22.0-27.0%             | 2.6-3.2%     | 2.8-3.4%      | 5.4-6.6%                    | <5 ppm           |
| US Pharmacopoeia22      | 22.0-27.0%             | 2.6-5.8%     | 2.8-6.2%      | 5.4-12.0%                   | <5 ppm           |
| Test article            | 31.2%                  | 6.94%        | 8.42%         | 15.4%                       | 10.45 ±2.40     |

As is obvious from Table A, the NTP test article fails to meet any of the listed
standards because the level of each of the described and quantified constituents
is higher in the test article than is accepted by any of the standards.

It must therefore be concluded that the 2-year gavage studies reported in Draft
TR 578 have relevance only to the test article itself, as provided by Shanghai
Xing Ling, and that these studies provide no information that has been shown to
be relevant to any Ginkgo biloba leaf extract that conforms to one or another of
these standards.

Use of corn oil as the vehicle for the test article raises questions
Draft TR 578 reports that corn oil was used as the vehicle for gavage
administration of the test article, a detail that raises significant concerns.

Ingredient and Product Monographs. Ottawa (ON): Health Canada, Natural Health Products Directorate
19 Blumenthal M, Busse WR, Goldberg A et al., editors. The Complete German Commission E
20 Upton R et al. American Herbal Pharmacopoeia and Therapeutic Compendium: Ginkgo Leaf; Ginkgo
Strasbourg, France: European Directorate for the Quality of Medicines & Healthcare.
The United States Pharmacopeial Convention.
Corn oil is commonly used as a vehicle for gavage administration of pure chemicals. No analysis has been done, however, to determine whether it is an appropriate vehicle for a "complex mixture of chemical constituents," as Draft TR 578 accurately describes *Ginkgo biloba* leaf and as is also accurate for the tested ginkgo leaf extract. There is little or no information, and no analytical data, to indicate that the test article was distributed in the corn oil vehicle in a manner that ensured absorption of the entire test article. For example, no analysis was done to determine whether some compounds within the specific *Ginkgo biloba* leaf extract used as the test article may have been enriched in this vehicle or whether other compounds, including potentially protective compounds, were present in a form in the corn oil vehicle that would allow for absorption.

Corn oil has frequently been used in toxicity studies as the dosing vehicle for lipophilic chemicals such as halogenated hydrocarbons. But ginkgo leaf extracts are usually produced by extraction with aqueous alcohols or acetone, and generally include lipophilic constituents, such as ginkgolic acids, as well as compounds that are water soluble and would not be dissolved in oil. It is therefore possible that the actual material fed to the test animals – that is, the mixture of corn oil and the specific *Ginkgo biloba* leaf extract used in these studies – was not precisely representative of the extract itself. Absent analysis of this extract/oil mixture there can be no certainty of the identity of the material actually consumed in these studies.

There is also concern related to the known potential for corn oil itself to have a toxicological effect on test animals. NTP produced a Technical Report in 1992 to evaluate the comparative toxicology of corn oil, safflower oil, and tricaprylin for use as a vehicle for gavage in studies in male F344/N rats.\(^\text{23}\) One of the conclusions in this report stated, "the use of corn oil as a gavage vehicle may have a confounding effect on the interpretation of chemical-induced proliferative lesions of the exocrine pancreas and mononuclear cell leukemia in male F344/N rats." An increased rate of mononuclear cell leukemia was observed in the male rats in the 2-year gavage studies of the tested ginkgo extract, yet there is no discussion in Draft TR 578 of the noted "confounding effect" of the vehicle on this finding of the study.

This concern is not entirely conceptual as there is some evidence that corn oil as a vehicle for gavage can have an effect on the toxicology of a studied material. For example, administration of chloroform by corn oil gavage for 90 days resulted in significantly greater hepatotoxicity in male and female B6C3F1 mice than with aqueous administration.\(^\text{24}\) It has also been suggested that oral consumption of corn oil enhances the toxicity and carcinogenicity of volatile organic compounds in rodents, and that this effect could be "due to induction of metabolizing


enzymes, thus increasing the generation of reactive intermediates." In addition, in a study that examined the effects of various levels of corn oil and lard fed during the initiation stage of azoxymethane-induced hepatocarcinogenesis in male Fischer 344 rats, an enhancing effect on hepatocarcinogenesis was observed with a corn oil diet compared with a lard diet.

In presenting this issue AHPA is not suggesting that all of the effects observed in the 2-year gavage studies were caused by the corn oil used as the gavage vehicle. Rather this information suggests that the conclusions presented in Draft TR 578 must be seen as inconclusive until the well established adverse effects of corn oil itself are addressed, and analysis is conducted to demonstrate that corn oil is a valid vehicle for administration of the complex mixture represented by the test article.

Potential safety concerns related to ginkgolic acids
As noted in Table A, it is a common practice to limit the level of ginkgolic acids in ginkgo leaf extracts to 5 ppm, even though both beneficial and harmful properties have been reported to be associated with these alkylphenol compounds. Reported negative associations have included "contact allergenic, cytotoxic, embryotoxic, immunotoxic, mutagenic and slight neurotoxic" properties, though it is also reported that "there is no conclusive evidence that oral consumption of Ginkgo leaves or full extracts containing as much as 22,000ppm (2.2%) of ginkgolic acids leads to allergic reactions or other serious side effects."

Nonetheless, the fact that the test article was measured to contain 10.45 ±2.40 ppm ginkgolic acid should be considered as a possible explanation for at least some of the results observed in the NTP studies.

Use of a CAS number to describe the test article is inaccurate
Draft TR 578 includes in its description of the identity of the test article a specific CAS number, number 90045-36-6. CAS No. 90045-36-6 is defined as:

"Extractives and their physically modified derivatives such as tinctures, concretes, absolutes, essential oils, oleoresins, terpenes, terpene-free fractions, distillates, residues, etc., obtained from Ginkgo biloba, Ginkgoaceae."

This definition is so broad as to include virtually any extractive or derivative obtained from Ginkgo biloba leaf, stem, root, bark, fruit or seed, regardless of significant variations in the chemical composition among all of these possible substances. To represent the test article used in the 2-year gavage studies by such a broad term is inaccurate, and could have the effect of implying that the results of these studies are relevant to any and all extractives and derivatives

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25 Ibid.
28 Ibid.
obtained from any part of the ginkgo tree. This term should be removed from any future revisions to Draft TR 578.

**Scientific and common names of the Ginkgo biloba tree are not synonyms of Ginkgo biloba extract**

Draft TR 578 presents Ginkgo biloba as the “botanical name” of the test article, and also lists several of the common name synonyms for the ginkgo tree as synonyms for the ginkgo extract used in the study. Use of these names for a Ginkgo biloba extract is not strictly accurate and AHPA recommends that these names be removed.

**Study reproducibility is compromised**

The National Center for Complementary and Alternative Medicine (NCCAM) at NIH has recognized the importance of proper characterization of study materials in order to fund replicable research on natural products, including complex botanical products. For animal studies that employ complex botanical products, such as extracts made from the leaves of Ginkgo biloba, NCCAM’s Policy on Natural Product Integrity requires, among other things, information relevant to the standardization process. That information should include a description of the manufacturing process with details of the extraction such as solvent(s), ratio of plant to solvent, extraction time and temperature, and data on batch-to-batch reproducibility.\(^{29}\)

Without this information it is not possible to reproduce the research on another batch of the specific Ginkgo biloba leaf extract from Shanghai Xing Ling, much less to apply the research to any dissimilar ginkgo extract such as EGb 761\(^{10}\) or any other ginkgo leaf extract.

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Addendum
In order to make the above comments most useful and to best clarify AHPA’s intended meanings herein, in several places within these comments AHPA has suggested that revisions be made Draft TR 578. These recommendations are clarified below in the form of proposed textual revisions. With each proposal, AHPA identifies language recommended for deletion with strikethrough text, and language recommended for addition in bold underline font.

This addendum should not, however, be viewed as an exhaustive list of changes that would need to be made to Draft TR 578 in order to take into account the comments submitted in this review and AHPA requests that complete review and revision of Draft TR 578 be undertaken in order to ensure that any final Technical Report on these 2-year studies is completely accurate and does not in any manner imply that these studies are relevant to any Ginkgo biloba leaf extract other than the specific test article.

Page P1:

NTP TECHNICAL REPORT
ON THE
TOXICOLOGY AND CARCINOGENESIS
STUDIES OF A SPECIFIC
GINKGO BILOBA LEAF EXTRACT
(CAS-No.-90045-36-6)

Page P3:

NTP TECHNICAL REPORT
ON THE
TOXICOLOGY AND CARCINOGENESIS
STUDIES OF A SPECIFIC
GINKGO BILOBA LEAF EXTRACT
(CAS-No.-90045-36-6)

Header on pages 3, 5, 6, 8, 9, 10, and wherever the term “Ginkgo biloba Extract” appears throughout the document:

A Specific Ginkgo biloba Leaf Extract, NTP TR 578

Pages 7 and 19:

A SPECIFIC GINKGO BILOBA LEAF EXTRACT
CAS-No.-90045-36-6
Synonyms: Ginkgo, Ginkgo biloba, fossil tree, maidenhair tree, Japanese silver apricot, baigou, bei gu yu, kew tree, yin hsing (yin hsing)

Botanical name: Ginkgo biloba

The Ginkgo biloba extract used in the current studies was procured from a supplier known to provide material to United States companies and contained 31.2% flavonol glycosides, 15.4% terpene lactones (6.94% bilobalide, 3.74% ginkgolide A, 1.62% ginkgolide B, 3.06% ginkgolide C), and 10.45 ppm ginkgolic acid.

Page 12:

Conclusions
Under the conditions of these 2-year gavage studies, there was some evidence of carcinogenic activity* of a specific Ginkgo biloba leaf extract in male F344/N rats based on increased incidences of thyroid gland follicular cell adenoma. The increased incidences of mononuclear cell leukemia and hepatocellular adenoma may have been related to Ginkgo biloba extract administration. There was some evidence of carcinogenic activity of the specific Ginkgo biloba leaf extract in female F344/N rats based on increased incidences of thyroid gland follicular cell neoplasms. Increased occurrence of respiratory epithelium adenomas in the nose may have been related to Ginkgo biloba extract administration. There was clear evidence of carcinogenic activity of the specific Ginkgo biloba leaf extract in male B6C3F1/N mice based on increased incidences of hepatocellular carcinoma and hepatoblastoma. The increased incidences of thyroid gland follicular cell adenoma were also related to the specific Ginkgo biloba leaf extract administration. There was clear evidence of carcinogenic activity of the specific Ginkgo biloba leaf extract in female B6C3F1/N mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma.

Administration of the specific Ginkgo biloba leaf extract resulted in increased incidences of nonneoplastic lesions in the liver, thyroid gland, and nose of male and female rats and mice and the forestomach of male and female mice. Increased severity of nephropathy in male rats was also due to administration of the specific Ginkgo biloba leaf extract.

Because the specific Ginkgo biloba leaf extract used in these studies may or may not be similar to other Ginkgo biloba leaf extracts sold in the U.S. or in other countries the conclusions given here should not be extrapolated to any other Ginkgo biloba leaf extract.

Page 20:

The main constituents of Ginkgo biloba leaves and their concentrations in standardized Ginkgo biloba extract (EGb 761®) and other commercially available preparations are shown in Table 1. The extract used in this study was not characterized to this extent and had significant chemical differences with respect to all quantified constituents including flavonol glycosides, terpene lactones, and ginkgolic acids.

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>Constituents of Ginkgo biloba</td>
</tr>
<tr>
<td>Extract/Class</td>
</tr>
<tr>
<td>-------------------------------</td>
</tr>
<tr>
<td>Terpene tri lactones</td>
</tr>
<tr>
<td>Flavonol glycosides</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Bisflavones</td>
</tr>
<tr>
<td>Fenanthrocyanidins</td>
</tr>
<tr>
<td>Alkylphenols</td>
</tr>
<tr>
<td>Carboxylic acids</td>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Flavonols-Catechins</td>
</tr>
<tr>
<td>Polyprenoids</td>
</tr>
<tr>
<td>Non-flavonol glycosides</td>
</tr>
<tr>
<td>High molecular weight</td>
</tr>
<tr>
<td>compounds</td>
</tr>
<tr>
<td>Impregnate constituents</td>
</tr>
<tr>
<td>Water-solvent</td>
</tr>
<tr>
<td>Various</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
</tbody>
</table>

Page 21-22:

In 1965, the German physician-pharmacist Dr. Willmar Schwabe III developed *Ginkgo biloba* leaf extracts (De Feudis, 2003). The final product, a standardized *Ginkgo biloba* extract (EGb 761®), has been subsequently marketed by Dr. Willmar Schwabe Pharmaceuticals under the trade names Ginkgold® (Nature’s Way™), Kaveri®, Rökan®, Tanakan®, and Tebonin®. EGb 761® is a quantified refined extract standardized to containing 24% flavonoid glycosides (primarily derivatives of quercetin, kaempferol, and isorhamnetin), 6% terpene lactones [3.1% ginkgolides (A, B, C, J) and 2.9% bilobalide], various organic acids (5% to 10%), and other constituents (Table 1). Many *Ginkgo biloba* components are biologically active, and it is believed that the action of multiple constituents contributes to the medicinal properties of the plant leaf extract. However, the standardization of EGb 761® and other *Ginkgo biloba* extracts is based on their flavonoid and terpene tri lactone contents (Figure 1), as these compounds are thought to be primarily responsible for the pharmacological activity associated with *Ginkgo biloba* extract.

In the United States, herbal formulations sold as dietary supplements such as *Ginkgo biloba* extract are regulated under the Dietary Supplement Health and Education Act of 1994 (DSHEA). As such, they are not subject to the same standards of pre-market testing as drugs intended to treat, cure, prevent, diagnose, or mitigate disease. In contrast, in Germany and France *Ginkgo biloba* dried leaf extract is regulated as a prescription drug and therefore, requires registration and adherence to specified content standards. For *Ginkgo biloba* dried leaf extracts, these are 22.0% to 27.0% flavonoid glycosides, 6% to 7% terpene lactones (2.82-6% to 3.43-2% ginkgolides A, B, C, and 2.6% to 3.2% bilobalide), and not more than 5 ppm ginkgolic acids, due to their cytotoxic and allergenic potential (Kressmann et al., 2002). In the United States, a wide range of component concentrations is observed in available *Ginkgo biloba* products (Table 1)
(Kressmann et al., 2002). However, analyses by independent investigators showed variation even in the composition of the standardized extracts (Woerdenbag and van Beek, 1997).

Page 23:

FIGURE 1
Structures of Flavonoid Glycoside Aglycone and Terpene Trilactone Contents of Standardized Ginkgo biloba Extract

Page 34:

The test article selection was based on availability of bulk product and market share of the manufacturer at the study initiation.

Page 35:

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

A Specific Ginkgo biloba Leaf Extract

The original intent was to use standardized extract EGB 761®, manufactured by Wilhelm Schwabe, due to its use in many human studies. However, this material was not available to the NTP because unformulated EGB 761® was exclusively sold to pharmaceutical companies at the time of procurement for the NTP studies. Through industry contacts, the NTP learned that Shanghai Xing Ling Science and Technology Pharmaceutical Company (Shanghai, China) produced an extract purported to be similar to the Schwabe extract that was said to be widely distributed in commerce (personal communication). NTP does not however know whether this ingredient is now or has ever been sold or offered for sale in the United States. A specific Ginkgo biloba extract made from leaves was nonetheless obtained from Shanghai Xing Ling Science and Technology Pharmaceutical Company, Ltd., in two lots (020703 and GBE-50-001003). Lot 020703 was used during the 3-month and 2-year studies. Lot GBE-50-001003 was used only for methods development. Identity, purity, stability, and moisture analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO); in addition, the study laboratory at Battelle Columbus Operations (Columbus, OH) confirmed the identity of the test article by infrared spectroscopy (Appendix I). Reports on analyses performed in support of the Ginkgo biloba extract studies are on file at the National Institute of Environmental Health Sciences.

Page 36:

Quantitation assays of α-glycosides in the hydrolyzed extracts using HPLC/UV indicated that the test material contained 16.71% quercetin glycosides, 12.20% kaempferol glycosides, and 2.37% isorhamnetin glycosides.
DISCUSSION AND CONCLUSIONS

Ginkgo biloba extract is a popular herbal supplement used to improve brain function. As with many natural products, there is significant variability in the contents of Ginkgo biloba extract available in the marketplace (Kressmann et al., 2002; Agnolet et al., 2010; Gawron-Gzella et al., 2010; Chandra et al., 2011). In a 2002 study analyzing Ginkgo biloba extract constituents from products available in the United States, Kressmann et al. (2002) found a range of concentrations for flavonol glycosides (24% to 36%), terpene lactones (4% to 11%), and ginkgolic acids (less than 500 ppm to 90,000 ppm). The Ginkgo biloba extract used in the present studies contained 31.2% flavonol glycosides, 15.4% terpene lactones (6.94% bilobalide, 3.74% ginkgolide A, 1.62% ginkgolide B, 3.06% ginkgolide C), and 10.45 ppm ginkgolic acid. These values do not reflect concentrations measured in the most common commercially available products in the United States and have a similar ratio of active ingredients to all exceed the specifications for the standardized Ginkgo biloba leaf extract known as (EGb 761®).

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was some evidence of carcinogenic activity* of a specific Ginkgo biloba leaf extract in male F344/N rats based on increased incidences of thyroid gland follicular cell adenoma. The increased incidences of mononuclear cell leukemia and hepatocellular adenoma may have been related to Ginkgo biloba extract administration. There was some evidence of carcinogenic activity of the specific Ginkgo biloba leaf extract in female F344/N rats based on increased incidences of thyroid gland follicular cell neoplasms. Increased occurrence of respiratory epithelium adenomas in the nose may have been related to Ginkgo biloba extract administration. There was clear evidence of carcinogenic activity of the specific Ginkgo biloba leaf extract in male B6C3F1/N mice based on increased incidences of hepatocellular carcinoma and hepatoblastoma. The increased incidences of thyroid gland follicular cell adenoma were also related to the specific Ginkgo biloba leaf extract administration. There was clear evidence of carcinogenic activity of the specific Ginkgo biloba leaf extract in female B6C3F1/N mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma.

Administration of the specific Ginkgo biloba leaf extract resulted in increased incidences of nonneoplastic lesions in the liver, thyroid gland, and nose of male and female rats and mice and the forestomach of male and female mice. Increased severity of nephropathy in male rats was also due to administration of the specific Ginkgo biloba leaf extract.

Because the specific Ginkgo biloba leaf extract used in these studies may or may not be similar to other Ginkgo biloba leaf extracts sold in the U.S. or in other countries the conclusions given here should not be extrapolated to any other Ginkgo biloba leaf extract.
CHEMICAL CHARACTERIZATION
AND DOSE FORMULATION STUDIES
PROCUREMENT AND CHARACTERIZATION
Ginkgo biloba Leaf Extract
Although the original study planned to use standardized extract EGB 761®, manufactured by Wilhelm Schwabe, this material was not available to the NTP because at the time of procurement for the NTP studies, this standardized extract was sold unformulated only to Pharma. Through industry contacts, the NTP learned that Shanghai Xing Ling Science and Technology Pharmaceutical Company (Shanghai, China) produced an extract purported to be similar to the Schwabe extract and that was said to be widely distributed in commerce. NTP does not however know whether this ingredient is now or has ever been sold or offered for sale in the United States. A Ginkgo biloba leaf extract was nonetheless obtained from Shanghai Xing Ling Science and Technology Pharmaceutical Company, Ltd. in two lots (020703 and GBE-50-001003). Lot 020703 was used during the 3-month and 2-year studies. Identity, purity, stability, and moisture analyses were conducted by the analytical chemistry laboratory at Midwest Research Institute (Kansas City, MO); in addition, the study laboratory at Battelle Columbus Operations (Columbus, OH) confirmed the identity of the test article versus a frozen reference of the same lot, shipped separately, by infrared spectroscopy. Reports on analyses performed in support of the Ginkgo biloba extract studies are on file at the National Institute of Environmental Health Sciences.

... For these assays, methanol:water (50:50) extracts of the specific Ginkgo biloba powdered leaf extract were partitioned with dichloromethane and dried over anhydrous sodium sulfate. The residue was reconstituted with methanol and analyzed using total ion current and single ion response mode following the methodology of Ndjoko et al. (2000) and Li et al. (2002). Further information on these methods can be found in Gray et al. (2005, 2007).

Page I-3:

Quantitation assays of α-glycosides in the hydrolyzed extracts using HPLC/UV indicated that the test material contained 16.71% quercetin glycosides, 12.20% kaempferol glycosides, and 2.37% isorhamnetin glycosides. ... HPLC/MS analyses for the presence of ginkgolic acids I, II, and III using standards from ChromaDex, Inc. (Irvine, CA), and for colchicine using the colchicine standard from Sigma-Aldrich, resulted in no observable peaks of ginkgolic acids or colchicine in the test material.