



## **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)  
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### **Table of Contents**

Introduction and summary .....	2
Identity of the specific <i>Ginkgo biloba</i> leaf extract test article .....	4
<i>Ginkgo biloba</i> leaf extracts in the U.S. market .....	6
Regulatory and pharmacopoeial standards for <i>Ginkgo biloba</i> leaf extract .....	7
Use of corn oil as the vehicle for the test article raises questions .....	8
Potential safety concerns related to ginkgolic acids .....	10
Use of a CAS number to describe the test article is inaccurate .....	10
Scientific and common names of the <i>Ginkgo biloba</i> tree are not synonyms of <i>Ginkgo biloba</i> extract .....	11
Study reproducibility is compromised .....	11
Addendum .....	12

## 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 ginkgo<sup>1</sup> leaf extract marketed by Dr. Willmar Schwabe GmbH & Co. as EGb 761<sup>®</sup> and that the levels of certain constituents of the test article “have a similar ratio” of these constituents as is found in EGb 761<sup>®</sup>. 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<sup>®</sup>. NTP’s analysis of the test article measured flavonol glycosides at 31.2%, terpene lactones at 15.4%,<sup>2</sup> and ginkgolic acids at 10.45 ±2.40 ppm.<sup>3, 4</sup> By comparison, EGb 761<sup>®</sup> 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<sup>®</sup> 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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<sup>1</sup> The common name for *Ginkgo biloba* is ginkgo and the two terms are used interchangeably throughout this document.

<sup>2</sup> 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 I-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.

<sup>3</sup> 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.

<sup>4</sup> Draft TR 578 states on page I-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<sup>®</sup>.

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.<sup>5</sup>, and that the company intended about a decade ago to seek approval of this proprietary extract as a drug in the U.S.<sup>6</sup> 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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<sup>5</sup> U.S. Patents 06030621, 06187314, 06475534, and 06632460.

<sup>6</sup> <http://www.quintiles.com/news/press-releases/1999-11-12/quintiles-signs-agreement-with-chinese-pharmaceutical-company-to-conduct-clinical-research-for-herbal-product-to-treat-s/> accessed January 12, 2012.

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<sup>®</sup>; 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 ( $10.45 \pm 2.40$  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  $10.45 \pm 2.40$  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.<sup>7, 8</sup> The company holds four U.S. patents, dated between 2000 and 2003, for a ginkgo leaf extract.<sup>9</sup> The most recent of these patents<sup>10</sup> 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<sup>®</sup>.
- 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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<sup>7</sup> Liu A, Zhang Z. [Effect of GBE50 on action potentials in normal and simulated ischemic guinea pig papillary muscles]. *Zhongguo Zhong Yao Za Zhi*. 2010 Sep;35(17):2342-5. [Article in Chinese].

<sup>8</sup> Liu AH, Zhang ZX, Wang XY. [Effect of GBE50 on delayed rectifier potassium current of ventricular myocytes in ischemic guinea pig]. *Zhongguo Ying Yong Sheng Li Xue Za Zhi*. 2010 Nov;26(4):444-8. [Article in Chinese].

<sup>9</sup> U.S. Patents 06030621, 06187314, 06475534, and 06632460.

<sup>10</sup> U.S. Patent 06632460, dated October 14, 2003.

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.<sup>11</sup>

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<sup>®</sup>. 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup>. 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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<sup>11</sup> <http://www.quintiles.com/news/press-releases/1999-11-12/quintiles-signs-agreement-with-chinese-pharmaceutical-company-to-conduct-clinical-research-for-herbal-product-to-treat-s/> accessed January 12, 2012.

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.<sup>12</sup> 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.<sup>13</sup> 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.<sup>14</sup> 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<sup>15</sup> and for the level of contained constituents, such as flavonol glycosides and terpene lactones.<sup>16</sup> But the U.S. law does not require every ginkgo leaf extract to be

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<sup>12</sup> 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.

<sup>13</sup> Baker AD, Brymer C, and Borrie MJ. *An analysis of Ginkgo biloba: Do you get what you pay for?* 2002. *Geriatrics Today* 5:13-16.

<sup>14</sup> ConsumerLab.com. Product Review: Supplements for memory & cognition enhancement (Ginkgo, Huperzine-A, and Aceyl-L-Carnitine). Last updated 12/30/09. Accessed January 29, 2012 at [https://www.consumerlab.com/reviews/Memory\\_Supplements\\_Ginkgo\\_Huperzine\\_Acetyl-L-Carnitine/GinkgoBiloba/](https://www.consumerlab.com/reviews/Memory_Supplements_Ginkgo_Huperzine_Acetyl-L-Carnitine/GinkgoBiloba/) (password required). One other product included in this report contained ginkgo leaf rather than an extract and is therefore not addressed here.

<sup>15</sup> 21 CFR 111.75(c).

<sup>16</sup> 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.<sup>17</sup>

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*

	1. Flavonol glycosides	2. Bilobalide	3. Ginkgolides	4. Terpene lactones (2 + 3)	5. Ginkgolic acids
Health Canada <sup>18</sup>	22-27%	-	-	5-7%	-
German Commission E <sup>19</sup>	22-27%	2.6-3.2%	2.8-3.4%	5-7%	<5 ppm
American Herbal Pharmacopoeia <sup>20</sup>	22-27%	-	-	5-7%	-
European Pharmacopoeia <sup>21</sup>	22.0-27.0%	2.6-3.2%	2.8-3.4%	5.4-6.6%	<5 ppm
US Pharmacopoeia <sup>22</sup>	22.0-27.0%	2.6-5.8%	2.8-6.2%	5.4- 12.0%	<5 ppm
<b>Test article</b>	<b>31.2%</b>	<b>6.94%</b>	<b>8.42%</b>	<b>15.4%</b>	<b>10.45 ±2.40</b>

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.

<sup>17</sup> 21 U.S.C. 343(s)(2)(D).

<sup>18</sup> Health Canada. Monograph: Ginkgo Biloba. In: *Natural Health Products Ingredients Database - Single Ingredient and Product Monographs*. Ottawa (ON): Health Canada, Natural Health Products Directorate (NHPD). 2009. Accessed on January 25, 2012 at: <http://webprod.hc-sc.gc.ca/nhp/nd/bdipsn/monoReq.do?id=100&lang=eng>.

<sup>19</sup> Blumenthal M, Busse WR, Goldberg A et al., editors. *The Complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines*. 1998. Austin (TX): American Botanical Council.

<sup>20</sup> Upton R et al. *American Herbal Pharmacopoeia and Therapeutic Compendium: Ginkgo Leaf; Ginkgo Leaf Dry Extract; Ginkgo biloba L*. 2003. Scotts Valley, CA: American Herbal Pharmacopoeia.

<sup>21</sup> *European Pharmacopoeia 7.0*. Monograph: Ginkgo Dry Extract, Refined and Quantified. 2010. Strasbourg, France: European Directorate for the Quality of Medicines & Healthcare.

<sup>22</sup> *United States Pharmacopoeia, Thirty-Fourth Revision: Powdered Ginkgo Extract*. 2010. Rockville, MD: The United States Pharmacopoeial 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.<sup>23</sup> 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.<sup>24</sup> 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

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<sup>23</sup> NTP Technical Report Series No. 426. Comparative Toxicology Studies of Corn Oil, Safflower Oil, and Tricaprylin (CAS Nos. 8001-30-7, 8001-23-8, and 538-23-8) in Male F344/N Rats as Vehicle for Gavage. 1992.

<sup>24</sup> Lilly PD, Simmons JE, and Pegram RA. *Effect of subchronic corn oil gavage on the acute toxicity of orally administered bromodichloromethane*. 1996. *Toxicol Lett*; 87:93-102.

enzymes, thus increasing the generation of reactive intermediates.”<sup>25</sup> 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.<sup>26</sup>

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.<sup>27</sup>

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.”<sup>28</sup>

Nonetheless, the fact that the test article was measured to contain  $10.45 \pm 2.40$  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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<sup>25</sup> Ibid.

<sup>26</sup> Rahman KMW. *Effect of type and amount of dietary fat during the initiation phase of hepatocarcinogenesis*. 2001. *Nutrition and Cancer*; 39(2):220-225.

<sup>27</sup> van Beek TA and Montoro P. *Chemical analysis and quality control of Ginkgo biloba leaves, extracts, and phytopharmaceuticals*. 2009. *J Chromatography A*; 1216:2002-2032.

<sup>28</sup> 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.<sup>29</sup>

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<sup>®</sup> or any other ginkgo leaf extract.

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<sup>29</sup> See <http://nccam.nih.gov/research/policies/naturalproduct.htm> (accessed on January 16, 2012).

## **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, kew tree, yinhsing (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 1

Constituents of <i>Ginkgo biloba</i>	Identified Chemical Constituents	Target Specification in EGb 761®
--------------------------------------	----------------------------------	----------------------------------

<b>Extract<sup>a</sup> Class</b>		<b>(range in other preparations)</b>
Terpene trilactones	Ginkgolides A, B, C, J, K, L, M (found in root only); bilobalide (sesquiterpene)	<b>6.0%</b> (0.2%-11%)
Flavonol glycosides	Major flavonoids <b>as their glycosides</b> : quercetin, kaempferol, isorhamnetin Minor flavonoids <b>as their glycosides</b> : apigenin, luteolin, myricetin	<b>24.0%</b> (24%-36%)
Biflavones	Bilobetin, ginkgetin, isoginkgetin, sciadopitysin	0%-(0.05%-1.7%)
Proanthocyanidins	Dimers of procyanidin and prodelfphinidin classes	<b>7.0%</b> (4%-12%)
Alkylphenols	Ginkgolic acids, cardanols	≤ 5 ppm (0.5%-4.8% in leaves; <500 to approximately 90,000 ppm)
Carboxylic acids	Non-phenolic acids (ascorbic acid, D-glucaric acid, quinic acid, shikimic acid), phenolic acids (protocatechuic, <i>p</i> -hydroxybenzoic, vanillic, caffeic, <i>p</i> -coumaric, ferulic and chlorogenic acids)	<b>13.0%</b> (N/A)
<b>Flavanols-Catechins</b>	Catechin, epicatechin, galocatechin, and epigallocatechin	<b>2.0%</b> (N/A)
Polyprenols	C85, C90, C95 polyprenols	0%-(1.9%-2.0% in leaves)
<b>Non-flavonol glycosides</b>	<b>N/A</b>	<b>20.0%</b> (N/A)
<b>High molecular weight compounds</b>	<b>N/A</b>	<b>4.0%</b> (N/A)
<b>Inorganic constituents</b>	<b>N/A</b>	<b>5.0%</b> (N/A)
<b>Water, solvent</b>	<b>N/A</b>	<b>3.0%</b> (N/A)
<b>Various</b>	<b>N/A</b>	<b>3.0%</b> (N/A)
<b>Unknown</b>	<b>N/A</b>	<b>13.0%</b> (N/A)

## 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% flavoneol 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 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% flavoneol glycosides**, **5% 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  $\alpha$ -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<sup>®</sup>, 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  $\alpha$ -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.