Memorandum

Date: May 24, 2012

From: [redacted] Ph.D., Toxicologist
Division of Biotechnology and GRAS Notice Review (DBGNR), HFS-255
Office of Food Additive Safety (OFAS)
Center for Food Safety and Applied Nutrition (CFSAN)

Through: [redacted] Ph.D., Supervisory Toxicologist, DBGNR, OFAS, CFSAN
[redacted] 05/30/2012

Through: [redacted] Ph.D., Division Director, DBGNR, OFAS, CFSAN, HFS-255
[redacted] 05/30/12

Through: [redacted] Ph.D., Office Director, OFAS, CFSAN, HFS-200
[redacted] May 30, 2012

To: [redacted] Office of Compliance, HFS-608, CFSAN

Subject: Review of available information pertaining to the safety of *Ginkgo biloba* extract for use in conventional foods.

Introduction

Ginkgo biloba extract is a dietary supplement used by many individuals for purported benefits in treating numerous ailments including cognitive and vascular insufficiencies. More complete lists of possible uses have already been extensively reviewed and discussed in other publications, i.e. Natural Standard Monograph, 2012; Natural Medicines Comprehensive Database, 2012; Health Canada Monograph, 2009. The use of *Ginkgo biloba* in conventional foods has prompted FDA to examine the publically available information to determine if there is evidence to support the safe use of *Ginkgo biloba* as an ingredient in foods.

Chemical Source and Identity

Ginkgo biloba is derived from the *Ginkgo biloba* tree. Generally, *Ginkgo biloba* is extracted from the leaf of the tree but, extractions can also be done on the seeds. Most scientific information pertains to studies done with a standardized *Ginkgo biloba* extract from the leaf. The most common standard extracts are EGb 761 [24% ginkgo flavone glycosides (primarily quercetin, kaempferol & isorhamnetin), 6% terpenoids (2.8-3.4% ginkgolides A, B and C, 2.6-3.2% bilobalide), ginkgolic acid (below 5ppm (5 mg/kg)), other constituents including: proanthocyandins, glucose, rhamnose, organic acids and D-glucaric acid], proprietary extract of W.

Schwabe Co. of Karlsruhe, Germany and LI1370 [25% ginkgo flavone glycosides, 6% terpenoids], produced by Lichtwer Pharma, Germany (German Commission E Monograph, 1994).

Data Availability

Until recently, the only source for any data from traditional animal toxicity studies was in selected scientific monographs. In the published scientific literature, there are limited data to address the safety of *Gingko biloba* extract from leaf, and even less data on seed components. There are no published animal data on acute toxicity, neurotoxicity or immunotoxicity.

German Commission E Monographs on Phytotherapy (1994)

The monograph discussed pharmacokinectic information including an absorption rate of 60 percent in rats exposed to radio-labeled standard *Ginkgo biloba* extract. The monograph also listed an LD_{50} in mice for the standard extract as being 7725 mg/kg after oral exposure and 1100 mg/kg via intravenous exposure. In addition, it stated that the standard extract showed no mutagenic, carcinogenic or reproductive effects. In the monograph, the composition of the standard extract that was utilized is not specified.

European Scientific Cooperative on Phytotherapy, ESCOP (2003)

This monograph reported 13-week gavage studies in mice and rats receiving a standardized *Ginkgo biloba* extract (EGb761) were administered 0, 65.5 (rats only), 125, 250, 500, 1000 (rats and mice) and 2000 (mice only) milligrams per kilogram per day (mg/kg/d). The study showed an increase in liver weight at all dose levels tested, and a dose-related increase in hepatocyte hypertrophy in male and female mice at and above 250 mg/kg/d and in male rats at all dose levels.

In addition, 6 month oral studies in dogs receiving more than 100 mg/kg of an unspecified standard extract showed a time- and dose-related mild temporary vasodilation in the cranial blood vessels. Also, no carcinogenic effects were noted in a 2-year rat study with dose levels of 4, 20, and 100 mg/kg of an unspecified standard extract. Finally, oral administration of an unspecified standard extract to rats (up to 1.6 g/kg/d) and rabbits (up to 0.9 g/kg/d) did not show embryotoxic, teratogenic or reproduction toxicity effects.

Recent Scientific Journal Articles Related To Reproductive-Associated Toxicity

Pinto et al. (2007) showed that treating pregnant Wistar rats with 7 and 14 mg/kg/d of *Ginkgo biloba* extract (chemical analysis revealed constituent levels similar to EGb 761) caused a significant decrease in fetal mean weight, while having no apparent toxicity on the mother. Zehra et al. (2010) reported that pregnant albino mice receiving 100 mg/kg of an unspecified standard *Ginkgo biloba* extract had fetuses with decreased weight and crown-rump length.

Another study shows the conflicted state of scientific data pertaining to *Ginkgo biloba* reproductive-associated toxicity. Fernandes et al. (2010) reported that treatment of pregnant Wistar rats with 3.5, 7.0 or 14 mg/kg/d *Ginkgo biloba* extract (chemical analysis revealed constituent levels similar to EGb 761) caused no toxic effect on the mother and did not induce embryonic death, growth retardation, or fetal malformations.

Ginkgolic Acid

Standard extracts of *Ginkgo biloba* contain ginkgolic acid, which has been shown to have cytotoxic, mutagenic, carcinogenic and immunotoxic properties. As a consequence, a maximum level for ginkgolic acid in standard *Ginkgo biloba* extracts was set at 5 mg/kg (World Health Organization Monograph, 1999). Unfortunately, one report indicates that ginkgolic acid levels in *Ginkgo biloba* extracts already on the market are often higher than the 5 mg/kg levels (Chiu et al., 2002).

National Toxicology Program (NTP) Studies (2012)

The *Ginkgo biloba* extract (NTP GBE) used in the 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. This extract was utilized in all studies undertaken by NTP.

For comparison the EGb 761 standard extract is 24% ginkgo flavone glycosides (primarily quercetin, kaempferol & isorhamnetin), 6% terpenoids (2.8-3.4% ginkgolides A, B and C, 2.6-3.2% bilobalide), ginkgolic acid (below 5ppm).

Genetic Toxicology Studies

As concluded by NTP, NTP GBE was mutagenic in *S. typhimurium* strains TA98 and TA100, and in *E. coli* strain WP2 *uvrA*/pKM101, with and without exogenous metabolic activation. Results of a peripheral blood micronucleus test in male and female B6C3F1/N mice administered NTP GBE for 3 months by gavage were negative in males but judged to be equivocal in females based on a significant trend test.

Rat - 90 day study

As reported by NTP, groups of 10 male and 10 female F344/N rats were administered 0, 62.5, 125, 250, 500, or 1,000 mg NTP GBE kg body weight in corn oil by gavage, 5 days per week for 14 weeks. Mean body weights of all dosed groups were similar to those of the vehicle control groups. Liver weights of all dosed groups of males and females were significantly greater than those of the vehicle control groups. The incidences of hepatocyte hypertrophy in all dosed groups of males and in 500 and 1,000 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, which consisted of small numbers of scattered midzonal hepatocytes having small to a few large, clear, discrete intracytoplasmic vacuoles that had the typical appearance of lipid droplets filling most or all of the cytoplasm, occurred in all dosed males. The incidences of thyroid gland follicular cell hypertrophy were significantly increased in 500 and 1,000 mg/kg males and in 1,000 mg/kg females. The incidences of pigmentation in the olfactory epithelium of the nose were significantly increased in 500 and 1,000 mg/kg males and in females administered 125 mg/kg or greater. Pigment accumulation was characterized by the accumulation of golden brown pigment within macrophages scattered throughout the basal aspect of the olfactory epithelium. The macrophages were more common in areas of olfactory epithelial atrophy but were also present in nonatrophied areas. Atrophy was characterized by thinning, decreased cellularity, and disorganization of the olfactory epithelium. Minimal pigment was apparent within the cells of the olfactory epithelium.

Mouse – 90 day study

As described by NTP, groups of 10 male and 10 female B6C3F1/N mice were administered 0, 125, 250, 500, 1,000, or 2,000 mg NTP GBE/kg body weight in corn oil by gavage, 5 days per week for 14 weeks. Mean body weights of 2,000 mg/kg females were significantly less than those of the vehicle control group. Liver weights of 250 mg/kg or greater males and all dosed groups of females were significantly greater than those of the vehicle control groups. Kidney weights of 2,000 mg/kg males were significantly less than those of the vehicle control group. The incidences of hepatocytic hypertrophy were significantly increased in males and females in the 250 mg/kg or greater groups. Significantly increased incidences of focal hepatocytic necrosis occurred in 1,000 and 2,000 mg/kg males.

The incidences of hepatocytic hypertrophy were significantly increased in males and females in the 250 mg/kg or greater groups. Significantly increased incidences of focal hepatocytic necrosis occurred in 1,000 and 2,000 mg/kg males. The incidences of hyaline droplet accumulation in the respiratory epithelium of the nose were significantly increased in 500 mg/kg males and 1,000 and 2,000 mg/kg females. In the olfactory epithelium of the nose, the incidences of hyaline droplet accumulation were significantly increased in the 125 (female only), 500, and 1,000 mg/kg groups. Incidences of atrophy of the olfactory epithelium were significantly increased in the 1,000 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 1,000 and 2,000 mg/kg females. Pigment accumulation was characterized by macrophages with an abundant amount of golden-brown granular cytoplasm. Macrophages were usually located immediately above the basal cell layer and below the nuclear layer of the sustentacular cells and olfactory neurons. Macrophages containing pigment were often noted near areas of atrophy of the olfactory epithelium, but the presence of the macrophages themselves did not appear to cause the atrophy.

Rat – 2 year study

As expressed by NTP, groups of 50 male and 50 female F344/N rats were administered 0, 100, 300, or 1,000 mg NTP GBE/kg body weight (bw) in corn oil by gavage, 5 days per week for 104 or 105 (females) weeks. Additional groups of 10 male and 10 female rats were administered the same doses, 5 days per week for 14 weeks. Survival of 1,000 mg/kg males was significantly less than that of the vehicle controls. Mean body weights of 300 mg/kg males and females were less (10% or more) than those of the vehicle controls after week 93, and those of 1,000 mg/kg males and females were less after week 89.

Liver weights were significantly increased in all dosed groups of special study rats at 14 weeks. In the liver at 2 years, incidences of hepatocellular adenoma were slightly increased in 100 and 300 mg/kg males. Significantly increased incidences of nonneoplastic lesions at 2 years included hepatocyte hypertrophy and bile duct hyperplasia in all dosed groups of males and females, focal fatty change in all dosed groups of females (characterized by a focal area of hepatocytes that displayed localized microvesicular and macrovesicular fatty change), cystic degeneration in 100 and 1,000 mg/kg males, and oval cell hyperplasia and necrosis in 1,000 mg/kg males.

At week 14, all dosed groups of males and 1,000 mg/kg females had increased levels of thyroid stimulating hormone compared to those of the vehicle control groups. There were no significant increases in the levels of triiodothyronine or total thyroxine. In the thyroid gland, incidences of follicular cell adenoma were slightly increased in 300 and 1,000 mg/kg males and 300 mg/kg females. Single incidences of follicular cell carcinoma occurred in the 300 and 1,000 mg/kg female groups. There were significantly increased

incidences of follicular cell hypertrophy in all dosed groups of males and females and follicle hyperplasia in all dosed groups of males.

In the nose, two incidences of adenoma of the respiratory epithelium occurred in 300 mg/kg females. Except for respiratory epithelium hyperplasia in 100 mg/kg females, the incidences of transitional epithelium and respiratory epithelium hyperplasia were significantly increased in all dosed groups of males and females. Except for olfactory epithelium respiratory metaplasia in 100 mg/kg females, the incidences of atrophy, respiratory metaplasia, nerve atrophy, and pigmentation were significantly increased in the olfactory epithelium of all dosed groups of males and females. Incidences of goblet cell hyperplasia in the respiratory epithelium were significantly increased in 300 and 1,000 mg/kg males and females, and incidences of chronic active inflammation were significantly increased in 1,000 mg/kg males and females. The incidence of submucosa fibrosis was significantly increased in 1,000 mg/kg males.

Furthermore, the incidences of mononuclear cell leukemia in 300 and 1,000 mg/kg males were significantly greater than that in the vehicle controls. Finally, dose-related increased severity of kidney nephropathy was noted in all dosed groups of males.

Mouse – 2 year study

As described by NTP, groups of 50 male and 50 female B6C3F1/N mice were administered 0, 200, 600, or 2,000 mg NTP GBE/kg bw in corn oil by gavage, 5 days per week for 104 weeks. Survival of 600 and 2,000 mg/kg males was significantly less than that of the vehicle controls; survival of 600 mg/kg females was significantly greater than that of the vehicle controls. Mean body weights of 600 and 2,000 mg/kg males were less (10% or more) than those of the vehicle controls after weeks 85 and 77, respectively; mean body weights of 2,000 mg/kg females were generally less than those of the vehicle controls between weeks 17 and 69 and after week 93.

In the liver, there were significantly increased incidences of hepatocellular adenoma in all dosed groups of females, hepatocellular carcinoma in all dosed groups of males and 2,000 mg/kg females, and hepatoblastoma in all dosed groups of males and 600 and 2,000 mg/kg females. The increased incidences of these neoplasms were primarily due to increased incidences of multiple adenoma, carcinoma, and hepatoblastoma. Except for the incidences of hepatocellular carcinoma or hepatoblastoma (combined) in 200 and 600 mg/kg females, the incidences of hepatocellular adenoma or carcinoma (combined), hepatocellular carcinoma or hepatoblastoma (combined), and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) were significantly increased in all dosed groups of males and females. Significantly increased incidences of nonneoplastic liver lesions included hypertrophy in all dosed groups of males and females, erythrophagocytosis in all dosed groups of males and in 600 and 2,000 mg/kg females, hematopoietic cell proliferation, inflammation, and necrosis in 600 and 2,000 mg/kg males, and cytoplasmic vacuolization, eosinophilic focus, and mixed cell focus in all dosed groups of females.

In the thyroid gland, two incidences each of follicular cell adenoma occurred in the 600 and 2,000 mg/kg male groups. The incidence of follicle hyperplasia was significantly increased in 2,000 mg/kg males, and the incidences of follicular cell hypertrophy were significantly increased in 2,000 mg/kg males and 600 and 2,000 mg/kg females.

In the forestomach, the incidences of inflammation, epithelium hyperplasia, and epithelium hyperkeratosis were significantly increased in all dosed groups of males and in 2,000 mg/kg females; the incidences of epithelium ulcer were significantly increased in 2,000 mg/kg males and females.

In the nose, the incidences of hyaline droplet accumulation in the olfactory epithelium were significantly increased in 2,000 mg/kg males and females; the incidences of pigmentation in the olfactory epithelium were significantly increased in 2,000 mg/kg males and 600 and 2,000 mg/kg females.

NTP Studies Conclusions

As summarized by NTP, under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of NTP GBE 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 NTP GBE 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 NTP GBE 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 *Ginkgo biloba* extract administration. There was *clear evidence of carcinogenic activity* of NTP GBE in female B6C3F1/N mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma.

Administration of NTP GBE 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 NTP GBE.

Comments Submitted Concerning NTP Studies

Multiple comments were submitted to NTP regarding the test article *Ginkgo biloba* extract, and the fact it may not be representative of other *Ginkgo biloba* extracts marketed in the United States. NTP clearly states the chemical composition of the *Ginkgo biloba* extract tested, and they state that it is comparable to other extracts in the market.

Conclusions

FDA is aware that the short-term uses of low doses of *Ginkgo biloba* extract in healthy people are ordinarily tolerated. Studies supporting such uses, however, are not adequate to establish the safety of *Ginkgo biloba* extract for use as an ingredient in food. It should be emphasized that, because a food ingredient in a conventional food may be consumed by the entire population over a lifetime, assurance of safety requires an evaluation of potential effects of long-term use. FDA is particularly concerned about *Ginkgo biloba* extract use as an ingredient in conventional food given the fact that NTP's 2 year study in mice showed *clear evidence of carcinogenic activity* of *Ginkgo biloba* extract, and NTP's 2 year study in rats showed *some evidence of carcinogenic activity* of *Ginkgo biloba* extract.

A safety determination for a substance that will be used as an ingredient in conventional food must be based on scientific studies appropriate to establish the safety of the substance under the conditions of its intended use. For the use of *Ginkgo biloba* extract as an ingredient in food, FDA considers that the data from NTP as

well as various other scientific literature reports, including those described above, raise safety concerns about the use of the substance. In light of these safety concerns, there is no basis to conclude that the use of *Ginkgo biloba* extract as an ingredient in conventional food is generally recognized as safe (GRAS).
Moreover, there is no food additive regulation in effect establishing safe conditions of use for *Ginkgo biloba* extract, and FDA is not aware of any information to indicate that *Ginkgo biloba* extract is the subject of a prior sanction. Therefore, FDA considers *Ginkgo biloba* extract an unapproved food additive when used as an ingredient in conventional food.

References:

Natural Standard Monograph (2012), http://www.naturalstandard.com/databases/herbssupplements/ginkgo.asp, Ginkgo (Ginkgo Biloba L.).

Natural Medicines Comprehensive Database (2012), Ginkgo Monograph http://naturaldatabase.therapeuticresearch.com/nd/Search.aspx?cs=CP&s=ND&pt=9&Product=Ginkgo.

Health Canada (2008), Natural Health Products Ingredients Database, Ginkgo Biloba Monograph, http://www.hc-sc.gc.ca/dhp-mps/prodnatur/applications/licen-prod/monograph/archive_mono_ginko_biloba-eng.php.

German Commission E Monographs (1994), Ginkgo Biloba Leaf Extract Monograph, http://cms.herbalgram.org/expandedE/GinkgoBilobaleafextract.html.

European Scientific Cooperative On Phytotherapy, ESCOP (2003), The scientific foundation for herbal medicinal products; 2nd Edition.

Pinto RM, et al. (2007), Intra-uterine growth retardation after prenatal administration of Ginkgo biloba to rats; Reproductive Toxicology 23:480-485.

Zehra U, et al. (2010), Ginkgo biloba induced malformations in mice; Journal of the College of Physcians and Surgeons Pakistan 20(2):117-121.

Fernandes ES, et al. (2010), Effects of Ginkgo biloba extract on the embryo-fetal development in Wistar rats; Birth Defects Research (Part B) 89:133-138.

World Health Organization, WHO (1999), WHO monographs on selected medicinal plants, Folium Ginkgo; Volume 1. p. 154-167.

Chiu AE, et al. (2002), Diffuse morbilliform eruption after consumption of ginkgo biloba supplement; J. Am. Acad. Dermatol. 46(1): 145-146.

National Toxicology Program, NTP, Technical Report 578 (2012), Toxicology and Carcinogenesis Studies of Ginkgo Biloba Extract in F344/N rats and B6C3F1/N mice; http://ntp.niehs.nih.gov/NTP/About_NTP/TRPanel/2012/February/DraftTR578.pdf.