Critical Review of the Results of the National Toxicology Program (NTP) Rodent Carcinogenicity Studies Conducted with a Specific *Ginkgo biloba* Leaf Extract

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EXECUTIVE SUMMARY

Intertek Cantox was requested by the American Herbal Products Association (AHPA) to provide an interpretive and critical review of the 2-year studies with a *Ginkgo biloba* leaf extract conducted by the National Toxicology Program (NTP) in F344/N rats and B6C3F1/N mice. Additionally, Intertek Cantox was requested to assess the relevance of the results of these studies to the safety of oral consumption of *G. biloba* extracts by humans. Intertek Cantox has been informed by AHPA that it believes that the specific *G. biloba* leaf extract used as the test article in these studies is dissimilar to commercially available *G. biloba* leaf extracts and that AHPA will be submitting comments to the NTP on this topic. It should be noted that the present report reviews only the actual studies conducted by the NTP with the specifically identified lot of *G. biloba* leaf extract and it is uncertain whether the results of the NTP studies can be extrapolated to assess the safety of other *G. biloba* leaf extracts.

Intertek Cantox reviewed the results of the NTP studies, as well as the conclusions of the NTP pertaining to the 'strength of evidence' for carcinogenic activity of the specific *G. biloba* leaf extract tested by the NTP based on the results of the studies. It is noted that the objective of the NTP is to only report the results of individual animal studies conducted to assess the potential toxicity of a chemical agent, rather than to assess the overall risk to humans from the potential exposure to a tested chemical. Positive results of carcinogenic activity in laboratory animals under the conditions of a particular study may indicate that exposure to the same chemical has the potential for hazard to humans.

A number of confounding study design issues were identified in this review that may have implications on the overall study results; these included:

- Absence of adequate demonstration of stability of the test material;
- Absence of heavy metal, mycotoxin, microbiology, polyaromatic hydrocarbon, and pesticide analysis for the test material; and
- Administration of high dose levels in the 2-year mouse study.

In addition, the following observations were made with respect to the study results and NTP's conclusions, as well as with respect to the relevance of these results to humans:

- Following gavage administration of the specific *G. biloba* leaf extract tested by the NTP to F344/N rats and B6C3F1/N mice at dose levels of up to 1,000 and 2,000 mg/kg body weight/day, respectively, increased incidences of non-neoplastic and neoplastic tumors of the thyroid gland and liver were observed in the animals.

- In mice, the results generated under the conditions of the study with the specific *G. biloba* leaf extract do provide *clear evidence of carcinogenic activity.* However, administration of the extract at the high dose levels selected in these studies, could have been predicted to produce effects on the liver, including the development of liver tumors, particularly in mice even at the lowest dose level tested.

- In rats, the NTP concluded *some evidence of carcinogenic activity* under the conditions of the study with the specific *G. biloba* leaf extract; however, in the absence of a statistically significant response in the increased incidence of rat thyroid gland tumors in comparison to concurrent control values, it could be argued that the strength of evidence for carcinogenic activity is only "*equivocal"*.

- The mechanism of action underlying the carcinogenic responses observed in the 2-year mouse and rat studies was likely related to the stimulatory effects of *G. biloba* extract on microsomal liver enzymes; the observed carcinogenicity was therefore considered likely to be a secondary response to non-genotoxic and threshold-dependent effects in the rodents.

- In light of the underlying non-genotoxic mechanism of action, likely related to enzyme induction, the responses observed in mice and rats might be deemed irrelevant to assessment of human safety. This is further corroborated by a number of studies demonstrating a lack of an enzyme stimulating effect of *G. biloba* leaf extracts in humans. The effects observed in these studies might therefore not be predictive of the extract exerting a similar response in humans at lower levels of exposure.

Critical Review of the Results of the National Toxicology Program (NTP) Rodent Carcinogenicity Studies Conducted with a Specific *Ginkgo biloba* Leaf Extract

1.0 OBJECTIVE

The National Toxicology Program (NTP) has released a draft Technical Report (NTP TR 578) on the 'Toxicology and Carcinogenesis Studies of *Ginkgo biloba* Extract in F344/N Rats and B6C3F1/N Mice' (NTP, 2011), scheduled for peer review on February 8th and 9th, 2012. Intertek Cantox was requested by the American Herbal Products Association (AHPA) to provide an interpretive and critical review of the NTP study results and specifically to determine whether NTP's conclusions regarding the 'strength of evidence' for carcinogenic activity of the tested *G. biloba* leaf extract based on the results of this study are appropriate. Additionally, Intertek Cantox was requested to assess the relevance of the results of these studies to the safety of oral consumption of *G. biloba* extracts by humans.

2.0 BACKGROUND

G. biloba, a slow-growing tree indigenous to Eastern Asia, has a long history of use in herbal medicines (Barrett *et al*., 1999; PDRHM, 2007). *G. biloba* leaf extracts are currently used for several indications related to improving cognitive function among others, and are commonly standardized to contain 24% flavonol glycosides and 6% terpene lactones (Barnes *et al*., 2007; PDRHM, 2007). The German Commission E recommends dose levels between 120 and 240 mg/day in 2 to 3 divided doses for a *G. biloba* leaf extract with an average material:extract ratio of 50:1 and standardized to contain 22 to 27% flavonol glycosides, 5 to 7% terpene lactones, and <5 ppm ginkgolic acid (Blumenthal *et al*., 1998). In Canada, the use of *G. biloba* leaf extracts in natural health products is limited to extracts that are standardized to contain 22 to 27% flavonoid glycosides and 5 to 7% terpene lactones (Health Canada, 2009). The recommended dose of standardized *G. biloba* extracts in Canada is between 4 and 12 g per day, calculated as dried leaves, which is equivalent to a dose level of 80 to 240 mg for an extract with a material:extract ratio of 50:1.

According to the NTP draft report, the National Institute of Environmental Health Sciences (NIEHS) nominated *G. biloba* leaf extract for evaluation by the NTP due to the widespread use of *G. biloba* extracts in herbal supplements to promote mental function and the limited availability of toxicity and carcinogenicity data. Specific reasons cited for its nomination included the positions that it is a well-defined product with biologically active ingredients, it is

consumed in rather large quantities over extended periods of time, and that some of its constituents are known mutagens (*e.g*., quercetin).

Following the nomination, the NTP initiated 2 dose-range determining 3-month studies, each conducted in mice and rats, as well as two 2-year carcinogenicity studies, also conducted with mice and rats in order to assess the safety and potential carcinogenicity of the specific *G. biloba* leaf extract used in the studies (hereinafter "the specific *G. biloba* leaf extract"). In parallel with the 2-year rat study, a special 14-week rat study was conducted to determine the potential effects specifically on the thyroid and liver.

Specifically, in the dose-range determining study, male and female F344/N rats (10/sex/group) were administered the specific *G. biloba* leaf extract at dose levels of 0 (vehicle control), 62.5, 125, 250, 500, or 1,000 mg/kg body weight/day in corn oil *via* gavage for 5 days a week for 3 months. Likewise, male and female B6C3F1/N mice (10/sex/group) were administered the specific *G. biloba* leaf extract at dose levels of 0 (vehicle control), 125, 250, 1,000, or 2,000 mg/kg body weight/day in corn oil *via* gavage for 5 days a week for 3 months to determine the appropriate dosing regimen for the 2-year carcinogenicity study. In the 2-year studies, male and female F344/N rats (50/sex/group) were administered the specific *G. biloba* leaf extract at dose levels of 0 (vehicle control), 100, 300, or 1,000 mg/kg body weight/day in corn oil for 5 days a week for 104 or 105 weeks (females and males, respectively) *via* gavage. Additional rats (10/sex/group) were administered the specific *G. biloba* leaf extract at the same dosage levels for 14 weeks for special analyses on thyroid hormones, the liver, and the thyroid gland. Male and female B6C3F1/N mice (50/sex/group) were administered the specific *G. biloba* leaf extract by gavage at doses of 0 (vehicle control), 200, 600, or 2,000 mg/kg body weight/day in corn oil for 5 days a week for 104 weeks.

3.0 EVALUATION OF STUDY DESIGN

3.1 Stability

The dose formulations were prepared by mixing the specific *G. biloba* leaf extract (Lot No. 020703) with corn oil. As described in the draft report, dose formulations were prepared 3 times during the 3-month studies and approximately every 4 weeks during the 2-year studies. The dose formulations were stored at room temperature in sealed plastic bottles enclosed in amber plastic bags for up to 35 days (3-month studies) or 41 days (2-year studies).

As noted in the draft report, homogeneity and stability were confirmed for at least 42 days under the described storage conditions (at room temperature in sealed plastic bottles enclosed in amber plastic bags), as well as at approximately 5°C for at least 3 hours under simulated animal room conditions. However, the lot of the material used to assess the homogeneity of the dose

formulations is not specified in the draft report, whereas the lot used to assess the stability of the dose formulations was indicated to be Lot No. GBE-50-001003 (lot used for the methods development), which was not the lot comprising the test material used to administer to the animals (*i.e*., Lot No. 020703). Furthermore, it is noted in the draft report that while the main 7 α-glycosides and terpenoids identified in the bulk material (Lot No. 020703) "appeared to be stable", when the material was stored away from light and under elevated temperatures (60°C), high-performance liquid chromatography analyses employed as part of the bulk stability testing, yielded highly variable results which consequently were deemed to be inconclusive. Therefore, the draft report does not provide conclusive support for the homogeneity or the stability of the dose formulations as used in the studies (*i.e*., dose formulations prepared with Lot No. 020703). Additionally, there is very limited data provided regarding the bulk stability of the test material lot (Lot No. 020703). The absence of clear chemical specifications for the tested material, and analysis to demonstrate conformity with such specifications following storage is noted herein.

It is also stated in the NTP draft report that each of the dose formulations was analyzed 3 times during each of the 3-month studies and every 12 weeks during the 2-year studies. All 15 dose formulations tested in the rat and mouse 3-month studies, and all 30 dose formulations in the rat and mouse 2-year studies, were determined to show no degradation based on the results showing that the dose formulations were within 10% of target concentrations. However, it is not clear how the concentration measures were obtained. It is likely that the concentration measures for the tested extract were based on measures of quercetin content only. Furthermore, it is unclear whether the changes in the concentrations, while within 10%, were simply due to methodological variability or due to the possibility that some of the constituents of the tested extract were indeed degrading to unidentified compounds.

Thus, the data to support the stability of the dose formulations over the course of the animal 3-month and 2-year studies, as provided in the NTP draft report, is limited and does not conclusively demonstrate that the dose formulations were stable over the course of the testing period.

3.2 Heavy Metal, Pesticide, Microbiology, Polyaromatic Hydrocarbons, and Mycotoxin Analysis

Considering that the specific *G. biloba* leaf extract is obtained *via* extraction of leaves, results of heavy metal, mycotoxin, pesticide, polyaromatic hydrocarbon, and microbiological analyses should be available for the test material to demonstrate absence of contaminants or compliance with acceptable limits. Such data, with the exception of polyaromatic hydrocarbon analysis, were compiled in the NTP draft report on the NTP-2000 rat and mouse rations provided to the animals during the studies, but not on the specific *G. biloba* leaf extract.

Thus, in the absences of such data for the specific *G. biloba leaf* extract it is not possible to exclude the possibility that the results observed in the NTP studies were not related to the specific *G. biloba* leaf extract per se, but rather to unidentified contaminants present in the test material.

3.3 Dose Selection for the 2-Year Mouse Study

For both rats and mice, the NTP conducted preliminary 3-month studies in order to establish appropriate doses for the 2-year studies. Specifically in the 3-month studies, the specific *G. biloba* leaf extract was administered at dose levels of 0 (vehicle control), 62.5, 125, 250, 500, or 1,000 mg/kg body weight/day to male and female F344/N rats and 0 (vehicle control), 125, 250, 1,000, or 2,000/kg body weight/day to male and female B6C3F1/N mice.

In female mice, statistically significant and dose-dependent increases in absolute and relative liver weights were observed at all dose levels tested (125 to 2,000 mg/kg body weight/day) compared to the concurrent control group. At the lowest dose level tested (125 mg/kg body weight/day), both absolute and relative liver weights were already 10% greater than the controls. Likewise in males, dose-dependent and statistically significant increases of at least 14% in comparison to the control group were observed in both absolute and relative liver weights at dose levels of 250 mg/kg body weight/day and greater; at the lowest dose level, only the increase in relative liver weight (6%) reached statistical significance. At the highest dose level (2,000 mg/kg body weight/day), relative liver weights were 37 and 38% greater in males and females, respectively.

In spite of the presence of significant increases in liver weights even at the lowest dose level tested in the mouse 3-month study (125 mg/kg body weight/day), the dose levels selected for purposes of the 2-year mouse assay were 200, 600, and 2,000 mg/kg body weight/day. The rationale for the dose selection for purposes of the 2-year mouse study was reported in the NTP report to have been based on the absence of an effect on survival. In a survey of 138 mouse carcinogenicity studies conducted over a period of 10 years, an association between a positive result for hepatocellular tumors in mice and an increase in liver weights at 1 year was shown (Carmichael *et al*., 1997). The authors suggested that an increase in relative liver weights might be a good indicator of having achieved a dose high enough to elicit a toxicological response and might be a suitable alternative to the typical determination of the maximum tolerable dose (MTD) based on body weight reduction/survival. Considering the overt increases in both relative and absolute liver weights in mice following only 3 months of administration of the test material at the lowest dose level tested (125 mg/kg body weight/day), in combination with the well-documented species-specific susceptibility of the B6C3F1 mouse to develop liver tumors (Drinkwater *et al*., 1989; Velazquez *et al*., 1996) (see Section 5.1), it is likely, especially in the mouse, that the lowest dose tested (200 mg/kg body weight/day) could be predicted in advance

to produce effects on the liver, including the development of liver tumors, and thus may have been inappropriate as the lowest dose level selected particularly for the purposes of a long-term carcinogenicity study.

Furthermore, the tumorigenic effects observed in the 2-year studies were likely secondary to non-genotoxic, threshold-dependent effects (see Section 5.1 for detailed discussion). In particular, the mechanism underlying the responses observed in both the liver and thyroid in the studies may have been related to enzyme stimulation at high dose-level. Since even the lowest doses applied in the 2-year studies likely mediated enzyme perturbations that could lead to effects on the liver and/or thyroid, the dose selection in the 2-year studies may have precluded the establishment of a no-observed-adverse-effect level (NOAEL) from the outset.

4.0 EVALUATION OF THE CONCLUSIONS PERTAINING TO THE STRENGTH OF EVIDENCE OF CARCINOGENIC ACTIVITY OF A SPECIFIC *GINKGO BILOBA* LEAF EXTRACT IN THE 2-YEAR RODENT NTP STUDIES

4.1 NTP's Definition of the Levels of Evidence for Carcinogenic Activity

Unlike organizations such as the International Agency for Research on Cancer (IARC), which evaluate all available data pertaining to the safety of a chemical, including pre-clinical and clinical data, as well as known human exposure estimates, and assess the overall risk to humans from exposure to the chemical, the NTP only reports the results of individual animal studies conducted to assess the potential toxicity of a chemical agent. The NTP notes that positive results of carcinogenicity obtained in a study demonstrate that the chemical is "carcinogenic for laboratory *animals* under the conditions of the study". Positive results in the studies "indicate that exposure to the chemical has the *potential* for hazard to humans"; however, in their evaluation of the study results, the NTP does not consider the relevance of the results to human safety *per se*. The NTP reports do, however, discuss the 'strength of evidence' from the study to support particular conclusions regarding the carcinogenic activity.

The 'strength of evidence' can be classified as *clear*, *some*, or *equivocal.* The different criteria required for each level of the 'strength of evidence' classification is summarized in Table 4.1-1. The study results may also be judged as showing *"no evidence*" of carcinogenic activity, meaning that no chemical-related increases in malignant or benign neoplasms were observed. Finally in cases of major qualitative or quantitative limitations (*e.g.*, excessive mortality, loss of tissue to autolysis, dosing errors, intercurrent disease, *etc*.), which would preclude the interpretation of the study results, a study can be considered to have been "*inadequate*".

Table 4.1-1 NTP's 'Strength of Evidence' Classification of Study Results and the Requirements Associated with Each Category	
Classification of the Evidence	**Criteria**
• *"Clear"*	Studies interpreted as showing a dose-related I. Increase of malignant neoplasms; II. Increase of a combination of malignant and benign neoplasms; or, III. Marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
• *"Some"*	Studies interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
• *"Equivocal"*	Studies interpreted as showing a marginal increase of neoplasms that *may be* chemical related.

4.2 Classification of the Strength of Evidence for Carcinogenic Activity of a Specific *Ginkgo biloba* Leaf Extract Based on the Results of the 2-Year NTP Studies

4.2.1 Classification of Evidence - Mice

The basis for NTP's conclusions regarding the strength of evidence of the specific *G. biloba* leaf extract carcinogenic activity in male and female B6C3F1/N mice is summarized in Table 4.2.1-1.

Table 4.2.1-1 Summary of the Basis for NTP's Conclusions Regarding the Strength of Evidence for Carcinogenic Activity of the Specific *Ginkgo biloba* Extract in B6C3F1/N Mice		
Test Animal	**Evidence Pertaining to Carcinogenic Activity**	**NTP's Overall Conclusion Regarding the Strength of Evidence**
Male B6C3F1/N mice	*Liver*: • Dose-dependent increases in incidences of hepatocellular carcinoma and hepatoblastoma. *Thyroid Gland:* • Incidences of thyroid gland follicular cell adenoma above historical controls.	*Clear evidence of carcinogenic activity*
Female B6C3F1/N mice	*Liver:* • Dose-dependent increases in incidences of hepatocellular carcinoma and hepatoblastoma; • Dose-dependent increases in incidence of hepatocellular carcinoma; and • Dose-dependent increases in incidences of multiple hepatocellular carcinomas and hepatoblastomas.	*Clear evidence of carcinogenic activity*

In B6C3F1/N mice, the determination of *clear evidence of carcinogenic activity* of the tested extract was predominantly based on the increased incidences of hepatocellular carcinoma and

hepatoblastoma in males and of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma in females. Incidence rates of hepatocellular neoplasms observed in male and female B6C3F1 mice under the conditions of the 2-year NTP study are summarized in Table 4.2.1-2.

Table 4.2.1-2 Summary of Hepatocellular Neoplasms in Male and Female B6C3F1 Mice in the 2-Year Study with the Specific *Ginkgo biloba* Extract

Tumor Type	Males				Females			
	Control	200	600	2,000	Control	200	600	2,000
Hemangiosarcoma	9/50 (18%)	3/50 (6%)	0/50 (0%)	3/50 (6%)	--	--	--	--
Adenoma (M: 24-78%/F: 2-78%)[1]	31/50 (62%)	46/50* (92%)	33/50 (66%)	33/50 (66%)	17/50 (34%)	37/50* (74%)	41/50* (82%)	48/50* (96%)
Carcinoma (M: 16-56%/F: 0-46%)	22/50 (44%)	31/50 (62%)	41/50* (82%)	47/50* (94%)	9/50 (18%)	10/50 (20%)	15/50 (30%)	44/50* (88%)
Adenoma or Carcinoma (M: 52-90%/F: 6-82%)	39/50 (78%)	46/50 (92%)	46/50 (92%)	49/50* (98)	20/50 (40%)	39/50* (78%)	41/50* (82%)	49/50* (98%)
Hepatoblastoma (M: 0-34%/F: 0-2%)	3/50 (6%)	28/50* (56%)	36/50* (72%)	38/50* (76%)	1/50 (2%)	1/50 (2%)	8/50 (16%)	11/50 (22%)
Carcinoma or hepatoblastoma (M: 18-58%/F: 0-46%)	24/50 (48%)	42/50* (84%)	45/50* (90%)	48/50* (96%)	10/50 (20%)	11/50 (22%)	18/50 (36%)	44/50* (88%)
Adenoma, carcinoma, or hepatoblastoma (M: 52-92%/F: 6-82%)	24/50 (78%)	42/50* (96%)	45/50* (96%)	48/50* (98%)	--	--	--	--

* P = <0.001.
[1] Overall historical control ranges for all routes.

In male mice, the dose-dependent increases in malignant hepatocellular tumors, reaching statistical significance at all dose levels for hepatoblastoma alone or when hepatocellular carcinomas or hepatoblastomas were combined, and when all malignant and benign tumors were combined, in comparison to concurrent controls, were consistent with *clear evidence of carcinogenic activity*. The increases also were significantly greater than concurrent controls at the mid- and high-dose levels when hepatocellular carcinoma was considered alone, at the highest dose levels when adenoma or carcinoma was combined, and at the low- and high-dose levels for hepatoblastoma alone. Likewise in females, dose-dependent increases in malignant tumors, reaching statistical significance at the highest does level (for carcinoma only and carcinoma or hepatoblastoma combined), were observed. Furthermore, significant dose-dependent increases in benign tumors (hepatocellular adenomas), as well as when benign and malignant tumors were combined (hepatocellular adenoma or carinoma) were observed at all dose-levels in females. The results in females, therefore, were also considered to show *clear evidence of carcinogenic activity.*

4.2.2 Classification of Evidence - Rats

Table 4.2.2-1 presents the basis for NTP's conclusions regarding the strength of evidence of the specific *G. biloba* leaf extract carcinogenic activity in male and female F344/N rats under the conditions of the 2-year study.

Table 4.2.2-1 Summary of the Basis for NTP's Conclusions Regarding the Strength of Evidence for Carcinogenic Activity of the Specific *Ginkgo biloba* Extract in F344/N Rats

Test Animal	Evidence Pertaining to Carcinogenic Activity	NTP's Overall Conclusion Regarding the Strength of Evidence
Male F344/N rats	*Thyroid Gland:* • Incidences of thyroid gland follicular cell adenoma above historical controls.	*Some evidence of carcinogenic activity*
Female F344/N rats	*Thyroid Gland*: • Incidences of thyroid gland follicular cell adenoma above historical controls; • Incidences of thyroid gland follicular cell adenoma or carcinoma above historical controls; • Single occurrences of follicular cell carcinoma (rare tumor).	*Some evidence of carcinogenic activity*

In rats, the NTP based their conclusion of *some evidence of carcinogenic activity* largely on the marginal increases in the incidences of benign follicular cell thyroid gland tumors (adenomas) in both males and females in comparison to control values and on the isolated occurrences of follicular cell carcinoma in females. The incidences of follicular cell thyroid non-neoplastic and neoplastic tumors observed in rats in the 2-year NTP study following administration of the specific *G. biloba* leaf extract are summarized in Table 4.2.2-2.

Table 4.2.2-2 Summary of Thyroid Neoplasms and Nonneoplastic Lesions in Male and Female F344/N Rats in the 2-Year Study with the Specific *Ginkgo biloba* Extract

Lesion Type	Males				Females			
	Control	100	300	1,000	Control	100	300	1,000
FC hypertrophy	13/50	37/50**	41/49**	41/45**	15/49	41/50**	45/49**	48/49**
Follicle hyperplasia	0/50	7/50**	9/49**	5/49*	3/49	3/50	1/49	5/49
FC Adenoma (M: 0-6%/F: 0-2%)[1]	2/50 (4%)	1/50 (2%)	3/49 (6%)	5/45 (11%)	1/49 (2%)	0/50 (0%)	3/49 (6%)	1/49 (2%)
FC Carcinoma (F: 0-4%)	--	--	--	--	0/49	0/50	1/49	1/49
FC Adenoma or carcinoma (F: 0-4%)	--	--	--	--	1/49 (2%)	0/50 (0%)	4/49 (8%)	2/49 (4%)

FC, Follicular cell.
* P = <0.05; ** P = <0.01.
[1] Overall historical control ranges for all routes.

Specifically, the incidence of follicular cell adenomas in males (1/50, 3/49, and 5/45 at 100, 300, and 1,000 mg/kg body weight/day, respectively) did exceed historical control values for corn oil gavage studies (0 to 4%) at the mid- and high-dose levels (6 and 11% at 300 and 1,000 mg/kg body weight/day, respectively) and historical control values for all routes of administration (0 to 6%) at the high-dose level. However, none of the increases reached statistical significance when compared to the concurrent control group (2/50 or 4%). In females, the incidence of follicular cell adenoma (0/50, 3/49, and 1/49 at 100, 300, and 1,000 mg/kg body weight/day, respectively) was increased above historical control values for corn oil gavage studies and all routes of administration, but only at the mid-dose level (6% *versus* 0 to 2%). As in males, none of the increases were statistically significant in comparison to the concurrent control group (1/49 or 2%). Although the single incidence of follicular cell carcinomas observed in each the mid- and high-dose females was deemed to be a rare occurrence, at 2% it fell within the historical control ranges for follicular cell carcinomas for corn oil gavage studies (0 to 2%) and for all routes of administration (0 to 4%). When both malignant and benign follicular cell tumors were combined (adenoma or carcinoma), only the incidence at the mid-dose level (4/49 or 8%) exceeded historical control values.

Non-neoplastic lesions of the thyroid gland in the 2-year study consisted of significantly increased incidence rates of follicular cell hypertrophy at all dose levels in both males and females, accompanied by significantly increased incidences of follicle hyperplasia in males only. The hypertrophy noted in both sexes, while occurring at greater levels than in the control group, was only of minimal to mild severity. Follicle cell hyperplasia in the males was considered as minimal at the low-dose level and between mild to moderate at the mid- and high-dose levels. In the 3-month dose selection study, a significant increase in the incidence of minimal to mild

follicular cell hypertrophy also was observed at the 2 highest dose levels in males and at the highest dose level in females. In the 14-week special study conducted in association with the 2-year rat study, low-dose males exhibited a significant increase in relative thyroid weight. Thyroid hormone level analysis in the 14-week special study revealed a dose-dependent increase in thyroid stimulating hormone (TSH), reaching statistical significance at all dose levels in males and at the highest dose level in females.

The changes observed in parameters assessing thyroid function in the 3-month study and the 14-week special study, together with the non-neoplastic changes in the thyroid of both males and females are indicative of a compound-related effect of the specific *G. biloba* leaf extract on the thyroid, and collectively support that the slight increase in follicular cell neoplasms was likely related to the administration of the extract. However, given that the incidences of benign follicular cell tumors were only increased in comparison to historical controls, and in females, only at the mid-dose level, it could be argued that the evidence for carcinogenic activity is only "*equivocal"* based on the absence of a statistically significant response in comparison to concurrent control values.

5.0 ASSESSMENT OF THE RELEVANCE OF THE NTP STUDY RESULTS FOR HUMAN CANCER RISK

5.1 Hepatocellular Tumors

In light of the high prevalence of hepatocellular tumors observed in mice, the relevance of the development of liver tumors in mice has been questioned with regard to human cancer risk (Maronpot *et al.,* 1987; Velazquez *et al*., 1996). The NTP has recognized the limitations of data pertaining to the development of liver tumors in the 2-year mouse bioassays, particularly in susceptible strains of mice (*e.g*., B6C3F1), with respect to extrapolating the results to humans in risk assessments and has noted that alternative rodent strains are being examined to supplement rat studies (Maronpot *et al*., 1987). The predictive value of mouse hepatocellular tumors with respect to human cancer risk has been repeatedly challenged (Velazquez *et al*., 1996; Carmichael *et al*., 1997). This is in part due to the fact that hepatocellular carcinoma in humans, particularly chemically-induced, is rare. In humans, the major risk factors associated with liver tumors are viral hepatitis, excessive alcohol consumption, and exposure to aflatoxin, in most cases accompanied by liver cirrhosis.

Most recently, the European Food Safety Authority (EFSA) reiterated that "hepatic tumors in mice are generally considered as irrelevant for human risk assessment" as part of their evaluation of a mouse dietary administration study conducted with aspartame (EFSA, 2011). Specifically, EFSA indicated in the report that there is general scientific consensus that

induction of hepatocellular tumors in mice by non-genotoxic compounds can be considered as irrelevant for human risk assessment (Holsapple *et al*., 2006; Billington *et al*., 2010). In their evaluation of the mode of action with respect to the relevance of rodent liver tumors to human cancer risk, Holsapple *et al*. (2006) concluded that in the case of chemicals displaying a phenobarbital-like P450 inducing mode of action, the observed hepatocarcinogenicity in rodents is not relevant to humans. Indeed, clinical use for over 80 years of phenobarbital, a known enzyme inducer in the rodent liver, has not been associated with an increased risk of tumor formation in the liver or any other organ in humans (McClain, 1990). In comparison to rats and humans, mice also are known to possess higher levels of monooxygenase activities, including increased levels of aryl hydrocarbon hydroxylase and biphenyl 2-hydroxylase, which are catalyzed by cytochrome P450 (Lorenz *et al*., 1984; Parke and Ioannides, 1990; Thorgeirsson *et al*., 1997). There are several examples of chemicals that are known to produce hepatocellular tumors in mice, but not in rats (Maronpot *et al*., 1987; Velazquez *et al*., 1996). Consideration also has been previously given to the possibility of a chemical with neoplastic effects confined to the mouse liver, inducing carcinogenic effects in humans in another organ. As noted by Carmichael *et al*. (1997), while there is an example of a genotoxic rodent (both mice and rats) liver carcinogen (*i.e*., benzidine) that is known to produce bladder tumors in humans, there is no example of a non-genotoxic mouse liver carcinogen that produces a tumorgenic response in humans in another organ. In the case of compounds inducing hepatocellular tumors *via* a cytotoxic mode of action, the carcinogenic response should be considered in the evaluation of human cancer risk if appropriate metabolism occurs in the animal model and in humans.

Although the specific *G. biloba* extract tested positive for mutagenic activity in the Ames assay, *in vivo* a negative response was obtained under the conditions of the micronucleus assay in B6C3F1/N male mice and in females the response was determined to be equivocal based on only a positive trend for increasing levels of micronuclei. While the response in females was determined to be equivocal based on trend analysis, none of the increases in the incidence rates of micronuclei in treated females attained statistical significance in comparison to controls and none of the increases were more than twice the control values at any dose level. The presence of a positive response *in vitro*, but not *in vivo* of the specific *G. biloba* extract is similar to the results of genotoxicity assays obtained for quercetin, which is one of the main constituents of the extract (it should be noted however that in the *G. biloba* extracts quercetin is glycosylated). The NTP draft report repeatedly indicates quercetin to be a mutagen; however, quercetin has been extensively studied for its mutagenic and genotoxic properties, both *in vitro* and *in vivo*, and while *in vitro* unequivocally positive results have been reported, *in vivo* quercetin has consistently tested negative (as reviewed by Harwood *et al.*, 2007). Furthermore, review of long-term animal studies with quercetin also demonstrated no carcinogenic activity for the compound. It has been shown that in the case of quercetin adequate metabolic processes, resulting in low bioavailability of unmetabolized quercetin, are operative *in vivo* that protect the

organism from genotoxic activity that may be apparent *in vitro*. Therefore, while the NTP draft report refers to quercetin as a known mutagen, this is not qualified in terms of *in vitro versus in vivo* effects. Furthermore, as already noted, *G. biloba* extracts contain almost exclusively quercetin glycosides and only trace amounts of flavonol aglycones (Upton, 2003). The flavonol glycosides undergo extensive first-pass metabolism and reach the blood and tissues neither as aglycones nor as glycosides. The glycosides are quickly deglycosylated to the aglycone and immediately conjugated with glucuronate or sulfate with or without methylation. Based on the negative *in vivo* results obtained in male mice following treatment with the specific *G. biloba* extract in the assays conducted by the NTP and the equivocal results in female mice, which interestingly showed a comparatively lower carcinogenic response in the 2-year study than males, the presence of quercetin glycosides in the extract, and the absence of quercetin-related *in vivo* genotoxicity or carcinogenic activity, the ability of the *G. biloba* extract to exert genotoxic properties *in vivo* is very questionable. It is more likely therefore that the effects observed in the 2-year mouse study were the result of non-genotoxic mechanisms. When considered cohesively, the carcinogenic responses observed in the NTP rodent studies are suggestive of a compound with enzyme stimulatory properties.

Several *in vitro* and *in vivo* studies examining the effects on rodent and human cytochrome P450 (CYP450) enzymes have been conducted. In rats, *G. biloba* extracts have been reported to induce CYP3A enzymes in a dose-dependent manner *in vitro* with no species difference between humans and rats (Deng *et al*., 2008a). *In vivo* studies conducted with mice and rats have confirmed the ability of *G. biloba* extracts and its constituents (specifically bilobalide) to induce CYP enzymes (Sugiyama *et al*., 2004; Umegaki *et al*., 2007; Deng *et al*., 2008b; Taki *et al*., 2009). In a study examining the ability of the 5 major constituents of *G. biloba* extract (bilobalide, ginkgolide A, B, quercetin, and kaempferol) on CYP enzyme induction, bilobalide dose-dependently increased the activity of CYP3A1 and CYP2E1, whereas ginkgolide A, B, quercetin, and kaempferol dose dependently increased the activity of CYP1A2 (Deng *et al*., 2008b). However, in humans, no induction of CYP450 enzymes has been observed based on the results of a number of clinical trials (Duche *et al*., 1989; Gurley *et al*., 2002; Markowitz *et al*., 2003; Izzo and Ernst, 2009; Zuo *et al*., 2010; Zadoyan *et al*., 2011).

In addition to the species-specific susceptibility of the B6C3F1 mouse to develop liver tumors, the levels at which the specific *G. biloba* extract was tested in the 2-year bioassay may be crucial to the interpretation of the liver tumors for purposes of human safety assessment. Most commonly, the highest dose level evaluated in a 2-year bioassay is set at the MTD in order to ensure that the assay is sensitive enough to detect a carcinogenic response without inducing excessive lethality (Chhabra *et al*., 1990; Gaylor, 2005). In the NTP studies on the specific *G. biloba extract*, the dose levels selected for purposes of the 2-year assays (*i.e*., 100, 300, and 1,000 mg/kg body weight/day in rats and 200, 600, and 2,000 mg/kg body weight/day in mice) were based on the results of preliminary 3-month studies. The 3-month studies were conducted

at dose levels of 62.5, 125, 250, 500, and 1,000 mg/kg body weight/day and 125, 250, 500, 1,000, and 2,000 mg/kg body weights/day in rats and mice, respectively. The rationale for the dose selection in both rodent species for purposes of the 2-year studies was based on the absence of an effect on survival, and in the case of rats also an absence of an effect on body weights. Hypertrophy of the liver and thyroid in rats and liver hypertrophy, along with body weight and organ weight changes in mice, were noted in the 3-month studies, but considered not to be "life-threatening". However, as discussed in Section 3.3, considering the overt increases in both relative and absolute liver weights following only 3 months of administration of the specific *G. biloba* extract at the lowest dose level tested (125 mg/kg body weight/day) in B6C3F1 mice with a demonstrated sensitivity for hepatocellular tumor development, and the association shown between liver tumor development and relative liver weight in mice (Carmichael *et al*., 1997), it is likely that especially in the mouse the lowest dose tested (200 mg/kg body weight/day) in the 2-year study could be predicted in advance to produce effects on the liver, including the development of liver tumors.

Administration of compounds at the MTD has been argued to result in repeated cell damage that is followed by reparative hyperplasia and ultimately uncontrolled cell growth (Velazquez *et al*., 1996; Carmichael *et al.,* 1997). Such a proliferative response at the MTD leading to tumor formation may not be relevant to an assessment of risk in humans at lower dose levels of exposure. A series of degenerative non-neoplastic changes were observed in the livers of male and female mice in association with the neoplasms, including hypertrophy, erythrophagocytosis, hematopoietic cell proliferation, inflammation, necrosis, and altered hepatic foci. In a report published by the National Academy of Sciences Committee on Risk Assessment Methodology (CRAM), it was stated that "[the MTD bioassay] does not provide…all the information useful for low-dose human risk assessment" (NRC, 1993) and for chemicals that do induce cancer at the MTD, it was suggested that additional data are necessary to determine the relevance of the response to human health risk assessment (Velazquez *et al*., 1996). Liver enzyme induction has also been suggested as an alternative non-genotoxic mechanism underlying liver tumor formation (Carmichael *et al.,* 1997). Liver tumors observed in long-term studies with compounds with a known proliferative effect on liver enzymes also are considered to be the result of high dose administration interfering with normal liver function. Overall, these non-genotoxic mechanisms are thought to be threshold dependent and it should therefore be possible to establish a dose below which an adverse response is not elicited, which can then be used in an assessment of human risk. However, since the lowest dose level tested in the 2-year mouse study was likely in excess of a level at which no neoplastic effects of the liver would be expected in mice, it is not possible to extrapolate the results to a dose level that would not induce liver cell hypertrophy and subsequent tumor development in B6C3F1 mice.

The results of the 2-year rat study provide additional support that the neoplastic hepatocellular effects observed in the mice were the results of treatment of a highly susceptible species at high

dose levels. In a less sensitive species, such as the rat, and at lower levels of use, these effects would likely not be observed. Although there were changes in a number of hepatic parameters noted in the 13-week rat study, as well as in the 14-week special rat study, along with non-neoplastic lesions in the 2-year study (hepatocellular hypertrophy, bile duct and oval cell hyperplasia, necrosis, cystic degeneration in males and hepatocellular hypertrophy, bile duct hyperplasia, and focal fatty change in females), none of these observed changes progressed to produce a statistically significant increase in liver tumors in the rat. In female rats no incidences of benign or malignant liver tumors were reported and in males only a slight increase in benign liver tumors above historical control values was noted. Specifically, although incidences of hepatocellular adenoma in male rats exceeded historical control values for corn oil gavage studies at the low- and mid-dose levels, the incidences were within the historical control ranges for all routes of administration. Furthermore, no adenomas were identified in male rats at the highest dose level tested.

It was noted that the hepatoblastomas observed in both males and females in the 2-year mouse study were considered relatively rare tumors. However, while hepatoblastoma is considered to be an infrequent tumor type of NTP 2-year studies, it is a variant of hepatocellular carcinoma and thus appears in animals that have also developed hepatocellular carcinoma (Maronpot *et al.*, 1987; Turusov *et al*., 2002). While they are rare, the occurrence of the hepatoblastomas in the 2-year *G. biloba* mouse study is not entirely unexpected in light of the overall increase in the incidence of hepatocellular lesions; however, the magnitude of the increase, given the rarity of the tumor, is somewhat unprecedented. Markedly higher levels of hepatoblastomas also have been observed following treatment of male D2B6F1 mice (11/30 or 37%) with phenobarbital, another enzyme stimulating compound, in the diet at 500 ppm (approximately 62.5 mg/kg body weight/day), for a period of up to 103 weeks (Diwan *et al*., 1995). Considering that *G. biloba* extract was administered at higher levels (≥200 mg/kg body weight/day), the incidence rates of 56, 72, and 78% for hepatoblastomas at 200, 600, and 2,000 mg/kg body weight/day, respectively, might not be completely unexpected for a compound with enzyme-inducing properties in the mouse.

Furthermore, there is indication that there are strain-specific differences in the susceptibility for the development of hepatoblastomas among mice strains, as supported by an example of a greater sensitive of B6C3F1 mice compared to Swiss-Webster mice for chemically induced hepatoblastoma (Turusov *et al*., 2002). Interestingly, an increase in the spontaneous incidence of hepatoblastomas in B6C3F1 mice in 2-year NTP studies has been reported (Turusov *et al*., 2002). Although Turusov *et al*. (2002) could not identify an explanation for the apparent increase in the background incidence of mouse hepatoblastoma, genetic drift in the NTP B6C3F1 mice was suggested. Also, as in the case of the specific *G. biloba* extract, 6 chemicals previously tested by the NTP (*i.e*., benzofuran, o-nitroanisole, oxazepam, pyridine, primidone, and anthraquinone) produced high incidences of hepatoblastomas in mice, but failed to produce

any increases in any liver tumors in rats. Additionally, differences pertaining to the onset in life and development of the tumor have been identified between hepatoblastomas in mice and humans (Turusov *et al*., 2002). Specifically, in humans hepatoblastomas are exceptionally rare in adults, but do develop in young children and sometimes *in utero*. In contrast, hepatoblastomas in mice have only been reported in aged animals, with only a limited number of cases of hepatoblastomas identified at 15-month necropsies. Moreover, unlike in mice in which hepatoblastomas appear to arise within preexisting hepatocellular adenomas and carcinomas, in humans the tumor type is formed *de novo*. It is worth noting however, that a common molecular alteration involving the β-catenin/Wnt signaling pathway has been reported in both mice and humans (Koch *et al.,* 1999; Anna *et al.*, 2000).

Considering that the etiology of hepatocellular tumors in humans is generally accepted to be different from that observed in murine test systems, combined with the fact that the testing of the specific *G. biloba* extract in the 2-year studies was likely conducted at excessive dose levels and that mice, particularly of the B6C3F1 strain are susceptible to the development of liver tumors, the relevance of the results of the 2-year mouse bioassay with respect to human risk characterization is questionable. This conclusion is further supported by the fact that the mechanism for the liver neoplasms in the study is likely to have been the result of prolonged liver enzyme induction with related hepatocellular hypertrophy. Chronic hepatocellular hypertrophy is recognized to lead to toxic and degenerative changes that in time result in necrosis and initiation of reparative processes. In rats, no significant increase in the incidence of liver tumors was observed, further corroborating that the occurrence of the liver tumors in mice was likely a species-specific response as a result of genetic predisposition, in combination with a high dose response associated with enzyme-induction and liver cell hypertrophy. Finally, while there is some evidence supporting the enzyme stimulatory properties of *G. biloba* extract in rodents, in humans, no induction of CYP450 enzymes has been observed based on the results of a number of clinical trials (Duche *et al*., 1989; Gurley *et al*., 2002; Markowitz *et al*., 2003; Izzo and Ernst, 2009; Zuo *et al*., 2010; Zadoyan *et al*., 2011).

5.2 Thyroid Tumors

As described in Section 4.2.2, incidences of follicular cell hypertrophy (minimal to mild severity) in male and female rats and follicle hyperplasia in male rats (mild to moderate) were significantly increased in comparison to control values. The increases in the incidence of follicular cell adenomas in both sexes of treated rats were slightly elevated above historical control values only. The single occurrences of follicular cell carcinoma in females at each the mid- and high-dose level, while rare, were not in excess of the historical control ranges (for either corn oil gavage studies or all routes of administration). In the 2-year mouse study, dose-dependent increases also were noted in follicular cell hyperplasia and hypertrophy (minimal to mild), reaching statistical significance in mid- and high-dose females (hypertrophy only) and in high-

dose males. With respect to the incidence of neoplasms, an increase only in the incidence of adenomas in mid- and high-dose males was noted and only in comparison to historical controls.

The etiology of follicular cell thyroid tumor formation in humans is generally thought to be similar to that of experimental animals, with perturbations in hormones of the thyroid and pituitary as the cause of most thyroid tumors (Velazquez *et al*., 1996). However, it is also recognized that while thyroid follicular cell tumors can develop in humans as a result of chronic imbalances in the thyroid-pituitary feedback mechanism, in humans, this is a threshold-dependant and much less sensitive response. Conversely, rodents are exceptionally more sensitive to thyroid hormone imbalances (Alison *et al*., 1994; McClain, 1995). Critical to this interspecies difference is the variability in the affinity with which the circulating thyroid hormones are bound to albumin in humans and other species. Specifically in rodents (mice and rats), the thyroid hormone plasma half-lives are approximately 10 times shorter than in humans, consequently resulting in a considerably greater turnover rate of the hormones in rodents.

Enzyme inducers, like phenobarbital, increase the metabolism and excretion of thyroid hormones (T_4 and T_3) and are known to promote thyroid tumor formation in rodents secondary to the effects on hormone balance (Alison *et al*., 1994). Extended induction of the thyroid by elevated levels of TSH in response to decreased levels of circulating thyroid hormones leads to follicular cell hypertrophy and hyperplasia, with eventual progression to thyroid tumors (Capen, 1997). Species that are generally more sensitive to such hormonal imbalance, like rodents, are particularly prone to secondary tumor formation as a result of chemically-induced (*via* for example enzyme inducers) disturbances in the feedback mechanism that regulates thyroid function. In rodents, only mild to moderate perturbations in thyroid hormone homeostasis will affect the incidence of thyroid tumor formation (McClain, 1995). Reportedly, only few compounds are known to result in a TSH-stimulating effect in humans (Alison *et al*., 1994). Epidemiological data corroborate that in humans chronic TSH-induced induction of the thyroid is only rarely associated with neoplasia (Paynter *et al*., 1988; McClain, 1995). Given the inherent predisposition of the rodent to thyroid tumor formation, thyroid tumors in rodent studies as a result of secondary, non-genotoxic mechanisms (*i.e*., hormone imbalance) bear little relevance to assessment of carcinogenic risk, particularly at dose levels that do not disrupt thyroid function in humans (Alison *et al*., 1994; McClain, 1995).

Considering that under the conditions of the NTP study, gavage administration of the specific *G. biloba* extract was associated with dose-dependent increases in levels of TSH in both male and female rats (as observed in the 14-week special study), combined with *in vivo* data in rodents demonstrating that *G. biloba* extracts possesses enzyme inducing properties, it is therefore reasonable to conclude that the thyroid tumors observed in rats were a secondary response to hormonal imbalances leading to prolonged stimulation of the thyroid. Furthermore, the increased incidence of the thyroid tumors observed in rats and mice did not reach statistical

significance over the course of the 2-year study periods, suggesting that while the specific *G. biloba* extract did have an effect on thyroid function, the imbalances, in a highly sensitive species, did not lead to an overwhelming tumor response in the thyroid. However, in this regard it is also important to consider whether the absence of a statistically significant response may have resulted from the occurrence of deaths prior to study termination as a result of non-thyroid related effects. A decrease in survival was observed in high-dose male rats as a result of mononuclear cell leukemia; however, the increase in early deaths was only notable beginning at Week 85 (600 days), which was not much earlier than the first incidence day for thyroid tumors in the high-dose male (Day 675). In low- and mid-dose male rats, as well as in all groups of female rats, survival rates were similar to the control group, so the absence of a statistically significant response is unlikely to have been the effect of animals dying prior to the development of thyroid gland tumors, particularly since the appearance of thyroid tumors while generally later in life, was with the exception of mid-dose males, similar between control and test animals. In fact, in mid-dose male rats in which the first incident day was considerably earlier than in the other test groups (Day 485) and in which survival was not affected, a statistically significant response in comparison to the control group also was not generated. Therefore, with the exception of perhaps the high-dose male rats, it is unlikely that the lack of a statistically significant response observed in rats in relation to the development of thyroid tumors was due to pre-term mortality of other causes.

6.0 CONCLUDING REMARKS

Review of the details pertaining to the identity and characterization of the specific *G. biloba* leaf extract demonstrated that the stability of the test material based on the data provided in the NTP draft report could not be adequately verified. Furthermore, the possibility of the presence of potential degradation products following storage could not be excluded based on the available data presented in the report. Also, it was noted that data related to heavy metal, microbiology, mycotoxin, polyaromatic hydrocarbon, and pesticide analysis, were not provided. In the absences of such data it is not possible to exclude the possibility that the results observed in the NTP studies were not related to the specific *G. biloba* leaf extract per se, but rather to other unidentified contaminants, impurities, or degradants present in the test material.

It was also noted that in the 2-year mouse study, the lowest dose level tested (200 mg/kg body weigh/day) was higher than the lowest dose tested in the dose-range determining 3-month study (125 mg/kg body weight/day), at which significant increases in liver weights of mice were observed. Considering that significant effects were observed at the lowest dose tested in the 3-month study, the rationale for the selection of a dose that is higher than the 3-month low-dose as the lowest dose for purposes of a 2-year study is questionable.

While the above noted study design issues may have implications on the overall study results that should be considered before any conclusions regarding the carcinogenic activity of the test material can be made, assuming that the results presented in the NTP draft report were indeed related to the specific *G. biloba* extract tested, oral administration of the specific *G. biloba* leaf extract at doses ranging from 200 to 2,000 mg/kg body weight/day under the conditions of the 2-year NTP study, was associated with *clear evidence of carcinogenic activity* in B6C3F1/N mice. In F344/N rats, the NTP determined that the results obtained following oral administration of the specific *G. biloba* leaf extract at doses ranging from 100 to 1,000 mg/kg body weight/day was indicative of *some evidence of carcinogenic activity*. However, while the increases in the incidence of thyroid tumors were in excess of historical control values, the increases were noted not to reach levels of statistical significance in comparison to the concurrent control group. Furthermore, the increases were limited only to benign neoplasms. Given therefore the absence of a statistically significant response in comparison to the concurrent control group and no increase in malignant tumor types, the results could be argued to be supportive only of *"equivocal"* evidence of carcinogenic activity in F344/N rats.

Furthermore, although under the conditions of the NTP studies the specific *G. biloba* extract tested was associated with varying levels of carcinogenic activity in rodents, the carcinogenic responses observed in these studies at relatively high dose levels are not necessarily predictive of the extract exerting similar responses in humans at lower levels of exposure. Specifically, the carcinogenic effects observed in the liver and thyroid in the rodent studies were likely observed as secondary effects of enzyme perturbations at high-dose levels, rather than as direct carcinogenic responses. Particularly, the ability of the extract to stimulate P450 enzymes should be considered as the mechanism underlying the effects observed in the 2-year studies. Indeed, the overall profile of the carcinogenic responses observed in the 2-year NTP studies is consistent with that of a chemical with enzyme stimulatory properties.

In light of the underlying non-genotoxic mechanism of action related to enzyme induction, the hepatocellular response in mice might be deemed to be irrelevant to assessment of human safety of the extract, whereas the response observed in the thyroid of both rats and mice is likely to be a threshold dependent effect that is considerably more pronounced in rodents than in humans. Although the increases in follicular cell tumors above historical control values were likely related to the administration of *G. biloba* in rats and mice, similar neoplastic changes of the thyroid in humans as a result of *G. biloba* consumption would be unlikely considering the differences in thyroid hormone homeostastis in humans and the lower levels of human exposure. Moreover, while in rodents there are data showing enzyme induction related *G. biloba* extract exposure, a limited number of studies have demonstrated a lack of an enzyme stimulating effect of *G. biloba* in humans.

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