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Peer-review Comments from the American Botanical Council on the Draft Technical Report from the National Toxicology Program on the Toxicology Studies of Green Tea Extracts in Rats and Mice

Re: Thakur SA, Blystone CR, Brix AE et al. NTP Draft Technical Report on the Toxicology Studies of Green Tea Extract in F344/NTac Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of Green Tea Extract in Wistar Han [Crl:WI(Han)] Rats and B6C3F1/N Mice (Gavage Studies) [NTP TR 585]. 2014.

The American Botanical Council (ABC) is an independent, international, nonprofit research and education organization comprised of research scientists, health professionals, educators, herb and dietary supplement industry members, and others in over 80 countries.* ABC appreciates the opportunity to submit comments on the NTP's draft report on the toxicology of a commercial green tea extract.

Comment Summary

Toxicological studies in mice and rats, such as the one carried out on green tea extract, can provide very useful data in the overall assessment of the safety and toxicity profile of ingredients used in a variety of consumer products including dietary supplements. This report[1] by the National Toxicology Program (NTP) is the 9th in a series of toxicology studies on botanical extracts used in dietary supplements. Based on an initial evaluation of the study, ABC finds that the report is problematic on a number of the following issues:

(1) the document does not clearly distinguish the use of green tea as a beverage and the use of green tea extracts as ingredients

in dietary supplements;
(2) there is an obvious issue of the applicability of findings in rodents to the safety of green tea extract in humans;
(3) there are questions about the appropriateness of the dosage levels used in the study and any suggestion that they have applicability with respect to the safety of the green tea at doses typically used as an extract or within a beverage during normal human intake; and
(4) there are inadequate data on the green tea extract itself, including the rationale for choosing the particular commercial source, the apparent disregard of the ingredient's expiration date, and the lack of adequate specific information on the solvents used in the preparation of this specific extract.

Safety of green tea as a beverage

The authors of this study have thus far failed to point out that the study material, green tea extract (hereinafter referred to as GTE in these comments) is different from dried green tea prepared as a water infusion, and that the observed toxicological effects of the GTE in the NTP study are not applicable to the very widespread use of green tea as a beverage. While the term "green tea extract" is consistently used throughout the draft report, the authors indicate that "green tea" can be used as a synonym for "green tea extract". This is confusing and potentially misleading, as the terms can be interpreted in such a way as to conflate the results for green tea extracts used in dietary supplements as if they could be applied to green tea as a beverage as well. Nowhere in the NTP draft report is it indicated that the study is not intended to examine in any way the use of green tea as a beverage, which has a long history of safe use in humans.

Accordingly, the systematic review on the safety of green tea *extracts* by the US Pharmacopeia (USP) Dietary Supplement Information Expert Committee (DSIEC) specifically excluded traditional green tea infusions or other beverage preparations due to the low incidence of reported adverse events associated with green tea beverages.[2] Further, the health Information web page on Green Tea on the National Center of Complementary and Alternative Medicine (NCCAM, at the US National Institutes of Health) states that "Green tea is safe for most adults when used in moderate amounts," and "There have been some case reports of liver problems in people taking concentrated green tea extracts. The problems do not seem to be connected with green tea infusions or beverages."[3] The authors of the NTP report have neither mentioned, clarified, nor emphasized this important distinction, and ABC believes that for the sake of clarity and avoiding any unnecessary confusion, it is in the public's best interests that they do so in the final report.

Applicability of the study's findings to humans

The evaluation of treatment effects or safety parameters in animal models is a well-established method to obtain preliminary data on a treatment regimen. However, as it is stated in the report, "The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports."[1] Nevertheless, the concern remains that the study results will be applied as such to the human population, even if the results from animal models often do not

concur with the results in human studies or sufficiently powered epidemiologic surveys (conducted on green tea beverage). This may be, for example, due to the fact that the animals may not adequately mimic the human physiology, and that the animals used in the studies most likely are young and healthy and may not adequately represent the typical human patient. There may also be other stressors to the animals involved during administration of a substance with physiologic or pharmacologic effects and when administered at such extreme doses. One example is the occurrence of nasal toxicity which was observed even at the lowest dosage of GTE given to mice (30 mg/kg). However, despite the relatively widespread use of GTE, not a single case of nasal toxicity or injury to the nasal epithelium in humans has been reported to our knowledge (and based on our searches for such on PubMed, Google Scholar, and other Internet databases).

Appropriateness of the dosage level

Initial doses for the current studies were selected based on a 3-month study in Harlan Sprague-Dawley rats conducted by the National Cancer Institute (NCI).[4] The abstract from that study described increased treatment-related deaths in the top dose group (1,000 mg/kg) to which green tea polyphenols were force-fed by gavage. Furthermore, the oral LD_{50} in Wistar rats for EGCG was determined in earlier studies to be 187 mg and 1868 mg EGCG/kg, respectively (in this study, no clinical signs were observed in Wistar rats after a single oral dose of 187 mg/kg EGCG; however all but one rat died within 72 hrs using 1868 mg EGCG/kg),[5] well within the dosage range used with the Wistar Han rats used the NTP study (48.4 – 484 mg EGCG/kg per day). Therefore, the increase in the occurrence of organ injury was to be expected in the treatment of Wistar rats at 484 mg EGCG/kg per day over the course of 2 years.

It is very challenging to determine a representative dosage level for GTE's since there is no official (e.g., from an official pharmacopeial monograph) dosage recommendation available, although Health Canada's Natural Products Ingredients Database[6] suggests dosages between 136 mg and 300 mg of EGCG or up to 690 mg of total catechins per day. A quick survey of human clinical studies found dosages from 250 mg per day up to 3 g/m^2 per day[7,8] (the average body surface area is reported to be 1.81 m^2 in normal weight adults[9]). Further, a relatively random survey of GTE-containing dietary supplements sold in the United States conducted on the Internet by ABC indicated that the recommended daily intake for these dietary supplements range from 250 mg to 2000 mg of GTE per day (corresponding to 125 mg – 500 mg EGCG per day based on product label information) (Table 1). This would indicate a daily amount of 4.2 mg – 33.3 mg/kg of GTE or 2.1 mg – 8.3 mg EGCG/kg in a human weighing 60 kg. As such, the highest dosage of GTE given to the rats, which is the basis of many of the authors' findings, is 5 to 39 times higher than dosage recommended and/or normally consumed to humans, using a factor of 6.2 to convert from the rat dose to the human equivalent dose.[10]

Table 1: Daily Intake Comparison among Commercially Available Green Tea Extract Brands[#]

Sample	Labeled amount of GTE extract/unit	Labeled standardized catechin[a]/EGCG content [%]	Daily Intake[b,c]	Daily amount of GTE	Max. daily amount catechins/EGCG
Product 1	equivalent to 1500 mg green tea	na/na	4 cps	na	600 mg/na
Product 2	na	na/na	90-120 drops	na	na/na
Product 3	500 mg	44.5/25	1 cps	500 mg	222.5 mg/125 mg
Product 4	500 mg	30/na	1 cps	500 mg	150 mg/na
Product 5	400 mg	40/na	1 cps	400 mg	160 mg/na
Product 6	na	na/na	6 mL	na	na/450 mg
Product 7	315 mg	15/na	4 cps	1260 mg	189 mg/na
Product 8	400 mg	50/na	1 cps	400 mg	200 mg/na
Product 9	250 mg[d]	75/55	2 cps	500 mg	na/na
Product 10	1000 mg	40/12	2 cps	2000 mg	800 mg/240 mg
Product 11	400 mg	40/na	1 cps	400 mg	160 mg/na
Product 12	400 mg	na/50	1 cps	400 mg	na/200 mg
Product 13	500 mg	80/50	1-2 cps	500-1000 mg	800 mg/500 mg
Product 14	500 mg	65/35	1 cps	500 mg	325 mg/175 mg
Product 15	725 mg	98/45	1 cps	725 mg	710.5 mg/326.25 mg
Product 16	250 mg	63/na	1-2 cps	250-500 mg	315 mg/na
Product 17	500 mg	80/50	1 cps	500 mg	400 mg/250 mg
Product 18	500 mg	na/35	1 tbl	500 mg	na/175 mg
Product 19	500 mg	na/na	1 cps	500 mg	na/na
Product 20	168 mg	na/90	1 cps	168 mg	na/150 mg
Product 21	250 mg	na/na	2-3 cps	500-750 mg	na/na
Product 22	250 mg	80/50	1 cps	250 mg	200 mg/125 mg
Product 23	250 mg	75/30	1-2 cps	250-500 mg	375 mg/150 mg
Product 24	750 mg	88/45	1 cps	750 mg	660 mg/337.5 mg

[#] Survey was conducted by the American Botanical Council from various randomly-chosen GTE-containing dietary supplement products located on the Internet between May 2 and May 6, 2014. This information is for research and comparison purposes only. This list is not intended to suggest an exhaustive review of such GTE-containing supplements.

[a]catechins or polyphenols depending on label information
[b]cps = capsules
[c]tbl = tablets
[d]product also contains 200 mg green tea leaf
na = not available

Selection criteria for the green tea extract

The criteria to select the specific commercial GTE used in the study are not well documented; the only information given in the draft report is the following: "The lot selected for testing was based upon concentration of EGCG, similarity to other products in the market, and availability in bulk quantity." It would have been helpful if the authors had explained more why they chose this specific extract, how the extract was manufactured, what the results were of the authors' market survey (since it is mentioned that the extract was similar to "other products on the market"). One could reasonably argue that a representative study on a GTE should have been carried out on a representative product, e.g., a leading product in terms of unit sales, if the eventual goal of the report was to provide a valid initial toxicological evaluation to be used as part of the basis for an eventual assessment by another party of the relative safety of GTE-containing dietary supplements used by the US consumer (i.e., as tested under the NTP protocols in mice and rats).**

Expiration date of the green tea extract

The Amax NutraSouce, Inc. certificate of analysis (CoA) of the GTE (50% EGCG lot # GTE50-A0302031114) used in the NTP study indicates that the extract was manufactured in November 2003. The CoA also notes that the GTE material had an expiration date of November 2006. Table I4 in the NTP report shows the dates when the animal feed for the NTP study was prepared and when it was analyzed for the 2-year gavage studies in mice and rats. According to this table, the initial preparation was made in July 2007, and the last preparation in June 2009. Therefore, the study was initiated with GTE material that was 7 months beyond its designated expiration date and the GTE material in the feed was used at least 30 months after the expiration date. The frequent analytical monitoring of the catechin composition of the extract by HPLC performed for NTP showed a stable level of catechins (apparently also in the animal feed, which is surprising based on the known susceptibility of green tea catechins to oxidation[11]), although it is not clear from the text if all the catechins were included in the stability evaluation; Nevertheless, ABC believes it is necessary and appropriate to question the ethical and possibly scientific validity of the results of the 2-year study if the material used was past the expiration date. Such practices would not likely be acceptable for selection and use of ingredients for drug studies and should not be considered best practices for this study.

Analytical data of the green tea extract

There are a number of concerns in regards to the analytical data that were provided. First, there are no data on the validation of the HPLC-UV method that was used to quantify the catechins in the commercial GTE. Since there was no evidence of such data, or a statement that the method had been validated according to acceptable standards, it is unclear if the method used actually did provide accurate data. Also lacking are data on residual solvents and pesticides, so the question remains if the observed toxicity at least to some extent was due to the presence of potential contaminants. It is unfortunate that the pesticide screen could not be performed due to matrix interferences. Federal government-mandated current Good Manufacturing Practices (cGMPs) for dietary supplements[12] require ingredients to be tested for limits on those types of contaminations that may lead to adulteration of the finished batch

of the dietary supplement. Therefore a pesticide test is required on every dietary supplement sold in the US, and it appears odd that this problem could not be solved by analytical means. It is also unclear why no test for residual solvents was performed. A solvent screen would have been appropriate, as class 2 solvents (The International Council on Harmonization Class 2 solvents are solvents that should be limited due to inherent toxicity)[13] have been found to be present in some GTEs. If the authors' rationale was that the presence of residual solvents was unlikely in an extract made with ethanol and water (which are the solvents listed in the manufacturer's CoA) or that they relied on the data on residual solvents provided by the manufacturer, this should have been clearly indicated in the NTP report. Providing data on pesticides and residual solvents would have allowed avoiding the uncertainty of the possible role of potential contaminants in the outcome of the study.

Conclusion

It is surprising that NTP would undertake a study on an ingredient which is investigated as a cancer chemopreventive agent and for which there are currently no epidemiological data or case reports of which we are aware suggesting carcinogenicity in humans. If the goal is use of the work as a preliminary screening tool to determine the need for future studies, it should be presented in that context as the study demonstrates that there is no evidence or equivocal evidence for carcinogenicity of green tea extracts in rodents as well. In addition, the authors identified liver, nose, and gastrointestinal tract (rats only) as the major targets for toxicity in Wistar Han rats and B6C3F1/N mice based on the 2-year studies. The authors note that liver toxicity is a major safety concern following GTE use in humans and that the "green tea extract-induced gastrointestinal toxicity was consistent with previously published studies in animals and humans". While there are indeed a number of case reports of liver injury and incidences of gastrointestinal toxicity in humans linked to the consumption of GTE, to our knowledge, no toxic effects of GTE's to the nose are known to date. Despite the explicitly stated limitations in the applicability of the report's findings to other animals including humans, the extrapolation of the results of such studies to the human population has happened on numerous occasions in the past and its occurrence based on this draft study is a major concern for the authors of this peer-review.
In addition, we believe that addressing our concerns with regard to a clear distinction between the use of green tea as a beverage and the use of green tea extracts as ingredients in dietary supplements, the dosage level used in the study, and inadequate data on the specific GTE itself (in particular the apparent disregard of the expiration date, and the lack of analytical data on potential pesticide levels and residual solvents) will improve the quality of the report and will make this a more helpful document for anyone with an interest in this study.

Sincerely,

[Redacted]

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Endnotes

* Founded in 1988, ABC produces peer-reviewed articles, white papers, summaries of published scientific studies, books, and other information related to history, ethnobotany, clinical research, quality control, conservation, and other aspects of medicinal and aromatic plants and fungi used for health purposes.
** The overarching vision of NTP is to expand the scientific basis for making public health decisions on the potential toxicity of environmental agents. In regard to the dietary supplement program, "NTP is conducting numerous studies in rodents to identify potential adverse effects of these agents after both short-term exposure and long-term exposure. These studies may provide toxicology data that can be used by FDA, the National Institutes of Health (NIH), the public, and other stakeholders in evaluating the safety of supplements and may lead to removal of unsafe products from the market." [14]

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