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Safety assessment of green tea based beverages and dried green tea extracts as nutritional supplements

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1. Abstract

The safety of green tea infusions and green tea extract (GTE)-based products is reviewed regarding catechins. Epigallocatechin 3-gallate (EGCG), the major catechin present in green tea, is suspected of being responsible for liver toxicity reported in humans consuming food supplements. Intake of EGCG with green tea infusions and GTE-based beverages is up to about 450 mg EGCG/person/day in Europe and higher in Asia. Consumption of green tea is not associated with liver damage in humans, and green tea infusion and GTE-based beverages are considered safe in the range of historical uses. In animal studies, EGCG's potency for liver effects is highly dependent on conditions of administration. Use of NOAELs from bolus administration to derive a tolerable upper intake level applying the margin of safety concept results in acceptable EGCG-doses lower than those from one cup of green tea. NOAELs from toxicity studies applying EGCG with diet/split of the daily dose are a better point of departure for risk characterization. In clinical intervention studies, liver effects were not observed after intakes below 600 mg EGCG/person/day. Thus, a tolerable upper intake level of 300 mg EGCG/person/day is proposed for food supplements; this gives a twofold safety margin to clinical studies that did not report liver effects and a margin of safety of 100 to the NOAELs in animal studies with dietary administration of green tea catechins.

Keywords: green tea; catechins; food supplements; safety assessment

2. Introduction

Green tea infusions are widely used as beverages and a number of chemical components present in green tea such as epigallocatechin 3-gallate (EGCG, see Fig. 1) are claimed to have chemopreventive actions on a variety of health-related endpoints in humans (Wickremasinghe, 1978; Singh *et al.*, 2011). Therefore, green tea-based products are marketed as "nutraceuticals". The marketed nutraceuticals are dried green tea extract (GTE)-based beverages to be consumed as ready-to-drink (RTD) and dried GTEs intended as food supplements in the form of capsules.

GTE-based food supplements have been associated with cases of liver toxicity in humans. Therefore, the Norwegian Food Safety Authority asked the Norwegian Institute of Public Health (NIPH) (NIPH *et al.*, 2015) to develop a safety assessment on the levels of EGCG in GTEs used in food supplements. The NIPH concluded that margins of safety between doses of GTEs or their major components causing adverse effects in animals and estimated human consumption are less than 10 and thus "higher than what is recommended by the MOS approach".

This manuscript provides a short overview on exposure of humans to green tea infusion, green tea extract-based beverages, and dried green tea extracts used as food supplements and includes separate considerations on health aspects and safety assessment of these products and specifically EGCG, since green tea extracts (GTEs) have been implicated in some cases of liver damage in humans. The safety assessment considers both animal toxicology and human clinical studies on GTEs to conclude on a tolerable upper intake level for GTEs used as nutraceutical supplements and for medicinal uses.

3. Safety of green tea infusions

Traditional green tea infusions use young leaves and leave buds from *Camellia sinensis* (L.) Kuntze to produce traditional "green tea" as the basis for traditional aqueous green tea infusions. Green tea is manufactured without fermentation and contains a number of catechins (for structures, see Fig. 1) (Nishitani and Sagesaka, 2004; EFSA, 2009).

The contents of catechins and other constituents in green tea show considerable variation due to growing conditions of the plant, age of leaves harvested, and processing. The contents of catechins in green tea infusions also vary with the ways to brew (Negishi, 2013; Bhagwat and Haytowitz, 2015). EFSA has derived intake estimates for green tea and EGCG intakes with means of 95.2 and 147.7 mg EGCG/day with a high intake of 288.5 and 447.6 mg EGCG/day for European consumers (EFSA, 2009). Based on a number of epidemiology studies and the concentration of EGCG in green tea sold in Japan, we have estimated mean EGCG consumption from green tea in Japan as 314 mg/day with a maximum of 734 mg/day (see Appendix A and (Khokhar *et al.*, 1997). Intake of EGCG may be even higher depending on the brewing conditions. While green tea infusion has sometimes been associated with liver damage in predisposed humans (EMA, 2013a,b), such events have not been observed in controlled studies and may be due to the presence of confounders (EMA, 2013a,b). Green tea infusions have a history of safe use in Asia and no alerts regarding liver toxicity are established for green tea infusions in Japan despite high consumption. Adverse effects of green tea consumption have not been reported in Japan despite high intakes (Appendix A). Therefore, it can be concluded that EGCG intake in the form of green tea infusions should be safe up to the maximum consumption of 734 mg EGCG/person/day.

4. Safety issues with GTE-based beverages

Green tea extract (GTE)-based beverages (for example, “Healthya green tea” and “Healthya water”) marketed in Japan include 540 mg of total green tea catechins (140 – 209 mg EGCG) in one serving which is the recommended daily consumption. The total intake of green tea catechins from one serving of such a beverage is equivalent for 4-6 cups of green tea and intake of green tea catechins and EGCG from this source at the recommended consumption are thus well within the historical daily consumption of green tea catechins in Asia. The GTE-based beverages marketed in Japan have been approved by the Japanese Consumer Affairs Agency (CAA) as “Foods for Specified Health Uses (FOSHU)” and the efficacy and safety of Kao Corporation’s GTE-based beverages “Healthya” have been assessed by the Japanese Ministry of Health Labor and Welfare in 2003 resulting in approval for marketing (now the authority of the FOSHU has been transferred to CAA), based on historical safe uses of green tea as food, absence of genotoxicity, several repeat-dose toxicity studies in rodents with GTE used by Kao Corporation (Chengelis *et al.*, 2008; Ogura *et al.*, 2008; Morita *et al.*, 2009a; Morita *et al.*, 2009b) and clinical trials conducted by Kao Corporation (see Nagao et al., in Appendix B). In addition, a number of clinical studies with beverages fortified with GTEs have been published with durations of up to one year (median duration of 12 weeks) and the highest intake of 498.6 mg EGCG/person/day without concerns for hepatotoxicity (for details of study design and outcome, see Appendix B). Liver function was assessed in 17 of the 20 publications without adverse effects of the GTE-based beverages on this endpoint. Treatment-related adverse effects on other endpoints were also not reported in these 20 publications. Therefore, GTE-based beverage can be considered as safe up to EGCG doses that are identical to those delivered by traditional green tea infusions based on the history of safe use of green tea and the absence of adverse effects induced by GTE-based beverages in clinical studies.

5. Safety issues with dried green tea extracts used as food supplements

As mentioned above, consumption of food supplements containing green tea extracts (GTEs) has been associated with adverse liver effects in humans in a number of case reports (NIPH *et al.*, 2015), but the causative agent responsible for the liver injury due to the consumption of these GTEs is

unknown (Navarro et al., 2017). In addition, observations made in the available toxicity studies on GTEs in experimental animals have raised concerns regarding safety of GTEs (Lambert *et al.*, 2010). These have been extended to include green tea catechins in general.

The human daily doses of GTEs from food supplements may vary widely due to different methods to obtain GTEs, different recommendations regarding intake by manufacturers, and consumer attitude. The Norwegian Institute of Public Health, based on data from food authorities in the Nordic countries, conclude that exposures of up to 1 944 mg/day for GTEs and up to 980 mg/day of EGCG (NIPH *et al.*, 2015) are possible. However, some consumers may have higher intakes of GTEs due to the easy palatability of capsule formulations.

An assessment of the safety of dried GTEs needs to consider both the results of liver function testing in the many available clinical studies on GTEs and the results of animal toxicity studies with dried GTEs. Well-designed human clinical studies are more relevant for a safety assessment than animal toxicity studies.

A large number of human studies have assessed possible health-protective effects of dietary supplementation with dried GTEs and many of these studies have also monitored "liver enzymes" in blood or determined other parameters indicative of liver function impairment in the dried GTE-exposed subjects (for details of study design and outcome, see Appendix B). Doses of EGCG applied ranged from 100 to 4000 mg EGCG/human subject/day with durations between one day and two years, median duration of the dried GTE-application was 90 days. Increased "liver enzymes" were only seen in studies that administered daily doses of EGCG > 800 mg/human subject/day. A systematic review of the literature published up to 2013 (Isomura *et al.*, 2016) concluded, based on an analysis of the reports from 34 randomized clinical trials, that liver-related adverse events were rare (seven cases in 1405 human subjects that had received dried GTEs versus one case in 1200 controls) and that liver-related adverse effects were generally "mild". In addition, an analysis of several databases regarding adverse effects after consumption of dried GTE-containing supplements also concluded "*Overall, the average incidence (of adverse effects) combined over the last ~10 years was approximately 0.00364998/10,000, which is considered extremely rare"* (Yates *et al.*, 2017). Studies published after the cut-off date for these analyses provide additional support for the conclusions (Dostal *et al.*, 2015; Lovera *et al.*, 2015; Navarro *et al.*, 2017).

The results of the most relevant animal toxicity studies with GTEs are summarized in Table 1. A comprehensive overview on the available animal toxicity studies with EGCG and GTEs is given in Appendix B.

The results of the studies show that the toxicity of EGCG after repeated administration depends on conditions of dosing with significantly lower NOAELs observed in "fasted" animals or after bolus doses administered by gavage. These differences indicate that kinetics of absorption of EGCG from the gastrointestinal tract plays a major role in the expression of liver toxicity in animals (Zhu *et al.*, 2000; Feng, 2006). Toxicokinetic data from humans after consumption of equal amounts of EGCG with green tea or capsules with dried GTEs indicated a higher C_{max} and a higher AUC when EGCG was delivered with capsules (Henning *et al.*, 2004) and intake of GTEs with food in humans resulted in significantly lower blood levels of EGCG as compared to intake after fasting. In addition, a comparatively higher bioavailability of EGCG was observed after an oral bolus dose of 800 mg/person as compared to 200 and 400 mg/person (Ullmann *et al.*, 2004). In dogs, plasma C_{max} was 10-fold higher in "fasted" dogs as compared to fed dogs after a single bolus dose of 500 mg green tea preparation/kg bw (Isbrucker *et al.*, 2006b). Apparently, toxicokinetics and toxicity assessment of EGCG are complicated by a variety of factors such as food intake (Chow *et al.*, 2005; Kapetanovic *et al.*, 2009; Wu *et al.*, 2012), previous intake of EGCG (James *et al.*, 2015), and species and dose

dependent differences in disposition and biotransformation (Ullmann *et al.*, 2004; Mata-Bilbao Mde *et al.*, 2008; Chen *et al.*, 2012). A variety of drug interactions has been observed with green tea polyphenols based on inhibition of enzymes of biotransformation and/or drug transporters. These interactions may have both beneficial and adverse outcomes (Teschke *et al.*, 2014; Jaiyen *et al.*, 2015; Kasture *et al.*, 2015; Cao *et al.*, 2016; Satoh *et al.*, 2016; Yang *et al.*, 2017).

GTEs did not induce neoplastic changes in two independent carcinogenicity bioassays and did not induce reproductive and developmental toxicity in an appropriately performed animal study (Isbrucker *et al.*, 2006c). In addition, GTEs did not result in genotoxic responses in *in vitro* and *in vivo* systems (Isbrucker *et al.*, 2006a; US-NTP, 2016).

6. Possible approaches to derive a tolerable upper intake level for EGCG in green tea and GTEs

The classical approach to risk characterization for chemicals marketed for technical purposes is to require a margin of safety (MoS) of > 100 based on the lowest NOAEL or a benchmark dose, usually derived from animal toxicity testing with the chemical considered. Applying this approach to EGCG intake from green tea and to intakes of GTEs results in a number of issues.

Studies using daily bolus administration. Animal studies with low NOAELs are the “fasted dog” study with a NOAEL of 40 mg EGCG/kg bw/day and the two-year carcinogenicity study on GTEs performed by the US NTP with NOAELs in the same range. Applying the MoS concept to define a tolerable upper intake level will result in tolerable intakes of < 0.4 mg EGCG/kg bw/day corresponding to 24 mg EGCG/day for a 60 kg adult. Such an intake limit for EGCG would already be exceeded by the consumption of a single cup of green tea. Moreover, there are a number of problems with interpretation of the study outcomes. The results of the “fasted dog” study are confounded by high toxicity and decreased food intake and survival. In the NTP study, pronounced adverse effects of the GTE on the gastrointestinal tract occurred in rats represented by dose-dependent increases in the incidences of gray to black focal to diffuse discoloration observed macroscopically in the stomach mucosa and small intestine of rats at terminal sacrifice. These gross changes coincided with significant dose-dependent increases in the incidences of necrosis in the mucosa of the glandular stomach and all segments of the small intestine in both males and females. These lesions in the gastrointestinal tract were primarily observed in the high dose groups and correlated with the low survival observed in these groups. Although the exact causes and mechanisms of observed gastrointestinal injury are not completely understood, injury could be attributed to administration of high doses of condensed tannins or catechin polyphenols in the GTE. Previous studies have demonstrated that exposure to high amounts of tannins could cause gastroenteritis and intestinal wall congestion (Dollahite *et al.*, 1962; Boyd *et al.*, 1965). Acute hepatic necrosis may therefore be secondary to septic shock or to hypoxia rather than to a direct hepatotoxicity of components in the GTE. Moreover, similar to the reported effects in lymphoid tissue, the observed effects on reproductive parameters may also represent secondary alterations as a response to gastrointestinal irritation and stress.

In addition, since regular green tea consumption at > 20-fold higher daily doses in humans is not associated with liver effects, a tolerable upper intake level for EGCG based on a minimum MoS of 100 and the NOAELs from the “fasted dog” study does not have support from real world observations. As

outlined above, the studies resulting in the low NOAELs for EGCG applied bolus doses to fasted animals and thus did not mimic recommended human exposures to EGCG from EGCG-containing beverages and food supplements. GTE-based food supplements are recommended to be taken with meals with the daily dose split over two administrations. Consumption of green tea or GTE-based beverages also does not result in uptake of GTEs/EGCG in the form of a bolus dose due to the large volumes of water to be consumed within a short time under such conditions.

Animal toxicity studies using administration in feed or several doses over a day. A tolerable upper intake level for EGCG is better justified by integrating studies that mimic the human exposure situation, i.e. intake of EGCG with food or with intake divided over several doses/day. There are two such studies available for GTEs with NOAELs of 460 mg EGCG/kg bw/day (90-day study in "fed" dogs) and 551.9 mg EGCG/kg bw/day (two-year rat study with application of GTE in diet) for EGCG. Using the NOAELs from these studies as a point of departure for risk characterization, a tolerable upper intake level of approximately 300 mg EGCG/person/day will give an MoS of > 100. In this context, it needs to be considered that some essential nutrients such as selenium or fluoride have an MoS < 100 between the daily intakes required to sustain health in humans and doses that induce adverse effects in experimental animals (EFSA, 2006). This clearly shows the limits of extending classical risk characterization procedures developed for chemical contaminants to essential nutrients or chemicals taken as food supplements/nutraceuticals.

Human studies and appraisal of the relevance of animal toxicity studies. To support a tolerable upper intake level of EGCG in humans, in addition to animal toxicity studies, results of a large number of clinical intervention studies with repeated administration of EGCG are available. Since many of these clinical studies have assessed liver function in the study populations, they are highly relevant for the endpoint of concern (liver toxicity) identified regarding intake of EGCG from dietary supplements. An evaluation of the outcome of the studies with dried GTEs administered as capsules showed that effects on liver enzymes were not observed when EGCG intake remained below 600 mg/person/day. Above this dose, occasional increases in "liver enzymes" were observed, but the changes were reversible and the extent of increase in "liver enzymes" was usually small. Based on these observations, a human NOAEL of 600 mg EGCG/day was derived and, by integration of a safety factor of two, a tolerable upper intake level of EGCG in humans was proposed as 300 mg EGCG/person/day (Yates *et al.*, 2017). This intake level also results in an MoS of approximately 100 (see above). Regarding green tea infusions and GTE-based beverages, adverse effects have not been reported and these products are considered safe to be consumed in the range of historical safe use. Thus, it should be pointed out that this tolerable upper intake level of EGCG is not appropriate for green tea infusion and GTE-based beverages containing similar EGCG level with green tea infusion.

Major issues limiting the utility of the case studies reporting liver effects after consumption of dried GTEs are the often inadequate exposure information, concomitant medication or alcohol use (Di Lorenzo *et al.*, 2015), presence of other plant extracts in addition to GTEs in the preparation implicated in the effect (Mazzanti *et al.*, 2015; Stickel and Shouval, 2015; Couturier *et al.*, 2016; Zheng *et al.*, 2016; Navarro *et al.*, 2017), changes in product composition over time (Lovera *et al.*, 2015); Stickel and Shouval, 2015), adulteration (Kanda *et al.*, 2003; Lau *et al.*, 2004; Di Lorenzo *et al.*, 2015; van Hunsel *et al.*, 2016), and product mislabeling (Navarro *et al.*, 2017). Although most of the case reports applied causality algorithms and documented an association between liver effects and consumed dietary supplements, an association of the reported effects with intake of EGCG remains unclear. Dose-response information to derive limits for intakes to be considered as safe is also not available from the case studies.

This review describes a safety assessment of green tea catechins in light of different intake forms based on the current knowledge. In conclusion, green tea catechins consumed as infusions and in the form of fortified beverages are safe to be consumed without adverse effects. While some of animal

toxicity studies with bolus administration of green tea extracts and case reports indicate concerns for liver injury after consuming green tea catechins with dietary supplements, green tea-based food supplements are considered safe at intake up to 300 mg EGCG/person/day based on the absence of liver toxicity in many well performed clinical studies.

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Figure legends

Fig. 1: Structures of green tea catechins and EGCG.

Table 1: Selected animal toxicity data for green tea extracts with long-term exposures expressed as doses of EGCG

Study	**Study design**	**Dosing condition/ doses of EGCG (mg/kg bw/day)**	**Toxicity**	**NOAEL/LOAEL on EGCG basis (mg/kg)**
(Isbrucker *et al.*, 2006b)	"Fasting" (no food for 15 h before application) Beagle dogs, 4 males and 4 females/dose group, **for 13 weeks**	0, 40, 120, and 400 mg as oral bolus applied in a capsule	Mortality, liver necrosis, reduced body weight gain	NOAEL of 40 mg EGCG/kg bw/day
(Isbrucker *et al.*, 2006b)	"pre-fed" Beagle dogs, Beagle dogs, 4 males and 4 females/dose group, **for 13 weeks**	0, 46, 275, 460 (oral in capsules, daily doses divided into two applications)	No adverse effects reported	NOAEL of 460 mg EGCG/kg bw/day
(Yoshida *et al.*, 2011)	Wistar rat, 10 males and 10 females/dose group, **for 12 months**	male: 0, 4.2, 63.7, 225.7, and 838.5 (feeding) female: 0, 6.2, 94.1, 333.9, 1101.3 (feeding)	No adverse effects reported	NOAEL of 838.5 mg EGCG/kg bw/day in males and 1101.3 mg EGCG/kg bw in females
(US-NTP, 2016)	F344 rats, 10 males and 10 females/group, B6C3F1 mice, 10 males and 10 females, **for 14 weeks**	Gavage of 0, 30.3, 60.5, 121.0, 242.0, and 484.0 in rats Gavage of 0, 30.3, 60.5, 121.0, 242.0, and 484.0 to mice	Reduced weight gain and increased mortality, changes in "liver enzymes",	Liver function parameters changed at higher doses, but little dose or time dependence; changes in "liver enzymes" were small
(Yoshida *et al.*, 2011)	Wistar rats, 50 males and 50 females/dose group, **for 2 years**	males: 0, 3.7, 53.5, 181.6, 551.9. Females: 0, 4.4, 64.7, 216.8, 671.4 (feeding)	No effects	NOAEL of 551.9 mg/kg bw/day, the highest dose administered

Study	Study design	Dosing condition/ doses of EGCG (mg/kg bw/day)	Toxicity	NOAEL/LOAEL on EGCG basis (mg/kg)
(US-NTP, 2016)	Wistar rats, 50 males, 50 B6C3F1 mice, 50 males, 50 females, **for 2 years**	Gavage of 0, 48.4, 145.2, 484.0 to rats; gavage of 0, 14.5, 48.4, and 145.2 to mice	Reduced body weight gain and survival, liver necrosis at 484 mg EGCG/kg bw/day in rats	Based on incidences of histopathologic changes, NOAEL for liver effects in rats was 48.4 mg EGCG/kg bw/day in females and 145.2 mg EGCG/kg bw/day in males In mice, NOAEL for liver was 14.5 mg EGCG/kg bw/day in males No increases in tumor incidences in rats and mice

Fig. 1