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The safety of green tea and green tea extract consumption in adults – Results of a systematic review

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ABSTRACT

A systematic review of published toxicology and human intervention studies was performed to characterize potential hazards associated with consumption of green tea and its preparations. A review of toxicological evidence from laboratory studies revealed the liver as the target organ and hepatotoxicity as the critical effect, which was strongly associated with certain dosing conditions (*e.g.* bolus dose *via* gavage, fasting), and positively correlated with total catechin and epigallocatechingallate (EGCG) content. A review of adverse event (AE) data from 159 human intervention studies yielded findings consistent with toxicological evidence in that a limited range of concentrated, catechin-rich green tea preparations resulted in hepatic AEs in a dose-dependent manner when ingested in large bolus doses, but not when consumed as brewed tea or extracts in beverages or as part of food. Toxico- and pharmacokinetic evidence further suggests internal dose of catechins is a key determinant in the occurrence and severity of hepatotoxicity. A safe intake level of 338 mg EGCG/day for adults was derived from toxicological and human safety data for tea preparations ingested as a solid bolus dose. An Observed Safe Level (OSL) of 704 mg EGCG/day might be considered for tea preparations in beverage form based on human AE data.

1. Introduction

Tea is the most commonly consumed beverage in the world after water, with total annual sales exceeding $43 billion globally, more than $11 billion of which is accounted for by green tea (*Camellia sinensis* (L.) Kuntze) (Euromonitor, 2015). A growing body of evidence continues to emerge demonstrating a variety of potential health benefits from consumption of green tea and its constituents (Cassidy et al., 2015; Jacques et al., 2013; Peng et al., 2014). A wide range of ready-to-drink green tea beverages and food supplement products in capsule and tablet forms have entered the market in recent years, resulting in additional exposure to the traditional consumption of brewed green tea. Indeed, their health benefits have led to discussions focused on the prospect of establishing dietary guidance or even recommended intakes for green tea and/or its bioactive constituents (Gaine et al., 2013; Lupton et al., 2014; Wallace et al., 2015).

Average green tea consumption was reportedly three cups per day among tea drinkers (Hakim et al., 2003; Kaegi, 1998; Khokhar and Magnusdottir, 2002), while in some countries it could be as high as ten cups per day (Muramatsu, 1991).[1] Green tea is rich in phenolic compounds and considered one of the major dietary sources of flavan-3-ols and flavonols (Song and Chun, 2008). According to the United States Department of Agriculture (USDA) Flavonoid Database, brewed green tea contains an average of 126.6 mg total catechins and 77.8 mg EGCG per 100 ml as consumed, on the basis of 1 g leaf/100 mL infusion. Consequently, each 240 mL serving of brewed green tea may provide an estimated 304 mg total catechins, with 187 mg EGCG. Therefore, the estimated daily intakes of catechins and EGCG through green tea consumption can reach approximately 912 and 560 mg/day, respectively, for individuals consuming an average of three 8 oz. cups of green tea daily. Various green tea preparations are also commonly used in food supplements and mostly by adults. A search of the U.S. Dietary Supplement Label Database using the words “green tea” as part of the dietary ingredient name produced 2373 products that contained the

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[1] It should be noted that brewing practices and cup sizes are not universal. The content of polyphenols in brewed tea and tea beverages can be affected by the types of preparation and techniques used, which vary considerably among different ethnic populations. In Japan and China, 2–3 g loose tea leaves or tea bags are typically used for tea brewing in a cup of 100–150 mL hot water, sometimes repeatedly, while American tea drinkers typically use 2.25 g (1 tea bag) in a cup of 180–240 mL water (Yang et al., 2007).

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ingredients named green tea, green tea leaf, green tea extract (GTE), green tea powder, green tea catechins (GTC), and/or green tea phytosome (NIH, 2015). Because these ingredients likely differ in their manufacturing processes, chemical compositions and recommended conditions of use, the amount of tea catechins delivered in these products can vary widely. The reported doses of tea catechins were between 25 and 750 mg per serving with daily intakes ranging from 25 mg to 1500 mg. The dosage forms were provided predominantly as capsules and tablets. In addition, green tea extracts are also widely used as flavoring agents *quantum satis* in various food applications in many markets.

Green tea catechins, including the well-known constituent EGCG, have been implicated as both beneficial (Fujiki et al., 2015; Legeay et al., 2015) and harmful (García-Cortés et al., 2016; Harrison-Dunn, 2016; Sarma et al., 2008). Many of the safety concerns stem from published case reports alleging a link between concentrated GTE consumption and liver injury (García-Cortés et al., 2016; Harrison-Dunn, 2016; Teschke et al., 2014). In 2009, the European Food Safety Authority (EFSA) Scientific Cooperation Project (ESCO) published a safety assessment on green tea, focusing on dried extracts and traditional infusions consumed as food including beverages and food supplements in the EU (EFSA, 2009). In the U.S., oral dosage of green tea catechins used in clinical trials for drug development is required to be taken with food in divided doses, and liver function is monitored during the trial with stoppage parameters to minimize hepatotoxicity risk (Dostal et al., 2015; Kumar et al., 2015). More recently in 2017, in response to a request from the European Commission, EFSA announced another safety review of green tea and its various preparations (EFSA, 2017).

To our knowledge a comprehensive systematic review on the safety of various green tea preparations has not yet been published to date. Notably, the ability to conduct such an assessment is hindered by the heterogeneity of published data, the lack of consistent chemical characterization of green tea materials tested in both toxicological and human intervention studies, and limited understanding of the mode of action (MOA) or adverse outcome pathway (AOP) for the observed toxicity. The broad array, wide use and lack of standardization or understanding of the composition of green tea preparations used in marketed products add further complexity to the risk assessment. The aim of the present analysis was to assess the safety of green tea in various preparations and under different conditions of use among the adult population, and if feasible, to identify a safe threshold intake level. Furthermore, specific emphasis was given to hepatotoxicity, which has been reported in animal models, and linked to adverse event case reports in humans.

2. Materials & methods

2.1. Literature search and study selection

The present review evaluated two main data sets: laboratory toxicology studies and human intervention studies. Searches for both data sets were performed primarily in the PubMed database with supplemental information from other relevant databases as detailed below. Additional studies were retrieved through reviewing the references cited in the publications identified through the original search.

2.2. Toxicological data

Laboratory studies evaluating the toxicity potential of green tea, GTE, or individual catechins from green tea were identified through a search conducted in PubMed (from inception through December 2016), ToxNet, the National Toxicology Program (NTP) website, and the Chemical Effects in Biological Systems (CEBS) Database. Search terms included "*Camellia sinensis*", "green tea", "green tea extract", "catechins", "flavan-3-ols", and "EGCG". Studies using oral administration were considered as the relevant route of exposure for hazard identification, except for genotoxicity assays. Studies conducted with other exposure routes, such as intravenous (*i.v.*), intraperitoneal (*i.p.*), or topical, were excluded from this analysis. The identified studies were in the form of either peer-reviewed publications or published study records. Detailed information including study design, animal strain and species, age, sex, dose, exposure route and duration, and toxicological findings were extracted from each study. If a publication reported more than one experimental condition, including but not limited to different dosing regimen, test article, animal model or exposure duration, then the data for each condition were counted and evaluated as a separate experiment.

2.3. Human safety data

A literature search was conducted in PubMed for human intervention studies published in English from inception through December 2016, using the search terms of "*Camellia sinensis* ", "green tea", "green tea extract", "catechins", "flavan-3-ols", and "EGCG" under the limits of "humans" and "clinical trial". Studies meeting the following criteria for inclusion were selected: 1) explicit reporting of AE outcomes, including self-reported events and/or measured safety related endpoints; or 2) explicit reporting of the absence of AE outcomes. Studies in the following categories were excluded: 1) non-ingestible delivery route of administration (such as topical application, inhalation, mouthwash, or chewing gum); 2) catechins from non-green tea sources (such as cocoa, red wine, cranberry, pine bark, grapeseed, or other botanicals); 3) studies testing green tea preparation in combination with other bioactive substances which were not derived from green tea; 4) study records lacking adequate description of experimental design, test substances, study conduct, or research data; 5) review articles; and 6) observational studies. In addition, if there was more than one peer-reviewed publication on the same intervention trial, only the publication reporting the details of experimental design, study conduct and safety outcomes was used in this assessment. Publications reporting the outcomes at different intervention durations of the same trial were treated as separate studies.

Based on the outcome from the toxicological data review, hepatotoxicity was selected as a specific outcome of interest and analyzed separately in this assessment. The clinical studies meeting the following criteria were included in this analysis: 1) intervention duration of at least one week; 2) explicit reporting of the following outcomes: elevation of liver function biomarkers from baseline level, serious hepatic-related adverse events (SAEs); discontinuation of the intervention due to elevated liver function tests or clinical hepatic-related events; or 3) explicitly reported absence of hepatic AE outcomes.

2.4. Assessment approach

2.4.1. Toxicological data

Identified laboratory toxicology studies were assessed for risk of bias according to the Office of Health Assessment and Translation (OHAT) Risk of Bias Rating Tool (OHAT, 2015). Each individual experiment was evaluated against nine questions applicable to experimental animal studies and *in vitro* assays, and rated on a 4-point scale ranging from low to high risk of bias options.

2.4.2. Human safety data

In order to ensure all potential adverse outcomes observed in human intervention trials were captured, all studies monitoring and reporting AEs were included in the analysis. Quality or risk of bias of individual studies was not considered because the majority of these studies were designed and powered to evaluate efficacy or pharmacokinetics as the primary outcome, rather than toxicity. Each type of reported AE was compiled and quantified to assess the overall nature of AEs associated with consumption of various green tea preparations.

For those studies monitoring and reporting liver-related outcomes,

Table 1
National Cancer Institute (NCI) common terminology criteria for adverse events (CTCAE version 5.0) specific to liver function (NCI, 2017).[a]

CTCAE Term	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)	Grade 4 (life-threatening)	Grade 5 (Death)
ALT increased	> ULN - 3.0 x ULN	> 3.0–5.0 x ULN	> 5.0–20.0 x ULN	> 20.0 x ULN	Death
AST increased	> ULN - 3.0 x ULN	> 3.0–5.0 x ULN	> 5.0–20.0 x ULN	> 20.0 x ULN	Death
AP increased	> ULN - 2.5 x ULN	> 2.5–5.0 x ULN	> 5.0–20.0 x ULN	> 20.0 x ULN	Death
Blood bilirubin increased	> ULN - 1.5 x ULN	> 1.5–3.0 x ULN	> 3.0–10.0 x ULN	> 10.0 x ULN	Death
GGT increased	> ULN - 2.5 x ULN	> 2.5–5.0 x ULN	> 5.0–20.0 x ULN	> 20.0 x ULN	Death

Note:
[a] Based on laboratory test results that indicate the level of increase of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), bilirubin, and gamma-glutamyl transferase (GGT) in the blood specimen; ULN refers to upper limit of normal.

the following data were extracted for each study: dosage form (beverage, food or solid dosage in tablet or capsule); description of test material preparation; dose per serving; conditions of use; daily intakes of total catechins, EGCG, catechin (C), gallocatechin (GC), epigallocatechin (EGC), epicatechin (EC), gallocatechin gallate (GCG), and epicatechingallate (ECG), and caffeine (if/when reported and/or based on extrapolation from the USDA Flavonoid Database); number of subjects, type of study population and subject demographics; study duration; and liver-related AEs, including incidence and severity. Only AEs that were reported as related specifically to the green tea intervention were recorded. The severity of liver-related AEs was graded according to National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (NCI, 2017). Grading for liver-related biomarkers was performed according to laboratory test results that indicated an increase in the level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), alkaline phosphatase (AP) or bilirubin in the blood specimen and assigned as Grade 1 to 5 based on the criteria described in Table 1. Those meeting the CTCAE criteria for Grade 3–5 were considered as SAEs.

Overall incidences of liver-related AEs and SAEs were calculated by dividing the number of events of green tea treatment-related liver AEs or SAEs (numerator) by the total number of subjects receiving green tea intervention in all the studies evaluating liver outcomes (denominator). The incidence rate of both liver-related AEs and SAEs was also calculated for each dosage form (i.e., solid dosage form in capsules or tablets versus beverages) and type of green tea preparation.

2.4.3. Weight of evidence analysis

The weight of evidence analysis was applied to each adverse outcome identified from the relevant animal and human studies, taking into consideration the quality of the study, dose-response, biological plausibility, and consistency across studies and species. Available toxico- and pharmacokinetic data and mechanistic evidence were also considered when evaluating the plausibility of reported toxicity. In brief, available human and animal studies on a particular adverse outcome were grouped and given an initial confidence rating based on key study design features (controlled exposure, exposure prior to outcome, individual outcome data, comparison group used), which was then potentially downgraded or upgraded based on the factors that may decrease confidence (risk of bias, unexplained inconsistency, indirectness, lack of applicability/human relevance) or increase confidence (large effect size, dose-response, cross-species/population/study consistency, consideration of residual confounding). Publication bias was not assessed and considered in the confidence rating of weight of evidence because not all identified datasets were from peer–reviewed publications.

2.4.4. Statistical analysis

To evaluate whether the chemical composition of green tea preparations may be useful in reflecting toxicity potential, concentrations of individual catechins on a dry weight basis in various green tea preparations were extracted from the publications if reported and subjected to principal component analysis (PCA) and hierarchical cluster analysis (HCA) using R 3.3.3 and R Studio 1.0.143. If not characterized and reported directly, their concentrations in a given experiment were estimated based on compositional information provided in other published sources on the same test material if/when available. Typical concentrations of catechins in green tea infusions were obtained from the USDA Flavonoid Database and also included in the PCA and HCA as a benchmark point (Bhagwat et al., 2014). The compositional variables included in the PCA and HCA were: total catechins, EGCG, GC, EGC, EC, GCG, and ECG. Catechin gallate (CG) and catechin were not considered in the analyses because their levels are reportedly in minute amounts in green tea compared to other catechins and often not reported in the publications. Further, any studies that did not report the information for all seven of the remaining compositional variables were excluded from the analysis. Compositional data from human studies were not subject to PCA or HCA because detailed chemical characterization of the test material was missing in a large number of these studies.

To characterize the relationship between compositional characteristics of green tea preparations and their hepatotoxicity potential, univariate linear regression was performed between the no observed adverse effect level (NOAEL) for hepatotoxicity identified from the animal oral toxicity studies with duration equal to or greater than four weeks against the concentrations (% w/w) of total catechins and EGCG in the test materials. Statistical significance was set at p value of 0.05.

3. Results

3.1. Toxicological data

3.1.1. Study characteristics and quality assessment

A total of 26 publications were retrieved through the literature search, reporting 49 individual experiments that were identified as relevant for this assessment. A summary of these studies is presented in Supplementary Tables 1–4, organized by study type, test material, duration, animal species with identified NOAELs, and quality rating. Among these, 13 evaluated acute and subacute toxicity, 22 evaluated subchronic toxicity, one evaluated chronic toxicity, six evaluated reproductive and developmental toxicity, four evaluated carcinogenicity, and four evaluated thyroid toxicity. The dosing routes included dietary or oral gavage in rats and mice, or oral capsules in beagle dogs. The test materials evaluated in these studies included brewed green tea, GTE, and purified individual catechins. The test material composition in these studies varied widely, ranging from simple aqueous (water)-extracted green tea with less than 40% (w/w) catechins, extracts comprised of highly concentrated catechins (up to 80% w/w, such as a branded green tea preparation, Polyphenon E®), to purified individual compounds such as EGCG. Among these studies, 17 experiments examined purified EGCG, nine examined GTEs with relatively low total catechin content (< 40% w/w) and 24 examined GTEs with highly concentrated catechins (> 60% w/w), compared to an average of

42.2% (w/w) total catechins reported for green tea infusion in the USDA Flavonoid Database. Among these experiments, 32 were rated as low risk of bias or probably low risk of bias in study quality, 15 were rated as not reported (NR) due to insufficient information, and three were rated as high risk of bias.

Nine published studies were identified in the literature reporting 28 individual experiments evaluating genotoxicity of green tea preparations or purified individual catechins. The study designs and outcomes are summarized in Supplementary Table 5. The quality of these studies was generally rated as low risk of bias or probably low risk of bias.

3.1.2. PCA and HCA of chemical compositions of green tea preparations

Composition of the catechins was reported for 32 test materials in 49 experiments. Among these, one study (Bun et al., 2006) reported only the concentrations of total catechins, EGCG and caffeine, but not the concentrations of the other five individual catechins (EC, EGC, ECG, and GCG, CG) for two tested green tea material. Another study tested only pure catechins (Chandra and De, 2010). Thus these two studies were excluded from the analyses. The PCA revealed that 83.5% of the variation was attributed to three principal components. PC1 explained 52.9% of total variance, PC2 and PC3 explained a further 21.5% and 12.9%, respectively. Fig. 1 shows the plot of PCA results, with markings identified by study materials as reported. The variations among different test materials were mainly due to the variation of EGCG content (−0.441 correlation with PC1), GCG content (−0.429 correlation with PC2), total catechins content (0.665 correlation with PC2), and EC content (0.478 correlation with PC2). The studies involving Polyphenon E and EGCG formed their own respective clusters and were clearly distinguished from other GTEs on the PCA plot, indicating significant differences in the catechin profile. In contrast, other GTEs dispersed widely. The HCA revealed three major clusters based on the linkage distance between various compositions (Fig. 2) which appeared to coincide with the PCA groups. Cluster A composition was characterized by the concentrations of both total catechins and EGCG similar or lower than that of the USDA green tea infusion, cluster B was comprised of purified EGCG, cluster C was made up by the GTEs containing highly concentrated total catechins (> 60% w/w). Although the test materials in cluster A were closer in linkage to green tea infusion in composition, none of the materials tested in these oral toxicity studies had the a catechin profile similar to the benchmark green tea infusion from the USDA Flavonoid Database.

3.1.3. Mutagenicity and genotoxicity

Nine studies examining genotoxity potential of green tea, GTE, or individual catechins were identified (Supplementary Table 5). The majority of studies demonstrated a lack of mutagenic activity of green tea, GTE and individual catechins as evidenced by negative responses in numerous bacterial reverse mutation tests (Ames tests) (Chang et al., 2003; Isbrucker et al., 2006a; Makena and Chung, 2007; Tewes et al., 1990; Wada and Matsumoto, 2009; Yamane et al., 1996). In addition, the findings from *in vivo* single dose or repeated dose micronucleus assays and the Big Blue transgenic rodent mutation assay confirmed the

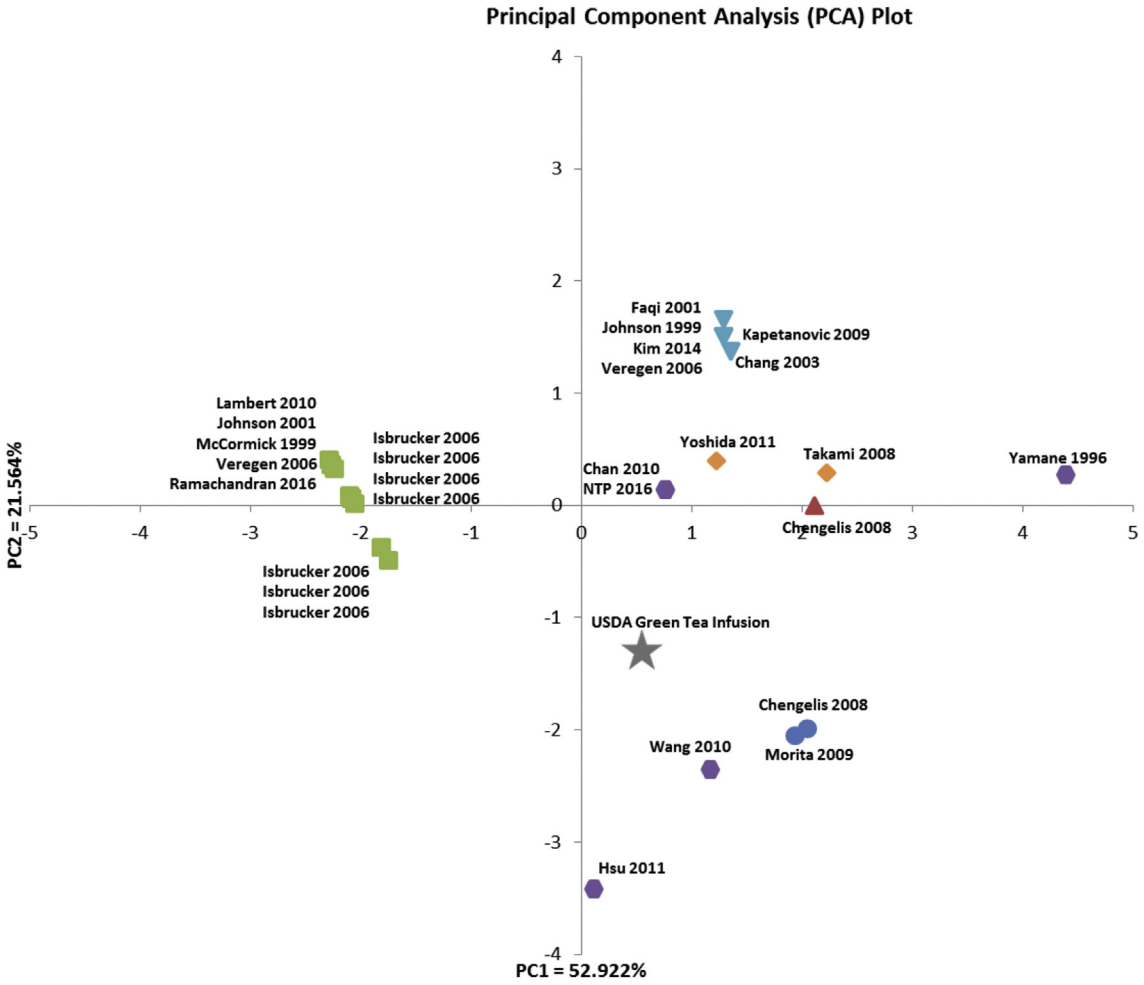

Fig. 1. PCA of catechins compositions of various green tea preparations used in animal toxicity studies. Different symbols represent the terms of green tea preparations identified in the studies. ■ Purified Epigallocatechin gallate (EGCG); ● Green Tea Catechins – Heated (GTC-H); ▲ Green Tea Catechins – Unheated (GTC-UH); ⬣ Green Tea Extract (GTE); ▼ Polyphenon E; ◆ Sunphenon; ★ USDA green tea infusion.

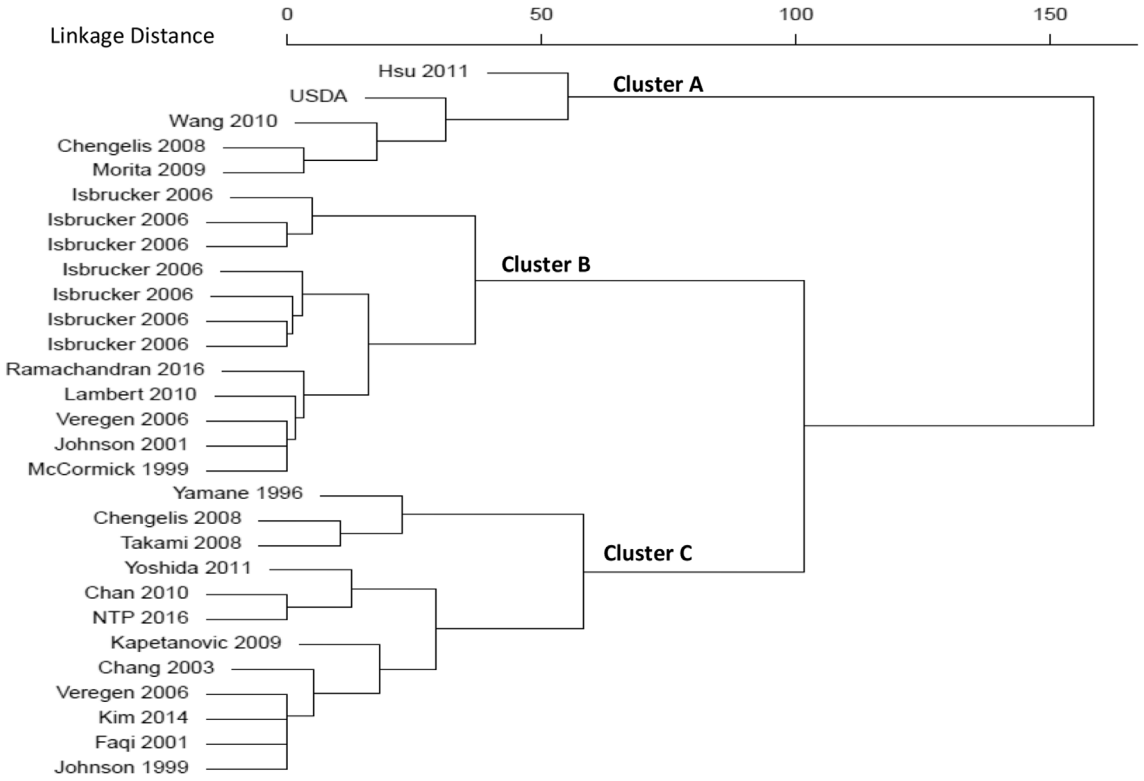

Fig. 2. HCA based on catechins compositions of various green tea preparations used in animal toxicity studies. **Cluster A** represents Green Tea Extracts (GTEs) with concentrations of catechins lower than or comparable to the USDA green tea infusion; **Cluster B** represents purified Epigallocatechin gallate (EGCG); **Cluster C** represents Green Tea Extracts (GTE) with concentrated catechins and Polyphenon E.

lack of mutagenic and clastogenic potential of green tea catechins (Chang et al., 2003; Isbrucker et al., 2006a; NTP, 2006; Ogura et al., 2008). Two studies specifically measured the plasma concentrations of catechins to ensure that the absence of genotoxic activity in the *in vivo* assays was not attributed to low absorption or rapid metabolism of catechins upon oral exposure. Appreciable concentrations of catechins were found in the plasma of ICR CD mice (total catechins of 173 ng/mL) and Sprague-Dawley rats (total catechins of 2324 ng/mL) following a single oral dose of 2000 mg/kg bw of green tea catechins in the micronucleus assays (Ogura et al., 2008). Isbrucker et al. (2006a) demonstrated that intravenously administered EGCG at 50 mg/kg bw dose, twice at 24-hour intervals to Wistar rats, led to plasma free EGCG levels up to 96 μM, a concentration at least 10 times greater than that reported in humans, without causing any reduction in the ratio of polychromatic erythrocytes (PCE) to normachromatic erythrocytes (NCE), or induction of micronucleated PCE in a micronucleus assay (Isbrucker et al., 2006a).

A few studies reported equivocal or positive genotoxic responses in the *in vitro* mammalian cell mutation assays, including the chromosomal aberration assay, Comet assay and L5178Y *tk* $^{+/-}$ mouse lymphoma assay (Chang et al., 2003; Isbrucker et al., 2006a; NTP, 2005; Ogura et al., 2008; Wada and Matsumoto, 2009). These responses were thought to be an indirect effect resulting from damage induced by reactive oxygen species (ROS) formed under the testing conditions, because green tea catechins have been shown to have pro-oxidant activity at high concentrations in cell culture systems. Takumi-Kobayashi et al. (2008) found that substantial amounts of H_2O_2 were generated when green tea catechins (400 or 600 μg/mL) were incubated under the testing conditions used in a chromosomal aberration assay, which appeared to coincide with increased structural chromosomal aberrations. In contrast, only very low amounts of H_2O_2 were detected when the same concentrations of catechins were incubated in water, and the addition of catalase to the chromosomal aberration assay media suppressed chromosomal aberrations (Takumi-Kobayashi et al., 2008). Isbrucker et al. reported that EGCG at 90–450 μM induced H_2O_2 production in a dose-dependent manner over three hours in the RPMI-1640 media used in the mouse lymphoma L5178Y *tk* $^{+/-}$ assay (Isbrucker et al., 2006a). Similar findings were reported in a primary rat hepatocyte culture where a biphasic dose-response of EGCG on ROS formation was observed, showing that EGCG at low concentrations ($\leq$15 μM) decreased ROS production while at high concentrations ($\geq$20 μM) significantly increased ROS in the culture (Kucera et al., 2015). This ROS-induced genetic toxicity associated with supraphysiological concentrations of green tea catechins *in vitro* has also been observed in other studies in which catechins were shown to induce oxidative DNA damage, including the formation of 8-hydroxy-2-deoxyguanosine (8-OHdG) and chromosomal damage, such as an increase in the number of micronucleated and binucleated cells (MNBN) (Bertram et al., 2003; Furukawa et al., 2003; Johnson and Loo, 2000; Oikawa et al., 2003; Sugisawa and Umegaki, 2002).

3.1.4. Acute and subacute toxicity

In the identified acute and subacute oral toxicity experiments, various GTEs or purified EGCG were evaluated in mice and rats at doses up to 5000 mg/kg/day with the duration ranging from a single dose to up to 28 days (Chang et al., 2003; Chengelis et al., 2008; Hsu et al., 2011b; Isbrucker et al., 2006b; Lambert et al., 2010; Ramachandran et al., 2016; Wang et al., 2010a; Yamane et al., 1996) (Supplementary Table 1). All experiments employed oral gavage administration except for one in which animals were dosed *via* dietary route. Adverse findings were mostly absent in the studies testing GTE with relatively low concentrations of catechins (< 40% w/w catechins), and when observed, appeared to be limited to reduced body weight gain and minimal gastrointestinal (GI) irritation (Chengelis et al., 2008; Hsu et al., 2011b; Wang et al., 2010a). Reduced weight gain was observed at a dose as low as 1000 mg/kg/day in several experiments, and GI irritation at 2000 mg/kg/day in one experiment. In contrast, incidence of mortality and morbidity, severe damage of the GI tract and

hepatotoxicity occurred dose-dependently and consistently when the GTE comprised of highly concentrated catechins (such as Polyphenol E) or purified EGCG was administered *via* oral gavage (Chang et al., 2003; Isbrucker et al., 2006a, 2006b; Lambert et al., 2010; Ramachandran et al., 2016). One study reported an LD_{50} for a GTE containing 74.5% (w/w) total catechins to be 3300 and 5000 mg/kg in female and male ddY mice, respectively, *via* oral gavage administration (Yamane et al., 1996).

3.1.5. Subchronic and chronic toxicity

Twenty-three published experiments investigated subchronic and chronic oral toxicity of various GTEs in rat, mouse or beagle dog models with durations of 13 weeks to 12 months (Supplementary Table 1). The NOAELs derived from these studies varied widely, ranging from 90 mg/kg/day to 1200 mg/kg/day for various GTEs or EGCG under reported experimental conditions. Suppressed body weight gain without decreasing food intake in mice and rats was reported in 12 experiments (Chan et al., 2010; Johnson et al., 1999, 2001; McCormick et al., 1999; Takami et al., 2008; Veregen, 2006; Yoshida et al., 2011; Kim et al., 2014). Local GI damage appeared to be a common toxicity observed across the studies and animal models (Isbrucker et al., 2006b; Johnson et al., 1999; Kapetanovic et al., 2009; McCormick et al., 1999; Veregen, 2006). The severity of GI toxicity was dose-dependent, ranging from asymptomatic minimal gastric erosion, occasional diarrhea and vomiting, to severe GI tract dilation, ulceration, hemorrhage and epithelial necrosis. The effects were more evident in the studies with oral gavage or in fasted animals, while minor or absent in the studies in which the GTE or EGCG was administered *via* dietary route or drinking water (in rodents) or under prefed conditions (in dogs). Treatment-related early death and severe toxicity in the liver, kidney, thymus, spleen and pancreas were reported in the studies involving highly concentrated green tea catechins or purified EGCG administered *via* bolus dose (gavage or capsules) as low as 150 mg EGCG/kg/day (Chan et al., 2010; Isbrucker et al., 2006b; Johnson et al., 1999; Kapetanovic et al., 2009; McCormick et al., 1999; Veregen, 2006). These systemic toxicities appeared to be consistent with the observations following repeated doses of EGCG (67.8 and 108 mg/kg/day) administered *via i.p.* route (Ramachandran et al., 2016). One study reported treatment-related toxicity in epithelia of the nasal-olfactory cavity and upper respiratory tract in the rats and mice receiving a GTE containing 76% (w/w) total catechins, and mice were shown to be more sensitive than rats (Chan et al., 2010). Nasal toxicity was reproduced in the 2-year carcinogenicity study of the same GTE conducted by the NTP (NTP, 2016), however, no other studies reported similar findings.

Long-term exposure to GTE *via* the diet was shown to be of low toxicity in a chronic toxicity study (Yoshida et al., 2011). A GTE with > 76.4% (w/w) total catechins was administered to Wistar Hannover GALAS rats *via* diet at 0, 0.02, 0.3, 1 or 3% concentrations for 12 months. No treatment-related toxicological findings were observed in mortality, clinical signs, hematology, clinical chemistry, urinalysis or gross necropsy. The exception was for suppressed body weight gain (*c.a.* 15%) in females of the highest dose group after week 25 and a dose-dependent increase in food consumption at doses above 0.3%, along with a statistically significant elevation in creatinine levels in the males of the 0.3% and 3% dose groups and the albumin/globulin ratio in males of the 3% dose group while the levels were still within their respective normal ranges. A statistically significant increase in relative liver weight accompanied by centrilobular hepatocyte hypertrophy and upregulation of CPY3A2 expression were found in the males exposed to the 3% dose but without other signs indicative of hepatotoxicity, including liver biomarkers. Considering the centrilobular hypertrophy in males and depressed weight gain in females at the 3% dose, the NOAEL for the GTE was determined to be 1% in both sexes, which reportedly delivered 395.5 and 585 mg total catechins/kg/day for males and females, respectively.

3.1.6. Carcinogenicity

Four individual carcinogenicity experiments with duration ranging from 26 weeks to up to two years were identified in three published studies (Supplementary Table 2). The test materials in these experiments contained high concentrations of total catechins (> 70% w/w). No long-term toxicity or carcinogenicity study of EGCG or other individual catechins was identified in the literature. No carcinogenic activity of GTE or Polyphenon E was observed under the experimental conditions of these studies. In the Yoshida et al. (2011) study, green tea catechins were administered through dietary route of exposure to Wistar rats at concentrations up to 3%. No treatment-related adverse changes were observed in mortality, clinical signs or macroscopic lesions at necropsy among all groups in the 2-year study. Although a dose-dependent increase in food consumption was observed in the groups exposed to doses above 0.3%, both males and females receiving the 3% dose had reduced body weight gain (*c.a.* 15%). No other abnormal changes were found except for centrilobular hypertrophy of hepatocytes in the males receiving the 3% dose. Tumor incidence and type were reportedly comparable between treated and control groups of male and female rats. In the study conducted with a GTE by the NTP (2016) and the study conducted with Polyphenon E by Veregen (2006), the test materials were administered *via* oral gavage. The NTP study reported significantly reduced survival in the rats receiving the GTE, and treatment-related growth retardation in both rats and mice. Non-neoplastic lesions were found in the GI tract and liver at the dose of 1000 mg/kg/day in rats and 300 mg/kg/day in mice. Dose-dependent non-neoplastic lesions in the nose and olfactory epithelium were found in rats and mice receiving the GTE. These findings are consistent with those reported by Chan et al. (2010), that orally administered GTE *via* gavage induces GI irritation and epithelial damage, and adverse changes at sites with high metabolic activity such as the nose and liver. The NTP (2016) study also reported non-neoplastic lesions in other sites including the lung, spleen, lymphoid, heart and bone marrow. Nonetheless, there was no clear evidence that GTE was carcinogenic in rats and mice under the experimental conditions. The carcinogenicity study of Polyphenon E was extracted from a FDA Drug Approval Package in which a summary of the study design and findings was reported (Veregen, 2006), however, no original study record was available in the public domain. Polyphenon E was administered up to 500 mg/kg/day to *p*53 transgenic heterozygous mice for 26 weeks, and no toxicologically significant findings were observed in clinical signs, hematology, clinical chemistry, gross pathology or histopathology, except for a slight reduction in body weight gain, food intake and thyroid weight (females only) at the 500 mg/kg/day dose, the highest dose tested. No treatment-related increase in tumor incidence was observed.

3.1.7. Hepatotoxicity

Occurrence of hepatotoxicity with varying degrees of severity was reported in a number of animal oral toxicity studies including mice (Chan et al., 2010; Chang et al., 2003; Lambert et al., 2010; NTP, 2016), rats (Johnson et al., 1999; McCormick et al., 1999), and fasted dogs (Isbrucker et al., 2006b; Kapetanovic et al., 2009), whereas absence of hepatic findings was also reported in rats (Bun et al., 2006; Isbrucker et al., 2006b; Johnson et al., 2001; Kim et al., 2014; Morita et al., 2009a) and pre-fed dogs (Johnson et al., 1999; McCormick et al., 1999). In the studies where hepatotoxicity was observed, the severity of toxicity progressed in a dose-dependent manner, ranging from centrilobular hypertrophy without pathological lesions, mild elevation of liver enzymes, to severe hepatocellular necrosis and bile duct hyperplasia.

Findings from animal toxicity studies revealed that GTEs and EGCG administered *via* dietary route were better tolerated than *via* oral gavage. This is illustrated in several rodent EGCG studies where an approximately ten-fold difference in the hepatotoxicity NOAELs was observed between dietary (500 mg EGCG/kg/day) and gavage (45 mg EGCG/kg/day) administration (Isbrucker et al., 2006b; Johnson et al., 2001; McCormick et al., 1999; Veregen, 2006). In addition, findings

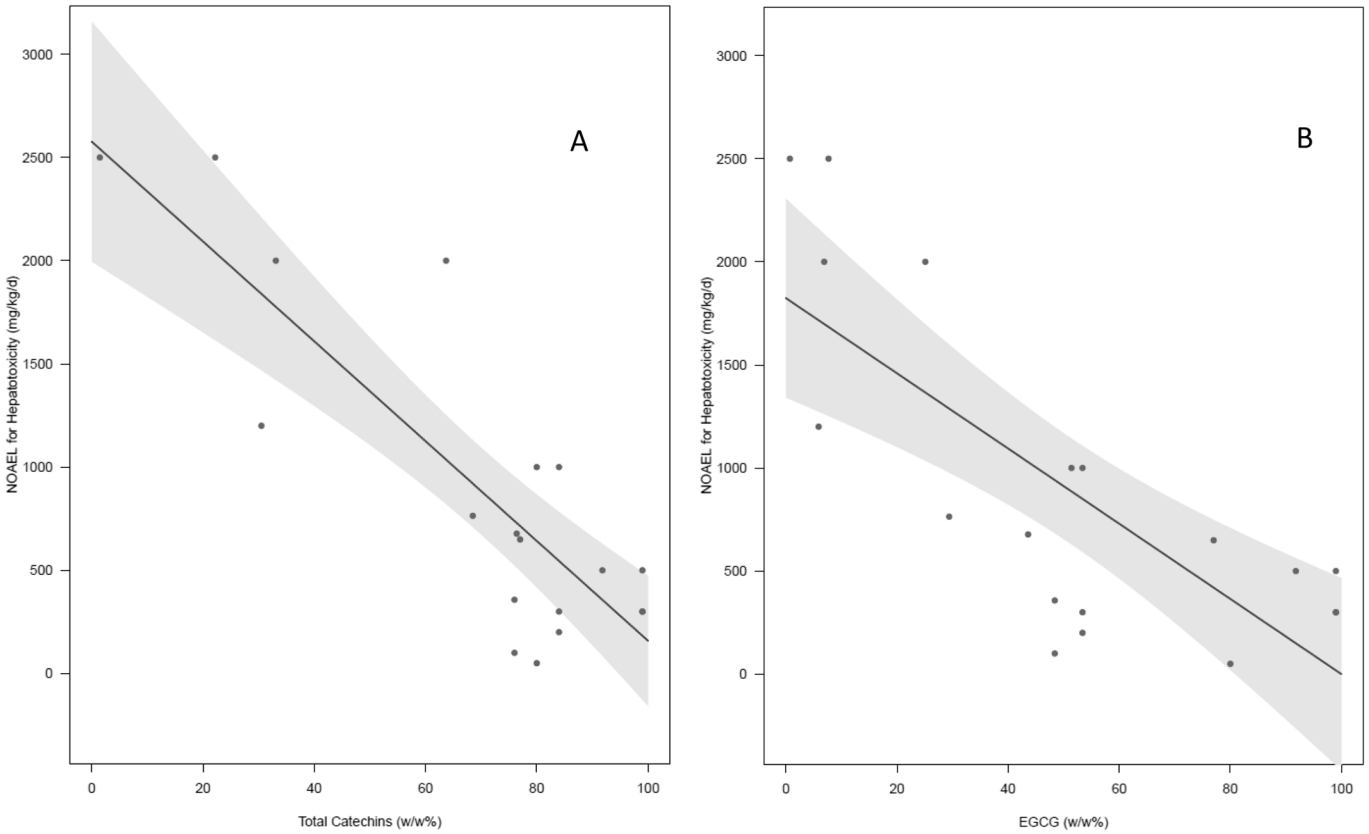

Fig. 3. Quantitative relationship between hepatotoxicity no observed adverse effect levels (NOAELs) and purity of total catechins ($r^2 = 0.7175$) and EGCG ($r^2 = 0.5786$) in the test materials of animal toxicity studies with duration ≥ 4 weeks. The shade area represents the 95% confidence interval of the linear regression line.
A – NOAELs vs. total catechins (%); B – NOAELs vs. EGCG. (%).

from the studies in beagle dogs showed that the fasted animals receiving a bolus dose of EGCG were more prone to severe liver damage than pre-fed animals receiving a comparable amount of EGCG in divided doses (hepatotoxicity NOAEL of 40 *vs.* 460 mg EGCG/kg/day in the fasted vs. pre-fed dogs) (Isbrucker et al., 2006b; Johnson et al., 1999). These findings support that dosing condition and food consumption may play a key role in deterring the harmful effect.

We further examined the relationship between compositional characteristics of test materials and their toxicity potential by plotting the NOAELs for hepatotoxicity identified from the toxicity studies with durations equal to or greater than four weeks against concentrations of total catechins and EGCG in the test materials (% w/w). As shown in Fig. 3 there was a directional inverse relationship between the NOAELs and the purity of total catechins in the test material ($R^2 = 0.7175$, $p < 0.001$), indicating the toxicity potential of GTE rose with increasing concentration of total catechins. A similar trend was also present between the NOAELs and the purity of EGCG in the test materials ($R^2 = 0.5786$, $p = 0.0002$), although the slope was less steep compared to that of total catechins ($\beta = -24.18$ and -18.24 for total catechins and EGCG, respectively).

3.1.8. Reproductive and developmental toxicity

Four published studies were identified reporting five experiments with reproductive and developmental toxicity outcomes (Supplementary Table 3) (Faqi et al., 2001; Isbrucker et al., 2006c; Morita et al., 2009b; NTP, 2016). Overall no adverse developmental effects were observed in the three teratogenicity experiments, including viable liter size, resorption rate, malformation, sex ratio, pre and post-implantation loss, and fetal weight. No evident maternal toxicity was found, though reduced maternal weight gain was reported in the SD rats receiving 600 and 2000 mg/kg/day of GTE in Morita et al. (2009b). The NTP (2016) study, though not designed for reproductive toxicity evaluation, did report decreased spermatid counts, reduced accessory sex organ weights in males, and increased estrous cycle length in female mice and rats after receiving 500 mg/kg and 1000 mg/kg, respectively, of GTE *via* oral gavage, five days a week for 14 weeks. However, no clear dose-response was observed (NTP, 2016). These findings have not been reported in other subchronic or chronic toxicity studies with similar experimental design. In a Segment III two-generation study of EGCG, reduced pup weight and growth rate for F_1 and F_2, and slightly delayed sexual maturation in both sexes of F_1 at the middle and highest doses (3600 and 12,000 ppm in the diet), was reported. Increased pup loss between day five to 21 postpartum in both F_0 and F_1 of the highest dose was also reported. No other signs of reproductive or developmental toxicity were found. A reproductive and developmental toxicity NOAEL was determined to be 100 mg EGCG/kg/day based on reduced growth rates in F_1 and F_2 rats (Isbrucker et al., 2006c).

3.1.9. Other toxicities

Four published experiments investigated the effect of green tea catechins on thyroid function in rat models (Supplementary Table 4), and reported that green tea catechins may have a goitrogenic effect (Chandra and De, 2010; Chandra et al., 2011; Sakamoto et al., 2001; Satoh et al., 2002). These studies showed that administration of GTEs or pure catechin at doses above 25 mg catechins/kg/day *via* oral gavage or 5% in the diet (delivering estimated 2334 mg catechins/kg/day) resulted in an increase in thyroid weight, induced hypertrophy and hyperplasia, decreased T3 and T4 and elevated thyroid-stimulating hormone (TSH) in rats. Reduced weight of testes and prostate glands and elevated luteinizing hormone (LH) and testosterone levels were also

observed in these studies. These adverse changes were inconsistent with other subchronic and chronic oral toxicity studies of GTEs and EGCG in which no pathological changes in thyroid function or tissue were noted.

3.1.10. Human intervention studies

3.1.10.1. Literature search results. A total of 159 individual studies were identified from the initial search, among which one study evaluated GTE delivered in meat patties and 53 studies examined brewed green tea or GTEs delivered in beverage form. The remaining studies examined green tea preparations administered in solid dosage *via* capsules, including 16 with Polyphenon E, 25 with EGCG, and 70 with other GTEs. The reported or estimated daily intake of EGCG from brewed green tea or GTE beverages ranged from amounts equivalent to one to 10 cups of green tea. The estimated intakes of total catechins and EGCG in these studies ranged from 96.3 to 1343 mg/day, and 29.5 to 4000 mg/day, respectively from green tea, GTEs and purified EGCG. There was a wide range of subject populations, including individuals with non-alcoholic fatty liver disease, diabetes, multiple sclerosis, prostate cancer, lung cancer, breast cancer, leukemia, uterine fibroids, metabolic syndrome, hypertension and hypercholesterolemia, along with overweight/obese and healthy individuals. All the studies were conducted in adult subjects except for the study by Matsuyama et al. which involved obese children (Matsuyama et al., 2008).

3.1.10.2. Adverse events. There were 104 studies that monitored and reported AEs and safety-related outcomes. The majority of studies (66 out of 104) demonstrated that green tea or GTE was generally well tolerated without adverse observations. Thirty-eight studies reported the occurrence of AEs following consumption of various green tea preparations, among which five employed green tea in beverage form, and 33 employed bolus dose administration *via* capsules (5 with EGCG, 14 with Polyphenon E, and 16 with other types of GTE). As shown in Fig. 4, reported treatment-related AEs were mostly related to GI disturbances, primarily nausea (22 studies), abdominal pain or discomfort (17 studies), diarrhea (14 studies), dyspepsia/indigestion (12 studies) and/or elevated liver enzymes (11 studies). Less frequently

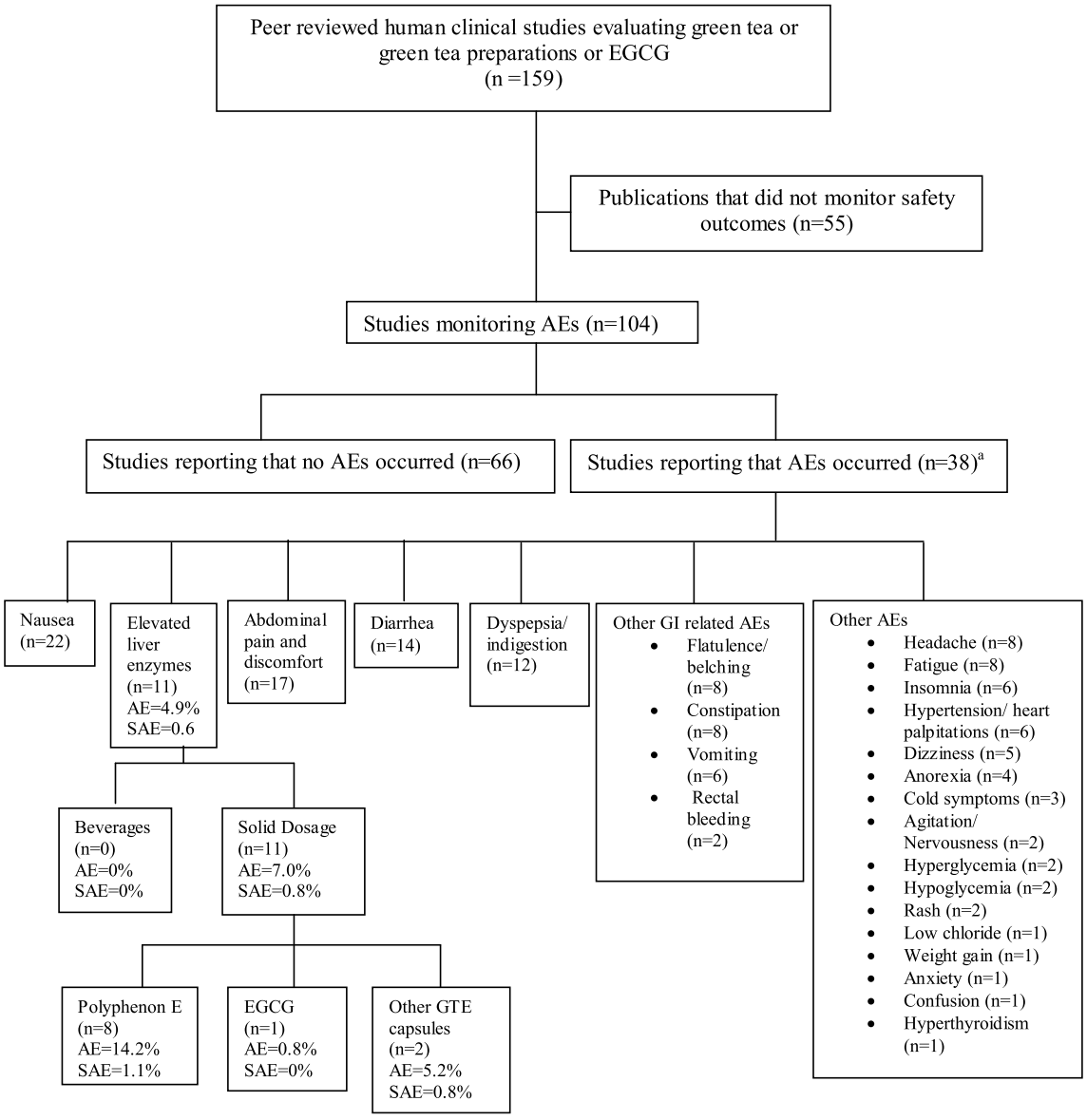

Fig. 4. Flow diagram of study selection process and adverse events (AEs). This figure describes the study selection process for determining which dosage forms have been associated with liver-related AEs; n represents number of studies; AE represents incidence rate within each category; serious adverse events (SAE) represents incidence rate within each category. [a] Several studies reported more than one type of AE.

reported AEs were cases of vomiting, constipation, and/or flatulence/belching. Occasionally AEs were related to stimulant effects attributed to caffeine content in the test material, including anxiety, nervousness and insomnia, as reported in the studies.

The reported doses where GI events were observed were in a range delivering 400 to 4000 mg EGCG/day. Nausea was predominantly reported in studies that employed solid bolus dose administration *via* capsules (19/22 studies: 2 with EGCG, 11 with Polyphenon E, and 7 with other GTEs). Abdominal pain and discomfort were reported only in the studies using solid bolus dosage in capsules (EGCG [1/17], Polyphenon E [7/17] and other GTEs [9/17]) except for one study using green tea beverage. Diarrhea was observed in five studies using Polyphenon E (5/14), one using EGCG (1/14), four using other GTEs (7/14), and one using a beverage form (1/14). Dispepsia/indigestion was observed in four studies using Polyphenon E (4/12), one using EGCG (1/12), six using other GTEs (6/12), and one using green tea in beverage form (1/12). Elevated liver functional biomarkers were only observed in the studies using solid dosage form and mostly associated with Polyphenon E (8/11).

Because the liver was identified as the target organ in animal toxicity studies of green tea preparations, AEs related to liver function in human studies were of particular interest in this assessment. Forty-eight studies met the inclusion criteria for hepatotoxicity review, among which 37 studies reported no treatment-related hepatotoxic events among the study participants receiving green tea preparations. Two studies (Ahn et al., 2003) evaluated effects of more than one green tea preparation. The study design, green tea preparation, dosage of catechins and EGCG, subject population, duration, and liver-related adverse outcomes are summarized in Table 2. Two studies (Chen et al., 2016; Wu et al., 2012) reported a statistically significant increase in mean ALT levels of all subjects consuming Polyphenon E or other GTE capsules compared to baseline. However, because these elevated levels were still within the normal range, these were not included as hepatic AEs. One study noted transient abnormal liver function after week 4 that returned to normal by week 8 and week 12 following continued treatment with Polyphenon E, and therefore this was not included as a hepatic AE (Ahn et al., 2003). None of the studies involving green tea beverage that monitored for liver related outcomes, including the Matsuyama et al., 2008 study conducted in children, observed any abnormal changes in liver function biomarkers (ALT, AST, GGT, AP, or bilirubin). In contrast, treatment-related elevation of serum liver enzyme levels was reported in 11 studies where test materials were administered in capsules, most of which involved Polyphenon E (8/11) (Chantre and Lairon, 2002; Crew et al., 2012; Dostal et al., 2015; Garcia et al., 2014; Joe et al., 2015; Lovera et al., 2015; Nguyen et al., 2012; Shanafelt et al., 2009, 2013; Ullmann et al., 2004).

Among the studies that employed a beverage or food dosing form, the highest intake level was a dose of GTE delivering 1519.7 mg/day total catechins and 704 mg/day EGCG, consumed in three divided doses before meals (i.e., in a fasted state) (Toolsee et al., 2013). No hepatic AEs were observed in these studies regardless of fed or fast state. The highest intake level in a solid dosage form at which no treatment-related hepatic AEs occurred across a diverse range of subject populations (adult healthy individuals and diseased patients) and durations, was a dose delivering 1633 and 676 mg/day of total catechins and EGCG, respectively, taken after a morning meal (i.e., in a fed state) (Laurie et al., 2005). Regardless of dosage form, none of the studies reported adverse liver effects at an EGCG equivalent dose equal to or less than 676 mg/day. With regard to the onset of hepatic AEs, abnormal changes in liver biomarkers were seen as early as in 10 days among healthy males consuming 800 mg/day EGCG under fasted conditions (Ullmann et al., 2004), while when subjects consumed the test material with or after meals, or in divided doses throughout the day, the occurrence of hepatotoxicity was mostly observed in the studies lasting 60 days or longer (Chantre and Lairon, 2002; Crew et al., 2012; Dostal et al., 2015; Garcia et al., 2014; Joe et al., 2015; Lovera et al., 2015; Shanafelt et al., 2009, 2013). There was a wide range of subject populations in these 11 studies, including individuals with multiple sclerosis (Lovera et al., 2015), prostate cancer (Nguyen et al., 2012), breast cancer (Crew et al., 2012), leukemia (Shanafelt et al., 2009, 2013), Barrett's esophagus (Joe et al., 2015) and low grade cervical intraepithelial neoplasia combined with HPV infection (Garcia et al., 2014), along with postmenopausal women with increased risk of breast cancer (Dostal et al., 2015), overweight/obese subjects (Chantre and Lairon, 2002), and healthy males (Ullmann et al., 2004). Most of these studies excluded individuals with abnormal liver function or diseases upon enrollment. There was no apparent pattern indicating any particular subject population was more susceptible than others to GTE-induced hepatotoxicity.

The overall incidence rate of hepatic AEs was determined to be 4.9% based on the number of events of elevated liver function biomarkers (111 events out of 2269 subjects consuming a green tea preparation in 48 studies that monitored hepatic AEs). However, there was a large amount of heterogeneity among these studies, including study population, sample size, test material composition, study dosage and duration. Among those subjects consuming green tea beverages, the incidence of liver-related AEs was 0% (0 events/675 subjects). Among the subjects receiving a bolus dose in capsule forms, the incidence was 7.0% (111 events/1594 subjects). The highest incidence occurred among the subjects receiving Polyphenon E (14.2%; 54 events/380 subjects) compared to 5.2% among those consuming other GTEs (56 events/1081 subjects) and 0.8% (1 event/133 subjects) among those consuming EGCG. Of these 56 AEs associated with consumption of other GTEs in capsules, 55 occurred in the Minnesota Green Tea Trial in which postmenopausal women received 1315 mg/day total catechins from the GTE (delivering 843 mg EGCG/day) for 12 months (Dostal et al., 2015).

All the hepatic AEs were graded on their severity following NCI criteria. The majority of hepatic AEs (98/111) were considered mild to moderate in severity (Grade 1 and 2), which involved elevated liver enzyme levels $< 5 \times$ ULN or bilirubin levels $< 3 \times$ ULN without other clinical symptoms. A total of 13 SAEs were reported from five studies, with nine resulting from consumption of 1315 mg/day of total catechins (843 mg/day EGCG) from the GTE in the Minnesota Green Tea Trial, and four from consumption of 800 mg/day EGCG from Polyphenon E in four other studies (Crew et al., 2012; Dostal et al., 2015; Garcia et al., 2014; Lovera et al., 2015; Shanafelt et al., 2013). These SAEs were comprised of 11 Grade 3 and two Grade 4 events, where liver enzymes were elevated $>$ 5 x ULN and/or bilirubin $>$ 3 x ULN. No deaths (Grade 5) were reported in any of the studies in this assessment. The incidence rate of SAEs was estimated to be 0.6% (13 events out of a total of 2269 subjects treated with green tea preparations from a total of 48 studies). Among the studies that employed solid dosage forms, the incidence rate of SAEs was 0.8% (13/1594), of which 0% (0/133) was for EGCG, 1.1% (4/380) was for Polyphenon E, and 0.8% (9/1081) was for other GTEs. Only three of these five studies that reported SAEs commented on resolution. Lovera et al. stated that the subject with a Grade 4 elevation of AST, ALT and bilirubin returned to normal after discontinuation of the intervention (Lovera et al., 2015). Garcia et al. stated that all abnormal liver function tests (one Grade 3 SAE; eight Grade 1 or 2) returned to baseline levels after discontinuation of Polyphenon E (Garcia et al., 2014). Dostal et al. (2015) reported that all 55 AEs returned to normal after discontinuing GTE treatment except for one case for which no information on the final resolution was reported. These findings suggest these hepatic AEs were reversible in nature. It should be noted that the authors of the Minnesota Green Tea Trial indicated the nine SAEs could be multifactorial because these subjects also experienced simultaneous infection, began the use of new medication, concomitant alcohol consumption, or self-reported past medical history of liver enzyme elevations (Dostal et al., 2015).

Overall, the results from human intervention studies of green tea preparations suggest that green tea preparations consumed in beverage forms are better tolerated than in solid bolus dose exposure. The most

Table 2
Summary of hepatic adverse event outcomes from clinical studies on various green tea, green tea extracts and EGCG.[a]

References	Description of green tea preparation	Condition of use[b]	Total Catechins per day	EGCG per day	Subjects on Intervention[c]	Duration	Findings related to the liver and severity rating
Beverage							
Panza et al., 2008	2 g green tea leaves infused in 200 ml hot water (80°C) for 3 min, followed by filtration	3X/day	NR (estimated 379.8 mg using USDA data)	NR (estimated 233.4 mg using USDA data)	14 healthy males; mean age 24.5 ± 0.8 yrs; mean BW 81.9 ± 3.9 kg	7 d	No adverse effects on liver reported
Kim et al., 2006	8 g powdered green tea dissolved in 1 L warm water	Daily (other conditions not specified)	741 mg	256 mg	20 Korean males who were chronic smokers; mean age 27.6 ± 3.6 yrs; mean BMI 23.6 ± 3.0	2 wks	No adverse effects on liver reported
Toolsee et al., 2013	One cup of green tea (1 green tea bag infused for 6 minutes in 200 ml hot water)	3X/day before meals	NR (calculated to be 1519.7 mg based on individual catechin data)	704 mg	65 subjects (33M/32F) at risk for diabetes; mean age 48.9 ± 6.9 (M) and 49.3 ± 6.6 (F) yrs; mean BMI 24.67 ± 3.69 (males) and 25.02 ± 3.66 (females)	14 d	No adverse effects on liver reported
Henning et al., 2015	6 cups of green tea	Daily (conditions not specified)	1010 mg (as green tea polyphenols)	562 mg	34 males with prostate cancer; mean age 62.1 ± 6.9 yrs; mean BMI 27.2 ± 3.8	3 - 8 wks	No adverse effects on liver reported
Yang et al., 2012	325 ml bottles (650 ml per day, prepared by extracting 28 g green tea leaves with 1 L hot water (70°C) for 8 min followed by filtration).	2X/day with meals	534 mg	NR	15 overweight subjects (8M/7F); mean age 27.6 ± 2.1 yrs; mean BMI 27.3 ± 0.9	6 wks	No adverse effects on liver reported
Basu et al., 2010	2 cups (4 cups per day, prepared by steeping 4 tea bags in 4 cups of boiled water for 10 min)	2X/day	928 mg	440 mg	13 obese subjects (10F/3M) with metabolic syndrome; age 28-59 yrs (mean 42.8 ± 2.6); mean BMI 28-45 (34.6 ± 1.5)	8 wks	No adverse effects on liver reported
Maki et al., 2009	500 ml bottle of green tea catechin beverage, also containing water sodium chloride, artificial citrus flavoring, glucose, erythritol, and sucralose.	1X/day, consumed within 30 min, at any time of the day, with or without food	625 mg	214.4 mg	65 obese subjects (32M/33F); mean age 47.0 ± 1.3 yrs; mean BMI 32.2 ± 0.05	12 wks	No adverse effects on liver reported
Nagao et al., 2005	One 340 ml bottle of high catechin beverage made by adding green tea catechins to an oolong tea, plus 170 mg ascorbic acid to avoid oxidation.	1X/day during supper	690 mg	135 mg	17 healthy males; age 24-46 yrs; mean BMI 24.9 ± 0.4	12 wks	No adverse effects on liver reported
Nagao et al., 2007	340 ml canned beverage prepared by brewing 9 g green tea leaves in 1 L distilled water for 5 min at 80°C	1X/day recommended to be consumed within one hour of a meal but not mandatory	582.8 mg	100.3 mg	123 Japanese subjects (51F/72M) with visceral fat type obesity; mean age 41.7 ± 9.9 yrs; mean BMI 26.9 ± 1.9	12 wks	No adverse effects on liver reported
Nagao et al., 2009	340 ml canned beverage prepared by brewing 9 g green tea leaves in 1 L distilled water for 5 min at 80°C	1X/day	582.8 mg	100.3 mg	23 overweight or obese Japanese subjects (15F/8M) with type 2 diabetes; mean age 64.9 ± 1.6 yrs; mean BMI 25.6 ± 0.8	12 wks	No adverse effects on liver reported
Sakata et al., 2013	700 ml green tea beverage	1X/day with meals	200 or 1080 mg	Not reported	12 subjects (sex data not specified) with non-alcoholic fatty liver disease; mean age 51.5 ± 14.8 yrs (200 mg group) and 47.1 ± 17.2 yrs (1080 mg group); mean BMI 29.1 ± 1.8 (200 mg group) and 28.0 ± 2.0 (1080 mg group)	12 wks	No adverse effects on liver reported. Significant reduction in AST and ALT compared to baseline.
Tsuchida et al., 2002	340 ml bottle	1X/day	588 mg	114.9 mg	39 healthy subjects; 20M of 30-62 yrs (mean 42.2) and 19 menopausal women; age 43-65 yrs (mean 54.8); mean BMI 26.43 ± 0.37	12 wks	No adverse effects on liver reported
Wang et al., 2010b	A) High catechin tea (HC): prepared by infusing 2.5 g tea leave in a tea bag in 250 ml hot water; Extra high catechin beverage (EH): B) prepared by infusing 1.25 g tea leaves enriched with catechin powder (0.3 g) and 0.45 g green tea powder	1) EH 1X/day 2) HC 2X/day 3) EH 2X/day	1) 458 mg, 2) 468 mg, 3) 886 mg	NR	139 overweight Chinese subjects (73% F overall, including control group but sex data for each group not reported); mean age for 3 groups: 1) 36.6 ± 9.1, 2) 37.5 ± 10.1, 3) 37.5 ± 9.1 yrs; mean BMI for 3 groups: 1) 27.1 ± 2.2, 2) 27.2 ± 2.5, 3) 26.8 ± 2.2	90 d	No adverse effects on liver reported
Matsuyama et al., 2008			576 mg	102.3 mg		6 m	

(*continued on next page*)

Table 2 (*continued*)

References	Description of green tea preparation	Condition of use[b]	Total Catechins per day	EGCG per day	Subjects on Intervention[c]	Duration	Findings related to the liver and severity rating
	340 ml canned beverage prepared by brewing 9 g green tea leaves in 1 L distilled water for 5 min at 80°C	1X/day consumed any time of day			19 overweight children (14M/5F); mean age 11.4 ± 0.5 yrs; mean BMI 27.2 ± 0.8		No adverse effects on liver reported
Yoneda et al., 2009	340 ml can green tea catechin beverage	1X/day with meals	588 mg	114.9 mg	77 healthy males; mean age 44.3 ± 0.05 yrs; mean BMI 24.8 ± 0.3	11-17 m	No adverse effects on liver reported
Polyphenon E Capsules							
Nguyen et al., 2012	4 capsules Polyphenon E (delivering 800 mg EGCG)	1X/day, consumed every morning with food	784-1000 mg	800 mg	24 male subjects with prostate cancer; mean age 63.4 ± 5.9 yrs; mean BMI 26.9 ± 3.4	3-6 wks	1 subject with Grade 1 ALT elevation (4%)
McLarty et al., 2009	4 capsules Polyphenon E (delivering 800 mg EGCG)	daily (conditions not specified) taken with meals	784-1000 mg	800 mg	26 males with prostate cancer, age 41-68 yrs (mean 58.5); mean BMI not reported	12-214 d, with a mean of 34.5 d	No adverse effects on liver reported
Dryden et al., 2013	1 or 2 capsules Polyphenon E (delivering 200 or 400 mg EGCG)	2X/day	392-500 or 784-1000 mg	400 or 800 mg	13 subjects (6M/7F) with mild to moderate ulcerative colitis; mean age 44.9 ± 15 yrs; BW 61-77 (mean 84.8 ± 14.2) kg	8 wks	No adverse effect on liver reported
Wu et al., 2012	1 or 2 capsules Polyphenon E (delivering 200 or 400 mg EGCG)	2X/day taken with meals	816-1000 mg (composition of highest dose provided)	800 mg (composition of highest dose provided)	71 overweight or obese females; mean age 59.6 ± 6.36 yrs (200 mg EGCG group) and 62.0 ± 9.42 yrs (400 mg EGCG group); BMI 27.2-31.8	2 m	A slight but stat. significant increase in ALT level in high dose group from baseline (20.6 vs. 23.7 IU/L) but remained in normal range.
Ahn et al., 2003	1 capsule Polyphenon E (delivering 200 mg EGCG)	1X/day	196-250 mg	200 mg	14 females with cervical lesions (age and BMI not reported)	12 wks	One subject with abnormal liver function at week 4 that returned to normal for week 8 and 12
Garcia et al., 2014	4 capsules Polyphenon E (delivering 800 mg EGCG)	1X/day with meals	NR	800 mg	41 females with persistent high risk HPV infection and low grade cervical intraepithelial neoplasia; age 19-58 yrs (mean 28.48 ± 8.78), BW 89-268 lbs (mean 149.78 ± 39.48)	4 m	4 subjects had elevated AST and 5 elevated ALT with one subject with Grade 3 ALT elevation
Shanafelt et al., 2009	2 -10 capsules (delivering 400 mg to 2000 mg EGCG)	2X/day with meals	NR	800-4000 mg	33 subjects (24M/9F) with chronic lymphocytic leukemia; age 41-76 yrs (median 62); BMI not reported	Up to 6 m	Transaminitis (33% of subjects, all with Grade 1 and reversible; dose associated with AEs not specified)
Shanafelt et al., 2013	5 or 10 capsules Polyphenon E (containing 1000 or 2000 mg)	2X/day with meals	5,278 mg for 7 days, then increased to 10,556 mg	2000 mg for 7 days, then increased to 4000 mg	42 subjects (30 M/ 11 F) with chronic lymphocytic leukemia ages 41-78 yrs (median 60); BMI not reported	6 m	13 subjects with Grade 1, 6 subjects with Grade 2 and 1 subject with Grade 3 transaminitis
Lovera et al., 2015	2 capsules Polyphenon E (delivering 400 mg EGCG)	2X/day with meals	NR	800 mg	10 subjects (9 F/1 M) with multiple sclerosis; ages 39-56 yrs (median 45); BMI not reported	6 m	One subject with Grade 1 abnormal liver function
Joe et al., 2015	1-3 capsules Polyphenon E (delivering 200, 400, or 600mg EGCG)	2X/day with meals	1500-1224 mg	1200 mg	33 subjects (10F/23M) with Barrett's esophagus; age 36-81 yrs (median 61); BMI not reported	6 m	Study discontinued due to adverse events. 2 cases of persistent Grade 1 elevated ALT (both in 1200-mg/day group)
Crew et al., 2012	2-4 capsules Polyphenon E (delivering 400, 600, or 800 mg EGCG)	2X/day consumed with food	NR	1600 mg	30 females with breast cancer; age 36-64 yrs (median 52); BMI 21.1-40.7 (median 28.2)	6 m	2 subjects with Grade 1 transaminitis and 1 subject with Grade 3 elevated ALT; 2 subject with Grade 1 high alkaline phosphatase
Kumar et al., 2015	1 capsule Polyphenon E (delivering 200 mg EGCG)	2X/day taken with meals	408-500 mg	400 mg	36 males with high grade prostitis intraepithelial neoplasia and/or atypical small acinar proliferation; mean age 62.0 ± 7.9 yrs; mean BMI 29.6 ± 4.9	1 y	Liver function was monitored but data not reported.
Lovera et al., 2015	2 capsules Polyphenon E (delivering 400 mg EGCG)	2X/day with meals	NR	800 mg		1 y	

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Table 2 (*continued*)

References	Description of green tea preparation	Condition of use[b]	Total Catechins per day	EGCG per day	Subjects on Intervention[c]	Duration	Findings related to the liver and severity rating
					7 subjects (5F/2M) with multiple sclerosis; age 33-59 yrs (median 47.5); BMI not reported		Study terminated early due to SAEs. 4 subjects had Grade 1 abnormal liver enzymes, 1 subject had Grade 4 abnormal liver function
EGCG Capsules							
Ullmann et al., 2004	1 capsule (200 mg, 400 mg, or 800 mg EGCG)	1X/day after fasting	800 mg (highest dose)	800 mg (highest dose)	27 healthy males; mean age range from 27.1 ± 5.7 yrs (800 mg group) to 28.78 ± 2.64 yrs (400 mg group); mean BW ranges from 70.67 ± 8.46 kg (800 mg group) and 71.94 ± 8.72 kg (400 mg group)	10 d	One subject of the 800 mg group presented with a slight and reversible increase of ALT (2-fold above NR at end of study) and recovery within 14 days after the study.
Widlansky et al., 2007	1 capsule (containing 150 mg EGCG)	2X/day with meals	300 mg	300 mg	21 subjects (15M/6F) with coronary artery disease; mean age 59 ± 8 yrs; BMI not reported	2 wks	No adverse effects on liver reported
Ahn et al., 2003	1 capsule delivering 200mg EGCG	1X/day	200 mg	200 mg	10 females with cervical lesions (age and BMI not reported)	12 wks	No adverse effects on liver reported
Mielgo-Ayuso et al., 2014	100 mg EGCG	3X/day with meals	300 mg	300 mg	43 obese females; ages 19-49 yrs; mean BMI 33.7 ± 2.6	12 wks	No adverse effects on liver reported
Hill et al., 2007	1 capsule (containing 150 mg EGCG)	2X/day right before meals	300 mg	300 mg	19 postmenopausal overweight or obese females; age 45-70 yrs; mean BMI 30.65 ± 0.59	12 wks	No adverse effects on liver reported
de la Torre et al., 2014	1 or 2 capsules delivering 200 or 400 mg EGCG with a mean dose of 9 mg/kg	1X/day	200 or 400 mg	200 or 400 mg	13 subjects (6F/7M) with Down's Syndrome, mean age 22.2 ± 4.2 yrs; mean BMI 25.4 ± 4.0	3 m	No adverse effects on liver reported
Other Green Tea Extract Capsules							
Yoshikawa et al., 2012	3 capsules contained 356.48 mg total catechins, 270 mg EGC, 6.78 mg GC, 71.39 mg ECG, 4.09 mg EGC.	3X/day	1169 mg	810 mg	20 healthy subjects (8M/12F); mean 49 ± 6.7 yrs; mean BMI 20.8 ± 2.7	1 wk	No adverse effects on liver reported
Frank et al., 2009	2 capsules, each containing 384 mg aqueous green tea extract	3X/day before meals	714 mg	150 mg	17 healthy males; age 18-55 yrs; mean BMI 26.7 ± 3.3	3 wks	No adverse effects on liver reported
Laurie et al., 2005	Green tea extract capsules delivering 4.86 g (3g/m^2)[d]	Taken with water after a meal in the morning	1633 mg	676 mg	16 subjects (8M/8F) with lung cancer; age 40-75 yrs (median 63); BMI not reported	4 wks	No adverse effects on liver reported
Brown et al., 2011	1 capsule containing 530 mg decaffeinated green tea extract	2X/day one hour before meals	796 mg	424-753 mg	63 healthy males; mean age 49.5 ± 5.6 yrs; mean BMI 31.7 ± 2.7	6 wks	No adverse effects on liver reported
Basu et al., 2010	1 capsule	2X/day	870 mg	460 mg	10 obese subjects (7F/3M) with metabolic syndrome; age 27-52 yrs (mean 39.5 ± 3.0); mean BMI 38.0 ± 2.3	8 wks	No adverse effects on liver reported
Hsu et al., 2008	1 capsule of 400 mg green tea extract	3X/day, 30 min after meals	614 mg	377 mg	41 obese Chinese females; mean age 43.0 ± 11.1 yrs; mean BMI 31.2 ± 3.5	12 wks	No adverse effects on liver reported
Tsao et al., 2009	500, 750 or 1000 mg/m^2 (body surface area) green tea extract capsules containing 350 mg GTE per capsule (26.9% total catechins, 13.2% EGCG, 8.3% EGC, 3.3% ECG, 2.2% EC, 6.8% caffeine). Dose was estimated to be 700, 1050, and 1400 mg[e]	3X/day after meals	564 mg, 847 mg and 1128 mg	277 mg, 416 mg, 554 mg	30 subjects (13M/17F) with high-risk of oral premalignant lesions; age 35-71 yrs; mean BMI not reported	12 wks	No adverse effects on liver reported
Chen et al., 2016	1 capsule green tea extract	3X/day, 30 min after meals	1345 mg	856.8 mg	39 obese females; mean age 44.1 ± 10.9 yrs; mean BMI 31.0 ± 3.8	12 wks	Liver function tests showed a significant increase in ALT from 27.2 ± 14.9 IU/ml to 33.6 ± 22.2 IU/ml, but remained within normal range.

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Table 2 (*continued*)

References	Description of green tea preparation	Condition of use[b]	Total Catechins per day	EGCG per day	Subjects on Intervention[c]	Duration	Findings related to the liver and severity rating
Chantre and Lairon, 2002	2 capsules (each capsule containing 375 mg of an 80% ethanolic dry extract standardized at 25% catechins expressed as EGCG)	2X/day	1500 mg	1080 mg	70 overweight to obese subjects (7M/63F); age 20-69 yrs (mean 44.7); BMI 25-32 (mean 28.9)	12 wks	1 subject with an increase in transaminase (Grade unspecified)
Pezeshki et al., 2016	1 tablet containing 500 mg green tea extract	1X/day	256 mg	157 mg	35 subjects (16M/19F) with nonalcoholic fatty liver disease; age 20-50 yrs; mean BMI 34.45 ± 4.62	90 d	No adverse effects on liver reported; significant reduction in AST, ALT, and AP compared to baseline
Roshdy et al., 2013	One 400 mg green tea extract capsule (95% polyphenols, 45% EGCG)	2X/day after meals	NR	360 mg	22 females with symptomatic uterine fibroids; ages 25-50 yrs (mean 41.5 ± 5.9); mean BMI 33 ± 6.9	4 m	No adverse effects on liver reported
Hsu et al., 2011a	1 capsule (containing 500 mg of green tea extract)	3X/day, 30 min after meals	1345 mg	856.8 mg	35 overweight or obese subjects (12M/23F); mean age 50.5 ± 9.2 yrs; mean BMI 29.7 ± 4.0	16 wks	No adverse effects on liver reported
Fukuzawa et al., 2014	2 green tea catechin tablets containing 125 mg green tea extract per tablet	3X/day after meals	600 mg	395 mg	26 subjects (13M/13F) with nonalcoholic steatohepatitis; mean age 53.9 ± 15.2 yrs; mean BMI 30.0 ± 4.7	6 m	No adverse effects on liver reported
Qian et al., 2012	1 capsule containing 250 mg green tea polyphenols	2X/day taken after meals	492 mg	232 mg	76 females with osteopenia; mean 56.5 ± 5.5 yrs (green tea only group) and 57.6 ± 6.7 yrs (green tea plus tai chi group); BMI not reported	6 m	No adverse effects on liver reported
de la Torre et al., 2016	3 or 4 capsules green tea extract (amount dependent on weight)	Daily (other conditions not specified)	NR	800 mg (highest dose)	43 subjects (24M/19F) with Down's Syndrome; mean age 23.1 ± 3.6 yrs; mean BMI 25.6 ± 4.2	12 m	No adverse effects on liver reported
Dostal et al., 2015	2 capsules (each capsule containing 375 mg of an 80% ethanolic dry extract standardized at 25% catechins expressed as EGCG)	2X/day with meals	1315 mg	843 mg	538 post-menopausal females at risk for breast cancer (based on breast density); mean age 59.9 ± 5.0 yrs; mean BMI 25.2 ± 3.7	1 y	53 incidences of ALT elevations in treatment group: 39 Grade 1, 7 Grade 2, 6 Grade 3, and 1 event of Grade 4 ALT elevation. Nine events related ALT or AST elevations in treatment group were classified as SAEs.

Note:

NR = not reported, F = female, M = male, wk = week, d = day, m = month.

[a] Listed in ascending order of intervention duration.

[b] Conditions of use recorded as reported by study. Feeding conditions were included if described in the study.

[c] Number of subjects on green tea intervention that completed the study. For those studies that reported AEs, this number also includes subjects that dropped out due to AEs related to green tea intervention. Sex, age (expressed as range, median or mean ± SD), and baseline body mass index (BMI) (expressed as range, median or mean ± SD kg/m^2) were also included. When available, baseline body weight (BW) was provided if BMI was not reported.

[d] Maximum tolerated dose determined in a dose escalation study, based on 1.62 m^2 as the average surface area of a 60 kg human.

[e] Dose estimated based on 1.62 m^2 as the average surface area of a 60 kg person, rounding downward to the closest dose that could be administered using one or more 350 mg capsules.

commonly reported AEs were related to GI disturbance. Hepatotoxic events were observed at a very low rate in these studies and were mostly mild or moderate in severity. Hepatic AEs appeared to be closely linked to consumption of green tea preparations with concentrated catechins and administered in bolus dose and capsule form, delivering a daily dose equivalent to or greater than 800 mg EGCG (1315–1500 mg catechins/day). Most of these studies administering a solid bolus dose form of GTE instructed subjects to consume the preparation with a meal or shortly after. No hepatic AEs occurred when the green tea preparation was consumed in beverage or food form in a fed or fasted state, or at the EGCG equivalent dose at or below 676 mg/day in a solid bolus dose in a fed or fasted state, despite the large variation in study designs and subject populations seen in this assessment.

3.1.10.3. Pharmacokinetic (PK) data. The hepatotoxicity induced by GTE or EGCG was dose-dependent and mostly influenced by dosing conditions such as exposure route (gavage *vs.* dietary) and feeding condition (fasted *vs.* fed), suggesting that internal exposure to these compounds in the body may be an important determinant in the pathogenesis. A close examination of kinetic data of these catechins from human and animal studies, especially their plasma kinetic parameters as indicators of internal exposure, may explain the relevance of hepatotoxicity within the context of human risk assessment. Because catechins have been shown to be eliminated after Phase II transformation in the body by forming glucuronide, sulfate and methylate conjugates, and for most xenobiotics the conjugated forms are typically less toxic than their parent forms, our review focused on the free form of catechins in the circulation. EGCG specifically was used as an example for the reasons that 1) more published toxicokinetic data are available for EGCG; 2) EGCG is one of the major catechins present predominantly in its free form in the systemic circulation in humans, and there is no apparent difference in its pharmacokinetics between purified EGCG and EGCG from GTE (Law et al., 2017); and 3) EGCG has been shown to cause hepatotoxicity both *in vitro* and *in vivo*.

Several toxicological studies reported plasma levels of catechins, particularly EGCG, in the tested animals (Isbrucker et al., 2006b; Kapetanovic et al., 2009; Ramachandran et al., 2016; Veregen, 2006). In these studies, plasma levels of catechins were found to be very low when no hepatotoxicity was observed. In the 13-week study of EGCG administered in the diet to SD rats, free EGCG was mostly not detectable in the plasma at the 50 and 150 mg EGCG/kg/day dose levels (detection limit was reportedly 0.007 μM) (Isbrucker et al., 2006b). At the 500 mg EGCG/kg/day dose which was the highest dose and NOAEL, the mean plasma levels of free EGCG was between 0.019 and 0.025 μM at week 1, and between 0.008 and 0.017 μM at week 13, showing that repeated dosing over 13 weeks did not cause elevated free EGCG levels in the rats' plasma, and there was no apparent accumulation of EGCG. These concentrations were at least 10-fold less compared to that reported in the 13-week study in which plasma EGCG levels were found in a range of 0.05–1.22 μM at week 7 and 0.12–0.62 μM at week 13 in Fisher rats receiving EGCG *via* oral gavage at the dose levels of 150 and 500 mg/kg/day and liver lesions were observed (Veregen, 2006). Consistent observations were also reported in the dog studies. In the 13-week study of EGCG in pre-fed dogs, the mean plasma Cmax of free EGCG ranged from 0.3 to 15.9 μM on day 78 at doses of 46, 275 and 460 mg EGCG/kg/day (Isbrucker et al., 2006b), and no liver damage was observed. In contrast, in the 13-week study in the fasted dogs, the mean plasma Cmax of free EGCG was found ranging from 6.7 to 121.3 μM at doses of 120 and 400 mg EGCG/kg/day on day 81, and serious hepatotoxicity occurred (Isbrucker et al., 2006b). A similar trend was also observed with the area under curve (AUC) values. In the pre-fed dogs without signs of liver damage, the mean plasma AUC_{0-6hr} was in a range of 3.9–192.6 μM × hr on day 78 at the 46, 275 and 460 mg EGCG/kg/day dose levels, whereas in the fasted dogs experiencing hepatotoxicity, the mean plasma AUC_{0-24hr} of free EGCG reached 57.1–811.8 μM × hr on day 81 at the 120 and 400 mg EGCG/kg/day dose levels. In the 13-week toxicity study of Polyphenon E conducted by Kapetanovic et al. (2009), with a daily dose of 200 mg/kg/day (delivering an estimated 128 mg EGCG/kg/day), the mean AUC_{0-24hr} of free EGCG in the pre-fed dogs was 32.4 μM × hr in which a lesser degree of liver damage was observed, compared to the mean AUC_{0-24hr} of 63.7 μM × hr in the fasted dogs, in which severe toxicity was observed (Table 3).

Pharmacokinetics of orally administered green tea catechins in healthy individuals has been extensively examined from various aspects, including the catechin source (Chow et al., 2001; Henning et al., 2004), dose-response (Chow et al., 2001; Yang et al., 1998), dosing frequency (Chow et al., 2005), and dosing condition (Chow et al., 2003; Naumovski et al., 2015). In general, green tea catechins are quickly absorbed upon ingestion and generally reach the peak plasma concentrations within one to five hours (Tmax) with a one-peak plasma concentration versus time course, followed by a multiphasic decrease consisting of a distribution phase and an elimination phase (Law et al., 2017). EGCG and ECG are mainly (60–90%) found in plasma in their free forms, while EGC and EC in plasma mostly exist in their glucuronide, methylate and sulfate conjugates. Most catechins are eliminated from plasma within 24 h of ingestion and the half-lives ($t_{1/2}$) of these catechins range from two to 10 h (Feng, 2006; Sun et al., 2009). Internal exposure to free EGCG, when expressed as plasma Cmax or AUC values, in human clinical trials is in a range of 0.0035–7.36 μM for Cmax, and 0.06–24.93 μM × hr for AUC_{0-24hr} following a single oral dose of 72.8 to 1200 mg EGCG (equivalent to 1.2–20 mg EGCG/kg for a 60 kg person) (Feng, 2006; Sun et al., 2009). With repeated oral dosing, the mean plasma Cmax values of free EGCG were reportedly in a range of 0.3–0.63 μM, and the mean AUC_{0-24hr} was 2.0–5.8 μM × hr following 800 mg EGCG/day from Polyphenon E taken with a meal for four weeks (Chow et al., 2003). Values could reach up to 6.10 μM for plasma Cmax and 19.7 μM × hr for plasma AUC_{0-t} following 800 mg EGCG/day under fasted conditions for 10 days (Table 3). Notably, the plasma Cmax and AUC of free EGCG were found to be significantly lower from green tea beverage than GTE or EGCG in capsules (0.08 *vs.* 0.15 μM for Cmax, and 0.27 *vs.* 0.62 μM × hr for AUC_{0-8hr}, respectively) (Henning et al., 2004). Generally the plasma concentrations of free EGCG in healthy human subjects under various dosing conditions resembled more closely the levels observed in the 13-week dietary study in rats as well as in the 13-week study in pre-fed dogs conducted by Isbrucker et al. (2006b), and were at least two orders of magnitude lower than those found in the fasted dogs by Isbrucker et al. (2006b) and Kapetanovic et al. (2009).

3.1.10.4. Weight of evidence. Overall strength of the body of evidence for each adverse outcome identified from animal toxicological and human intervention studies was graded based on the consideration of consistency, temporal and dose-response, biologic plausibility and human relevance (Table 4). The consideration for the confidence ratings are summarized in the narrative below.

There is moderate evidence demonstrating that oral exposure to green tea preparations or EGCG is associated with reduced body weight or weight gain. The results from animal toxicity studies present a pattern of findings consistent across species and studies and with a clear dose-response effect. Although suppressed weight gain with or without reduced food intake could be a secondary effect to other toxicities, when observed in the absence of other toxicological findings as shown in some studies, reduced weight gain by itself may be a pharmacological effect of green tea catechins rather than adverse to health. This observation is supported by the findings from human studies reporting a positive effect of green tea or GTE consumption on weight loss (Johnson et al., 2012; Jurgens et al., 2012). Mechanistically, green tea catechins have been shown to inhibit *de novo* lipogenesis, stimulate lipid oxidation and increase thermogenesis in animal models and humans (Grove and Lambert, 2010; Hursel and Westerterp-Plantenga, 2013), which supports the biologic plausibility of this effect. This effect

Table 3
Mean $AUC_{0\text{-}t}$ and Cmax of free EGCG in plasma after oral administration of polyphenon E or EGCG in healthy human volunteers and beagle dogs under fasted and fed conditions.[a]

	Human				Dogs		
Duration	Single dose	Single dose	10 days	4 weeks	13 weeks	13 weeks	13 weeks
Dose	213.6 mg EGCG/ person from green tea infusion (estimated 3.56 mg EGCG/kg [b])	400, 800, 1200 mg EGCG/ person from Polyphenon E (estimated 6.67, 13.3, 20 mg EGCG/kg [b])	200, 400, 800 mg EGCG/ person/d (estimated 3.3, 6.67, 13.3 mg EGCG/kg/d [b])	800 mg EGCG/person/d from Polyphenon E (estimated 13.3 mg EGCG/kg/d [b])	200 mg Polyphenon E/kg/d (estimated 128 mg EGCG/kg/d)	40 – 400 mg EGCG/ kg/d in one bolus dose	46 – 460 mg EGCG/kg/d in two divided doses
Fasted Condition (in humans or dogs) or Gavage Route (in rats)							
AUC (μM × hr)	0.27	4.62 – 24.9	2.42 – 19.7	not reported	63.7 – 98.5	20.04 – 811.83	not reported
Cmax (μM)	0.08	1.74 – 7.36	0.57 - 6.1	not reported	not reported	3.9 –121.2	not reported
Fed Condition [b]							
AUC (μM × hr)	not reported	1.33 – 10.89	not reported	5.8 ± 3.3 *q.d.*; 2.0 ± 0.9 *b.i.d.*	32.4	not reported	3.9 – 192.6
Cmax (μM)	not reported	0.31 – 2.01	not reported	0.63 ± 0.27 *q.d.*; 0.34 ± 0.14 *b.i.d.*	not reported	not reported	0.3 –15.9
Reference	Henning et al., 2004	Chow et al., 2005	Ullmann et al., 2004	Chow et al., 2003	Kapetanovic et al., 2009	Isbrucker et al., 2006b	Isbrucker et al., 2006b

Note:
[a] The values were converted to μM if the data were reported in different unit (*e.g.*, ng/ml).
[b] The dose was estimated based on a 60-kg body weight person.
[c] The fed condition includes the scenario that either the test article was taken with the meal, or taken after a meal.

of green tea catechins may also explain the observations of reduced pup weight and growth rate in F_1 and F_2 rats in the two-generation reproductive toxicity study of EGCG, which may in turn contribute to the observed delay in sexual maturation in these rats (Isbrucker et al., 2006c).

The results of our review support the general pattern that consumption of high dose GTE and EGCG is associated with GI toxicity as evidenced by the findings of animal toxicity studies as well as the AEs reported in human clinical trials. The severity and incidence rate of GI toxicity were dose-dependent, increased under the experimental conditions where GTE or EGCG was ingested in large bolus doses *via* oral gavage (in rodents) and in capsules (in dogs and humans). Compared to more frequent occurrences of GI disturbance in human studies involving solid dosage of GTEs, only two studies involving beverage application reported GI events. One of these studies delivered a very large dose (6 g/day) of pulverized green tea powder which was divided in 1 g/serving and dissolved in an unknown amount of water, taken as six servings daily (Jatoi et al., 2003). The second study did not mention if brewed green tea was consumed in a fasted or fed state (Kalus et al., 2010). The adverse effects were shown to be exacerbated under fasted conditions in both animal and human studies. Mechanistically, high concentrations of tea catechins have been shown to be cytotoxic and induce oxidative stress, which may explain the damage to GI epithelial lining associated with high oral doses of green tea catechins, while ingestion with or after food may help dilute their concentrations in the gut.

Our review supports that consumption of GTE or EGCG as a bolus dose, but not green tea consumed as a beverage or part of the diet, is causally associated with hepatotoxicity. Collectively, the evidence from published human and animal studies revealed a consistent pattern of hepatic adverse effects based on the incidences of abnormal liver function biomarkers (in both human and animals) and/or histopathological lesions (in animals) following the oral exposure to bolus doses of GTE or EGCG in a dose dependent manner. The hepatotoxicity risk appears to be influenced by several factors. Evidence from animal studies demonstrated that hepatotoxicity risk rose with increasing concentrations of catechins and EGCG as shown by the inverse correlation between hepatotoxicity NOAELs and purity of catechins and EGCG in the test material (Fig. 3). This suggests that GTEs containing highly concentrated catechins may be more likely to induce liver damage than those with catechins at concentrations similar to a traditional green tea infusion. The incidence and severity of hepatotoxicity increased when GTE or EGCG was administered under fasted conditions, while exposure to green tea, GTE or EGCG as part of the diet, drinking water or under fed conditions appeared to alleviate such risk. The findings from published human studies corroborate those from animal studies. Hepatotoxicity events linked to green tea preparations occurred at a relatively low rate in the human studies reviewed. Treatment-related abnormal changes in liver function biomarkers were mostly reported in the studies where GTE or EGCG was consumed in solid dosage form (as capsules), with the highest incidence observed in studies involving Polyphenon E. The majority of hepatic AEs were mild-to-moderate in nature while several SAEs occurred with the exposure to GTE at 800 mg EGCG/day or above. This is consistent with sporadic AE case reports published in recent years linking consumption of products containing GTE to liver injury (Gloro et al., 2005; Mazzanti et al., 2009; Molinari et al., 2006; Sarma et al., 2008). Furthermore, the causality for GTE-induced liver injury was affirmed in a *post hoc* analysis of the Minnesota Green Tea Trial, reporting an OR of 7.0 (95% CI, 2.4–20.3) for developing liver function abnormalities as compared with those in the placebo arm, and a rise–fall pattern of liver enzyme levels following the challenge–dechallenge cycles of GTE consumption (Yu et al., 2017). Hepatotoxicity was clearly absent in the animal studies in which GTE or EGCG was administered in the diet or drinking water despite concentrations of catechins in the test materials (> 68% w/w) greater than that of a traditional green tea infusion. Consonantly, among the human

Table 4
Weight of evidence analysis for the observed adverse effects.

Outcome	Body of Evidence[a]	Consistency across studies and species	Temporal /dose response	Biologic plausibility	Human relevance	Confidence rating[b]
GI toxicity	Animal (11) Human (22)	yes	yes	strong	high	high
Hepatotoxicity	Animal (16) Human (11)	yes	yes	strong	high	high
Reduced body weight or weight gain	Animal (23) Human (4)	yes	yes	medium	limited	moderate
Nasal-olfactory toxicity	Animal (4) Human (3)	no	no	medium	limited	very low
Thyroid toxicity [c]	Animal (4) Human (1)	no	yes	medium	no	very low
Cardiotoxicity	Animal (5) Human (6)	no	yes	weak	limited	low
Pancreatic toxicity	Animal (5) Human (0)	no	no	weak	limited	very low
Renal toxicity	Animal (4) Human (0)	no	yes	weak	limited	very low
Thymic toxicity	Animal (4) Human (0)	no	yes	weak	limited	very low
Reproductive & developmental toxicity	Animal (3) Human (0)	no	yes	weak	limited	very low
Lung, spleen and lymphoid, bone marrow toxicity	Animal (6) Human (0)	no	no	weak	limited	very low

Note:
[a] The counts refer to the numbers of individual animal experiments or human intervention trials in which the outcome was observed and reported and were included in this systemic review.
[b] High Confidence = The true effect is highly likely to be reflected in the apparent relationship. Moderate Confidence = The true effect may be reflected in the apparent relationship. Low Confidence = The true effect may be different from the apparent relationship. Very Low Confidence = The true effect is highly likely to be different from the apparent relationship.
[c] Thyroid toxicity observed in animal toxicity studies was hypothyroidism, while the AE reported in the human study was a case of hyperthyroidism.

studies with a wide range of subject populations in which hepatic events were monitored and reported, none of the studies involving green tea beverages reported any liver-related AEs regardless of the composition of the GTE, dose of individual catechins, or feeding state of the subjects. These findings are in agreement with the long history of safe consumption of large quantities of green tea as a beverage by humans without any documented detrimental health effects (Muramatsu, 1991).

Evidence from pharmaco- and toxicokinetic data of green tea catechins as the indicator of internal exposure further supports the notion that hepatotoxicity is a dose-dependent event, and GTE and EGCG consumed in a bolus dose and/or under fasted conditions elevate the toxicity risk in that it may significantly increase free catechin concentrations in systemic circulation and the liver. Plasma kinetic data of free EGCG reported in human subjects resemble more closely those found in the animal toxicity studies in which green tea catechins were administered *via* dietary route in rodents or *via* capsules in pre-fed dogs. Particularly the plasma Cmax and AUC values of free EGCG from drinking brewed green tea were found to be substantially lower compared to those observed following ingestion of GTE or EGCG in capsule form in humans. Consumption of tea catechins with food or under fed conditions results in significantly lower oral bioavailability and plasma levels of free catechins (Naumovski et al., 2015).

It is probable that severe GI tract damage observed in the animal studies may cause free catechins to bypass enterohepatic circulation and directly leak into systemic circulation due to a damaged GI barrier, resulting in greater plasma levels of free catechins. Additionally, sustained fasting has been shown to lower levels of uridine 5′-diphosphoglucuronic acid (UDPGA), a cofactor of glucuronosyltransferase (UGT) (Parkinson and Olgilvie, 2007), which might in turn reduce the rate of glucuronidation, a major detoxification pathway of catechins, leading to more free catechins in the blood. Under ordinary conditions of green tea consumption as a beverage, or consumption of GTE or EGCG in capsules with or after a meal, the amounts of free catechins in circulation are unlikely to reach supraphysiological levels associated with hepatotoxicity in animal models. However, when GTE or EGCG is ingested as large bolus doses under fasted conditions, more free catechins may enter systemic circulation, and thus increase the risk of hepatotoxicity and other systemic toxicity. One factor that has yet to be studied is the concentration of catechins in the portal vein and liver tissue. This is presumed to be higher than that found in systemic circulation due to the fact that ingested catechins are absorbed and transported *via* portal vein to the liver for the first pass elimination (Ferruzzi, 2010; Xie et al., 2012). Further research may be warranted to understand the amount of catechins reaching the liver as the target organ under various dosing conditions and the implications for hepatotoxicity risk.

Mechanistic evidence related to green tea catechins and hepatotoxicity does not appear to impact the overall confidence rating because no clear MOA or AOP has been established. Several potential mechanistic targets have been proposed in the literature to explain the biologic plausibility of the hepatotoxic effect associated with GTE and EGCG. Lambert et al. (2010) postulated that liver damage may be, at least in part, due to oxidative stress induced by high concentrations of free catechins and their metabolites in the liver, which may lead to increased hepatic lipid peroxidation (Lambert et al., 2010). Furthermore, intraperitoneally administered EGCG (75 mg/kg) was found to suppress hepatic antioxidant enzymes in mice, which could exacerbate oxidative damage in hepatocytes (Wang et al., 2015). Emoto et al. confirmed in an *in vivo* study that IGS rats dosed with 200 mg/kg GTE intraperitoneally showed increased lipid peroxidation and oxidative DNA damage in hepatocytes (Emoto et al., 2014). Genetic polymorphisms have also been suspected to play a role in the pathogenesis. It has been suggested that individuals with a low catechol-*O*-methyl transferase (COMT) activity, one of the main enzymes responsible for the detoxifying biotransformation (methylation) of tea catechins, may be more prone to green tea catechin-induced hepatotoxicity based on *in vitro* and animal data (Forester and Lambert, 2015; Lambert et al.,

2007; Wu et al., 2003). However, this mechanism has not been corroborated by the findings from human studies where no differences in plasma or urinary levels of catechins and their metabolites were observed between the homozygous high-activity and homozygous low-activity COMT genotype after green tea catechins consumption (Miller et al., 2012; Perry, 2014). Church et al. (2015) also explored the role of genetic background in a study using genetically diverse out-bred mice in contrast to genetically homogenous inbred animals typically used in toxicological testing. It was found that the severity of hepatotoxicity induced by EGCG administered intraperitoneally was highly variable with a small subset (16%) developing severe hepatic inflammation and necrosis, whereas the majority (65%) experienced only nil to mild hepatic damage in these outbred animals (Church et al., 2015). More recently several studies reported EGCG-induced damage in hepatic mitochondria. In *in vitro* assays, EGCG was shown to potentiate Ca-induced mitochondrial membrane damage and uncouple oxidative phosphorylation in rat hepatocyte mitochondria with compromised membrane permeability but not in normal mitochondria (Kucera et al., 2015; Weng et al., 2014). Since mitochondrial dysfunction has been linked to certain diseases and drug-induced toxicity (Brenner and Moulin, 2012), this mechanism, if proven *in vivo*, could indicate that pre-existing health conditions or concomitant medication use affecting mitochondrial membrane integrity may predispose certain individuals to green tea catechin-induced hepatotoxicity.

The specific component(s) in green tea preparations responsible for liver injury remain uncertain to date; however, catechins (particularly EGCG) have been implicated to play a key role (Galati et al., 2006; Goodin et al., 2006; Isbrucker et al., 2006b; Johnson et al., 1999; Lambert et al., 2010; Schmidt et al., 2005). EGCG administered *via ip.* at 50 mg/kg dose has been shown to induce severe hepatotoxicity in both inbred Swiss Webster mice and Diversity outbred mice, while ECG at the same dose did not (Church et al., 2015; Goodin et al., 2006). EGCG has also been found to be a more potent cytotoxic agent (LC_{50} of 200 μM) compared to other catechins (LC_{50} > 2000 μM) in isolated rat hepatocytes, and induced mitochondrial membrane collapse and ROS formation at a concentration of 200 μM (Galati et al., 2006). Lambert et al. (2010) found that oral administration of 750 mg EGCG/kg/day to male CF-1 mice for two days significantly increased the levels of plasma 8-isoprostane, hepatic malondialdehyde (MDA), and positive staining for 4-hydroxynonenal (4-HNE) in liver samples, and elevated hepatic expression of metallothionein and γ-histone 2AX protein (Lambert et al., 2010).

Our review does not support a causal relationship between consumption of green tea preparations and thyroid toxicity. The observations of thyroid dysfunction were inconsistent across animal toxicity studies, and there is lack of reported hypothyroidism AEs in the human studies. This is further corroborated by an absence of epidemiological evidence that links green tea consumption to any adverse health effect on thyroid functions despite the prominence and long history of tea consumption. It is well documented that rodents are highly sensitive to goitrogenic agents in comparison to humans because they lack high-affinity thyroxine-binding globulin which is present in humans, and the plasma half-life of T_4 in rats (12–24 h) is much shorter than in humans (5–9 days). Male rats are especially sensitive to thyroid toxicants because of their higher circulating levels of TSH than female rats (Capen, 1996, 1997; Dohler et al., 1979; Jahnke et al., 2004; McClain, 1989). The reported thyroid toxicity associated with GTE consumption in a few rodent studies may not bear direct relevance to normal green tea consumption in humans considering the known interspecies difference in thyroid physiology. The reported changes in the absolute and relative weights of the testes and prostate glands and the levels of LH and testosterone in those studies are likely secondary effects to hypothyroidism in that hypothyroidism leads to growth retardation, reduced testicular and prostatic weights, and altered levels of reproductive hormones in laboratory animals (Choksi et al., 2003).

Our review does not support a causal relationship between consumption of green tea preparations and nasal and olfactory toxicity that was reported in two published rodent studies where GTE was administered *via* oral gavage (Chan et al., 2010; NTP, 2016). There was a lack of consistency in animal toxicity studies, the majority of which (including the studies with duration up to two years) did not observe such effects. In published human studies in which AEs were monitored and reported, a few isolated cases of sinusitis and rhinitis among the subjects receiving GTE were reported in two studies (Maki et al., 2009; Tsao et al., 2009) but were not reported in the remaining 102 studies. Mechanistically, the evidence does not support the biologic relevance to human risk assessment due to the known species differences between humans and rodents regarding the amount and expression pattern of metabolizing enzymes, including cytochrome P450 enzymes (CYP450s), in the nasal mucosa. Green tea catechins are known substrates of CYP450s, and the susceptibility of rodents to GTE-induced nasal toxicity observed in these studies suggests metabolic activation may play a role in toxicity induction. However, it is well documented that the metabolic activity of CYP450s in the nasal epithelium of rodents is much higher relative to that of humans, and nasal cytotoxicity in rodents does not necessarily correlate with similar toxicity in humans as shown for a number of known chemicals (Jeffrey et al., 2006). Additionally, the nasal lesions were reported only in the two studies conducted by the NTP, and the investigators of which suggested gavage-related reflux of GTE or stomach contents could be a potential inducer of nasal toxicity in these rats and mice. Therefore, the available evidence does not appear to support a conclusion of toxicity in the nose and olfactory epithelia as a potential risk to humans consuming green tea or GTE.

Our review suggests that the toxicological findings in the heart, kidney, pancreas, spleen lymphoid, bone marrow and/or reproductive organs in a number of animal toxicity studies may be secondary in nature resulting from severe liver and GI toxicities. Adverse findings in these organs were primarily histopathological lesions and typically accompanied by other signs of toxicity in multiple organs and/or early death (Chang et al., 2003; Isbrucker et al., 2006b; Kapetanovic et al., 2009; NTP, 2016; Takami et al., 2008), whereas largely absent in studies in which no or low GI and hepatotoxicity was observed. No green tea treatment-related AEs related to these organ functions were seen in the human studies except for three cases of hypertension, two of which were considered attributable to increased caffeine intake from consumption of non-decaffeinated green tea preparations (Choan et al., 2005; Laurie et al., 2005; Maki et al., 2009). In the human studies where blood pressure, heart rate and/or renal function were monitored, no significant differences in blood pressure or heart rate were found between the group that received green tea preparation or EGCG and the control, and no adverse changes in renal function parameters were observed. In the absence of any known biologic plausibility supporting that green tea catechins may adversely affect these organs, the body of evidence is considered of low confidence for any direct relationship between green tea catechins consumption and toxicity in these organs.

4. Discussion

The evidence summarized in this systematic review of animal toxicological studies suggests that the signs and severity of adverse effects associated with oral exposure to green tea, its extracts or individual catechins (such as EGCG) vary widely and are dependent upon the internal dose in systemic circulation and at the target organ, which can be influenced by the dose level, composition of test material, dosing route and feeding state of the animals. The observed toxicities include a wide spectrum of effects, such as reduced weight gain, GI toxicity, hepatobiliary toxicity ranging from elevated liver enzyme levels to hepatocellular necrosis and bile duct hyperplasia, epicardium inflammation and myocardial necrosis, pancreatic degeneration and necrosis, renal proximal tubular necrosis, nasal and olfactory toxicity, and thyroid dysfunction. The assessment of AEs from human intervention studies

involving various green tea preparations or EGCG which monitored safety outcomes revealed that the most prevalent AEs were GI in nature, and hepatotoxicity did occur though at a low rate. There is clear evidence that green tea catechins are not genotoxic or carcinogenic based on the results from carcinogenicity and genotoxicity assays and corroborated by the lack of documented evidence from human epidemiological studies reporting any association between green tea consumption and increased cancer risks.

As green tea and GTE are widely consumed in foods and supplements for their various health benefits, and consumption of GTE under certain conditions is associated with some adverse health effects, there is clearly a need to establish an evidence-based safe intake level for GTE to inform the public and regulators for risk management purposes. However, because of the large heterogeneity in the composition among different GTE preparations used in the animal and human studies, it was not feasible to identify the safe intake level on the basis of GTE as a whole entity. Since mechanistic evidence points to EGCG as a key determinant in the toxicity potential of GTE and possibly a more potent cytotoxic agent relative to other catechins, we considered EGCG as a suitable surrogate marker of GTE for the establishment of an acceptable daily intake (ADI).

Furthermore, we consider adults without liver disease as an applicable population of this safe intake level. Multiple potential mechanisms of action on hepatotoxicity of GTE have been reported in the literature, some of which indicated that oxidative stress, genetic polymorphisms, pre-existing health conditions or concomitant medication use affecting mitochondrial membrane integrity may predispose certain individuals to green tea catechin-induced hepatotoxicity. In the case of GTE, we did not observe any clear pattern in the human safety dataset generated from clinical studies involving diverse subject populations. However, considering that most clinical studies only enrolled subjects with normal liver function at baseline, the safe intake levels of EGCG identified herein are more appropriate for those with normal liver function.

Hepatotoxicity is identified as the critical effect[2] considering the consistency between animal and human data with a clear dose-response, biologic plausibility and that its occurrence appeared to proceed to other systemic toxicities. Although GI toxicity was observed at lower dose levels compared to hepatotoxicity in most animal studies and all human studies reporting AEs, it was not considered a critical effect because it is a local effect and could be readily prevented through consumption of GTE with or after a meal. The point of departure (POD) for the critical effect can be identified based on the dose-response from both animal and human data. Because dosing and feeding conditions are important considerations in relation to the hepatotoxic potential of GTEs, POD was considered for both fed and fasted conditions. For the GTE ingested in bolus doses under fed conditions (*i.e.*, with food or after a meal), a hepatotoxicity NOAEL of 500 mg/kg/day for an EGCG preparation of 91.8% purity administered to pre-fed dogs in capsules from a high quality 13-week study conducted by Isbrucker et al. (2006b) was selected as the most relevant. Under the fasted condition, a hepatotoxicity NOAEL of 50 mg/kg/day for an EGCG preparation of 80% purity administered to fasted dogs in capsules from the same publication of Isbrucker et al. (2006b) was selected as the most relevant. Applying a default 100-fold uncertainty factor (UF), a safe intake level of 4.6 mg EGCG/kg/day was derived for GTE consumed in bolus doses under fed conditions, equivalent to 322 mg EGCG/person/day for a 70 kg adult. The safe intake level would be much lower for GTE consumed under fasted conditions, calculated as 0.4 mg EGCG/kg/day, equivalent to 32 mg EGCG/person/day for a 70 kg adult. The composite UF of 100 is typically used to account for interspecies (10-fold) and inter-individual (10-fold) differences in toxicokinetics and toxicodynamics (Renwick, 1993; IPCS, 1994; Aggett, 2007).

The human safety data revealed that green tea or GTEs consumed as a beverage have a quite different toxicity threshold from those in solid dose form, thus the safety intake levels were considered separately. For GTE or EGCG consumed in a solid dosage form (*i.e.* in capsules or tablets), regardless of fed or fast state, the highest dose of GTE at which no treatment-related hepatic AEs occurred across a diverse range of healthy adult individuals and diseased patients and durations was a dose delivering the equivalent of 676 mg EGCG/day (Laurie et al., 2005). Hepatic AEs were observed at higher doses in some, but not all studies. The GTE or EGCG in solid dosage form was administered with or after a meal in the majority of human interventions studies. One study clearly reported EGCG capsules were ingested before a meal and resulted in a hepatic AE from a dose of 800 mg EGCG/day (Ullmann et al., 2004). Accordingly, 676 mg EGCG/person/day was selected as an appropriate NOAEL for solid dosage form independent of feeding state based on human safety data. Given that EGCG is similar to a nutrient in that it provides health benefits, a safe level of intake for EGCG was determined by using an approach similar to that used by the Institute of Medicine (IOM) for nutrient risk assessment. Since nutrient risk assessment is more likely to be based on human data, relatively smaller UFs (1–10) are typically used (Taylor and Yetley, 2008). IOM applied UFs between 1 and 2 for several nutrients based on human data, such as manganese and fluoride (UF of 1), niacin and iron (UF of 1.5), and vitamin B6 and choline (UF of 2) (IOM, 1997, 1998, 2001). These low UFs were applied to NOAEL values due to the fact that they were derived from human data. According to Hathcock and Shao (2008), a robust data set of human clinical trials can justify application of a UF of 1. Because the NOAEL for EGCG was derived from a large set of human clinical studies with diverse designs, durations and subject populations, it could be argued that a UF of 1 may be acceptable. We chose to apply a UF of 2 to account for uncertainties due to inter-individual variability in metabolism of EGCG, limited understanding in the MOA, and that these human studies were designed to establish benefit as the primary outcome while not necessarily designed or powered to detect AEs. A default UF of 10 for inter-individual variability is likely overly conservative as the safe intake level is established for only adults with normal liver function, rather than the general population. Application of a UF of 2 results in a safe intake level of 338 mg EGCG/person/day for GTEs consumed in solid bolus dosage form by adults, which is comparable to the value derived from animal data under fed conditions (322 mg/day) and consistent with the NOAEL and UL recently proposed by Yates et al. and Dekant et al. (Dekant et al., 2017; Yates et al., 2017). With regard to the NOAEL for exposure *via* beverage form, the highest intake level reported was a dose of GTE delivering the equivalent of 704 mg EGCG/day, consumed in three divided doses before meals (fasted state) (Toolsee et al., 2013). Given the complete absence of liver-related AEs in the studies of green tea beverages regardless of the feeding state, this intake level could be considered as an Observed Safe Level (OSL) for a green tea preparation consumed in beverage form by adults (Hathcock and Shao, 2008). Whether this OSL could be applied to other green tea preparations administered in other food matrices is unclear at this time. However, results of the present analysis suggest that matrices that result in a dilution and/or slower systemic delivery of the catechins from green tea would tend to be better tolerated.

We recognize the challenge in determining a safe intake level for GTE as a complex mixture of bioactive constituents. Typically, a whole mixture approach is recommended by risk assessors because evaluating chemical mixtures of concern as a whole (as opposed to an individual component-based approach) is necessary to account for the unidentified fraction(s) and precludes the need to assume additivity among identified constituents. Because botanical materials used in research often vary substantially in their constituent profile and concentrations, Rider and Gennings (2015) introduced the concept of applying a statistical "sufficient similarity" approach to risk assessment of botanical mixtures

[2] Critical effect usually refers to the hazard of human relevance or a precursor to the effect that occurs at the lowest dose level in a sensitive species with the assumption that if the critical effect is prevented from occurring, then no other adverse effects of concern will occur (Nielsen et al., 2013).

(Rider and Gennings, 2015). This approach compares the NOAELs or benchmark doses (BMDs) of a reference mixture which is chemically characterized and experimentally evaluated in a dose-response toxicology study (*i.e.*, data rich) to a candidate mixture (Marshall et al., 2013). It is considered reasonable to use the RfD derived from the reference mixture study as a surrogate for the candidate mixture that is considered sufficiently similar. In the present analysis we were able to examine chemical similarity among different green tea test materials showing three clusters. However, biological similarity was not assessable because the toxicity outcomes were influenced not only by chemical composition, but also variability in dosing conditions. Nevertheless, this approach may be of use in assessing the safety of other poorly defined GTEs without having to repeat toxicity testing if it can be identified as sufficiently similar in chemical composition to a well-characterized, well studied GTE preparation.

Although outside the scope of this analysis, we speculate, based on knowledge related to extraction processes, that highly concentrated catechin-containing extracts are likely produced using particular solvent extraction systems, such as organic solvents which are proficient at extracting lipophilic constituents from plants (Blumberg et al., 2015). Although this review did not specifically compare different extraction methods, the limited AEs overall and complete absence of liver-related AEs from brewed green tea or green tea beverages suggests that aqueous preparations of tea are safe across a wide range of intakes and conditions. To our knowledge, the United States Pharmacopoeia (USP) recently convened a panel of experts evaluating this aspect in relation to GTE hepatotoxicity (https://callforcandidates.usp.org/node/4097, accessed August 17, 2017).

One of the strengths of the present systematic review is the comprehensive assessment of AEs from human studies involving green tea, GTE and EGCG interventions. To our knowledge no such analysis has been published to date. A recently published safety assessment of green tea and GTE and a meta-analysis reviewed liver-related AEs related to green tea interventions from human clinical studies (Dekant et al., 2017; Isomura et al., 2016). The present analysis quantitatively reviewed all types of AEs reported in relevant human clinical trials inclusive of the incidence and severity of hepatic AEs, and thus provided a more complete view of the spectrum of AEs associated with consumption of green tea preparations. Regarding hepatotoxicity, our results are in agreement with the findings of Isomura et al. concluding that incidence of liver-related AEs is very low in published clinical trials involving green tea preparations (Isomura et al., 2016). However, our criteria for selection of human studies was different from the Isomura et al. study which included studies testing a mixture of green tea in combination with other bioactive substances that were not derived from green tea, and excluded studies that were not placebo controlled. As observed in our analysis, treatment-related hepatic AEs were reported in several clinical trials which did not employ a placebo control. Excluding non-placebo controlled studies in the Isomura et al. study likely led to an underestimation of the prevalence of liver AEs in clinical trials, which may explain the much lower incidence (0.5%) reported by Isomura et al. as compared to 4.9% found in the present analysis. Furthermore, the more inclusive selection criteria in the present analysis provided a more robust dataset allowing us to examine the patterns of AEs. Our results are also consistent with the recent safety assessment by Dekant et al., which also concluded that liver-related AEs are dependent on the dosage form and conditions of use of green tea preparations, and proposed a 300 mg/day limit for EGCG consumed in supplemental form (Dekant et al., 2017). Although both reviews reached similar conclusions, our review included a PCA analysis and statistical correlation between reported green tea preparation composition and identified NOAELs. The results of our analysis provide additional evidence on the impact of GTE composition and conditions of use on toxicity.

The present analysis has some inherent limitations. Most notably for human intervention studies was the lack of consistent monitoring and reporting of safety-related endpoints and chemical composition of the green tea preparations. In our review, only two-thirds of clinical studies clearly reported in the publication that AEs or safety-related endpoints were monitored. In the studies which monitored and reported safety related outcomes, results were typically reported in terms of subject's self-reported AEs or as reasons for dropouts. Only a minority of studies in our analysis systematically monitored safety through measurements of hematology and clinical chemistry parameters. Many studies did not specify the onset of AEs or if there was resolution upon removal of the intervention. A number of studies failed to note the dose level that was associated with the observed AEs, or many did not assess the severity according to CTCAE criteria. In those cases, the lowest dose used in the study was assumed to have caused the AE, and was assigned a severity grading based on the description of the AE in the publication. Further, the causality of AEs in relation to the intervention reported in the studies was often not articulated by the authors. As noted by Dostal et al., the SAEs that occurred could be multifactorial in nature, because these subjects also experienced simultaneous infection, began use of new medication, disclosed alcohol consumption, or self-reported past medical history of liver enzyme elevations (Dostal et al., 2015). These inherent limitations in the data from human intervention studies may potentially impact the precision of the present analysis of incidence rates and severity grading. It is important to note that although most clinical studies of dietary bioactive substances are not designed or powered to evaluate safety, safety-related measures integrated and more thoroughly reported in clinical efficacy studies would be of great value to inform conclusions about their safety in target populations at efficacious levels.

Regarding chemical composition of green tea preparations, there were inconsistencies in what compounds were reported. Most, but not all, human studies reported total catechin and EGCG levels and therefore these components were included in the present analysis (Table 2). Some studies also reported EGC, ECG, EC, C and other catechins, however, not enough clinical studies reported levels of these compounds to enable meaningful PCA and HCA analyses. In some studies, the specific dose of the test material was based on the subject's body weight or surface area and thus an average dose was estimated based on 60 kg as the weight of an average person. Some publications did not provide details of conditions of use, such as dosing regimen or if the product was consumed in a fed or fasted state. There was also wide heterogeneity in study design, duration, population and outcome measures. These limitations preclude firm conclusions regarding the specific green tea preparation, constituent(s) and/or conditions of use that may be responsible for the observed effects. Nonetheless, overall the published human intervention data were directionally consistent with the animal toxicology data. Lastly, our literature search was limited to the PubMed, ToxNet, the NTP website and CEBS databases. It is possible there were relevant published studies from other databases that may be missing from this review. However, considering the consistency in the totality of the body of evidence between animal toxicology data and human clinical data, any missing studies from present systematic review would unlikely change the overall conclusion.

Our review identified a few gaps in research examining the association between oral exposure to green tea catechins and potential hazards. In particular, an important gap in the evidence base is a lack of information regarding the concentrations of tea catechins in the portal vein and liver, at a minimum, in animal toxicology studies. The information if available may lead to a better understanding on how the levels at a target site compare to those observed in systemic circulation, and determine relevant catechin concentrations in *in vitro* mechanistic studies. Additionally animal and mechanistic studies are needed that employ green tea preparations with a catechin composition similar to brewed tea. The PCA analysis of test material composition revealed that the majority of toxicology studies of green tea were for GTEs with highly concentrated catechins or purified EGCG. As the most common exposure to green tea in humans is through ingestion of brewed green

tea, using relevant study material in green tea toxicological research may help improve the evidence base, and better elucidate any link between drinking brewed green tea and adverse health outcomes. Lastly, impact of chronic consumption of GTEs, particularly in conjunction with additive effects of other pathophysiological conditions and environmental exposures that may be occurring simultaneously on the liver, may merit further research. The information may help identify potential risk factors and sensitive populations, and thereby inform regulatory authorities for appropriate risk management policies.

In summary, the present systematic review revealed that green tea is safe across a wide range of intakes and preparations. Under certain circumstances, there is a consistent relationship between green tea preparation in solid dosage form, GTE concentration and constituent level consumed as bolus doses and under fasting conditions and gastrointestinal irritation and liver injury. While there are limitations due to heterogeneity in the identified studies, the findings are quite consistent between animal toxicology and human intervention data. The results of the present analysis, combined with the previously published report from Dekant et al. (2017) suggest that the composition of green tea preparations that most closely reflects that of a traditional infusion is safe. Preparations based on concentrated extracts, containing high levels of individual constituents, such as EGCG, and consumed in solid dosage form, may require health-based guidance values to assure their safe use. Considering hepatotoxicity as the critical effect, for adult individuals with normal liver function, a safe intake limit of 338 mg EGCG/day (in a fed or fasted condition) delivered in solid dosage form (derived in the present review or the previously published conservative limit of 300 mg/day) might be considered. An OSL of 704 mg/day for an EGCG equivalent dose (fed or fasted) might be considered for a green tea preparation consumed in beverage form.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.yrtph.2018.03.019.

Transparency document

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