



## Review and Perspective on the Composition and Safety of Green Tea Extracts

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### Authors' contributions

This work was carried out equally in collaboration between all authors. All authors read and approved the final manuscript.

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### ABSTRACT

The growing body of evidence regarding the putative health benefits of green tea (*Camellia sinensis*), including reduced risk of cancer and cardiovascular disease, has led to an increase in the consumption of brewed green tea and the formulation of green tea extracts (GTE) into a variety of food and beverage products and food supplements. The principal bioactive ingredients in green tea beverages and GTE are polyphenols, particularly the flavan-3-ols, which have been shown to act on antioxidant, anti-inflammatory, glucoregulatory, and cell signaling pathways. Some experimental evidence and case reports suggest the use of green tea and GTE is associated with the potential for inducing liver injury. The ability to extrapolate findings from *in vitro* and animal model studies is always limited and the available results on green tea- and GTE-induced liver injury in humans have presented clinical and regulatory challenges due to the difficulty of demonstrating a causal relationship between intake and harm. Attention to the risk for hepatotoxicity has largely been focused on GTE. Existing data are insufficient to identify the causative agent in the preparation or composition of GTE or its dose or duration of use as well as nutrigenetic, medical, and other factors that may contribute to the risk of hepatotoxicity. Responses by different government regulatory agencies regarding the safety of GTE are inconsistent with one another, including the dosage and derivation of its bioactives from aqueous versus hydro-alcoholic extracts. Restrictions on the production of GTE limit the application of innovative extraction

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technologies that may improve the efficiency, quality, and cost-effectiveness of production and minimize the presence of endogenous or contaminant heavy metals as well as mycotoxins, pesticides, and other unwanted constituents. The objective of this review is to characterize the phytochemical profile of green tea and GTE, provide an updated description of the methods that could be used to extract green tea constituents, and discuss recent considerations on the potential for harm from consumption of GTE and products containing GTE.

**Keywords:** Green tea extract; flavan-3-ols; hepatotoxicity; cancer; cardiovascular disease; extraction; regulations.

## 1. INTRODUCTION

Based on archeological evidence, the consumption of tea appears to have first occurred about 5000 years ago during the Paleolithic period, predating the legend of its discovery by the mythical Chinese emperor, Shen Nung. By the fourth century, tea was an important part of Chinese life because of its perceived value as a medicine for the treatment of a variety of ailments [1]. Today, after water, tea is the most widely consumed beverage in the world. Tea is made from leaves of *Camellia sinensis* (L.) O. Kuntze and is cultivated in more than 30 countries [2,3]. The usual amount of dry green tea leaves used to brew a cup (237 mL) is 1.8-2.5 g. The global average consumption of tea is about 0.5 kg *per capita*, though amounts (in kg *per capita*) are greater where tea drinking is common such as India (0.73), China (0.95), Japan (0.96), Ireland (1.90), United Kingdom (1.97), Turkey (2.04), and Libya (1.90) [4]. Tea is especially rich in flavan-3-ols (also referred to as catechins) but also serves as an important source of flavonols contributing over 90 and 40% of these flavonoids, respectively, to the American diet [5]. Data from the USDA Continuing Survey of Food Intake by Individuals reveals that at the 90<sup>th</sup> percentile of intake, American men and women consume 3.5 and 3.0 cups (or 830 and 711 mL) daily providing 454 and 393 mg of flavan-3-ols, respectively [6]. These data, together with reports of green tea drinkers in other countries suggest a range of intake of 500-2360 mL daily providing up to 931 mg of flavan-3-ols [7-12].

Numerous research studies suggest that regular consumption of tea is associated with an array of bioactivities, including antibacterial, antioxidant, anti-inflammatory, antiviral, glucoregulatory, and immune-stimulant actions, and putative health benefits, including a reduced risk of chronic conditions like arthritis, some forms of cancer, cardiovascular disease, dental caries, type 2 diabetes mellitus, dyslipidemia, and

neurodegenerative diseases [2,13-17]. This diversity of actions appears largely related to the flavonoid constituents of tea via mechanisms partly overlapping with those of other dietary polyphenols, including the modulation of protein kinases, growth factors and transcription factors as well as the modification of intracellular signaling by activation of membrane receptors or molecular targets within cells [15,18,19]. These actions may result directly or indirectly from the antioxidant capacity of green tea *in vivo* to reduce oxidative stress [20-22] and associated DNA damage and lipid peroxidation [23-25].

Given the wide use of tea at 4,624,625 tonnes or 2.3 trillion cups per year, even modest benefits on health could have significant implications for the promotion of public health [4]. An effort to further broaden these benefits beyond drinking tea infusions has led to the extraction of green tea polyphenols for use in functional foods and food supplements. A growing body of research on dried green tea extracts (GTE) suggests similar benefits can be derived from the use of food products containing GTE [26]. Specifications for GTE apply to its constituent polyphenols (especially (-)-epigallocatechin gallate or EGCG) as well as caffeine and L-theanine, other major bioactive components. Green tea consumption has a long, safe history of use [27-30] and green tea preparations such as essential oils, solvent-free oleoresins, and natural extractives (including distillates) are deemed Generally Recognized As Safe (GRAS) [31] for use in foods. GTE are currently formulated into foods, beverages, candy and gums, chocolates, and dietary or food supplements as well as toothpaste. However, concerns have been expressed about the potential for harm of GTE preparations consumed at high doses in supplement form, particularly an idiosyncratic hepatotoxicity [32-38]. The objective of this review is to characterize the phytochemical profile of green tea and GTE, provide an updated description of the methods used to extract green tea constituents, and

discuss recent considerations on the potential for harm from consumption of GTE and products containing GTE.

## 2. COMPOSITION OF PHYTOCHEMICALS AND HEAVY METALS IN GREEN TEA LEAVES AND GREEN TEA

On a dry weight basis, the main constituents of tea leaves are polyphenols [39], particularly flavan-3-ols (Table 1). Other constituents of the tea leaf include fiber, carbohydrates (cellulose, pectin, glucose, fructose, sucrose), proteins (enzymes constituting an important fraction), amino acids (L-theanine or 5-N-ethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, lysine), alkaloids (principally caffeine and smaller amounts of theophylline and theobromine), minerals and trace elements, pigments (chlorophyll and carotenoids), volatile compounds (aldehydes, alcohols, esters, lactones, hydrocarbons), lipids (linoleic and  $\alpha$ -linolenic acids), sterols (stigmasterol), and vitamins (vitamins A, C, E, and K as well as several B vitamins)[40-43]. The composition of tea leaves varies widely, being substantially dependent on agricultural, botanical and genetic factors – such as climate, season, growing location, horticultural practice, cultivar, age of the leaves and the plant – and post-harvest factors like the conditions and duration of storage.

The flavan-3-ols in tea leaves, green tea infusions and extracts include EGCG, (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), (+)-catechin (C), and (+)-gallocatechin (GC) [39] (Table 2). The chemical structures of these compounds are presented in (Fig. 1). Other flavonoids and phenolic acids are present at <1% of dry weight. The major flavonols in tea are glucose or rhamnose conjugates of quercetin and kaempferol [44]. Phenolic acids in tea leaves include quinic acid esters of gallic acid and caffeic acid [44]. The polyphenols are not evenly distributed in the tea plant with the leaf bud and first leaves generally containing the highest concentrations [45]. Flavan-3-ols are concentrated in mesophyll cells of the leaf structure in close proximity to epidermal cells [46].

EGCG is the most abundant flavan-3-ol in tea leaves and green tea infusions, but with its concentration varying about 8-fold between teas and tea preparations [47-54] (Table 2). Though

showing less variation in concentration, the next most abundant tea flavan-3-ols are EGC and ECG. In green tea infusions, only EGCG and EC are present consistently. While the content of EGC and ECG in green tea is lower than EGCG, their values in the infusion appear disproportionately larger. The range of caffeine concentrations in green tea infusions is comparable to that of EGCG, suggesting that flavanols and alkaloids leach out the tea matrix in different degrees during steeping or brewing. These variations can be attributed not only to intrinsic factors but also to the methods employed in extraction of flavonoids from tea leaves and green tea for quantification.

Tea trees accumulate significant amounts of trace metals in their leaves [55]. While some of these minerals are essential for human health, some might be associated with toxicity when consumed in large amounts or for long durations if the leaves are consumed directly or dissolved in the infusion. Among the metals and elements listed in the (Table 3) [55], aluminum, arsenic, cadmium, fluoride, and lead can induce acute or chronic toxicity if sufficient exposure is achieved. However, while the amount of these metals in fresh tea leaves and green tea can exceed recommended safe levels for these elements, they are not fully extracted by aqueous or alcoholic solvent, and are present in much lower amounts in infusions [55]. The level of arsenic in green tea infusions is 1000-fold larger than the level generally found in U.S. drinking water (2.0  $\mu\text{g/L}$ , although levels of 0.138 to 1,700  $\mu\text{g/L}$  have been measured in surface water in the U.S.) [56]. As the minimal risk level (MRL) for acute-duration oral exposure ( $\leq 14$  d) to inorganic arsenic is 5  $\mu\text{g/kg bw/d}$ , daily consumption of 230 mL green tea infusion containing 1.53  $\mu\text{g/L}$  arsenic (the highest identified arsenic content [55]) for 14 d would not attain the MRL for a 70 kg adult. The U.S. Environmental Protection Agency (EPA) requires water suppliers to limit the cadmium concentration in water to <5  $\mu\text{g/L}$ , which is 7- to 47-fold larger than cadmium concentrations reported in green tea infusions. The content of lead in green tea infusions is comparable to levels in surface water and groundwater in the US at 0.005 and 0.03  $\mu\text{g/mL}$ , respectively [57]. Most drinking water supplies in the U.S. contain <5  $\mu\text{g/L}$  of chromium [58]. Green tea infusions may become a significant source of chromium among those who drink a substantial volume daily; the Adequate Intake for chromium established by the Institute of Medicine is 25 and 35  $\mu\text{g/d}$  for women and men, respectively. The

majority of chromium in tea is the less-toxic trivalent form, whereas water-soluble hexavalent chromium ranges from 0-10% of total chromium from green tea leaves, depending on its origin; the highest amount of hexavalent chromium found in a cup of green is ~70-fold lower than the maximum acceptable concentration [59]. Importantly, the level of metals and elements in GTE can be readily identified and controlled by parameters in its specification.

**Table 1. Approximate composition of tea leaves<sup>1</sup>**

Constituents	% dry weight
Polyphenols	30
Fiber	26
Protein	15-20
Carbohydrate	5-7
Minerals	4-9
Alkaloids (caffeine)	2-4
Amino acids	1-4
Pigments	Trace
Lipids and sterols	Trace
Volatile compounds	Trace
Vitamin	Trace

<sup>1</sup>Chaturvedula and Prakash [39]

**Table 2. Range of flavonoids and alkaloid contents in tea leaves, green tea, and green tea infusion<sup>1,2</sup>**

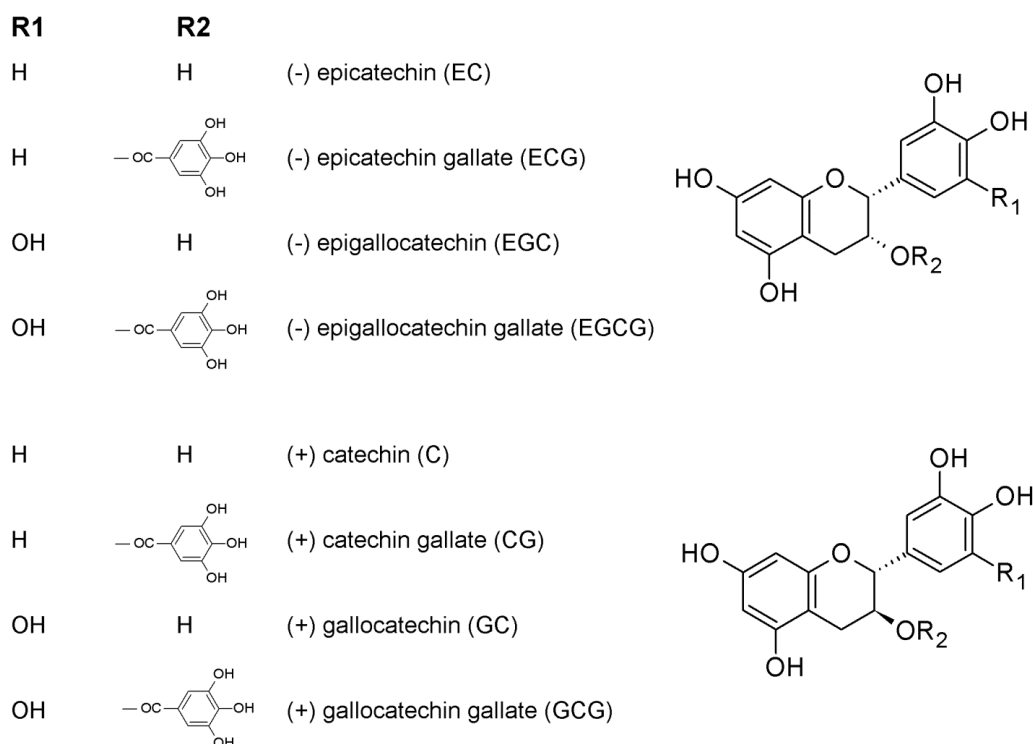
Constituents	Fresh tea leaves	Green tea leaves <sup>3</sup>	Green tea in fusions <sup>4</sup>
	mg/g dry weight		mg/L
<b>Flavonoids</b>			
(-)-EGCG	68 - 134	13 - 113	95 - 712
(-)-EGC	22 - 53	ND - 45	ND - 471
(-)-ECG	5.9 - 18	5.7 - 51	ND - 1508
(-)-EC	1.9 - 11	1.9 - 21.1	25 - 216
(+)-GCG	ND - 2.5	ND - 7.1	ND - 9.3
(+)-CG	ND	ND - 3.7	
(+)-C	ND - 1.2	ND - 1	ND - 688
EGCmG	NR	0.3 - 1.2	NR
Quercetin-rhamnoside-galactoside	trace - 1.0	NR	NR
Quercetin-rhamnoside-glucoside	0.5 - 1.9	NR	NR
Rutin	0.6 - 2.6	NR	NR
Kaempferol	NR	NR	3.6 - 6.4
Myricetin	NR	NR	4.8 - 7.3
Quercetin	NR	NR	13.4 - 20.7
Thearubigins	NR	NR	ND - 221
<b>Alkaloids</b>			
Caffeine	16 - 28	26 - 39	99 - 338
Theobromine	0.2 - 1.6	NR	7.6 - 86

<sup>1</sup>Abbreviations: C, catechin; CG, catechin gallate; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; EGCmG, epigallocatechin 3-O-methylgallate; GCG, galocatechin gallate; ND, not detected; NR, not reported

<sup>2</sup>Compiled from Bhagwat et al. [47]; Cabrera et al. [48]; Friedman et al. [49]; Komes et al. [50]; Nishitani and Sagesaka [51]; Peterson et al. [52]; Wang et al. [53]; Xu et al. [54]

<sup>3</sup>Includes data from loose tea and tea bags

<sup>4</sup>Tea infusions made by brewing 2 g tea in 200 mL at 80°C for 3 min



**Fig. 1. Chemical structure of main flavan-3-ols in tea leaves**

**Table 3. Range of trace metal and elemental content in tea leaves, green tea, and green tea infusion<sup>1</sup>**

Trace metals	Fresh tea leaves	Green tea	Green tea infusion <sup>2</sup>
	$\mu\text{g/g}$ dry weight		$\mu\text{g/mL}$
Aluminum	13 - 11,981 (177) <sup>3</sup>	211 - 4074 (41)	0.7 - 6 (24)
Arsenic	0.021 - 0.073 (13)	trace - 1.66 (536)	$0.02 \times 10^{-3}$ - $1.53 \times 10^{-3}$ (17)
Cadmium	ND <sup>4</sup> - 1.27 (63)	0.013 - 0.114 (141)	$0.04 \times 10^{-3}$ - $0.24 \times 10^{-3}$ (17)
Chromium	trace - 3.41 (103)	0.45 - 0.99 (2)	ND - $6.91 \times 10^{-3}$ (17)
Copper	9.4 - 127 (59)	4.7 - 36.5 (169)	0.033 - 0.191 (50)
Fluoride	49 - 808 (33)	49 - 344 (14)	0.59 - 2.52 (20)
Lead	ND - 14.5 (181)	0.11 - 1.93 (141)	0.004 - 0.032 (17)
Manganese	ND - 3154 (56)	211 - 2081 (33)	0.52 - 10.09 (54)
Nickel	6.6 - 14 (43)	ND - 9.35 (28)	0.04 - 0.269 (21)

<sup>1</sup>Summarized from Karak and Bhagat [55] <sup>2</sup>The range of trace metal and other element contents in green tea infusion is due partly to the different brewing methods, including tea:water ratio, water temperature, and brewing duration; <sup>3</sup>Values in parentheses are the number of samples tested; <sup>4</sup>ND, not detected

### 3. ADVANCED EXTRACTION TECHNOLOGIES FOR PLANTS/BOTANICALS

To obtain natural products from plant materials, effective extraction techniques are required to remove unwanted constituents and concentrate bioactive compounds. Traditional techniques, such as Soxhlet extraction, have been used for many decades, while novel techniques have

been developed more recently to improve extraction efficiency and the cost-effectiveness of the process. It is worthwhile noting that pretreatment of the raw plant material, e.g., breaking, crushing, and milling, can significantly affect the kinetics of solvent extraction [60]. Extraction efficiency is related to the solvent to leaf ratio and depends substantially on polarity of the constituent compounds and the type of solvent. For example, acetone, ethyl acetate, acetonitrile, isopropanol, ethanol, methanol, and

water are preferred for extraction of the more amphipathic compounds, such as polyphenols and their glycosides and hexane, petroleum ether, benzene, and chloroform are utilized to maximize extraction of lipophilic constituents, such as fats and essential oils [61]. Extraction solvents can be chosen based on solvent selectivity, capacity, reactivity, stability, solubility, regeneration capability, and toxicity [62]. Several additional novel extraction technologies have emerged that may be useful for the extraction of green tea constituents. For example, ionic liquids have been used for extraction of the stilbene resveratrol from the dried rhizome of *Polygonum cuspidatum* [63]. A surfactant and micelle approach has been used to extract flavonoids from licorice root [64]. Supercritical CO<sub>2</sub> or dimethyl ether with solvent modifiers can be used to decaffeinate tea and extract flavan-3-ols [65]. This section provides a brief overview of novel extraction methods suitable for consideration of plant polyphenols.

### 3.1 Accelerated Solvent Extraction (ASE)

ASE is also known as pressurized solvent extraction, subcritical solvent extraction or pressurized liquid extraction [66]. ASE is an automated process and widely used in the extraction of functional components from botanicals and plants [67-71]. The high temperature and pressure applied in this protocol not only facilitate extraction efficiency but also reduce solvent use and extraction time by increasing the solubility of target compounds and the rate of solvent diffusion into the plant matrix as well as decreasing solvent viscosity and surface tension [69,72]. Extraction efficiency for thermally labile components can decrease when temperatures are set too high, resulting in a lower quality extract [71]. Since ASE is a relatively fast process, prolonged extraction time does not raise the yield [70-74]. Importantly, ASE often allows for the use of environmentally safe solvents, such as water and aqueous ethanol to achieve more exhaustive extractions of plant materials relative to use of these same solvents under normal extraction conditions. In general, smaller particle sizes of the plant material combined with larger volumes of solvent can increase the amount of functional components dissolved, resulting in greater extraction efficiency [73,75,76].

### 3.2 Supercritical Fluid Extraction (SFE)

Supercritical fluid is a phase between gas and liquid, and its temperature and pressure are

above the critical point. Its density is similar to liquid but its viscosity is similar to gas, resulting in a higher diffusion coefficient than liquid. Due to its high dissolving capacity for many compounds, including phytochemicals, supercritical fluid can be used as an efficient extraction solvent. The principal SFE solvent is CO<sub>2</sub> due to its relatively low critical conditions (30.9°C and 73.8 bar), profile of safe use, and relatively low cost. Thus, SFE with CO<sub>2</sub> presents a good method for the extraction of thermally labile phytochemicals [77,78]. Supercritical CO<sub>2</sub> has a very high diffusion coefficient, leading to a much faster extraction rate than conventional solvent extraction [79]. Due to its non-polarity, CO<sub>2</sub> may not be an efficient solvent for extraction of polar bioactives such as phenolic compounds. However, the efficiency of SFE for polar compounds is significantly increased when combined with organic solvent modifiers like ethanol [80,81]. While extracted phytochemicals can be easily recovered by decreasing the pressure of the supercritical CO<sub>2</sub> [78], the high cost and sophisticated operation conditions limit the broad utilization of SFE. However, it is noteworthy that SFE is the method of choice for the commercial production of quality decaffeinated tea.

### 3.3 Ultrasound-Assisted Extraction (UAE)

Ultrasound waves can change the physical and chemical properties of plant material and induce cavitations causing an increase in pressure and temperature close to the plant surface, disrupting cell walls and enhancing the release of intracellular compounds into the extraction solvent [82,83]. UAE can also cause swelling of the plant material that can facilitate the extraction of phytochemicals. UAE is a simple and cost-effective method which has been shown to improve the extraction of polyphenols, though extraction efficiency is dependent on the nature of the plant matrix [66,72]. UAE is also suitable for thermally labile compounds because satisfactory extraction can be achieved at relatively low temperature [72].

### 3.4 Enzyme-Assisted Extraction (EAE)

The principle of EAE is use of enzymes, such as cellulases, pectinases and hemicellulase, to break the plant cell wall to facilitate the extraction of targeted constituents from plant matrices [84]. EAE has been used for extraction of proteins, phenolics, oils, and carotenoids [85-89]. Although EAE may offer better extraction efficiency in

comparison with conventional extraction methods, this method extends considerably the time for completion of conventional extraction protocols and may not be better than other novel techniques using pre-extraction treatment, e.g., UAE [89,90]. During EAE, specific enzymes are needed for different raw materials, which require the information on the exact composition of the cell walls. In one study, UAE pretreatment led to a better and faster extraction of phenolic compounds from acerola than an EAE-added protocol [90]. Additional factors that limit the application of EAE to green tea include the relatively high cost of enzymes and environmental sensitivity regarding the use of these enzymes.

### 3.5 Pulsed Electric Field (PEF) Assisted Extraction

PEF assisted extraction uses an electrical field to induce electroporation of cell membranes which results in an increased permeability of extraction solvent into the plant matrix and extraction efficiency [66,91,92]. PEF treatment improves the extraction of phytochemicals, including polyphenols like anthocyanins and tannins [91]. Compared to other pre-treatments such as UAE, PEF assisted extraction requires less time and lower energy costs [93]. PEF has been used as a pre-treatment before pressing to enhance the yield and quality (turbidity and odor intensity) of juices from different fruits and vegetables [94,95]. PEF may yield comparable or better extraction efficiency than UAE and requires less time (seconds vs. minutes) and energy than UAE [91,96].

### 3.6 Microwave Assisted Extraction (MAE)

As electromagnetic radiation, microwaves can penetrate plant materials and interact with polar molecules such as water to generate heat [72]. The heat generated internally in the plant materials disrupts cell structure and facilitates the dissolution of phytochemicals from the plant matrices [95]. Moreover, homogeneously increased temperature of extraction solvent and plant materials by MAE generally leads to enhanced extraction efficacy. MAE also reduces extraction time in comparison to conventional extraction methods [97-99]. However, in certain cases, unwanted products can be generated during MAE, especially when the temperature is too high [100]. The major advantage of MAE is

that it offers significant reductions in extraction time and solvent usage with a similar or even better extraction efficacy in comparison to the conventional extraction methods [66].

## 4. TECHNOLOGIES FOR GREEN TEA EXTRACTION

On a commercial scale, extractions of green tea for beverages or food supplements are typically conducted using aqueous, ethanolic or supercritical fluid solvents with either batch or continuous processes [101]. A variety of laboratory-scale methods for GTE preparation have been reported and demonstrate that various solvents, temperatures, and other process variables contribute to a wide range of constituents in the final product. Market studies of commercial GTE have reported a marked variation in the content of EGCG (11.9-fold), polyphenols (2.6-fold), and caffeine (6.5-fold) [102-104] (Table 4). It is noteworthy that the variation in the phytochemical content of GTE in these studies is equivalent or less than that reported in surveys of commercial green teas used for infusions.

### 4.1 Continuous or Batch Aqueous Extraction

A single aqueous batch extraction for GTE most closely mimics the typical home preparation of green tea. Phytochemical yields have been reported for both batch and continuous extraction processes, including reverse flow continuous extraction. For batch processes, time, temperature, and tea:water ratios have been manipulated in laboratory studies. Total flavan-3-ol content plateaus from 30 to 120 min during aqueous batch extractions of green tea [50,105]. Vuong et al. [105] reported that EC content plateaued at 30 min, but catechin content continued to increase to 120 min in a batch aqueous process. Similarly, L-theanine aqueous batch extraction was maximized at 80°C for 30 min [106]. Aqueous shaking extraction of flavan-3-ols from dry green tea leaves at 25°C peaked at 2 h, but had only minor differences from 0.5 to 24 h with a maximum 8% difference between 2 and 6 h [107]. Reflux extraction of green tea leaves beyond 30 min up to 3 h at 80°C did not yield significantly more flavan-3-ols or caffeine [107]. Longer aqueous extraction resulted in lower caffeine:flavan-3-ol ratios [108].

**Table 4. Fold-variation in phytochemical content of GTE and commercial green tea samples on a weight/weight basis**

GTE constituent	GTE dietary supplements, fold variation (n=10) <sup>1</sup>	Commercial green teas, fold variation (n=23) <sup>2</sup>
Caffeine	6.5	79.7
EGCG	11.9	24.1
EGC	18	142
ECG	16.9	22.7
EC	7	26
Theophylline	- <sup>3</sup>	47.5
Total Polyphenols	2.6	-

<sup>1</sup>Seeram et al. [103] <sup>2</sup>Friedman et al. [104] <sup>3</sup>not analyzed

The water:tea ratio also impacts the yield of flavan-3-ols and L-theanine. L-theanine yield is maximized at water:tea ratios >20:1 and may introduce differences of ≥25% [106]. In contrast, water:tea ratios of ≥50:1 yield a maximum content of flavan-3-ols, 80% greater than 10:1 ratios [105].

A continuous or semi-continuous aqueous extraction of green tea leaves increases polyphenol yields beyond that obtained with batch extraction. Following 3 min steeping of green tea leaves, re-extraction slightly increased the proportion of flavan-3-ols, as a third 3 min extraction had 33, 22, and 15% of flavan-3-ols, phenolic acids, and methylxanthines, respectively, of the first extract [50]. Similarly, successive aqueous extraction yielded 11% more caffeine and theobromine at the third extract of loose green tea leaves relative to the first [109]. Flavan-3-ols had a cumulative 91% extraction efficiency compared to an 80% efficiency for caffeine at 95°C following four sequential 10 min extractions at 95°C [110]. This result is significantly greater than the 65% and 59% yield of flavan-3-ols and caffeine, respectively, after the first 10 min of steeping [110]. While fluorosis from tea consumption is extremely rare, a semi-continuous extraction process can be utilized to reduce fluorine content of GTE. Discarding the initial 20 s infusion of green tea at 50°C and then extracting in boiling water for 5 min reduced the fluorine content by 26% [111]. Successive 20 min boiling aqueous extractions of green tea yielded ~30% less aluminum after each additional extract [112].

Since the flavan-3-ol content peaks at 0.5 to 2 h in batch extractions, increasing yields of semi-continuous extraction are expected. Although not compared in the reports discussed above, re-extraction likely provides an advantage of

increasing rates of diffusion of solutes from leaves to water. Ultimately, time and temperature of aqueous extractions can be manipulated in either continuous or batch processes to change the yield of the constituent phytochemicals and minerals.

#### 4.2 Extraction Temperature

Extraction temperature plays an important role in the yields of flavan-3-ols from green tea with efficiency varying dependent on the individual compound [113]. Perva-Uzunalić [110] obtained maximum aqueous extraction efficiency of flavan-3-ols after 20 min at 80°C and 10 min at 95°C, suggesting an interactive effect of temperature and time on extraction efficiency. Response surface methodology, which is generally employed to generate an optimal response by conducting a sequence of designated experiments, has been used to characterize the yield of bioactive components of GTE. Zhang et al. [114] reported that steeping 1 g tea powder in 16 mL water for 40 min at 96°C extracted the most EGCG, theanine, and total phenols. A potential degradation of flavan-3-ols may occur when a protocol includes both high temperature and extended extraction. For example, aqueous extraction of green tea for 7 h at 98°C reduced the flavan-3-ol content of GTE by 20% [115]. Increasing temperature from 80 to 95°C augmented epimerization of EGCG to (-) gallic catechin gallate (GCG) by 52% from 0.25 g/100 g green tea leaves [115]. The caffeine content of aqueous extracts reaches a plateau at 80°C from 20-40 min extraction, and then decreases slightly at 90°C [113]. Thus, temperature employed during extraction not only affects extraction efficiency of flavan-3-ols but can also contribute to an altered flavonoid profile through epimerization.



### 4.3 Extraction Solvent

Laboratory-scale preparations of GTE have utilized a variety of solvents with various effects on extract yields [107,110,116] (Table 5). Friedman et al. [104] compared an 80% ethanol extraction to an aqueous extraction of 24 green teas and found that aqueous alcohol extraction yielded 36% more flavan-3-ols, 12% more caffeine, ~50% more theobromine, and 30-200% more theophylline than water alone. As shown in (Table 5), the percentage of ethanol in the extraction solvent is crucial to yield of flavan-3-ols, but not necessarily caffeine, with 40% ethanol in water being the best as compared to all other aqueous ethanol solvents and methanol, acetonitrile, and acetone [107,110,116]. Similarly, Rusak et al. [117] noted the yield of flavan-3-ols from loose green tea extracted for 30 min was highest with 40% ethanol compared to 10 and 70% ethanol, and water. The 40% ethanol extract recovered 18% EGC, 74% EGCG, 40% GCG, and 97% ECG more than water extraction. Thus, aqueous ethanol provides a larger ratio of flavan-3-ols to caffeine than other extraction solvents.

In contrast, the flavonol and proanthocyanin yield from green tea leaves appear only marginally affected by ethanol or aqueous ethanol extraction [110]. However, acetonitrile and acetone extraction provides 33 to 83% flavonols and proanthocyanidins compared to water extraction. Recovery of caffeine and theobromine was greatest with 70 and 15% methanol in water, respectively, following three 10-min sequential extractions of green tea leaves [118]. Theophylline was only recovered in the 70% methanol extract, but not in methanol alone, water, acetonitrile or acetone [118].

Less information is available about how solvents affect other green tea constituents. Mossion et al. [119] reported that mineral content of water

decreased extraction of aluminum, total organic carbon, and total polyphenols. Kanrar et al. [120] demonstrated an ethyl acetate-cyclohexane mixture (9:1, v/v) was effective at extracting pesticide residues from green tea. Pollution associated with industrialization near tea cultivation areas can increase the presence of heavy metals, particularly lead, in raw tea leaves. Different solvent extractions vary in their capacity for extracting lead from tea plants [121,122]. Sullivan et al. [123] have noted that while hydroethanolic solvents are not completely efficient in the extraction of heavy metal accumulations, they can be an effective decontamination step in herbal product processing.

### 4.4 pH

The pH of aqueous extraction solvents varies from ~5.5 to 7.2 based on temperature and the quantity of green tea leaves utilized for extraction [124]. Yoshida et al. [124] reported that the aqueous pH was nearly constant at ~6.5 when the ratio of green tea leaves to water was between 0.5 and 3%. Stability of tea flavan-3-ols decreases with increasing pH above pH 3, at which they are quite stable [107]. Thus, the pH of extraction solvent strongly determines extraction efficiency of these flavonoids from green tea leaves. The effect of pH varies during extraction, with a pH of 4.5 providing a maximum recovery of gallic acid, EGC, and EGCG, whereas ECG and EC are stable up to pH 6.5 [102]. Extraction at pH 7 results in greater proportions of EC and ECG to other flavan-3-ols [102]. GTE extraction held at 80°C for 20 min at pH 8, led to twice the epimerization of EGC to GC compared to pH 6 [124]. The mineral content of GTE is also affected by the acidity of extraction solvents. Water extraction with 0.12 µmol/L HCl provides a yield with 44% aluminum, 19% calcium, and 44% magnesium of the original amount in the green tea leaves [112].

**Table 5. Effect of extraction solvent on green tea extract phytochemical yield, as fold-difference of water extract<sup>1</sup>**

Phytochemical	Aqueous ethanol				Methanol	Acetonitrile	Acetone
	20%	40%	50%	80%			
EGCG	2.76	3.32	3.26	1.92	1.3-3.2	-	-
Catechins	1.46	1.61	1.46	0.79 - 1.15	1.17	1.24	0.63
Flavonols	<sup>2</sup>	-	0.91	1.02	0.97	0.39	0.33
Proanthocyanidins	-	-	0.97	0.96	0.91	0.83	0.83
Caffeine	1.03	1.03	0.97	-	-	-	-

<sup>1</sup>Compiled results from Perva-Uzunalić et al. [110]; Meterc et al. [116]; Chung and Lee [107].

<sup>2</sup>not determined

#### 4.5 Other Extraction Technologies

Other extraction technologies for preparing GTE may modulate the yield of flavan-3-ols. In a semi-continuous SFE using CO<sub>2</sub>, Sosa et al. [125] tested whether encapsulation with a poly-ε-caprolactone, a biodegradable particle, applied during the solvent removal step would affect the constituent profile of the final GTE. They found that total phenols varied 60-100% of the maximum theoretical composition, but caffeine content was reduced to 13%. Ultrasonic extraction of green tea leaves for 1 h had 5% less flavan-3-ols, mainly as EGC and ECG, and the same caffeine content relative to a 2 h aqueous extraction [107]. Conventional hot-water steeping of green tea yielded 55% magnesium, 260% calcium, and a similar percentage of aluminum compared to focused microwave oven heating [112]. Ionic liquids have also been used to extract caffeine and theophylline from commercial tea drinks [126].

#### 4.6 Procedures for Solvent Removal

GTE is dried prior to distribution. Spray drying, drum drying, or lyophilization can be used to process GTE. Excessive heat during a high pressure spray drying process of 145°C pre-expansion temperature and 79°C resulted in 88% less polyphenols relative to optimized conditions at ≤130°C [116]. Temperatures during spray drying between 33 and 65°C did not affect the amount of total polyphenols but the residual water content increased with lower temperatures, a condition which is unacceptable for commercial applications.

### 5. POSTPRODUCTION HANDLING OF GREEN TEA

#### 5.1 Grinding

The size of the dry tea leaf particles also affects the yield of extracted compounds as smaller particle sizes increase surface area available for extraction. Aqueous extraction of green tea leaves with a particle size ≤1 mm have ~10% greater yields of flavan-3-ols [105]. Gains in fluoride yields with smaller particle sizes relative to flavonoids may be exploited to limit fluoride in GTE [111]. Caffeine and total flavonoids, but not EGCG, are dependent on particle size for supercritical CO<sub>2</sub> decaffeination [65].

#### 5.2 Storage

The duration that green tea leaves are stored before use may affect GTE composition.

Friedman et al. [104] found that after 6 mo storage at 20°C, EGCG concentration in green tea leaves decreased by 28% and total flavan-3-ols decreased by 32%. Storing tea leaves under vacuum or nitrogen and/or low humidity and temperature can minimize flavonoid oxidation.

#### 5.3 Decaffeination

Green tea may be decaffeinated prior to extraction. At optimal conditions for supercritical CO<sub>2</sub> decaffeination of ground green tea leaves, 99.6% caffeine, 40.5% flavan-3-ols, and 43.1% chlorophyll are extracted using ethanol as a co-solvent [127]. Ethanol is superior to water in this regard, and a 50% aqueous ethanol modifier for supercritical CO<sub>2</sub> decaffeination resulted in 70% caffeine and 6% flavan-3-ols being removed from green tea powder [65]. Perva-Uzunalic et al. [110] noted that supercritical CO<sub>2</sub> reduces flavan-3-ols by 17% and flavonols by 10% from the starting material. Up to 43% of chlorophylls are removed after 120 min of supercritical CO<sub>2</sub> decaffeination with an ethanol co-solvent [128]. While the loss of polyphenols can be minimized during the decaffeination process, commercial decaffeinated green teas generally have a lower content of flavan-3-ols than non-decaffeinated green tea [102].

### 6. POTENTIAL HEALTH BENEFITS OF GREEN TEA INFUSIONS AND EXTRACTS

Before discussing the reported potential for adverse effects of GTE, it is worth briefly noting the potential health benefits associated with green tea and GTE. A wealth of basic research as well as evidence from observational studies and randomized clinical trials indicate the consumption of green tea infusions or GTE to be associated with an improvement in intermediary biomarkers of chronic disease and/or a reduced risk of some forms of cancer, cardiovascular disease, type 2 diabetes mellitus, neurodegenerative conditions like Alzheimer's disease and Parkinson's disease, and other age-associated diseases. These putative benefits appear due principally to the array of bioactivity of the flavonoid constituents of green tea, particularly the flavan-3-ols, including antioxidation, anti-inflammation, glucoregulation, anti-proliferation, energy regulation, and modulation of signal transduction pathways [16,129-135]. A brief summary of selected systematic reviews and meta-analyses of the association between green tea and health outcomes are presented in (Tables 6 and 7). In

addition, green tea infusions and GTE have a modest effect on weight loss and/or weight management, though this benefit appears due principally to an interaction between the constituent flavonoids and caffeine. Randomized clinical trials indicate green tea flavonoids have a positive impact on blood glucose, suggesting a potential benefit to people with impaired gluco-regulation and an increased risk of type 2 diabetes. Evidence from clinical studies also indicates a modest effect of green tea in decreasing serum total cholesterol and LDL-cholesterol [155]. Data from observational studies showing green tea is associated with a reduced risk of stroke is consistent with results from clinical trials. While rodent model studies reveal a marked chemopreventive effect of green tea on several forms of cancer, results from observational studies and from clinical trials examining intermediary biomarkers are mixed and dependent on cancer type, sex, and study design.

## 7. SAFETY PROFILE OF GREEN TEA EXTRACTS

Green tea consumption has a long, safe history of use as a beverage and green tea preparations are deemed GRAS for use in foods; however, concerns about the potential for hepatic toxicity of GTE prepared by aqueous solvent extractions, particularly hydro-alcoholic extracts, have been raised. As discussed above, the polyphenol profile of alcohol extractions of green tea leaves appears to affect overall GTE composition, and the extraction solvent causes a wider effect on more than processing steps or raw materials. Based on laboratory analysis of 17 to 54 samples of quality grade commercial green tea infusions (Table 3), aqueous and hydro-alcoholic extracts of green tea are expected meet guidelines for the suggested safe levels of metals in herbals, food ingredients, and dietary supplements (Table 8). For example, the aluminum content reported in green tea infusions (0.7 - 6 mg/mL) is ~33-fold less than CODEX standards. Depending on the location that a tea is grown, the content of arsenic, cadmium, copper, and lead in dry tea may exceed recommendations for exposure to these elements, but are not fully extracted by aqueous or alcoholic extraction, and are present in much lower amounts in infusions [55]. Reports on the heavy metal content of commercial or laboratory-prepared GTE are lacking in the literature, however, commercial products are commonly controlled for contaminants inclusive of heavy metals through established quality

specifications in accordance with applicable current Good Manufacturing Practices (cGMP). Upon drying, residual solvents are not expected to be at concentrations above toxic threshold due to the drying process (Table 9). While tea leaves and infusions are unlikely to have residue solvents as their processing typically does not include water or organic solvents, data from these sources and GTE are not readily available from literature. While decaffeinating tea with methylene chloride may leave residual traces, it is excluded from import into the United States and some other countries. In contrast, ethanol is regarded as a solvent with low toxic potential. There is no evidence that hydro-alcoholic processes extract significantly different proportions of bioactive or toxic compounds from green tea that would lead to toxicity. However, components of GTE other than polyphenols and caffeine are rarely reported in animal experiments or case studies of toxicity, so data on these constituents of GTE are quite limited.

Green tea flavonoids consumed in a phospholipid complex are better absorbed than in a free form but do not appear to be associated with toxicity [160]. Nanoparticles and other technologies for enhancing the bioavailability and/or distribution of tea flavonoids have been tested in animal models without evidence of harm but remain untested in humans. In humans, green tea flavan-3-ols appear similarly bioavailable from infusions and GTE. Importantly, foods co-consumed with tea may impair maximum plasma flavonoid status and the time required to achieve maximal concentrations [161]; such actions would likely reduce any potential toxicity associated with tea polyphenols [162]. Indeed, consumption of GTE in a fed state resulted in lower and less variable toxicity than found under fasted conditions in dogs fed GTE at 50 to 1000 mg/kg body weight (bw)/d [163,164]. Further, clinical toxicokinetic studies by Ullmann et al. [165,166] confirmed that GTE under fasting conditions or following a single bolus led to more marked increases in bioavailability and plasma concentrations of EGCG than administration with split doses or with food. Rarely, moderate consumption of aqueous green tea infusions, aqueous GTE, and hydro-ethanolic GTE have been associated with liver injury in humans but confounding factors and effect modifiers make it impossible to assign a direct causal relationship to individual or combined tea flavonoids or related polyphenols [167]. Evidence regarding toxicity from tea and tea polyphenols is described below.

**Table 6. Meta-analyses and systematic reviews of randomized clinical trials on the health benefits of green tea or green tea catechins in anthropometry, glucoregulation, lipid profile, blood pressure, and coronary heart disease risk<sup>1</sup>**

Authors	Number of study (n)	Subject (n)	Duration	Dosage in RCT	Objectives	Findings
<b>Anthropometry</b>						
Jurgens et al. [132]	RCT (14)	1562	12-13 wk	140.85-1206.9 mg GTC/d	BW in overweight or obese adults	BW: ↔ outside Japan; ↓ in Japan. WC: ↔
Hursel et al. [136]	RCT (11)	1188	12-13 wk	270-1207 mg GTC/d	BW	BW: ↓ Effect modification by caffeine and ethnicity
Phung et al. [137]	RCT (15)	1243	8-24 wk	141-1207 mg GTC/d	BMI, BW, WC, WHR	GTC with caffeine BMI: ↓ BW: ↓ WC: ↓ WHR: ↔  GTC alone: ↔
<b>Glucoregulation</b>						
Zheng et al. [138]	RCT (22)	1548	3-24 wk	236-1207 mg GTC/d	Glucoregulation	FG: ↓ FI: ↔ HbA1C: ↔ HOMA: ↔
Liu et al. [139]	RCT (17)	1133	4-14 wk	208-1207 mg GTC/d	Glucoregulation	FG: ↓ FI: ↓ HbA1C: ↓
<b>Lipid profile</b>						
Kim et al. [133]	RCT (20)	1415	3-24 wk	145-3000 mg GTC/d	Lipid profile	TC: ↓ LDL-C: ↓ HDL-C: ↔ TG: ↔
Zheng et al. [140]	RCT (14)	1136	3-24 wk	150-2500 GTC mg/d	Lipid profile	TC: ↓ LDL-C: ↓ HDL-C: ↔ TG: ↔
<b>Coronary heart disease</b>						
Hooper et al. [141]	RCT (170) [12 green tea]	6557 (515 green tea)	0-52 wk (0-12 wk for green tea)	Not available	CHD risk	LDL-C: ↓ BP: ↔
Taubert et al. [142]	RCT (10) [1 green tea]	516 (343 green tea)	2 mo	1 tea bag/d, 544 mg phenols	BP	BP: ↔

<sup>1</sup>Abbreviations: BMI, body mass index; BP, blood pressure; BW, body weight; CHD, coronary heart disease; FG, fasting blood glucose; FI, fasting insulin; GTC, green tea catechins; HbA1C, glycated hemoglobin; HDL-C, high density lipoprotein cholesterol; HOMA, homeostatic model assessment; LDL-C, low density lipoprotein cholesterol; RCT, randomized clinical trial; TC, total cholesterol; TG, triglyceride; WC, waist circumference; WHR, waist and hip ratio; ↓, decrease; ↔, no change

## 7.1 Evidence from Cell Studies

Primary hepatocytes from adult male Wistar rats were used to investigate the potential toxicity of hydro-ethanolic GTE constituents [168]. Hepatocellular necrosis was observed at doses of 1 to 3 mg GTE/mL medium. However,

individual flavan-3-ols, caffeine, and theanine were not cytotoxic up to 3 mg/mL medium except for the observation that EGCG reduced cell viability at the 1 mg/mL dose only. The lipid-soluble fraction did not appear to account for increased cytotoxicity. With an LD<sub>50</sub> of 200 μM, Galati et al. [169] reported that EGCG was the

most potent of green tea phenolics in inducing mitochondrial membrane potential collapse in isolated rat hepatocytes. In contrast, Nishikawa et al. [170] found a benefit of EGCG at 50-100  $\mu\text{g/mL}$  in stimulating apoptosis of human hepatocellular carcinoma cell lines. However, the

direct relevance and ability to extrapolate these findings is limited as only sub-micromolar concentrations are observed *in vivo* following purified flavan-3-ol and flavonol administration to rodents and humans [171].

**Table 7. Meta-analyses and systematic reviews of observational studies on the health benefits of green tea or green tea catechins in coronary heart disease, stroke, and cancer risk<sup>1</sup>**

Authors	Number of study (n)	Subject (n)	Duration	Objectives	Findings <sup>2</sup>
<b>Coronary heart disease</b>					
Wang et al. [143]	Cohort (12), Case-control (6) [5 green tea]	416,676 (13,978 cases)	5-20 y follow up	CAD (CHD and MI)	RR=0.72 (0.58-0.89) 1 cup/d: ↓ 10% CAD risk
<b>Stroke</b>					
Arab et al. [144]	Cohort (8), Case-control (2), Cross-sectional (1)	213,897 (5,537 cases)	4-15 y	Total stroke	RR=0.79 (0.73-0.85, $\geq 3$ vs. $< 1$ cup/d)
<b>Cancer</b>					
Zheng et al. [145]	Case-control (20), Cohort (4)	7,376 cases, 487,894 control in case-control; 8,874,734 person-y in cohort	NA <sup>3</sup>	Esophageal cancer	Cohort: RR=0.77 (0.57-1.04, highest vs. non/lowest) Case-control: RR=0.70 (0.51-0.96)
Zheng et al. [146]	Case-control (8), Cohort (2)	3,731 (3,557 cases)	0-9 y	Esophageal cancer	Overall: $\leftrightarrow$ Case control: RR=0.32 (0.10-0.54, high), 0.43 (0.21-0.66, medium), 0.45 (0.10-0.79, low) vs. non-drinker in female only
Kang et al. [147]	Cohort (7), Case-control (11)	223,044 (6814 cases)	NA	Stomach cancer	Overall: RR=0.86 (0.74-1.00) subgroup analysis (6 studies): RR=0.68 (0.53-0.87, $\geq 5$ cup/d vs. lowest)
Myung et al. [148]	Case-control (8), Cohort (5)	254,869 (6,636 cases)	NA	Stomach cancer	RR=1.10 (0.92-1.32, highest vs. lowest consumption)
Wang et al. [149]	Cohort (6)	352,275 (1,675 cases)	NA	Colorectal cancer	Overall RR=0.90 (0.72-1.08) Shanghai: RR=0.70 (0.55-0.85, highest vs. lowest) Singapore: RR=1.36 (1.06-1.74)
Tang et al. [150]	Cohort (5), Case-control (7)	106,069 (5,495 cases)	NA	Lung cancer	RR=0.78 (0.61-1.00) in highest intake

**Table 7 Continued.....**

Ogunleye et al. [151]	Case-control (9)	164,943 (5,617 cases)	NA	Breast cancer	RR=0.81, (0.75-0.88, >4 vs. ≤1 cup/d)
Tang et al. [152]	Cohort (2), Case-control (5)	107,764 (3,487 cases)	NA	Endometrial cancer	Overall: RR=0.85 (0.77-0.94) Cohort: RR=1.05 (0.85-1.28) Case control: RR=0.81 (0.71-0.93)
Zheng et al. [153]	Cohort (6), Case-control (7)	111,499 (3,608 cases)	NA	Prostate cancer	Overall: RR=0.72 (0.45-1.15) Case control: RR=0.43 (0.25-0.73) Cohort: RR=1.00 (0.66-1.53)
Nagle et al. [154]	Case-control (3)	6,092 (2,567 cases)	NA	Ovarian cancer	RR=0.58 (0.33-1.01), ≥1 cup/d vs. never/seldom

<sup>1</sup>Abbreviations: CAD; coronary artery disease; CHD, coronary heart disease; MI, myocardial infarction; RR, relative risk <sup>2</sup>Values in parenthesis is the 95% confidence interval <sup>3</sup> NA: not available

**Table 8. Established safe levels of metals in herbals, foods, and food ingredients, and as provided by dietary supplements**

Metal	Herbal products (w/w)	Foods and ingredients (w/w)	Dietary supplements (dose)
Aluminum	<sup>1</sup>	0.2 mg/mL <sup>3</sup>	-
Arsenic	-	0.1-0.5 mg/kg <sup>4</sup>	15 µg/d (inorganic) <sup>5</sup>
Cadmium	0.3 mg/kg <sup>2</sup>	0.3-400 µg/kg <sup>4</sup>	5 µg/d <sup>5</sup>
Copper	-	0.1-0.4 mg/kg <sup>4</sup>	-
Lead	10 mg/kg <sup>2</sup>	0.1-2 mg/kg <sup>4</sup>	10 µg/d <sup>5</sup>
Mercury	-	1-100 µg/kg <sup>4</sup>	15 µg/d <sup>5,6</sup>

<sup>1</sup>Abbreviations: -, no established guidelines <sup>2</sup>World Health Organization (WHO) guidelines for assessing quality of herbal medicines with reference to contaminants and residues, 2007 [156] <sup>3</sup> EPA 40 CFR part 143 [157] <sup>4</sup> Joint FAO/WHO Food Standards Programme, CODEX Committee on Contaminants in Foods, Sixth Session, Maastricht, The Netherlands. Working document for information and use in discussions related to contaminants and toxins in the GSCFTT (General Standard for Contaminants and Toxins in Foods and Feeds), March 26-30, 2012 [158] <sup>5</sup>United States Pharmacopeial Convention Chapter 2232 Elemental Contaminants in Dietary Supplements, USP 36-NF31 S1 [159] <sup>6</sup> Hg in total as methylmercury, 2 µg/d

**Table 9. Recommended limits for residual solvents in U.S. dietary supplements with relevance to GTE<sup>1</sup>**

Solvent category	Residual solvent	Recommended limits† (mg/d)
Class 2 (solvents to be limited)	acetonitrile	4.1
	chloroform	0.6
	dichloromethane	6
	hexane	2.9
	methanol	30.
Class 3 (solvents with low toxic potential)	acetic acid‡	50
	acetone	50
	ethanol‡	50
	ethyl acetate	50

<sup>1</sup>American Herbal Product Association Guidance Policies [159]. †Based on a 10 g dose; ‡ Higher levels permitted when intended as part of the product formulation

## 7.2 Evidence from Animal Models

In rodents, the No Observed Adverse Effect Level (NOAEL) with oral ingestion ranges from

65.5 to 2500 mg GTE/kg body weight (bw), providing intakes of flavan-3-ols of 120 to 3542 mg/kg bw/d and EGCG of 50 to 1500 mg/kg bw/d over periods ranging from 4 to 26 weeks

[163,172-178]. Applying an uncertainty factor of 100, Isbrucker et al. [175] suggested an acceptable daily intake (ADI) for EGCG, when adjusted to 100% purity, of 5.3 mg/kg bw, which would be approximately equivalent to 322 mg/d for the average individual. The lowest level of GTE (72% flavan-3-ols) causing liver toxicity in female F344/NTac rats was 1000 mg/kg bw/d following 14 wk of consumption *via* gavage [172]. However, at 65.5 mg/kg bw/d, nasal toxicity was observed in B6C3F1 mice, manifested by chronic inflammation of the olfactory epithelium, nerve atrophy, and metaplasia [172], though the relevance to humans of these signs is not clear. In contrast, consumption of 2500 mg/kgbw/d of an aqueous (but not fully characterized) GTE *via* gavage for 28 d did not cause any adverse effects on body or organ weights, serum enzymes, or histopathology in male or female ICR mice [173]. Chengelis et al. [179] also reported that male and female Sprague Dawley rats consuming up to 2 g/kg bw/d GTE *via* gavage for 28 d did not exhibit any adverse liver effects. They also reported no adverse effects of heat- and non-heat sterilized green tea flavan-3-ols, but noted that decaffeinated purified green tea flavan-3-ols reduced body weight. This animal model contrasts with human studies indicating that caffeine is an important contributor to weight loss associated with green tea and GTE [180]. Toxicological studies using rodents have not investigated the difference between hydrophilic and lipophilic components of GTE.

Highly purified flavan-3-ols have been observed to induce hepatotoxicity in rodents at lower doses than GTE. For example, in an acute study using male CF-1 mice, Lambert et al. [181] administered a single oral dose of EGCG at 1500 mg/kg bw and observed liver injury and death. The observed hepatocellular necrosis and apoptosis was associated with increased biomarkers of oxidative stress in plasma and liver. Plasma alanine transaminase was found to be increased 24 and 48 h after EGCG administration. Based on allometric scaling, Lambert et al. [181] calculated the minimum equivalency to this dose to be the EGCG amount from 10.5 cups of green tea, and noted this amount in humans would be typically consumed over the course of a day without any apparent adverse effect. Consistent with an apparent greater sensitivity of mice to flavan-3-ol toxicity, Takami et al. [178] fed male and female F344 rats a semi-purified GTE (81% polyphenols) at 5% (w/w) of the diet for 90 d and observed only an increase in plasma alkaline phosphatase and

aspartate transaminase but no histopathological changes in the liver or other tissues.

Bun et al. [182] directly tested the effect of GTE derived using different extraction solvents on liver function in rats in two experiments. A preliminary 6-wk trial compared administration by gavage of 2500 mg/kg bw/d of GTE prepared with methylene chloride (from which potential non-polar hepatotoxins might be concentrated) and its distilled water vehicle (with 3% Tween 80) (control). A second 12-wk experiment compared the effects following gavage administration of an aqueous green tea extract (1400 mg/kg bw), an 80% hydro-ethanolic green tea extract (2000 mg/kg bw), or the vehicle (control). Signs characteristic of hepatotoxicity, including serum levels of lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and  $\gamma$ -glutamyl transferase, were not significantly affected by either aqueous or hydro-ethanolic extracts.

Long-term exposure to a concentrated GTE material (c.a. 76% catechins) has also been examined in rodents in a 2-y carcinogenesis study conducted by National Toxicology Program [183]. Only at the highest dose tested for each species (300 and 1000 mg/kg bw/d dose for B6C3F1 mice and Wistar rats, respectively), non-neoplastic lesions in the liver were observed, including hepatocyte necrosis, chronic inflammation, oval cell hyperplasia and hematopoietic cell proliferation. However, it is noteworthy that no increase in liver tumors was observed after chronic exposure to GTE in either species and that the manifestation of liver damage did not appear to progress to a more severe level when compared to those observed in the 14-wk study by Chan et al. [172].

Two studies of GTE toxicity studies have been conducted in dogs. Isbrucker et al. [174] fed Beagles doses of 50, 150 or 500 mg/kg bw/d of GTE (80% EGCG) and observed no adverse effects when administered over 13 wk in divided doses during a pre-fed state. Mortality resulted when the two higher doses were administered in a single bolus dose to fasted dogs; however, this model was considered an unrealistic comparison to the human condition. Kapetanovic et al. [163] also tested oral doses of GTE (56-72% EGCG) in Beagles at 200, 500, and 1000 mg/kg bw/d for 6.5 mo. Similar to the findings of Isbrucker et al. [174], they found that dosing in a fed state resulted in lower and less variable exposure as well as less frequent and lesser severity than

found under fasting conditions. Importantly, Chow et al. [162] reported the same relationship in humans wherein volunteers consuming an acute dose of 400 to 12000 mg of GTE showed a >3.5-fold increase in the average maximum plasma concentration of EGCG when taken in a fasting condition than when taken with food.

In contrast to evidence from animal models of hepatotoxicity, several reports have shown a hepatoprotective action of green tea preparations. For example, Chen et al. [184] found a protective effect of intraperitoneal EGCG for 3 d at 50 and 75 mg/kg bw against acute carbon tetrachloride hepatotoxicity in mice and Bruno et al. [185] found GTE reduced serum alanine transaminase and aspartate transaminase activity as well as hepatic steatosis in obese mice. Fiorini et al. [186] found EGCG at 85 mg/kg bw administered via intraperitoneal injection or drinking water protected against hepatic ischemia/reperfusion injury in steatotic mice. Dobrzynska et al. [187] and Zhang et al. [188] both reported that feeding green tea polyphenols to rats reduced the adverse effects of ethanol toxicity in the liver.

### 7.3 Evidence from Human Studies

As it is unethical to attempt to induce harm in human studies, there is no direct evidence of toxicity from green tea or GTE. Clinical toxicity from GTE or purified tea polyphenols can only be deduced from animal model data and/or observational evidence. Similar to rodents, humans extensively metabolize flavonoids to glucuronidated, methylated, and sulfated forms for relatively rapid excretion through bile, urine or feces and do not appear to accumulate these compounds in plasma or tissues for long periods [189]. In general, EGCG, EGC, and EC reach their maximum plasma concentrations within 1.5-2.5 h after consumption and return to the baseline values within 24 h [190]. Flavan-3-ol bioavailability is also dose-dependent in acute oral administrations, e.g., at 50, 100, 200, 400, 800 or 1600 mg EGCG [165]. Plasma and urine concentrations of flavan-3-ols are absent 24 h after an acute dose and their accumulation is essentially undetectable even after repeated doses of 200, 400, or 800 mg EGCG daily for  $\geq 10$  d [166,191]. However, these data do not imply an absence of bioactivity, as repeated provision of 800 mg/d green tea polyphenols resulted in an inhibition in the capacity of phase II enzymes in plasma and a consequent increase in the maximum of free EGCG concentration in

plasma, but the kinetics of circulating EGCG were not altered.

Only a few, minor adverse side effects have been reported from randomized clinical trials of GTE, though most of these studies have had durations of less than 6 mo. Reports of minor to moderate side effects of GTE consumption include gastric irritation, diarrhea, nausea, and vomiting [192]. These untoward reactions appear unrelated to the chemical composition of GTE [28,130,132].

In 2008, up to 34 cases of liver damage associated with GTE had been reported worldwide and were summarized by Sarma et al. [28]. For most case reports, it is usually unclear whether the toxicity was due to GTE *per se* or related to other chemicals introduced during the extraction process, to concomitant medications, to other intentional or adulterant ingredients in the product or other factors. Though of variable quality, 27 of these reports were categorized according to the Naranjo causality algorithm scale as possible causality and 7 as probable causality. These cases were associated with the use of 6 different products varying markedly in product composition, solvent extraction, dose, and duration of use. The reported hepatotoxicity was coincident with increases in the activity of serum alanine transaminase and/or alkaline phosphatase or acute hepatitis-like syndrome [193]. However, the Naranjo scale has been found to lack validity and reproducibility when evaluating hepatotoxicity and is no longer recommended for such assessments [194]. The NIH Liver Tox database includes case reports of GTE extract and indicates no dose-response relationship between GTE intake and hepatotoxicity [193]. Many of the cases of liver toxicity associated with GTE were identified in Europe and linked to the use of alcohol extracts; however, hepatotoxicity has been reported for both aqueous and alcoholic GTE [28]. Liver injury has also been reported in at least 3 individuals drinking green tea infusions: an adult consuming 4-6 cups/d for 6 mo; an individual drinking an unspecified amount of green tea daily; and an individual consuming 6 cups/d of a commercial green tea infusion for 4 mo [33,194-196]. More recently, Teschke et al. [197] analyzed 185 case reports, spontaneous reports, review articles, and comments regarding liver injury associated with the use of 60 herbs, herbal drugs, and herbal dietary supplements, including GTE, and concluded that convincing causality assessment was rarely provided. The most robust current



approach for structured, quantitative, and validated hepatotoxicity cases is the Council for International Organizations of Medical Sciences (CIOMS) scale for initial causality assessment [198]. While not without limitations, the CIOMS scale compiles liver specific criteria for challenge, de-challenge, risk factors, exclusion of unrelated diseases, and co-medication [199,200].

In contrast to case reports, randomized clinical trials of GTE do not provide evidence of hepatotoxicity. For example, in a 3-wk randomized clinical trial testing a GTE daily containing ~670 mg flavan-3-ols in healthy men, Frank et al. [201] found no untoward effects on  $\gamma$ -glutamyl-transpeptidase, alanine transaminase or aspartate transaminase. In a 12-wk phase II randomized clinical trial of patients with oral premalignant lesions, Tsao et al. [202] found no adverse effects, including on hematological or blood chemistry outcomes, from GTE supplementation at 500 to 1000 mg/m<sup>2</sup> (~14.7 to 29.4 mg/kg bw or ~1034 to 2068 mg/d). Similarly, Bettuzzi et al. [203] reported no adverse effects following the administration of green tea flavanols at 600 mg/d for 1 y in a safety assessment study among men with high-grade prostate intraepithelial neoplasia. In a randomized clinical trial of 40 overweight and obese Japanese children, 6 to 16 y, Matsuyama et al. [204] tested the effect of 575.9 vs 75.4 mg daily of green tea flavanols and found no significant effects on  $\gamma$ -glutamyl-transpeptidase, alanine transaminase, aspartate transaminase or albumin. In contrast, supplementation with a GTE at 1300 mg/d for a mean of 34.5 d in men with prostate cancer and scheduled for a radical prostatectomy was associated with statistically significant decreases in aspartate transaminase and alkaline phosphatase; non-significant decreases in alanine transaminase,  $\gamma$ -glutamyl-transpeptidase, and lipase were also reported [205]. While these results indicate no adverse effect on liver function and suggest a favorable trend, it is important to note that all these values were within normal limits throughout the study.

Hepatotoxicity from herbal products and pharmaceuticals is not uncommon and has often raised concerns about product quality [206-208]. Navarro et al. [38] assayed 97 herbal dietary supplements and found that 29 of 73 products that did not identify GTE or any of its constituents did contain flavan-3-ols, suggesting that packaging and labels of such products seem to be unreliable as regards GTE content. In their study matching clinical histories with the analysis

of tea flavonoids, among patients with confirmed hepatotoxicity, no statistically significant association was observed between the presence of flavan-3-ols or the dose consumed and liver injury causality score, severity, or pattern of liver injury [38]. Liver injury associated with green tea infusions and GTE is consistent with the definition of idiosyncratic drug-induced hepatotoxicity based on its rare occurrence and lack of dose-dependency [38,209]. While green tea infusions and GTE appear well tolerated in most all individuals, they have been associated with liver injury in rare circumstances, possibly as a result of specific environmental, genetic, and/or medical conditions.

## 8. AUTHORITATIVE REVIEWS OF GREEN TEA EXTRACTS

In 2003, the French Health Product Safety Agency (AFSSAPS) and the Spanish Food Safety and Nutrition Agency suspended the marketing authorization of an 80% ethanolic GTE (Exolise<sup>®</sup>) due to reports of hepatic disorders [210]. This product was indicated at 375 mg/d of GTE, standardized to 25% EGCG, and obtained using 80% ethanol as the solvent. More recent cases were reported to AFSSAPS in 2006 with aqueous GTE and AFSSA, the French Food Safety Agency, in 2009 [32,211,212]. In 2007, the U.S. Pharmacopeia (USP) determined GTE was a Class 2 substance and issued a press release suggesting specific cautionary wording be added to any dietary supplements containing GTE as an ingredient: "Take with food. Discontinue use and consult a healthcare practitioner if you have a liver disorder or develop symptoms of liver trouble such as abdominal pain, dark urine, or jaundice" [213]. However, two years later, the USP undertook a follow-up comprehensive review and, based on updated information, reclassified GTE as an article falling within Class A (Admitted into the Compendia – does not require a caution/warning statement in labeling) and canceled the published proposal for a cautionary labeling statement [214]. Additional expert reviews based on case reports, observational studies, and clinical trials confirm the general safety of green tea and GTE consumption [28,30,130,132,215-217] (Table 10).

In 2009, the European Food Safety Authority Scientific (EFSA) Cooperation Group (ESCO) published guidance for the safety assessment of botanicals in which green tea was considered based upon knowledge of the risk of

hepatotoxicity and the worldwide, long-term consumption of traditional tea infusions; intakes of brewed green tea from the mean to 95<sup>th</sup> percentile of intake at 362 to 1097 g/d, respectively, were noted in China and Japan and were associated with mean and 95<sup>th</sup> percentile intakes of EGCG at 95 to 289 and 148 to 448 mg/d and of total flavan-3-ols at 186 to 565 and 307 to 931 mg/d, respectively [216]. The ESCO concluded that traditional green tea infusion is assumed to be safe on level A (safety presumed based upon available knowledge derived from long-term use). Dried aqueous GTE which have a similar composition and do not exceed the concentration of polyphenols compared to a traditional green tea infusion were also classified to be on level A when consistent to EFSA guidance on the safety assessment of botanicals intended for use in the preparation of beverages

[216,218]. In marked contrast, this EFSA report [216] questions the safety of use of GTE for use in food supplements and recommends application of a margin of safety (MOS) – the ratio between the NOAEL (determined from animal studies) and daily intake for EGCG – of 100. Though absent specific information, the Norwegian Food Safety Authority recently issued a warning about using highly concentrated GTE supplements based upon some cases of hepatotoxicity reported by the Norwegian Medicines Agency [219].

Similar to EFSA guidelines, France has published restrictions on the use of preparations of green tea in food supplements limiting them to traditional extracts (i.e., aqueous extracts at a maximal amount equivalent to 30 mg/d of EGCG

**Table 10. Summary of systematic reviews that include safety assessment of green tea or GTE**

Review	Date	Conclusions
US Pharmacopeia, Safety of Green Tea Extract <sup>1</sup>	2008	Assigned Class 2 safety rating, suggested labeling: “Take with food. Discontinue use and consult a healthcare practitioner if you have a liver disorder or develop symptoms of liver trouble such as abdominal pain, dark urine, or jaundice”
Green tea consumption and liver disease; interventional and observational studies with liver cancer, cirrhosis, and fatty liver disease as outcomes <sup>2</sup>	2008	Increased green tea consumption may reduce the risk of chronic liver diseases.
US Pharmacopeia, Green Tea Extract Monograph <sup>3</sup>	2009	Class A safety rating, no cautionary label statement
Green tea for the prevention of cancer <sup>4</sup>	2009	Green tea consumption appears safe at moderate, regular, and habitual use (3-5 cups/d, providing ~250 mg catechins/d)
EFSA ESCO advise on the EFSA guidance for the safety assessment of botanicals <sup>5</sup>	2009	Aqueous GTE for food supplement use given A except for weight reduction purposes which received grade B, other GTE (e.g. hydro-alcoholic preparations) given grade B
Green tea for weight loss and weight maintenance in overweight or obese adults <sup>6</sup>	2012	Assessed randomized control trials of at least 12 wk duration of green tea preparations. Most adverse effects reported appeared unrelated to green tea or control intervention.
French Agency for Food, Environmental and Occupational Health Safety (ANSES) <sup>7</sup>	2012	Based on case reports, hepatitis severity following GTE consumption could not be conclusively associated with GTE consumption, dose response could not be achieved.

<sup>1</sup>Sarma et al. [28]; <sup>2</sup>Jin et al. [215]; <sup>3</sup>Sarma. [30]; <sup>4</sup>Boehm et al. [130]; <sup>5</sup>EFSA J. [215]; <sup>6</sup>Jurgens et al. [132]; <sup>7</sup>ANSES, 21011-SA-0108 [217]

for a person weighing 60 kg) and excludes hydro-alcoholic extracts and powders [220]. In some contrast, the French Agency for Food, Environmental and Occupational Health Safety recently evaluated 17 case reports of hepatitis associated with GTE consumption and found no conclusive evidence for a causal relationship because the data on quantity of green tea and GTE consumed were lacking so that no dose-response relationship could be established between intake and severity of hepatitis [217]. Belgium allows a maximum dose of EGCG at 1600 mg/d and a maximum amount of 25% ethanol permitted in the extraction process [221]. Currently, the Plant LIBRA project, co-financed in the context of the 7<sup>th</sup> European Union Framework Program, is evaluating the safety of GTE using several assessment approaches, including the Observed Safe Level [222], and Minimum Anticipated Biological Effect Level [223] and Estimation of the Optimal Range [224,225].

More recently, Cochrane database reviews have confirmed the general safety of consuming green tea infusions at 3-5 cups/d [130] and the safety of infusions and GTE associated with weight loss studies [132]. The safety of GTE was reviewed by the US Pharmacopeia and assigned as a Class A substance that does not require a caution or warning statement [28,30]. GTE prepared as essential oils, solvent-free oleoresins, and other natural extractives are currently classified as GRAS for food and ingredient use by the U.S. Food and Drug Administration [226]. Further, purified tea flavan-3-ols, an aqueous GTE, and L-theanine from green tea have self-determined GRAS status (Table 11).

**Table 11. Industry-reported GRAS status of GTE and green-tea derived ingredients**

Product	Manufacturer	Date
Teavigo, green tea extract standardized to 94% EGCG <sup>1</sup>	DSM	2005
Purified green tea catechins <sup>2</sup>	Kao, Inc.	2007
L-theanine extracted from tea leaves <sup>3</sup>	Blue California	2010
AssuriTEA aqueous green tea extract <sup>1</sup>	Kemin	2012

<sup>1</sup>AIBMR Life Sciences, GRAS Self-determination Inventory Database [227] <sup>2</sup>Assuming maximum use of 540 mg catechins/350 mL, with up to 1 to 1.5 g green tea catechins presumed safe; FDA GRN No. 225 & 259 [228] <sup>3</sup>FDA GRN No. 338 [229]

## 9. SUMMARY AND PERSPECTIVE

The interest in the putative health benefits of green tea has led to an increase in the consumption of brewed green tea and the application of GTE to a variety of food and beverage products and food supplements. With some experimental evidence of toxicity from green tea and GTE and a number of case reports of hepatotoxicity, the concept of a safety threshold for green tea and GTE has been raised. While there are several approaches to determining safety thresholds [222,224,225, 230,231,232], some suggest a conservative threshold for nutrients and food supplements should be equivalent to at least 10-fold less than the lowest dose associated with toxicity observed in humans. In the case reports of GTE-associated hepatotoxicity summarized by Bonkovsky [34], the reported range of intake was 10-29 mg GTE/kg bw/d. using the lower 10 mg/kg bw dose, an estimated acceptable intake would be 1.0 mg/kg bw/d or 70 mg EGCG daily for a reference 70 kg person. However, such a calculation is in marked contrast to usual intakes and the GRAS status of green tea and GTE and the expert and systematic reviews on the safety of consuming moderate levels of GTE and green tea infusions [28,30,130,132,215]. For example, the 90<sup>th</sup> percentile of EGCG intake associated with green tea consumption in the U.S. is 189 and 218 mg/d for women and men, respectively; the 95<sup>th</sup> percentile of EGCG intake associated with green tea consumption in China and Japan is 288 and 447 mg/d [4,8]. These values are about 3- to 6-times higher than a 10-fold safety threshold of 1.0 mg/kg bw/d and suggest an inappropriate application of the precautionary principle, particularly in the absence of established causality. Although most individual studies have been relatively short-term, recent Cochrane database reviews confirm the general safety of green tea and GTE [130,132]. Nonetheless, it is noteworthy that Bettuzzi et al. [203] found no untoward responses in a year-long randomized, placebo-controlled trial of a GTE providing 311 mg/d EGCG and 142 mg/d of other flavan-3-ols. The noted gap could be attributed to the factors such as dosing frequencies and conditions, as well as the medical conditions, concomitant use of other products and sensitivities of individuals.

Responses by government regulatory agencies to the experimental data and case reports of hepatotoxicity associated with use of GTE are contradictory, e.g., with EFSA stating that GTE

for food supplement use cannot be considered safe and suggesting a MOS (calculated with data from animals) of at least 100 [216] and the USP admitting GTE into its Compendia without a requirement for any caution/warning label (though USP's Dietary Supplement Information Expert Committee continues to monitor clinical case reports) [213]. After an accountability assessment of 17 cases of hepatitis linked to green tea and GTE reported between 2009 and 2011, the French Agency for Food, Environmental and Occupational Health Safety concluded that these cases could not be clearly associated with green tea due to confounding by other likely causes and an inability to obtain adequate information on intake [217]. This contrast between approaches and recommendations arises in part from the rare and idiosyncratic nature of the injury and the apparent absence of a dose-response relationship between GTE and hepatotoxicity [36,37,193]. The sensitivity and extremely low occurrence of hepatotoxicity with GTE may have a nutrigenetic basis, e.g., polymorphism(s) in pathways for flavonoid metabolism [35,233]. This relationship is highlighted by the recent report of Navarro et al. [38] which indicated no statistically significant association between GTE dose or content of flavan-3-ols and liver injury causality score, severity, or pattern of liver injury. Despite concern raised in some reports about a great risk for harm induced by GTE derived from hydro-alcoholic versus aqueous extractions, specifically hepatotoxicity, there is no evidence that the former provides significantly different proportions of bioactive or toxic compounds than the latter. This work is essentially confirmed by the observation that hepatotoxicity associated with GTE have been reported from cases following use of variety of products varying markedly in composition, solvent extraction, dose, and duration of use. Although the pathogenesis of hepatotoxicity associated with GTE is unknown, it is important to note that EGCG, the component of GTE most often identified as the cause of hepatotoxicity, is readily soluble in both water and alcohol-water mixtures. Regulations ranking the potential hepatotoxicity of GTE via its extraction with water versus hydro-alcoholic solvents are not based on the available evidence. Rather than an extraction process, it appears that the greater number of cases linked to a limited number of GTE products may be largely due to their greater sales and indication for chronic use, such as for weight loss.

The evidence derived from observational studies and clinical trials over the last three decades suggest that flavan-3-ols from green tea may contribute importantly to reducing the risk of prevalent age-related chronic diseases. The biological plausibility underlying this association is derived from a large body of experimental studies revealing several relevant molecular mechanisms of action of these flavonoids. However, the application of this information, e.g., through the development of innovative dietary products containing GTE, requires careful consideration of the safety profile of its constituent phytochemicals. Clinical case reports of hepatotoxicity associated with use of GTE and products containing GTE have raised concern and generated responses from regulatory agencies. In addition to the usual limitations associated with the extrapolation of evidence from *in vitro* and animal model experiments, data on liver injury induced by GTE and its specific flavan-3-ols constituents revealed by these approaches are mixed in that some demonstrate hepatotoxicity while others show hepatoprotective actions. Case reports are important but also limited, especially with rare and idiosyncratic reactions, in their ability to reveal causality due to confounding medical and lifestyle factors of the patients.

## 10. CONCLUSION

The scientific evidence regarding the potential benefits and risks of green tea polyphenols and GTE continues to emerge. Existing data are insufficient to identify in the preparation or composition of GTE the causative agent, its dose or duration of use as well as nutrigenetic, medical, and other factors contributing to the risk of hepatotoxicity. While reference to quantitative equivalents associated with traditional dietary intakes of green tea, its constituent flavonoids, and related dietary polyphenols is important in evaluating GTE products, opportunities should be allowed for innovation to improve the efficiency, quality, and cost-effectiveness of their production through extraction technologies. This approach can also prove useful in minimizing the presence of endogenous or contaminant heavy metals as well as mycotoxins, pesticides, and other unwanted constituents.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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