Annex A and Annex B

Annex A

Discussion papers presented to the COT on the hepatotoxic effects of green tea catechins

Table 1 – A table of discussion papers that have been presented to the COT on the hepatotoxic effects of green tea catechins.

Discussion Paper reference and Date	Paper Title
TOX/2021/47 (07/09/2021)	The Safety of Green Tea Catechins (Reserved).
TOX/2022/51 (06/09/2022)	The safety of green tea catechins – First draft statement.
TOX/2023/05 (07/02/2023)	The safety of green tea catechins – Second draft statement.
TOX/2023/26 (16/05/2023)	The safety of green tea catechins – Third draft statement.

Annex A to COT Statement 08/2024

November 2024

Annex B

Statement on the Hepatotoxicity of Green Tea Catechins – Summary tables of cited toxicity studies

1. This Annex is to be read in conjunction with the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment's (COT) Statement on the Hepatotoxicity of Green Tea Catechins available at https://doi.org/10.46756/sci.fsa.wii944.

2. The European Food Safety Authority (EFSA) published a scientific opinion in 2018 (EFSA, 2018)concluding that catechins from green tea prepared in the traditional way of infusion, or reconstituted drinks giving the equivalent composition of catechins as green tea infusions were, in general, safe; however, at the time EFSA were unable to determine a dose of epigallocatechin-3-gallate from green tea extracts that would be considered safe.

3. To determine whether any new data have become available since the publication of the EFSA Opinion that might be relevant to the safety of the use of GTEs and hepatotoxicity, a literature search was conducted spanning the duration of 2018 to September 2022. Databases searched included PubMed, Google Scholar and LIVERTOX. Search terms used included (green tea extract and hepatotoxicity); (green tea extract and liver toxicity); ("green tea" and hepatotoxicity); ("green tea" and liver damage); (epigallocatechin-3-gallate OR EGCG) AND hepatotoxicity).

4. The purpose of this annex is to provide summary information on the cited toxicological studies within the statement. There are four tables:

i. Table 1 summarises the data obtained from the literature that describes the possible Mode of Action for green tea catechins toxicity.

ii. Table 2 summaries the in vitro toxicity studies that were not previously reported in the EFSA, 2018 Opinion (EFSA, 2018).

iii. Table 3 summarises the in vivo animal data obtained from new reports and studies published in the literature since the publication of the EFSA Opinion (EFSA, 2018).

iv. Tabe 4 summarises the human data on hepatotoxicity reported in the literature since the publication of the EFSA Opinion (EFSA, 2018).

Annex B to COT Statement 08/2024

November 2024

Table 1 - Table summarising toxicological data obtained from the literature that describes the possible Mode of Action for green tea catechins hepatotoxicity.

Test item	Model	Concentration	Length of exposure	Results	Reference
dGTE 1 (decaffeinated GTE, 70% EGCG).	HepG2 cells.	0.001 to 1000 µg/ml dGTE 1.	24 hours	Protective against hydrogen peroxide-induced apoptosis and cell death by attenuating oxidative stress pathways, similar to EGCG itself.	Shil et al., (2022)

dGTE 2 (decaffeinated GTE, 45% EGCG).	HepG2 cells.	0.001 to 1000 µg/ml dGTE 2.	24 hours	Increased cellular and mitochondrial oxidative stress and apoptosis in addition to hydrogen peroxide.	Shil et al., (2022)
Green tea.	See Hoofnagle et al., (2021) summary on pp.16.	See Hoofnagle et al., (2021) summary on pp.16.	See Hoofnagle et al., (2021) summary on pp.16.	Green-tea related liver injury was found to be strongly associated with the HLA-B*35:01 allele.	Hoofnagle et al., (2021)*
EGCG (98% purity).	Female C57BL/6J mice (n=8/group).	**Free diet: on the 6^{th} day, mice were split into 3 groups: 0, 400, 800 mg EGCG/kg bw per day.	6 days feeding; exposed to EGCG for 24 hours.	EGCG enhanced lipid metabolism pathways but did not cause liver injury.	Shi et al., (2021)

EGCG (98% purity).	Female C57BL/6J mice (n=8).	Fixed diet: 50% of the average food intake, limited to 2g each mouse per day. On the 6^{th} day, mice were randomly divided into three groups: dieting, dieting + 400 mg/EGCG/kg bw per day and, dieting + 800 mg/EGCG/kg bw per day.	6 days feeding; exposed to EGCG (intragastric) for 24 hours.	EGCG caused dose-dependent hepatotoxicity, associated with overactivation of linoleic and arachidonic acid oxidation pathways, which increased the accumulation of pro-inflammatory lipid metabolites, which thus contributed to liver injury.	Shi et al., (2021).
NA	In silico docking.	NA	NA	The binding free energy calculations showed that some EGCG metabolites exhibited strong predicted binding affinity to NQO1 and would thus lead to inhibition.	Pandey et al., (2020).

NA	Literature review of EGCG toxicity in children.	‘High doses’ is not further defined. However, the 0.8 g EGCG/day EFSA TDI was cited.	Length of exposure not further defined.	EGCG toxicity observed at high concentrations was related to pro-oxidative properties attributed to catechol structures, which are able to form a superoxide anion radical.	Sergi (2020).

				Categorised cases into three groups: idiosyncratic HILI, intrinsic HILI or liver adaptation.	
GTE	Literature review on suspected liver injury associated with GTE from 1999 to June 2019.	GTE containing 25-90% EGCG with other constituents (in dietary supplements).	Length of exposure not further defined.	Mechanistic steps leading to liver injury have not been elucidated, although there is evidence that GTE may cause idiosyncratic HILI in susceptible users, as well as intrinsic HILI that is dose dependent. Authors noted that idiosyncratic HILI can be: a) metabolic (1 week to several months exposure), lacking hypersensitivity issues with delayed response to re-exposure to GTE or; b) immunologic type (few weeks exposure),	Teschke and Xuan (2019).

EGCG (93% purity).	C57BL/6J mice (n=11-18/group).	Mice were dosed at up to 750 mg/kg bw per day via intragastric administration.	3 days.	Hepatic inflammation, necrosis and haemorrhage were observed; associated with increased oxidative stress and decreased superoxide dismutase and glutathione peroxidase levels.	James et al., (2018).
EGCG	Literature review on the modulation of DNA methylation by GTCs	In vitro cells (MCF-7 and MDA-MD-23; breast cancer cell lines): 0 – 50 EGCG µmol/L. In vitro cells (KYSE 510; human oesophageal cancer cell line): 5 – 10 EGCG µmol/L.	In vitro cells (MCF-7 and MDA-MD-23; breast cancer cell lines): 3 or 6 days. In vitro cells (KYSE 510; human oesophageal cells): 12-144 hours.	EGCG modulates DNA methylation by attenuating the effect of DNMT1; however, the exact mechanism of DNMT1 inhibition is not fully understood.	Yiannakopoulu (2015)

*These results are based on a systematic review by Hu et al., (2018). Regulatory Toxicology and Pharmacology 95 (2018) 412-433.

**Concentrations were assumed to have been expressed per kg body weight, but the methodology did not clarify this in detail.

Abbreviations: dGTE – Decaffeinated green tea extract; DNA - Deoxyribonucleic acid; DNMT1 - DNA-methyltransferase 1; EGCG - epigallocatechin-3-gallate; GTC –

Green tea catechins; GTE – Green tea extract; HepG2 - human liver cancer cell line; HILI – Herb-induced liver injury; NA – Not applicable; NQO1 - NAD(P)H dehydrogenase [quinone] 1.

Table SEQ Table * ARABIC 2 – In vitro toxicity studies that were not previously reported in the EFSA, 2018 Opinion.

Test item	**Model**	**Concentration**	**Length of exposure**	**Results**	**Reference**
EGCG	Bovine thymus DNA	Incubated with EGCG (0, 1, 2, 3, 4 and 5 µM) and 20 µM metal ions.	1 hour at 37ºC.	Oxidative damage under the action of metal ions and H2O2-induced oxidative stress.	Furukawa et al., (2003)
EGCG	HL-60	0, 50, 100, 150, 200 and 250 µM	1 hour at 37ºC.	Low concentration of EGCG can cause oxidative DNA damage in human cells and H2O2 plays a critical role in EGCG-induced DNA damage.	Furukawa et al., (2003)
EGCG (>98% purity)	Human lymphocytes	Increasing concentrations: 10-100 µM.	24-hour incubation.	At the maximum dose, the survival rate decreased by 25%.	Bertram et al., (2003)
EGCG (>98% purity)	Nalm6 cells	Increasing concentrations: 10-100 µM.	24-hour incubation.	At the maximum dose, Survival rate decreased by 50%.	Bertram et al., (2003)

Abbreviations: DNA - Deoxyribonucleic acid; EGCG - (-)- Epigallocatechin-3-gallate ; H2O2 – Hydrogen peroxide; HL – Human leukaemia cell.

Table SEQ Table * ARABIC 3 – Toxicological table summarising in vivo animal data obtained from new reports and studies published in the literature since the publication of the EFSA Opinion (EFSA, 2018).

Test item	Model	Concentration	Length of exposure	Results	Reference
Green tea fat burner capsule	Male and female wild type mice (n=3-4 per dose group)	150 mg EGCG per capsule: 250 or 500 mg/kg bw per day via oral gavage.	6-week period	Did not result in a significant elevation of ALT levels over the treatment period.	Cho et al., (2021)
Green tea fat burner capsule	Male and female PD-1 -/- (C57BL/6J) mice (model for IDILI) (n=3-4 per dose group)	150 mg EGCG per capsule: 250 or 500 mg/kg bw per day via oral gavage in conjunction with anti-CTLA-4 antibody at a dose of 300 µg on days -3 and -1 prior to treatment and then weekly to sustain CTLA-4 inhibition.	6-week period	In the high dose female mice, GTE induced a delayed onset increase in serum ALT levels and an increase in CD8+ T cells. Whilst in the high dose male mice, a smaller increase in ALT was observed in day 7.	Cho et al., (2021)

Green tea polyphenols in Polyphenon® (29.2% EGCG; total sum of catechins 65.4%) with ebulin f from dwarf elder fruits	37 Swiss female mice (four treatment groups)	Group 1 (n = 16) was treated intraperitoneally with 2.5 mg/kg body weight of ebulin f; Group 2 (n = 7) received one oral (p.o.) dose of Pol60; Group 3 (n = 11) was administered with both treatments at the same day; Group 4 (n=3) littermates which received no treatment to serve as controls.	2-week period	Combined treatment resulted in a reduction in mouse survival by 70% with darkened areas in the internal organs (presumed to be bleeding). Hypothesised the GTEs enhance the apoptotic effect of ebulin f.	Rojo et al., (2020)*

Decaffeinated GTE (180 mg EGCG/capsule; total sum of catechins 225 mg/capsule)	Lean male B6C3F1 mice (n=5 per dose group)	Doses of either: 1x (equivalent of 1.5 mg total catechins delivered in 300 µL of gavage solution); 3x (4.5 mg total catechins) or 10x (15 mg total catechins) mouse equivalent doses by gavage.	Up to two weeks (Monday – Friday)	No significant alterations to the liver tissue following administration of decaffeinated GTE. However, there was no group receiving a caffeinated preparation for comparison, the study used historical data from a different study for comparison.	Gurley et al. (2019)

EGCG	Literature review on the toxicological effects of green tea	Acute oral gavage toxicity in rats: single administration 1,868 EGCG mg/kg bw. Acute oral gavage toxicity in mice: single administration of 1,500 EGCG mg/kg bw. Subacute oral gavage toxicity in rats: 0.5 and 1 g/kg bw. Subacute oral gavage toxicity in mice: 1,500 EGCG mg/kg bw per day. Subacute oral gavage toxicity in dogs: 300 EGCG mg/kg bw per day, and 500 EGCG mg/kg bw per day.	Acute oral toxicity in rats: death observed 72 hours following administration. Acute oral toxicity in mice: 48 hours. Subacute oral gavage toxicity in rats: Subacute oral gavage toxicity in mice: 5 days. Subacute oral toxicity in dogs: low dose for 14 days and higher dose for 28 days.	The most important side effects reported were hepatotoxicity and gastrointestinal disorders especially when consumed on an empty stomach. Limited data on using green tea and its components during pregnancy, consideration should also be taken when co-administrating with drugs.	Bedrood et a (2018)

Multi-treat (dietary supplement), 300 mg GTE per tablet (30% polyphenol)	Male albino rats (strain not specified) (n=9 per dose group)	Control group, paracetamol (2 g/kg, orally for one week), GTE (8.5 mg/kg, orally for one month), paracetamol followed by GTE, paracetamol recovery (for one month) and, paracetamol followed by GTE recovery (for one month).	Varies – one week, one month, and/or with one month recovery.	Administration of paracetamol or GTE resulted in biochemical and histopathological alterations that indicated hepatotoxicity including augmented concentrations of AST and ALT, hepatocellular necrosis and degeneration and degeneration, congestion, haemorrhage, inflammation and fibrosis.	El-Bakry et a (2017)
EGCG (100% purity)	Adult female Swiss albino mice (n=5 per group)	Control (0), 217, 67.8, 21.1 and 6.6 EGCG mg/kg/day.	Oral gavage for 14 consecutive days followed by 14 days of observation without treatment.	EGCG induced hepatotoxic effects, reversible following cessation of 14 days after treatment.	Ramachand et al., (2016

EGCG (100% purity)	Adult female Swiss albino mice (n=5 per group)	Control (0), 108, 67.8, 21.1 and 6.6 EGCG mg/kg/day.	Either through oral or intraperitoneal route for 14 consecutive days followed by immediate termination after 24 h of the last dose.	Hepatotoxicity. A 14-day tolerable dose of 21.1 and 67.8 EGCG mg/kg for intraperitoneal and oral routes were identified, respectively.	Ramachand et al., (2016
EGCG (100% purity)	Adult female Swiss albino mice (n=5 per group)	Control (0), 108, 67.8, 21.1 and 6.6 EGCG mg/kg/day.	Intraperitoneal route for 14 consecutive days followed by 14 days of observation without treatment.	EGCG induced hepatotoxic effects, reversible following cessation of 14 days after treatment.	Ramachand et al., (2016

*The COT were of the opinion that the relevance of these findings to the effects of consumption of green tea or its extracts is questionable.

Abbreviations: ALT - Alanine transaminase; ASR - Aspartate aminotransferase; dGTE – Decaffeinated green tea extract; DNA - Deoxyribonucleic acid; DNMT1 - DNA-methyltransferase 1; EGCG - epigallocatechin-3-gallate; GTC – Green tea catechins; GTE – Green tea extract; HepG2 - human liver cancer cell line; HILI – Herb-induced liver injury; IDILI – idiosyncratic drug0induced liver injury; NA – Not applicable; NQO1 - NAD(P)H dehydrogenase [quinone] 1.

Table SEQ Table * ARABIC 4 – Human data on hepatotoxicity reported in the literature since the publication of the EFSA Opinion (EFSA, 2018).

Test item	**Model**	**Concentration**	**Length of exposure**	**Results**	**Reference**

EGCG	Women of reproductive age (≥18 to ≤40-years old) with or without uterine fibroids (n=39; 13 per dose group)	(i) 800 mg of EGCG daily; (ii) 800 mg of EGCG daily with clomiphene citrate* 100 mg for 5 days; (iii) 800 mg EGCG daily with letrozole* for 5 days.	30–35 days (after the onset of their next menstrual cycle).	No subject demonstrated signs of drug-induced liver injury and no subject showed serum folate level outside the normal range. Authors suggest that a daily dose of 800 mg EGCG alone or in combination with clomiphene citrate or letrozole (for 5 days) is well-tolerated and is not associated with liver toxicity or folate deficiency in reproductive-aged women.	Siblini et al., (2023)

GTE	Post-menopausal women (n=1,075)	843 mg EGCG per day or placebo capsules.	12 months.	Clinically relevant serum AST and ALT elevations were found within 6-9 months of the women in the treatment group with the UGT1A4† heterozygous genotype.	Acosta et al., (2022)
Green tea infusions and GTE dietary supplement	Analysed cross-sectional data from 2009-2014 of the USA National Health and Nutrition Examination Survey	690 – 1,315 GTE mg/day (supplements).	Six weeks – 12 months.	Investigated the association between green tea infusions and GTE supplement consumption and liver biomarkers. Authors observed green tea consumption was associated with reducing the probability of having one or more abnormal liver biomarkers. GTE supplement consumption had no significant effect.	Fallah et al., (2022)

Green tea (drink) and royal jelly with magnesisum	48-year-old-woman	Green tea (unknown), royal jelly with magnesium twice a day.	~1.5 L of green tea per day for 5 years, royal jelly with magnesium for 3 months.	Presenting with symptoms suggestive of gastroenteritis, her AST and ALT levels were 8x the ULN. Condition rapidly worsened and underwent liver transplant for fulminant hepatitis.	Percevault et al., (2022)
ANACA3+® dietary supplement (containing green tea leaf powder at 160 mg/dose)	28-year-old woman	160 mg green tea leaf powder/dose from four capsules/day.	Consumed dietary supplement for 1 year.	Abdominal pain associated with elevated AST >100x the ULN and ALT >200x the ULN. Patient discontinued supplementation; liver function normalised over 1 month after onset of symptoms.	Percevault et al., (2022)

Dietary supplements containing green tea	“Middle-aged women and adults”	Either unavailable or does not provide detailed granularity on the % of EGCG for each dietary supplement.	4-52 weeks.	Hepatocellular lesions.	Assis et al., (2022
GTE and a dietary supplement that also contained GTE	47-year-old woman	Unknown; dietary supplements containing varying amounts of GTEs. Levels undisclosed as part of proprietary blends.	“Years”	Drug-induced liver injury.	AZ Big Media, (2021)
GTE	90 patients (mean age 44, m = 22, f = 68)	Not further described.	Not further described.	Main symptoms were jaundice, fatigue, nausea, and abdominal pain. The HILI patterns were mainly hepatocellular, cholestatic and mixed.	Ballotin et al., (2021)

GTE containing supplements	8/29 reports of DILI	Composition of supplements were not detailed in this review; however, consumption of supplements was concomitant with use of medicine.	Varied; ranged between 15 and 175 days, with a latency period of between 7 and 175 days.	8 case reports of DILI were attributed to herbal supplements containing GTE (7 were weight loss and 1 energy support supplement).	Bessone et al., (2021)
GTE containing supplements	40/1,414 cases; aged 17 to 69 years of age	Catechin per serving ranged from 6.6 – 384 mg; EGCG per serving ranged from 1.6 – 219 mg. Total estimated daily doses ranged from 50 to 2,000 mg GTE (median = 800 mg)	Symptoms developed between 15 to 448 days (median = 72 days).	40 cases of liver injury were directly attributed to green tea consumption of which 16 products were linked to GTE induced liver injury. Liver injury was typically hepatocellular, with marked increases in serum ALT and AST concentrations.	Hoofnagle et al., (2021)

GTE containing supplements	Not further described.	Median intake if 720 mg EGCG/day	At least 2 weeks.	The reported GTE-related hepatotoxicity in the majority of cases were acute hepatitis with a hepatocellular injury pattern.	Woo et al., (2021)
Hydroxycut® brand dietary supplement	22-year-old obese female	Specific product used by the patient was unknown. Previous formulations have been listed to contain 91 mg per 2 capsules serving of GTE.	2 capsules daily for ~3 months.	Presented with chest-pain fatigue and shortness of breath and was diagnosed with drug-induced liver injury. Cessation of Hydroxycut intake reduced ALT and AST levels.	Khetpal et al., (2020)

				Review showed a correlation between the occurrence of severe hepatotoxicity and the consumption of GTEs.	
GTE	75 individual cases associated the GTE intake	500 to 3,000 mg GTE per day (equating to ~250 – 1,800 EGCG mg/day)	Varied.	Typically, liver injury due to GTE exposure manifests within 3 months, but the latency to the onset of symptoms ranges from 10 days to 7 months. Most cases present with symptoms of acute hepatitis accompanied by marked hepatocellular enzyme elevations.	Oketch-Rabah et al., (2020)

Green tea infusions	2-year-old child	2-3 cups of green tea infusions; each cup provided 80-106 mg of polyphenols (equivalent to 36 -47.7 g of polyphenols in 5 months).	5 months	Presented with rash and diarrhoea for 5 days and a fever that persisted for 10 days. Final presumptive diagnosis was severe acute hepatitis secondary to green tea infusion toxicity.	D’Agostino et al., (2019)
Vital Stem™ dietary supplement	50-year-old woman	Unknown; contained GTE, L-leucine, blueberry powder, L-carnosine and Vitamin D3. Levels undisclosed as part of proprietary blend.	3.9 g dissolved in pomegranate juice daily for one month.	Presented with constriction around the common bile duct, elevated ASR and ALT levels.	Surapaneni et al., (2018)

Concurrent consumption of Evlution Nutrition Lean Mode Stimulant-Free Weight Loss Supplement™ and Evlution Nutrition Trans4orm Thermogenic Fat Burner™	21-year-old man	3 capsules twice per day of the weight loss supplement containing 250 mg GTE (EGCG content unknown) and, 2 capsules twice per day of the fat burner containing 500 mg GTE (standard minimum of 50% EGCG).	8-weeks.	Acute hepatitis. It was found that the weight loss supplement also contained Garcinia cambogia, which has been reported to cause hepatotoxicity, which according to authors may have had a synergistic effect.	Popovic et al., (2018)
Commercially available fat burner containing GTE	52-year-old woman	Product contained GTE with unknown concentration of EGCG.		Presented with hepatitis and cholestatic idiosyncratic liver injury.	Gavrić et al., (2018)
Chili Burn™	57-year-old woman	972 mg GTE (standardised to ECCG 30%) per 2 tablets.	10 weeks; consumed 85 pills in total.	Presented with hepatitis idiosyncratic liver injury.	Gavrić et al., (2018)
SlimCut	The same woman (as above) now 62-year-old	GTE supplement containing 45% EGCG.	One month; consumed 60 pills in total.	Presented with the same type of liver injury as at the previous admission.	Gavrić et al., (2018)

EGCG	>30 years old (n=92; n=47 in EGCG treatment group and n=45 in placebo group)	400 mg EGCG per capsule in treated group; 400 mg mannitol in placebo group.	Orally once daily for 4-weeks, then one capsule twice daily for 4-weeks, and then one capsule three times daily for 40 weeks. After 48 weeks, all patients underwent a 4-week wash-out period.	EGCG was overall well tolerated but was associated with hepatotoxic effects in some patients (n=8/47). The authors state that doses of more than 1,200 mg should not be used.	Levin et al., (2018)

*Clomiphene citrate and letrozole are ovarian stimulation medication which was started between cycle days 2-5 for subjects randomised into these treatment groups.

† UGT1A4 – uridine 5’-diphospho-glucuronosyltransferase 1A4 is an enzyme of the glucuronidation pathway that transforms small lipophilic molecules such as steroids, bilirubin, hormones and drugs, into water-soluble, excretable metabolites.

Abbreviations: ALT - Alanine transaminase; ASR - Aspartate aminotransferase; dGTE – Decaffeinated green tea extract; DNA - Deoxyribonucleic acid; EGCG - epigallocatechin-3-gallate; GTC – Green tea catechins; GTE – Green tea extract; HILI – Herb-induced liver injury.