Safety assessment on levels of (-)-Epigallocatechin-3-gallate (EGCG) in green tea extracts used in food supplements
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Background and terms of reference as provided by the Norwegian Food Safety Authority

Green tea in food supplements

Green tea extracts are popular ingredients in food supplements. In the past few years there have been several reports linking the consumption of food supplements containing green tea extracts to negative health effects. The food administrations in Norway, Sweden and Denmark have over the past years received several case reports of liver toxicity after consumption of food supplements containing green tea extracts. In 2014 alone, the Regional Medicines Information and Pharmacovigilance Center in Norway (RELIS) received four case reports linking the consumption of food supplements containing green tea extracts to hepatitis.

With this background, The Norwegian Food Safety Authority (NFSA) requests the Norwegian Institute for Public Health (NIPH) to draft an opinion on green tea extracts in food supplements based on the enclosed reports on green tea and green tea extracts and to respond specifically to the following question:

What levels of (-)-Epigallocatechin-3-gallate (EGCG) are considered safe for daily use in food supplements?

In this opinion we also request NHP to highlight other possible safety concerns associated with consumption of green tea extracts.

Literature as provided by the Norwegian Food Safety Authority


6. Safety of green tea powder in food supplements – ANSES, France (2012). *This document was translated to English using Google translate.*
7. Risk of hepatotoxicity associated with the consumption of foods containing green tea – ANSES, France (2012)
   *This document was translated to English using Google translate.
   *This document was translated to English using Google translate.
Introduction

Green tea is produced from the leaves from *Camellia sinensis* (L.) Kuntze. Mainly three different traditional tea products are prepared from the leaves and the leaf buds: Green tea, black tea and oolong tea. Green tea is produced without fermentation and thus preventing oxidation of the polyphenolic components. Black tea manufacture is carried out by fermentation ensuring a high degree of enzymatically catalyzed aerobic oxidation of the polyphenols followed by a series of chemical condensations. Oolong tea is a semi-fermented tea, where polyphenols are partially oxidized (EFSA, 2009).

Traditional green tea infusion is prepared as follows: To preserve the leaf catechins (a type of polyphenols) after harvest, an enzyme deactivation is performed by rapid steaming (Japanese green tea) or pan firing/roasting (Chinese green tea) the leaves before rolling and high temperature air drying. Depending on the quality of green tea, the recommendations for preparing traditional green tea infusions vary in amounts of green tea and water used (usually 0.75-1.5 g green tea/100 ml), temperature of water (50-100 °C, usually sub-boiling), brewing time (30 sec–3 min) and the possibility of a repeated extraction (e.g. recommendation to discard the first and consume the second extraction) (EFSA, 2009).

Commercial preparations of green tea extracts use various extraction techniques and manufacturing procedures and are not uniform. They may differ from the traditional green tea infusion, not only in the deprivation of water, but also e.g. in the solvent being different from water, in the source (e.g. fresh leaves instead of green tea), in extraction conditions (e.g. degree of comminution, concentration ratios, temperature, duration, stirring) and in fractionation procedures concentrating active compounds. Some tea extract powders or dry extracts are made by spray drying strong infusions obtained by soaking tea leaves in ethanol/water mixtures after they have been concentrated to 40-50% solids (EFSA 2009).

This safety assessment of (-)-Epigallocatechin-3-gallate (EGCG) from green tea extracts used in food supplements is based on the literature provided by the Norwegian Food Safety Authority. Thus, this assessment is not a complete risk assessment on the use of ECGC from green tea extract in food supplements, nor an exhaustive review of toxicological studies on ECGC from green tea extract. The assessment has been performed by the Department of Food, Water and Cosmetics at the Norwegian Institute of Public Health.

Oslo, 12.11.2015

Ragna Bogen Hetland, Senior Scientist

Hubert Dirven, Department Director
Chemical composition of green tea

It has been demonstrated that the content of green tea shows great variability. Plant variety, growing environment, season, age of leaves and manufacturing conditions of the traditional green tea have a pronounced impact on the composition.

Traditional green tea leaves contain a diversity of polyphenolic compounds, which account for up to 30% of the dry weight of the leaves. Most of the polyphenols in green tea are flavonoids of the subclass flavan-3-ols, commonly known as catechins. The primary catechins in green tea are (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG). Furthermore, (+)-catechin (C), (+)-gallocatechin (GC), (-)-gallocatechingallate (GCG), (+)-catechingallate (CG) occur in green tea. An overview of the major flavonol-3-ols in the leaves and in the infusion is listed in table 1. The main purine alkaloid in green tea leaves is caffeine (2.9-4.2%). Small amounts of the purine alkaloids theobromine (0.15-0.2%) and theophylline (0.02-0.04%) are also present. The total amino acids content in green tea leaves amounts to 4%, including the tea characteristic L-theanine as a major component (2% of green tea) (EFSA 2009).

Table 1. Mean and range (in brackets) of flavonoid content in Green tea leaves and Green tea infusion (EFSA, 2009).

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Green tea leaves (mg/100 g)</th>
<th>Green tea infusion (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-Epigallocatechin-3-gallate (EGCG)</td>
<td>7116 (1600 - 20320)</td>
<td>77.8 (2.31 - 203)</td>
</tr>
<tr>
<td>(-)-Epigallocatechin (EGC)</td>
<td>2058 (100 - 5477)</td>
<td>16.7 (1.0 - 54.4)</td>
</tr>
<tr>
<td>(-)-Epicatechin-3-gallate (ECG)</td>
<td>1491 (340 - 4630)</td>
<td>19.7 (4.3 - 140)</td>
</tr>
<tr>
<td>(-)-Epicatechin (EC)</td>
<td>812 (190 - 2000)</td>
<td>8.29 (1.9 - 26.0)</td>
</tr>
<tr>
<td>(+)-Gallocatechin-3-gallate (GC)</td>
<td>7.1 (0 - 14.1)</td>
<td>Not reported</td>
</tr>
<tr>
<td>(+)-Gallocatechin (GC)</td>
<td>258 (69.5 - 447)</td>
<td>1.54 (no range reported)</td>
</tr>
<tr>
<td>(+)-Catechin (C)</td>
<td>57.1 (0 - 253)</td>
<td>2.55 (0 - 44.4)</td>
</tr>
</tbody>
</table>

The concentration of components in traditional green tea infusion is strongly dependent on how the infusion is prepared by the consumer (amount of tea and water, brewing time and agitation). Furthermore, the content is affected by the grade of comminution of the tea leaves and if they are contained in a tea bag (EFSA, 2009).

The concentrations of components in dried green tea extracts vary widely, depending on the source material and the extraction procedure (e.g. extraction solvent). EGCG is a polar substance, and it is soluble both in water and in ethanol-water mixtures. The chemical structure of EGCG is shown in figure 1. Commercial preparations that contain enriched quantities of polyphenolics (60 – 80% or more of dry weight), with EGCG particularly prominent in the mixture, are available. No specifications for green tea extracts are available. It is suggested that the future specification for green tea extracts should include the different catechins (above all to EGCG), caffeine and L-theanine.)
Use of green tea extract in food

Dried green tea extracts are used in food as food supplements and in beverages. With respect to food supplements, the exposure to green tea components may vary considerably depending on the composition of the actual product and the daily dose recommended by the food supplement manufacturers/providers (EFSA, 2009).

Data from food authorities in the Nordic countries suggest that the daily doses recommended by the food supplement manufacturers/providers may be up to 1944 mg for green tea extracts and up to 980 mg for the major catechin EGCG (DTU, 2015).

Absorption, distribution, metabolism and excretion (ADME) of EGCG

In general, the metabolism of catechins follows the same pathway in mice, rats and humans. The observed differences between the species are on quantitative differences between individual metabolites. After oral absorption, the main biotransformation pathways of EGCG are glucuronidation, sulphatation and O-methylation (EMA, 2013).

Human data

After ingestion of green tea extract by human volunteers, plasma EGCG was mainly found in the free form (64 – 77%) whereas EGC and EC were conjugated to a higher degree. Data on tissue distribution and excretion of EGCG (and other green tea catechins) in humans were not available (EFSA, 2009).

In a randomized, placebo-controlled study, EGCG or decaffeinated extract of green tea containing 60% EGCG was orally administered once daily to human volunteers at a dose of 800 mg EGCG/day for 4 weeks. Adverse events reported during the treatment period included nausea, stomach ache, dizziness and muscle pain. However, all events were rated as mild, and the daily dose of 800 mg was regarded as safe and well tolerated. Both catechin formulations demonstrated similar EGCG kinetics on the last treatment day (e.g. for the decaffeinated extract $C_{\text{max}}$ 287.6 ± 124.2 ng/ml; $t_{\text{max}}$ 248 ± 184.9 minutes; half-life 163.0 ± 56.2 minutes). Furthermore, after 4 weeks of chronic administration of a high daily bolus dose (800 mg EGCG or the decaffeinated extract), there was an > 60% increase in the systemic availability of free ECGC (measured as area under the plasma EGCG concentration-time curve, AUC) as compared to the onset of the study. A similar increase in bioavailability was not observed when 800 mg
EGCG/day was administered in two doses/day (400 mg twice a day). The elimination half-lives of EGCG did not indicate accumulation (Chow et al., 2003; in EFSA, 2009).

Other studies in healthy volunteers also showed that decaffeinated extract administered as a single dose (800 mg EGCG/day) was rather well tolerated (reports of mild and transient nausea) when the extract was taken with food. Notably, the $C_{\text{max}}$ of free EGCG in the fasting condition was more than five times that obtained after administration of the same dose with food (Chow et al., 2005; in EFSA, 2009).

The kinetic parameters of EGCG (50 mg, 100 mg, 200 mg, 400 mg, 800 mg, 1600 mg) or placebo was investigated in 50 healthy male subjects in a randomized, double-blind, placebo-controlled study. It was reported that 1600 mg EGCG administered under fasting conditions resulted in a peak plasma concentration of 3300 ng/ml after 1.3 – 2.2 hours (Ullmann et al., 2003, in EFSA 2009).

In a second randomized, double-blind, placebo-controlled study with repeated oral administration, male test persons received one capsule with EGCG (purity 94%) every day (doses: 0, 200, 400 or 800 mg/day) for ten days after fasting overnight. Kinetic parameters were determined on treatment day 1 and day 10. The EGCG was quickly absorbed, and a linear increase in systemic availability depending on the dose was observed for all tested doses at day 1. After repeated administration (day 10), a dose proportionality was only observed for the two lower dose groups, whereas increasing the dose to 800 mg/day led to a steeper increase in systematic availability. The results indicated a dose-dependent saturation of capacity-limited excretion routes or an increase in hepatoduodenal re-circulation. However, it was assumed that EGCG did not accumulate, since an accumulation factor < 1 was calculated for all dose groups. Comparison of the kinetic parameters measured on day 1 and day 10 showed a decrease in mean values of systemic availabilities and maximum plasma concentrations for free EGCG at the dose of 200 mg/day ($C_{\text{max}}$ (ng/ml): 327.4 (day 1); 259.5 (day 10)), whereas the corresponding values increased at the doses of 400 mg/day ($C_{\text{max}}$ (ng/ml): 504.0 (day 1); 704.5 (day 10)and 800 mg/day ($C_{\text{max}}$ (ng/ml): 2268.8 (day 1); 2800.2 (day 10)). The authors indicated that the doses were well tolerated by the test persons (Ullmann et al., 2004, in EFSA 2009).

**Animal data**

When beagle dogs were given an oral, single dose of 250 mg/kg body weight of radiolabeled EGCG, approximately 20% of EGCG was absorbed systemically compared with 1.6% - 14% in rats (EMA, 2013, EFSA, 2009).

The effect of food intake on green tea availability and related safety parameters has been investigated in a study on beagle dogs (Isbrucker et al., 2006a, in EFSA, 2009). No adverse effects were noted when pre-fed dogs were administered 500 mg green tea preparation (91.8% EGCG)/kg body weight per day, in divided doses, for 13 weeks (6/gender and group). However, when 500 mg green tea preparation (80% EGCG)/kg body weight per day was administered as a single bolus to fasted dogs (4/gender and group), severe liver damage (liver necrosis) and lethality was demonstrated. Furthermore, maximum plasma levels of free EGCG were approximately ten times higher in fasted than in pre-fed dogs at the dose level of 500 mg/kg body weight per day ($C_{\text{max}} = 55.6 \mu g/ml$ versus $C_{\text{max}} = 5.75 \mu g/ml$). It was also demonstrated that systemic availability of EGCG, particularly in the highest dose group, was higher at the end of the study than in the beginning.
In an additional study, fasting and pre-fed beagles were given an EGCG preparation (78% purity) at a dose of 300 mg/kg body weight per day for two weeks. On day 14 of treatment, the observed plasma concentration of EGCG was 10-fold higher in the fasted animals than in the pre-fed animals (Isbrucker et al., 2006, in EFSA, 2009).

The kinetics of EC, EGC and EGCG has been studied after repeated oral administration in male Sprague-Dawley rats for 28 days. Green tea extract (86, 76 and 590 mg/g EC, EGC and EGCG, respectively) was given in drinking water at 0.1% and 0.6% (w/v) ad libitum. Although EGCG was the major catechin in the dosing solution (about 7-times more EGCG than EC or EGC), considerable lower systemic total EGCG concentration was achieved. After administration of the 0.6% solution, it was also noted that no EGCG was detected in the urine over the entire observation period of 21 days. However, EGCG was the dominating catechin in faeces (Kim et al., 2000, in EMA, 2013).

When rats were given an oral dose 14C-labelled EGCG at a dose of 50 mg/kg body weight, 11.9% of cumulative radioactivity was found in the urine and 78.3% in the faeces within 96 hours (EFSA, 2009).

Biodistribution data obtained after gastric intubation of mice with (3H)EGCG demonstrated that EGCG is widely distributed to various organs and that liver is one of the target organs of EGCG. A second administration after a 6 hours interval enhanced the concentration of radioactive EGCG in blood, liver, pancreas, bladder and bone 4 -6 times compared to values after a single administration. It has also been observed that excretion of EGCG in mice predominantly occurs via faeces, only about 0.6% was excreted via the urine (EMA, 2013, EFSA, 2009).

In summary, the metabolism of catechins follows the same pathway in mice, rats and humans. The provided literature did not refer to any data on absorption after oral exposure in humans. However, absorption of approximately 20% was demonstrated in beagle dogs after an oral, single dose of 250 mg/kg body weight of radiolabeled EGCG (information if fasted or pre-fed was not reported). In rats, absorption has been reported to be 1.6% - 14%. It has also been observed that the excretion of EGCG in rats and mice predominantly occurs via faeces. Data on tissue distribution and excretion is not available for humans. However, bio-distribution data from mice demonstrated that EGCG is widely distributed to various organs, and the liver is one of the target organs. Comparison of plasma concentrations of free EGCG in humans demonstrated a dose-dependent linear increase in systemic availability for all tested doses at day 1 of treatment. After repeated treatment (10 days or 4 weeks), however, a steeper increase in systematic availability was demonstrated at the higher concentrations, thus indicating an increased bioavailability at higher doses. The above mentioned human data also show that administration of green tea extract under fasting conditions, and as a bolus, leads to a significant increase in plasma concentration and bioavailability of EGCG compared to administration with food and in split doses, respectively. This effect is supported by studies in beagle dogs showing a ten-fold increase in plasma concentration of EGCG after administration of EGCG to fasting dogs compared to pre-fed dogs.
Toxicological data on EGCG

Human studies

According to a randomized, placebo-controlled study in healthy volunteers (eight subjects per group) EGCG or a decaffeinated extract of green tea containing 60 % EGCG, orally administered once daily at a dose of 800 mg EGCG/day for 4 weeks was regarded as safe and well tolerated. Adverse events reported during the 4-week treatment period include nausea, stomach ache, dizziness, and muscle pain. All of the reported events were rated as mild events. No significant changes were observed in blood counts or blood chemistry profiles after repeated administration of green tea polyphenol products (Chow et al., 2003, in EFSA 2009). Other studies described in the previous paragraph on ADME of EGCG also reported mild effects at 800 mg/day (Chow et al., 2005, Ullmann et al., 2004; in EFSA 2009). However, results also showed that administration of concentrated green tea extracts under fasting conditions and as a bolus lead to a significant increase of plasma concentrations and bioavailability of EGCG compared to administration with food or in split doses respectively (Chow et al., 2005, Ullmann et al., 2003; in EFSA, 2009). The ESCO Working Group noted that the design of the referred studies (e.g. small number of persons per group, short exposure period) did not allow the detection of any adverse effects other than those very common. The Working Group also noted that, although these clinical trials with dose levels up to 800 -1600 mg EGCG /day did not reveal serious adverse effects, the studies were not designed to investigate the safety of EGCG.

Case reports

The food administrations in Norway, Sweden and Denmark have received several case reports on liver toxicity after consumption of food supplements containing green tea extract, as presented in a recent report by The National Food Institute, Technical University of Denmark (DTU, 2015). Four of these case reports were received by the Regional Medicines Information and Pharmacovigilance Center in Norway (RELIS) in 2014 alone. The reported cases where the content of EGCG was reported are summarised in table 2. For more information on the supplements, see Annex 1.
<table>
<thead>
<tr>
<th>Description of Food supplement</th>
<th>Recommended intake</th>
<th>Reported intake – total daily dose</th>
<th>Observations</th>
<th>Comments</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Aqueous extract from dried, heat-treated leaves from <em>C. sinensis</em> (only ingredient).</td>
<td>Up to 3 tablets per day (up to 210 mg EGCG)</td>
<td>2 tablets per day (total of 140 mg EGCG) for 10 months (61-year-old woman).</td>
<td>Nausea and deterioration of general condition. Elevated ALAT, LDH and bilirubin. Returned to normal six weeks after cessation of intake</td>
<td>Tablets also contained 40 mg caffeine/tablet</td>
<td>DTU, 2015</td>
</tr>
<tr>
<td>B (version 1): Water/ethanol extract from leaves of <em>C. sinensis</em> L. (1944 mg with 30% EGCG).</td>
<td>2 tablets twice a day for 14 days (583.2 mg EGCG), thereafter 1 tablet twice a day (taken with water in connection with a meal)</td>
<td>4 tablets per day (583.2 mg EGCG) for 14 days (one woman).</td>
<td>Fatigue, fever with shivering, feeling cold, elevated liver counts when hospitalised</td>
<td>4 tablets also contained 194 mg caffeine</td>
<td>DTU, 2015</td>
</tr>
<tr>
<td>B (version 2): Extract from leaves of <em>C. sinensis</em> L. (972 mg with 30% EGCG).</td>
<td>2 tablets per day (291.6 mg EGCG)</td>
<td>1 – 2 tablets per day (145.8 – 291.6 mg EGCG) for 3 weeks to 6 months (six women 32 – 66 years of age, one man 65 years of age).</td>
<td>Toxic hepatitis in a total of 7 cases in 2014, two additional cases after publication in 2014. Probable causal relationship was considered in four cases, possible causal relationship was considered in one case.</td>
<td>Causality based on the Roussel Uclaf Causality Assessment Method (RUCAM) in Drug Induced Liver Injury</td>
<td>RELIS 2014 in DTU, 2015</td>
</tr>
<tr>
<td>B (version 3): Extract from leaves of <em>C. sinensis</em> L. (600 mg with 30% EGCG).</td>
<td>2 tablets per day (180 mg EGCG)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C: Green tea extract</td>
<td>1400 mg green tea extract per day (980 mg EGCG)</td>
<td>1400 mg green tea extract (980 mg EGCG) for 9 weeks (50-year-old woman)</td>
<td>Stomach pain, highly elevated liver enzymes and elevated EBV-viral antibodies IgG. Chronic active hepatitis was suggested based on liver biopsy.</td>
<td>The woman had been on a low-carb-diet and also been taking other supplements.</td>
<td>DTU, 2015</td>
</tr>
</tbody>
</table>

As referred by EFSA (EFSA, 2009), the Dietary Supplements Information Expert Committee (DSI-EC) critically analysed the adverse event reports on liver damage using the Naranjo causality algorithm to assess the likelihood that exposure to green tea products caused hepatotoxicity. Analyses of events were performed according to several defined criteria in order to range causation as doubtful or unlikely, possible, probable and definite or certain (Naranjo *et al.*, 1981, Sarma *et al.*, 2008; in EFSA, 2009). Case reports where levels of EGCG were reported are summarized in table 3.
Table 3. Other case reports of liver injury after intake of food supplements containing known levels of EGCG from green tea extract (as described by EFSA, 2009; DTU, 2015).

<table>
<thead>
<tr>
<th>Description of food supplement</th>
<th>Number of cases and duration</th>
<th>Intake</th>
<th>Observations</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exolise® (hydroalcoholic green tea extract, standardised to 25% EGCG). Exolise was the only therapy in four cases.</td>
<td>12 females, 27-59 years, 1 male, 32 years 9 days-5 months. Average 50 days.</td>
<td>2-5 capsules/day (~187.5-468.75 mg EGCG/day) Daily dose recommended by provider corresponds to 375 mg EGCG.</td>
<td>Liver injury. 6/13 cases were hospitalized. One case needed liver transplantation (concomitant intake of several medications and alcohol use). Recovery (data from 9 cases): 15 days-3 months.</td>
<td>Naranjo scale rating possible causality: probable causality (2 cases), possible causality (11 cases) The product was marketed as a weight-loss product (suspended in 2003).</td>
<td>Sarma et al. 2008 (in EFSA, 2009)</td>
</tr>
<tr>
<td>Green LiteTM, Polyphenon® (hot water extract of green tea, decaffeinated) used for weight loss. Medication: medroxyprogesterone injections, 150 mg every 3 month over the previous few years.</td>
<td>1 female, 42 years 6 months</td>
<td>600 mg catechines with 65% EGCG (~390 mg EGCG/day)</td>
<td>Jaundice, biopsy: toxic hepatitis. Her condition deteriorated. A liver transplant was performed 17 days after admission. The patient recovered.</td>
<td>Naranjo scale rating: possible causality Abnormal liver function can be a serious side effect of the treatment with medroxyprogesterone.</td>
<td>Sarma et al. 2008, Canadian Adverse Reaction News-letter, 2007 (in EFSA, 2009)</td>
</tr>
<tr>
<td>Mega Green Tea Extract (decaffeinated green tea leaf extract standardized to 98% polyphenols (710.5 mg) and to 45% EGCG (326.25 mg). Oral medication: anastrozole (1 mg for 5 years), ramipril (2.5 mg for 3 years), oxibutynine (5 mg for 15 years), vitamin D3 supplementation (for 1 year).</td>
<td>1 female, 63 years, weight 72 kg 45 days</td>
<td>710.5 mg polyphenols, 326.25 mg EGCG daily</td>
<td>Jaundice, lassitude, mild pruritus and discoloration of stool and urine. Cessation of all medication and food supplement intake 4 days prior to hospitalization. Liver biopsy: indicative of a toxic mechanism. 40-fold elevated transaminases. Patient’s condition improved. Seven month after discharge, medication was re-started (apart from anastrozole). Liver enzymes stayed within normal levels. No re-exposure to the food supplement.</td>
<td>RUCAM1 score: causality probable.</td>
<td>Pillukat et al. 2014 (in DTU, 2015)</td>
</tr>
</tbody>
</table>
In France, a health monitoring system, The French Nutrivigilance system, which aims to identify potential adverse effects associated with consumption of food supplements, and some other product categories, has been established under the responsibility of the French Agency for Food, Environment and Occupational Health Safety (ANSES). During the period 2009 – 2011, seventeen alerts were received related to hepatitis following consumption of products containing green tea. Of the reported cases, sixteen were related to food supplements and one to green tea consumed as tea. The cases were critically examined by ANSES in order to determine whether green tea was the cause of the reported hepatitis. Causality score was based on method described by ANSES (ANSES 2011, in ANSES, 2012). In seven of the reported cases, causality was classified as probable, in four cases causality was considered possible and in five cases causality was considered doubtful (ANSES, 2012).

In summary, the referred clinical studies with dose levels up to 800 -1600 mg EGCG /day did not reveal serious adverse effects; only mild effects not related to liver toxicity were reported. However, the results showed that administration of concentrated green tea extracts under fasting conditions and as a bolus, lead to a significant increase of plasma concentrations and bioavailability of EGCG compared to administration with food or in split doses, respectively. With respect to the case reports, consumption of food supplements resulted in effects on the liver at dose levels of EGCG from 140 mg/day to 980 mg/day. Thus, the two lowest dose levels reported to result in liver injury were 140 and 187.5 mg EGCG/day, corresponding to 2.33 and 3.13 mg EGCG/kg bw per day for a 60 kg person. The commercial preparations were quite different with respect to manufacturing procedures and the chemical composition, but all contained EGCG. Furthermore, the conditions under which the supplements were taken, e.g. with food or in a fasting state, are not disclosed.

**Animal and in vitro studies**

**General toxicity**

Summaries of toxicity studies in beagle dogs, rats and mice, where a NOAEL has been derived, are presented in tables 4a, 4b and 4c. Studies included in the tables are described by EFSA (EFSA, 2009) and/or EMA (EMA, 2013).
Table 4a. Summary of subchronic toxicity studies in beagle dogs where a NOAEL for EGCG is determined.

<table>
<thead>
<tr>
<th>Material tested</th>
<th>Type of study and doses of EGCG</th>
<th>Observations</th>
<th>NOAEL (mg EGCG/kg body weight per day)</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGCG preparation (91.8% purity)</td>
<td>Given at 0, 50, 300 or 500 mg/kg bw per day</td>
<td>13 weeks oral study in pre-fed beagles (6/group). Doses divided into two doses per day.</td>
<td>460</td>
<td>Extraction method not specified.</td>
<td>Isbrucker et al., 2006a (in EFSA, 2009 and EMA, 2013)</td>
</tr>
<tr>
<td>EGCG preparation (80% purity)</td>
<td>Given at 0, 50, 150 or 500 mg/kg bw per day</td>
<td>13 weeks oral study in fasting (for 15 hours) beagles (4/group). Doses given as single boluses.</td>
<td>40 (based on the observed effects in animals who died or were killed)</td>
<td>Extraction method not specified.</td>
<td>Isbrucker et al., 2006a (in EFSA, 2009 and EMA, 2013)</td>
</tr>
<tr>
<td>Polyphenol fraction of green tea containing 53.4% EGCG, 11.4% EGC, 9.1% EC, 5.1% GCG and 4.9% ECG</td>
<td>13 weeks oral study in beagle dogs (4/group).</td>
<td>No effects specified in EFSA, 2009 or EMA, 2013.</td>
<td>≥ 320</td>
<td></td>
<td>Johnston et al., 1999, abstract only (in EFSA, 2009 and EMA, 2013)</td>
</tr>
</tbody>
</table>
Table 4b. Summary of subchronic toxicity studies in rats where a NOAEL for EGCG is determined.

<table>
<thead>
<tr>
<th>Material tested</th>
<th>Type of study and doses of EGCG</th>
<th>Observations</th>
<th>NOAEL (mg EGCG/kg body weight per day)</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green tea extract composed of GC (0.52%), EGC (2.26%), catechin (0.51 %), EC (2.83%), CG (0.45%), caffeine (4.99%), EGCG (48.4%), and ECG (12.8%). Given at 0, 62.5, 125, 250, 500 or 1000 mg extract/kg bw per day 5 days a week</td>
<td>14 weeks feeding study in rats (10/gender and dose). 0, 30.25, 60.5, 121.0, 242.0 or 484.0 mg EGCG/kg bw 5 days per week by gavage.</td>
<td>Treatment related histopathological changes were seen in the liver, nose, mesenteric lymph nodes and thymus. 242 (based on liver effects in both males and females). 30.25 for males and &lt; 30.25 (lowest dose) for females (based on histopathological changes in the nose cavity in females)</td>
<td>242 (based on liver effects in both males and females). 30.25 for males and &lt; 30.25 (lowest dose) for females (based on histopathological changes in the nose cavity in females)</td>
<td>Extracted in deionized water.</td>
<td>Chan et al., 2010 (in EMA, 2013)</td>
</tr>
<tr>
<td>EGCG preparation (77% purity)</td>
<td>13 weeks feeding study in rats (10/gender and dose). 0, 50, 150 or 500 mg EGCG/kg bw per day in food.</td>
<td>No treatment related adverse effects recorded. Increased levels of bilirubin were observed at the highest dose. 500 (150 if increased bilirubin is regarded as treatment related)</td>
<td>500 (150 if increased bilirubin is regarded as treatment related)</td>
<td>Bioavailability in rats referred to be 0.003 – 0.45 % of the ingested dose. Extraction method not specified.</td>
<td>Isbrucker et al., 2006a (in EFSA, 2009 and EMA, 2013)</td>
</tr>
<tr>
<td>Polyphenol fraction of green tea containing 32.1% EGCG, 17.7 % EGC, 8.5 % EC, 3.3 % GCG, 10.7% ECG, and 1.4% CG. A total of 66.2% catechins were administered.</td>
<td>13 weeks oral study in rats (10/gender and group). 0, 141, 283, 566 or 1132 mg EGCG/kg bw per day by the diet</td>
<td>Increase in mean thyroid weight and marked histological lesions at the highest dose. Hypertrophy and hyperplasia in thyroid follicles also at 283 and 566 mg EGCG/kg the in males and at 566 mg EGCG/kg in females. 141 (males) and 283 (females) based on histological changes of the thyroid</td>
<td>141 (males) and 283 (females) based on histological changes of the thyroid</td>
<td></td>
<td>Sakamoto et al., 2001 (in EFSA, 2009)</td>
</tr>
<tr>
<td>EGCG (purity not specified)</td>
<td>13 weeks oral study in rats (20/gender and group). 0, 45, 150 or 500 mg EGCG/kg bw per day by gavage.</td>
<td>Body weight gain, food consumption, relative and absolute weight of thymus and spleen were dose-related reduced. 48</td>
<td>150 (both males and females) based on histopathology (liver, pancreas, thymus) 45 (males) based on reduced body weight gain and decreased absolute and relative thymus weights</td>
<td></td>
<td>McCormic et al., 1999, abstract only (in EFSA, 2009 and EMA, 2013)</td>
</tr>
<tr>
<td>Polyphenol fraction of green tea containing 53.4% EGCG, 11.4% EGC, 9.1 % EC, 5.1 % GCG and 4.9% ECG</td>
<td>13 weeks oral study in rats (20/gender and group). 0, 48, 160 or 534 mg EGCG/kg bw per day by gavage.</td>
<td></td>
<td>The basis for NOAEL is not specified</td>
<td></td>
<td>Johnson et al., 1999, abstract only (in EFSA, 2009 and EMA, 2013)</td>
</tr>
</tbody>
</table>
Table 4c. Summary of a subchronic toxicity study in mice where a NOAEL for EGCG is determined.

<table>
<thead>
<tr>
<th>Material tested</th>
<th>Type of study and doses of EGCG</th>
<th>Observations</th>
<th>NOAEL (mg EGCG/kg body weight per day)</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green tea extract composed of GC (0.52%), EGC (2.26%), catechin (0.51%), EC (2.83%), CG (0.45%), caffeine (4.99%), EGCG (48.4%), and ECG (12.8%). Given at 0, 62.5, 125, 250, 500 or 1000 mg extract/kg bw per day 5 days a week</td>
<td>14 weeks feeding study in mice (10/gender and dose). 0, 30.25, 60.5, 121.0, 242.0 or 484.0 mg EGCG/kg bw) 5 days per week by gavage.</td>
<td>Treatment related mortality occurred in both genders at highest dose. Histopathological changes were seen in the liver, nose, mesenteric lymph nodes and thymus, as well as changes in Peyer’s patches, spleen and mandibular lymph nodes.</td>
<td>242 (based on liver effects in both males and females). &lt; 30.25 (lowest dose) (based on histopathological changes in the nose cavity in males)</td>
<td>Extracted in deionized water.</td>
<td>Chan et al., 2010 (in EMA, 2013)</td>
</tr>
</tbody>
</table>

As listed in tables 4 a - c, NOAELs derived for EGCG in subchronic animal studies range from 40 mg/kg bw per day in fasted beagle dogs up to ≥ 460 mg/kg bw per day in pre-fed dogs and in rats. The design of the different studies are not, however, completely comparable. In some studies, the daily dose of EGCG was given through the diet, in other studies the total daily dose was given in one or two administrations (gavage or capsule(s)). Furthermore, EGCG was in some studies given as a purified preparation, in other studies EGCG was part of an extract from green tea. It is also noted that in dogs, studies have been performed in both pre-fed and fasting animals. Results from these studies demonstrated that fasting reduced the derived NOAEL with a factor of 10 (from 460 to 40 mg/kg bw per day), indicating an increased susceptibility to green tea extract and EGCG under fasting conditions. It is also seen that when the daily dose of EGCG is given as a bolus, effects seem to occur at lower levels than when given in the general diet or in divided daily doses. With respect to liver toxicity, a NOAEL of 40 mg/kg bw per day was derived from the study in fasting beagle dogs given EGCG preparation as one daily oral administration. In two studies in rats given EGCG by gavage, NOAELS of 48 mg/kg bw and 45 mg/kg bw were derived.

**Reproduction and developmental toxicity**

EGCG preparations of >91% purity were administered to pregnant rats during organogenesis and development. Diets supplemented at 1400, 4200 or 14,000 ppm EGCG preparation during organogenesis were non-toxic to dams or foetuses. In a two-generation study where rats were fed 1200, 3600 or 12,000 ppm EGCG preparation, no adverse effects on reproduction or fertility were demonstrated. At the highest dose, growth rate of the offspring was reduced, and there was a slight increase in pup loss. A growth effect among pups was also seen at 3600 ppm, but in the second generation only. The authors derived a NOAEL equivalent to 200 mg/kg body weight per day EGCG preparation (Isbrucker et al., 2006b, in EFSA 2009).
**Genotoxicity and carcinogenicity**

Several studies on reproductive toxicity and genotoxicity have been reported. With the exception of a mouse lymphoma cell assay, the other *in vitro* and *in vivo* studies on the mutagenic potential of EGCG have not shown any evidence of mutagenic activity (EMA, 2013).

In a combined toxicity (12 months) and carcinogenicity (24 months) study, rats were fed a diet containing 0 to 0.3% catechins (of which EGCG constituted 43.6%). No significant increase in the incidence of any specific tumors was observed. After 12 months, no liver damage was observed for doses of catechins corresponding to 2000 mg/kg bw per day in rats (Yoshida *et al.* 2011, in ANSES, 2012).

**Exposure**

The daily consumption of green tea extracts from food supplements may vary considerably. Proposed levels of green tea preparations in food supplements offered for weight reduction purposes, may give rise to a daily intake of 150 mg caffeine, 115 – 270 mg EGCG and 375 mg catechins or higher (EFSA, 2009). For a 60 kg person this corresponds to an intake of 2.5, 1.9 – 4.5 and 62.5 mg /kg bw per day of caffeine, EGCG and catechins, respectively.

Data provided by NFSA suggests that the daily doses recommended by manufacturers/providers of food supplements may result in an intake up to 1944 mg of green tea extracts and up to 980 mg of EGCG (DTU, 2015). For a 60 kg person these levels correspond to 32.4 mg/kg bw per day of green tea extract and 16.3 mg/kg bw per day of EGCG.

**Assessment**

This assessment is based on the literature provided by NFSA, thus it is not a complete risk assessment on EGCG from food supplements.

The included human studies did not reveal any adverse effects on liver at dose levels up to 800 – 1600 mg EGCG /day, corresponding to 10 – 26.7 mg EGCG/kg bw per day. With respect to the case reports, causality of various grades between EGCG and liver toxicity was suggested. Such effects were reported at daily consumption of food supplements containing from 140 and up to 980 mg EGCG, with the lowest consumption level corresponding to 2.33 mg EGCG/kg bw per day for a 60 kg person.

**The proposed use levels of green tea extract reported by EFSA and NFSA may give rise to intakes of EGCG up to 4.5 mg/kg bw per day and 16.3 mg/kg bw per day, respectively. These values exceed the lowest level (2.33 mg/kg bw per day) at which liver toxicity caused by EGCG was reported in humans with a factor of 1.9 and 7.0.**

As suggested by EFSA (EFSA, 2009), the safety of extracts for use in food supplement may be assessed by applying the MOS (margin of safety) approach. The MOS, which is defined as the ratio between the NOAEL and the daily intake of the compound in question, should at least be 100, this being the uncertainty factor routinely used for non-genotoxic compounds. With respect to liver toxicity, a NOAEL of 40 mg/kg bw per day was derived from the study in fasting beagle dogs given EGCG preparation as one daily oral administration. In studies in rats given EGCG by gavage, NOAELS of < 30.25 - 48 mg/kg bw were derived (based on other observations than liver toxicity).
Based on the NOAEL of 40 mg/kg bw per day obtained in fasting dogs, and the proposed intake levels reported by EFSA and NFSA, the derived MOS is 8.9 and 2.5, respectively. This indicates that an intake of EGCG from food supplements at the proposed levels is higher than what is recommended using the MOS approach. In order to obtain a MOS of 100, daily exposure to EGCG should be 0.4 mg/kg bw per day or lower.

**Uncertainties**

Direct comparison between results from the different human studies and case reports are not possible. The commercial preparations containing EGCG differed widely, including manufacturing procedures, extraction solvent and variation in type and content of components additional to EGCG.

In the reported human studies, conditions under which the supplements were taken, e.g. with food or in a fasting state, were not disclosed.

There are indications from human experimental studies that plasma concentration and bioavailability of EGCG are considerably higher when taken in a fasting state than after food intake. This means that correspondingly lower doses have a toxic effect, which may be of great importance if EGCG is used as part of weight-loss programmes or in situations with reduced food intake.

There is little information about intestinal absorption of EGCG in humans when EGCG is given under various situations with respect to food intake. There is also an uncertainty with respect to absorption rates, metabolism and clearance in mice and rats compared to humans.

From the referred studies in dogs, it is noted that fasting reduced the derived NOAEL with a factor of 10 (from 460 to 40 mg/kg bw per day), indicating an increased susceptibility to green tea extract and EGCG under fasting conditions. It is also seen that when the daily dose of EGCG is given as one bolus, effects seem to occur at lower levels than when given in the diet or when the daily dose is divided in multiple doses.

The composition of the green tea extract/purity of EGCG in the commercial products is often unknown; therefor potential toxic effects of contaminants or combined toxic effects due to the presence of other components cannot be assessed.

**Conclusion**

Based on evaluation of the provided literature and taking into account the uncertainties mentioned, it is estimated that an intake of more than 0.4 mg EGCG/kg bw per day from food supplements taken as a bolus administration may cause adverse biological effects.

A major concern associated with consumption of green tea extracts is the increased plasma concentration and bioavailability of EGCG that has been demonstrated in humans and dogs taking food supplements during fasting conditions. There are indications on increased susceptibility to toxic effects when green tea extract is given after hours of fasting.

Commercial preparations containing EGCG differ widely, and the composition of the green tea extract or the purity of EGCG is often not known. This gives rise to uncertainty about potential toxic effects of contaminants which may be present, or potential combined toxic effects due to the presence of other components in the preparation.
References


Annex

Some more information on food supplements referred in Table 1: Case reports of liver injury reported to Nordic food authorities (as described in DTU, 2015).

Supplement A:

Content of recommended daily dose (up to 3 tablets): Aqueous extract from dried, heat-treated leaves from *C. sinensis* (only ingredient), up to 210 mg EGCG.

Supplement B (version 1):

Content of recommended daily dose (4 tablets): Water/ethanol extract from leaves of *C. sinensis* L. (1944 mg with 30% EGCG). Other ingredients: aqueous extracts of dill (600 mg), ginger (200 mg), fruits of *Capsicum annuum* L. (48 mg) and peppermint (18 mg).

Supplement B (version 2):

Content of recommended daily dose (2 tablets): Extract from leaves of *C. sinensis* L. (972 mg with 30% EGCG). Other ingredients: extract from *Capsicum annuum* L. (24 mg), vitamins (vit. B1, B2, B6, B12, B3, B5 and minerals (magnesium and chrome).

Supplement B (version 3):

Content of recommended daily dose (2 tablets): Extract from leaves of *C. sinensis* L. (600 mg with 30% EGCG). Other ingredients: extract from *Capsicum annuum* L. (28.8 mg), choline, vitamins (vit. B1, B2, B6, B12, B3, B5 and minerals (magnesium and chrome).