Key studies described by EFSA in their Opinion

In this guide

Intervention studies

46. The EFSA Panel evaluated a total of 49 intervention studies on green tea preparations, which included green tea infusions (4 studies) and GTEs (45 studies). No intervention studies in pregnant women, breastfeeding infants or children were identified. Following a review of the 49 studies evaluated, 9 studies reported elevated liver transaminases. However, two of the studies considered did not include a control group and were thus excluded. In the 7 remaining studies showing a higher incidence of abnormal liver parameters, doses of EGCG were 800 mg or above. The total number of treated participants receiving doses at or above 800 mg EGCG per day in the studies was 724. Of the reviewed studies, six covered a duration of between 4 and 12 months. Reported EGCG content in catechin extracts used ranged from 56 – 72% in Polyphenon E a decaffeinated extract of green tea containing 64% EGCG (Dostal et al., 2015, Yu et al., 2017) to pure EGCG (Ullmann et al., 2004).

47. Of the studies that reported no effects on liver parameters (26 studies: 1 used infusion, and 25 used extracts), the EGCG doses ranged from 10 to 857 mg per day ranging over a period of 10 days to 12 months. Of the studies using GTEs, 14 of the studies were at a dose of 316 mg EGCG per day or less (n=756 subjects) and 377 mg – 800 mg EGCG per day in 4 studies.

48. The COT considered that despite the weighting of data, with most studies using 300 mg dosage of EGCG per day or less, it is sufficient to show that the use of green tea herbal infusions is relatively safe compared to GTEs.

49. 756 subjects across 29 studies treated with GTEs at levels of 316 mg EGCG per day or less, exhibited no elevation in serum transaminase levels. The EFSA Panel noted that in many cases, elevated transaminase levels returned to normal after dechallenge and increased following rechallenge, suggesting a relationship between exposure to GTE and liver effects.

50. With regard to the consumption of GTCs from herbal infusions, the EFSA Panel noted “The sparse data on green tea exposure from traditional green tea infusions and noted that there was no evidence of elevated ALT levels at a consumption of green tea infusion of ≥ 5 cups per day or containing 700 mg EGCG (–)- per day.” Elsewhere in the EFSA Opinion (pp.44), it is clarified that in order to consume 700 mg EGCG from green tea infusions, the consumption would be greater than or equal to 5 cups of green tea per day.

Human case reports of liver toxicity

51. The EFSA ANS Panel considered reports on the association of GTEs (in supplements) with hepatotoxicity, noting that many studies were focused on the purported beneficial effects of green tea on the liver. Such studies were outside the remit of the Panel and the scope of the mandate for the Opinion in question and thus, were not considered.

52. Several cases of liver toxicity were reported with the use of weight loss supplements containing GTE. In 2003, the weight loss supplement Exolise® was withdrawn from the market following 13 cases of hepatotoxicity (Sarma et al., 2008; Mazzanti et al., 2009, 2015; Navarro et al., 2017). The GTC EGCG was extracted using 80% ethanol (as an extraction agent) and standardised to 25% EGCG. The supplement also contained 5-10% caffeine. The recommended dose was two capsules twice a day, corresponding to the equivalent of 375 mg EGCG (ESCO, 2009). Liver toxicity was estimated to occur in one case per 100,000 boxes sold and appeared on average after 50 days of use (Sarma et al., 2008).

The COT noted that a daily dose corresponding to 375 mg EGCG is somewhat lower than the ‘safe’ limit of 800 mg described by EFSA.

53. In 2009, fat burner supplement Hydroxycut® was withdrawn from the market, following 23 cases of hepatotoxicity reported to the US FDA (Livertox, 2012). Prior to 2009, Hydroxycut® formulations contained GTE, as well as caffeine, and ephedra.

54. SLIMQUICK® weight loss products, of which some contain GTE, have also been linked to six cases of acute liver injury between 2007 and 2011 (Zheng et al., 2016). Furthermore, some SLIMQUICK® products have caffeine content through the ingredients: guarana and yerba mate.

55. The presence of additional ingredients, in combination with GTEs further complicates the interpretation of data and the ability to attribute observed adverse effects to a specific chemical. A study found that 40% of herbal and dietary supplements linked to hepatotoxicity, contained catechins despite not identifying them as an ingredient (Navarro et al., 2013).

56. There was large variability in dose, composition, duration of exposure to GTE and incidence of hepatotoxicity as a result of consumption of green tea products, where doses ranged from three cups of green tea herbal infusions to 1,800 mg GTE per day. The EFSA Panel concluded that cases of hepatotoxicity associated with the consumption of green tea herbal infusions were very low. However, the COT noted that eight of 22 cases of hepatotoxicity from exclusive use of green tea products were reported to occur after consumption of green tea infusion. The EFSA Panel concluded that many of the cases of liver injury were as a result of idiosyncratic reactions.

57. Overall, in terms of human studies, the COT considered the “safe” limit of 800 mg per day defined by EFSA is realistic. However, the Committee recognised that there were a number of unknowns and uncertainties as discussed in the EFSA Opinion (see Uncertainties section).

Data from animal studies

58. Animal studies indicate that the liver is the target organ for EGCG toxicity in mice as demonstrated by higher incidences of elevated ALT levels and liver toxicity following high oral bolus doses or parenteral administration. The method of administration of green extract tea gives rise to differences in the magnitude of the effects observed. Daily oral bolus doses of 750 mg/kg bw EGCG

(2 doses/day for up to 7 days) induced hepatotoxicity in mice, whereas doses of 100 mg/kg EGCG administered intraperitoneally were enough to induce liver injury (Lambert *et al.*, 2010).

59. In a 14-week toxicity study (n=10/sex/group) in which rats were administered a GTE (ethanol:water extraction of green tea leaves, resulting in an EGCG content of 48.4%) by oral gavage, 5 days/week, the NOAEL for liver toxicity was 500 mg GTE/kg bw per day – equivalent to 242 mg EGCG/kg bw per day (Chan et al., 2010).

60. In a study on fasted dogs treated with Polyphenon E (GTE) containing 63.3–64.8% EGC, animals were administered 0, 200, 500 and 1,000 mg/kg bw per day in gelatine capsules (equivalent to approximately 0, 128, 320 and 640 mg/kg bw per day of EGCG) on an empty stomach. The study was terminated early (after 6.5 months instead of the intended 9-month period), due to extensive morbidity and mortality in all treated groups. Hepatic centrilobular necrosis and chronic active inflammation with infiltration of neutrophils and mononuclear cells were reported in the liver together with brown intracytoplasmic pigment in Kupffer cells (not described in relation to dose).

61. In a follow-up 13-week study in fed and fasted dogs receiving 200 mg/kg bw per day of Polyphenon E, corresponding to 128 mg EGCG/kg bw per day, increased levels of ALT were observed in one fasted dog and increased levels of AST was observed in another of the dogs. No effects on liver parameters were observed in fed dogs. Fasted dogs showed mild liver damage (haematopoiesis and presence of pigmented macrophages), whereas no changes were observed in the livers of fed dogs. Exposure (in terms of the area under the curve (AUC) and maximum concentration values (Cmax)) to EGCG was considerably lower in fed dogs than in fasted ones at the end of the 13-week period, which might explain, at least in part, the difference in toxicity in the fasted state (Kapetanovic et al., 2009).

62. In a 13-week study in dogs, an EGCG preparation (80% EGCG) was administered by capsule at doses of 0, 50, 150 and 500 mg/kg bw per day (corresponding to 0, 40, 120 or 400 mg EGCG/kg bw per day) to groups of fasted male and female animals (Isbrucker et al., 2006). Three dogs in the high-dose and two in the intermediate-dose group died or had to be terminated prematurely for humane reasons. Serum bilirubin levels were elevated in all high-dose animals, and some of these animals had increased ALT and AST values. The NOAEL was 50 mg/kg bw per day (equivalent to 40 mg/kg bw per day of EGCG).

63. In a second, 13-week study reported in the same paper (Isbrucker et al., 2006), groups of fed male and female dogs received capsules containing re-crystallized EGCG (91.8% purity) at doses of 0, 50, 300, or 500 mg/kg bw per day. No adverse effects were observed in any dose group. The NOAEL was therefore 500 mg/kg bw per day, the highest dose tested. This paper also showed that there was an appreciable increase in systemic exposure to EGCG in the fasted animals compared to that in the fed ones. Therefore, the NOAEL in fasted dogs was 10 times lower than the NOAEL identified in fed dogs.

64. A commentary was provided by Wu *et al*., as mentioned in the EFSA Opinion (pp. 50). Wu *et al.*, (2011) pointed out that the estimated AUC was lower at the NOAEL in fasted dogs (9.2 and 12.1 µg h/mL in female and male, respectively) than at the NOAEL in fed dogs (39.9 and 88.3 µg h/mL in female and male, respectively). In their commentary, they pointed out, that if the biological exposure is the primary factor for toxicity, the NOAEL exposure levels should be similar to one another when studies are conducted in the same species. Based on this argument, they speculated that fasting had increased the susceptibility of target organ systems to the effects of green tea extract. The EFSA Panel considered that an additional component contributing to increased susceptibility to EGCG in fasted dogs could be due to reduced hepatic glycogen.

65. Animal and human studies were considered in terms of other systemic end points. In the 13-week follow-up to the above study by Kapetanovic et al. (2009) , where one dose of 200 mg/kg bw per day of Polyphenon E, corresponding to 128 mg EGCG/kg bw per day was given to fasted (n=9; lots A, B and C; 3/lot) or fed (n=3, lot A) male dogs, severe toxicity, mainly in the gastro-intestinal tract, was observed in fasted dogs, whereas no adverse effects were observed in fed dogs. Additional observations in the fasted animals included vomiting, mild diarrhoea and/or red material in the faeces. No such signs were observed in the fed animals (Kapetanovic et al., 2009).

66. Based on histopathological effects (no clinical chemistry was performed in the study) in the liver in male and female rats, the Panel identified a possible NOAEL of 145 mg EGCG/kg bw per day (administered by gavage, 5 days/week, for 14 weeks and up to 105 weeks). Based on liver effects in male mice only, the NOAEL identified was 48.4 mg EGCG/kg bw per day (NTP, 2016).

67. The COT were of the opinion that EGCG appears to be directly hepatotoxic, at least in experimental animals, with limited evidence in humans, but there is also evidence for idiosyncratic liver toxicity in humans. The relationship with dose is more obvious with animals than humans.

Pyrrolizidine alkaloids

68. Pyrrolizidine alkaloids (PA) are known hepatotoxicants, as well as genotoxic and carcinogenic (COT, 2008; EFSA, 2017), and their contamination has also been suggested as a contributing factor to the hepatotoxic potential of green tea. This is thought to be the result of the co-harvesting of PA-producing plants.

69. It is thought that 1,2-unsaturated PAs can be activated by CYP enzymes, namely CYP3A4, to form hepatotoxic reaction products (EFSA, 2011, 2016, 2017; Stegelmeier et al., 2016, Robertson and Stevens, 2017).

70. PAs have been well documented to induce acute liver toxicity. However, as noted above, this is most often hepatic veno-occlusive disease, which differs somewhat from the pathology profile observed with GTCs. The lowest dose of PA known to induce acute/short-term effects in human poisoning cases is 1– 3 mg/kg bw per day, based on the onset of hepatic veno-occlusive disease in a child after 2 weeks exposure and lethality in a 2-month infant after 4 days exposure.