

Safety studies on epigallocatechin gallate (EGCG) preparations. Part 2: Dermal, acute and short-term toxicity studies

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Abstract

Green tea extract and its principal active ingredient, epigallocatechin gallate (EGCG), are gaining attention and increased usage due to their healthful properties. Despite the increasing demand for these products, few studies have examined their safety. The toxicity of purified green tea extracts containing high concentrations of EGCG have been evaluated in a series of studies in order to define the safety of Teavigo™, a high-concentration EGCG extract produced by the same novel method. Topical EGCG preparations caused minor dermal irritation in rats and guinea pigs, but not rabbits, and was a moderate dermal sensitizing agent in the guinea pig maximization test. A rabbit eye irritation test produced a strong enough response to not warrant any further testing in this assay. An oral dose delivering 2000 mg EGCG preparation/kg was lethal to rats; whereas, a dose of 200 mg EGCG/kg induced no toxicity. The dietary administration of EGCG preparation to rats for 13 weeks was not toxic at doses up to 500 mg/kg/day. Similarly, no adverse effects were noted when 500 mg EGCG preparation/kg/day was administered to pre-fed dogs in divided doses. This dose caused morbidity when administered to fasted dogs as a single bolus dose, although this model was considered an unrealistic comparison to the human condition. From these studies a no-observed adverse effect level of 500 mg EGCG preparation/kg/day was established.

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1. Introduction

The regular consumption of green tea made from *Camellia sinensis* leaves has long held value among traditional medical practices of promoting overall health. Epidemiology studies have, in general, substantiated these beliefs and illustrated reduced risks for various cancers among tea drinkers (Sasazuki et al., 2004; Zhang et al., 2002). Investigations into these beneficial effects have demonstrated that green tea, and its extracts, are rich in polyphenolic compounds with strong antioxidant and free

radical scavenging properties. Studies in animals and humans have also suggested that green tea extract, and its principal compound, epigallocatechin gallate (EGCG), can reduce lipid levels, inhibit the actions of carcinogens, and have cardioprotective effects (for reviews, see Higdon and Frei, 2003; Ioannides and Yoxall, 2003). Together, these favorable reports have led to an increased demand for products enriched with green tea polyphenols.

Despite the favorable data supporting the virtues of a diet rich in green tea and its associated polyphenols, few researchers (Chang et al., 2003; McCormick et al., 1999; Stratton et al., 2000) have investigated their potential toxicity when administered at high doses, as a concentrated product, or as a nearly-pure compound free from interactions with other chemicals normally found in green tea extracts. DSM Nutritional Products Ltd. has recently

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developed a unique patented process for purifying EGCG from a hot water extract of *C. sinensis* leaves to produce Teavigo™, a crystallized product containing greater than 90% EGCG. In a previous paper we demonstrated that this form of purified EGCG was non-genotoxic in various in vitro cell and in vivo animal studies (Isbrucker et al., in press). In this second study, we report on the acute, dermal and ocular effects of EGCG preparations, as well as its systemic safety after repeated dosing in rats and dogs.

2. Methods

2.1. Materials

EGCG was purified from a hot water extraction of *C. sinensis* leaves as described previously (Isbrucker et al., in press). Briefly, catechins were isolated from the initial hot water extract with ethyl acetate and subjected to chromatographic separation of EGCG followed by spray drying. Recrystallization of spray-dried EGCG was used in some studies. This novel purification method is the basis for the production of Teavigo™, a preparation comprising greater than 90% EGCG derived from *C. sinensis* extract. Several isolates of EGCG were used for these studies (Table 1), which presented varying concentrations of EGCG, all greater than 77% purity. However, preparations used within an individual study remained consistent. Although other catechins, residual solvents from processing, caffeine and additional unidentified compounds were found in each EGCG preparation, they are not expected to affect the outcome of the assays at the concentrations detected. The unidentified compounds would be those occurring naturally in green tea extracts.

All animal studies and all EGCG formulations for administration were conducted or prepared in accordance with Good Laboratory Practice (GLP) and based on standardized regulations as indicated.

2.2. Animals

Animals were acquired from suppliers as indicated. Rodents were separated by sex, housed 2–5 per cage and acclimatized for a minimum of 5 days prior to any treatments. In all studies, the animals were housed singly in rooms maintained at 22 ± 3 °C with 12 h light/dark cycles and provided with fresh water and standardized diets ad libitum, unless otherwise noted. All dogs were at least 5 months of age upon arrival and acclimatized for 18–21 days prior to entering the study. Dogs were fed pelleted complete commercial diet on schedule according to the experimental protocols. Tap water was available to the dogs ad libitum. All of the animal studies were conducted under strict ethical guidelines of the nations in which they were conducted.

2.3. Dermal toxicity and irritation studies

An acute dermal toxicity study in rats (Wolz et al., 2002a) was conducted in accordance with the Organization for Economic Co-operation and Development (OECD) guideline number 402 (OECD, 1987a). One day prior to commencing the study on acute dermal toxicity, the backs of HanBrl:WIST (SPF) rats (5/sex; RCC Ltd., Fullinsdorf, Switzerland) were clipped with an electric clipper to expose an area of approximately 10% of the total body surface. Only those animals without injury or irritation of the skin were used in the test. On the test day, an EGCG preparation (93% EGCG) dissolved in distilled water, was applied evenly to the exposed skin at a dose of 2000 mg/kg body weight (1860 mg EGCG/kg; 4 ml/kg) and covered with a semi-occlusive dressing. The dressing was removed 24 h later and the skin flushed with lukewarm tap water and dried. The animals were observed twice daily for 15 days for signs of irritation and toxicity. Macroscopic examination of all animals was performed at the termination of the study on day 15.

A primary skin irritation study (Wolz et al., 2001c) was also conducted with male New Zealand White albino rabbits (SPF-quality; Charles River Deutschland, Kisslegg, Germany) based on the guidelines described in the EC Commission Directive 92/69/EEC, B.4, “Acute Toxicity—Skin Irritation” and OECD guideline number 404 (OECD, 1992a). The dorsal fur of three male rabbits was removed with electric clippers 24 h prior to the application of EGCG. Each animal was treated with 0.5 g of EGCG preparation (93.4% EGCG) dissolved in 0.3 ml distilled water and applied to the skin of one flank using a Metalline semi-occlusive patch (Lohmann GmbH, Neuwied, Germany). The patch was held in place with a bandage for 4 h, after which the dressing and patch were removed and the skin cleaned of residual EGCG with water. Skin reactions and irritation effects were assessed at approximately 1, 24, 48 and 72 h after the removal of the dressings. Adjacent areas of untreated skin from each animal served as controls. Erythema and edema were scored on a scale of 0–4, with 0 showing no effect and 4 representing severe erythema or edema. Dermal histopathology was not performed.

2.4. Dermal sensitization studies

An open epicutaneous test for skin sensitization by EGCG (Csato, 2001) was conducted using female GOHI (SPF) guinea pigs (BRL, Fullinsdorf, Switzerland) in a procedure adopted from OECD guideline number 406 (OECD, 1992b). During the induction phase of this assay, an EGCG preparation (80% EGCG) was applied to the shaved right-hand flanks of animals at concentrations of 5, 10 or 30% in ethanol (equivalent to 4%, 8% or 24% EGCG) at a dose of 100 μ l/8 cm². Applications were conducted daily, 5 days per week, for 4 weeks, to 6 female animals per dose. Control animals were treated with ethanol alone with the same dosing regimen. Treatment sites were left open between applications. During this induction phase new treatment sites were chosen and treated

Table 1
Analytical evaluation of EGCG preparations used

Assay	Analytical results (%)			
	EGCG	Other catechins	Water	Others ^a
Rat acute dermal toxicity	93.0	1.77	4.1	1.11
Rabbit primary skin irritation	93.4	1.85	4.4	0.4
Guinea pig dermal sensitization	80	5.28	4.3	10.43
Guinea pig maximization test	93.4	1.85	4.4	0.4
Rabbit eye irritation study	93.0	1.77	4.1	1.11
Rat acute oral toxicity	93.4	1.85	4.4	0.4
Rat 13-week study	77	3.7	5.6	13.7
Dog 13-week study (fasting)	80	5.28	4.3	10.43
Dog 14-day tolerance study	80	5.28	4.3	10.43
Dog graded dose study	91.2	3.67	3.9	1.24
Dog 13-week study (pre-fed)	91.8	2.06	4.7	1.42

^a Citric acid, caffeine, and other naturally occurring substances normally present in green tea extracts.

with EGCG whenever the irritation became considerable. Immediately following the 4-week induction period, the animals were challenged with 0%, 1%, 3%, 5%, and 10% EGCG on the left flank at a dose of 25 $\mu\text{l}/2\text{ cm}^2$. A second similar challenge was conducted 2 weeks later at concentrations of 0%, 0.1%, 0.5%, 1%, 3%, 5% and 10%. During the induction period the animals were observed daily, 5 days/week, for signs of erythema and edema on each test site. Challenge reactions were assessed at 24 and 48 h after application. The intensity of all skin reactions was graded on a scale of 0–4, with 0 showing no reaction and 4 representing a severe skin reaction which included erythema and edema with eventual deeper skin damage.

2.5. Guinea pig maximization test

Female Himalayan strain albino guinea pigs (RCC Ltd.) were used to assess the dermal contact sensitization potential of EGCG (Wolz et al., 2001b). A preliminary study was done in order to select the maximum non-irritant concentration. The maximization test was conducted according to the methods of Magnusson and Kligman (1969) and based on OECD (1992b) guideline number 406. The EGCG preparation (90% EGCG) used for this study was dissolved in distilled water. On the first day of the induction period the scapular region of 10 animals was clipped and three pairs of intradermal injections (0.1 ml/site) were made: a 1:1 mixture of Freund's Complete Adjuvant with water; 0.1% EGCG preparation; and a 1:1 mixture of Freund's Complete Adjuvant with 0.2% EGCG preparation. Five (5) control animals were administered saline in place of EGCG. Dermal irritation reactions were monitored and scored for a period of 3 days. On day 7 the area between the injection sites was re-clipped and rubbed with 10% sodium dodecyl sulfate to provoke a mild inflammatory reaction. The following day this same area was treated with 0.5 ml of a 50% EGCG solution and kept under a semi-occluded Metalline patch for 48 h. The skin was then washed and assessed for irritation. A test challenge was conducted 22 days after the initial injection of EGCG. One flank of each of the animals was clipped and treated by epidermal application of a 50% EGCG solution (0.15 ml) under patch test plasters and held in place for 24 h using tape and an elastic bandage. The treated sites were washed and assessed for challenge reactions 24 and 48 h after removal of the dressing. A similar re-challenge was done on the contralateral flank approximately 1 week after the first challenge. Skin reactions in both the irritation and challenge portions of the study were graded on a 0–4 scale, with 0 indicating no reaction and 4 representing severe erythema with slight eschar formation or severe edema.

2.6. Eye irritation study

The primary eye irritation potential of EGCG was investigated in New Zealand rabbits (Charles River Deutschland) (Wolz et al., 2002b) in compliance with OECD (1987b) guideline number 405. Both eyes of the animals were examined at the beginning of the study. On the day of treatment, 0.1 g of EGCG preparation (93% EGCG) was placed in the conjunctival sac of the left eye of one female rabbit. The lids were then briefly held together, but the EGCG was not rinsed from the eye. The animal was monitored for ocular irritancy for a period of 17 days. As it was suspected that EGCG might be an ocular irritant, a single animal was treated first and observed to recovery. Due to the results from this preliminary study, subsequent rabbits were not tested.

2.7. Acute oral toxicity

This study was carried out based on guidelines described in EC Commission Directive 96/54/EC, Part B.1 "Acute Toxicity—Oral Acute Toxic Class Method" and OECD (2001) guideline number 423. Wistar (CrI:WI)BR (outbred, SPF-quality; Charles River Deutschland, Sulzfeld, Germany) rats of both sexes were administered a single dose by oral gavage of 2000 or 200 mg/kg body weight EGCG preparation (90% EGCG) (Wolz et al., 2001a). All animals were observed daily for 15 days and body weights were recorded on days 0, 7 and 15. Macroscopic

examination was performed on the day of death or at termination on day 15.

2.8. Repeated dose studies in rats

This study was conducted in accordance with OECD guideline number 408 for the subchronic oral toxicity testing in rodents (OECD, 1998). CrI:CD(SD)IGS BR Sprague–Dawley rats (10/sex/dose; Charles River Laboratories, Raleigh, NC) were fed EGCG preparation (77% EGCG) admixed into Purina Rodent Chow (PMI Feeds Inc., St Louis, MO) to deliver nominal doses of 50, 150 and 500 mg EGCG/kg/day for 13 weeks (Pfannkuch et al., 2000). Control animals (10/sex) were fed basal diet without EGCG. An additional 10 rats/sex were included in the control and high-dose groups and allowed to recover for 4 weeks on basal diet without EGCG following the 13-week treatment period. All animals were observed daily for morbidity and mortality, and were examined weekly for toxicity. Body weight and food consumption were also determined on a weekly basis. A complete ophthalmologic examination was conducted on all animals prior to treatment initiation, and during the last week of the 13-week feeding period, which consisted of examining both eyes of all animals using an indirect ophthalmoscope following mydriasis with Tropicamide Ophthalmic Solution. Blood levels of unconjugated EGCG were determined after one day of feeding and again during week 13. Blood samples for EGCG analysis were stored with EDTA, but not ascorbic acid. Hematology, coagulation and serum chemistry were evaluated at the end of the treatment and recovery periods. All surviving animals were subjected to a complete necropsy at the time of sacrifice and weights obtained for major organs. Detailed histopathological examination was performed on tissues from the control and high-dose animals sacrificed after 13 weeks. Gross lesions were examined from the animals in the low- and mid-dose groups sacrificed after 13 weeks and from the recovery animals.

Test diets were mixed weekly during the study, and fresh diet was provided to the animals on a weekly basis. To approximate each dose, the dietary level of EGCG was adjusted during weeks 4, 7, and 10 of the study. Dietary levels of EGCG during each period were determined based on food consumption and body weight data. Stability analysis showed EGCG to be stable in the feed under room temperature storage conditions for up to 23 days. Diets prepared for week 12 were inadvertently mixed using Teklad Rodent Diet (Teklad Test Diets, Madison, WI). These diets were fed for the entire week 12 period, but were mixed with the same concentrations of EGCG. Use of this diet for 1 week of this study was not expected to affect the toxicological or pathological outcome, as both diets are nutritionally complete and deliver similar levels of calories, protein, fat, fiber, nutrients and minerals.

Statistical processing for this study consisted of analysis of variance (ANOVA) followed, where appropriate, by the post-hoc Dunnett's test for comparing multiple treatment groups to a single control. A minimum significance level of $p \leq 0.05$ was used in all comparisons.

2.9. Repeated dose and tolerance studies in dogs

Two 13-week toxicity studies in Beagle dogs were adapted from the EC Commission Directive 91/507/EEC and OECD (1981) guideline number 409. In the first study, groups of four male and four female dogs (Harlan France, Gannat, France) were administered a spray-dried form of EGCG preparation (80% EGCG) in capsules at doses of 50, 150 or 500 mg/kg/day for 13 weeks (Pfannkuch et al., 2001). These doses delivered 40, 120 or 400 mg EGCG/kg/day, respectively. Control dogs were administered empty capsules. The administration occurred after a minimum 15 h fasting and 3–4 h prior to feeding the animals. Mortality, clinical condition, body weight and food consumption were monitored throughout the study. Clinical laboratory analyses, cardiovascular investigations and ophthalmologic examinations were performed before the start of treatment and at the end of the study. Blood samples were taken at various time points for toxicokinetic determinations on days 1 and 81 of treatment. All dogs dying or sacrificed in a moribund condition during the study were submitted to

necropsy. The surviving dogs were necropsied at the end of the treatment period, selected organs were weighed and tissue samples were submitted for histopathological examination.

Based on the results of the above study, a short-term tolerance study was conducted in dogs to evaluate any relation between feeding status and the oral toxicity of EGCG (Pfannkuch et al., 2002). Female dogs were randomly distributed into three groups of 6 dogs/group. Group 1 was dosed after fasting over night and at least 4 h prior to feeding. Group 2 was dosed approximately 2 h after feeding. A spray-dried preparation of EGCG (78% purity) was administered to these groups to deliver 300 mg/kg/day EGCG. The third group received a dose of 500 mg EGCG/kg/day 2 h after feeding. EGCG was administered in gelatin capsules to all groups. The dogs were observed twice daily for mortality, moribundity and adverse clinical signs for 14 days. Group 3 animals were studied for 28 days. Body weights and physical examinations were performed weekly on all animals. Blood samples were collected on days 14 (Groups 1, 2 and 3) and 28 (Group 3) for clinical chemistry and hematology. Kidneys, liver, lymph nodes, spleen and thymus were removed at necropsy for further histology analysis. Blood samples for determination of plasma EGCG levels were collected during day 14 from all animals at 0, 0.5, 1, 2, 4, 8 and 24 h after dosing. A second kinetic study was performed in the group 3 animals on day 28.

A preliminary, 14-day, graded-dose study was conducted in 3 Beagle dogs (Biological Research Laboratories, Fuellinsdorf, Switzerland) to determine the tolerance to re-crystallized EGCG (91.2% purity) prior to the second repeated-dose study (Schlaeppli and Pfannkuch, 2001). This product was generally well tolerated at doses up to 500 mg/kg/day with a moderate occurrence of vomiting at the highest level (results not shown).

In a second 13-week study 48 Beagle dogs (Covance Research Products, Inc., Kalamazoo, MI) were randomly assigned to one of four dose groups, each consisting of 6 dogs per sex, administered re-crystallized EGCG (91.8% purity) at doses of 0, 50, 300, or 500 mg/kg/day (Pfannkuch et al., 2003). The total daily doses were divided into twice-daily administrations of 0, 25, 150 and 250 mg/kg, respectively. EGCG was administered in gelatin capsules, which were prepared weekly based on body weights. The capsules were administered approximately 1 h after each feeding, which occurred twice daily, for 13 weeks. All dogs were monitored twice daily for signs of toxicity and evidence of moribundity. Detailed clinical observations and body weight were measured weekly. Food consumption was measured daily. Ophthalmic and electrocardiographic evaluations were conducted at the beginning and end of the study. Blood samples for clinical pathology assessments were obtained from all dogs twice during the pre-test period and again during weeks 4, 8 and 13. Plasma concentrations of EGCG were measured on the first day of dosing, and again during week 12 of the study at pre-dose, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 24 h. Neurological evaluations based on physical and behavioral tests were conducted at the onset of the study and during weeks 2, 4, 8 and 12. All dogs, including those found dead or euthanized moribund, received a complete necropsy. Selected organs were weighed and tissue samples were prepared for histopathological evaluation.

3. Results

3.1. Dermal toxicity and irritation assays

No systemic signs of toxicity were observed in any of the animals following the dermal application of a 93% EGCG preparation. However, a slight to moderate erythema was noted in all dosed rats after removal of the dressing at 24 h and this persisted for up to 5 days. Body weights remained within standard range for this strain and age of rat and were unaffected by the dermal treatment. There were no abnormal macroscopic findings observed at necropsy. The acute dermal LD₅₀ to rats of both sexes was found to be greater than 1860 mg EGCG/kg. In contrast

to these findings, no irritation was noted following the 4 h dermal exposure of rabbits to 0.47 g EGCG under semi-occluded patch.

3.2. Dermal sensitization studies

The daily application of an EGCG preparation (80% purity) to guinea pig skin caused slight to well defined, erythema responses (grades 1–2) during the induction period of the open epicutaneous test. The occurrence of irritation responses increased through the application period, with the 30% dose group (24% EGCG) having the greatest frequency, starting by the fifth application. Erythema did not become evident in the 10% and 5% groups until the 13th and 16th applications, respectively. In the 10% dosed group a slight erythema was noted in two (of six) animals on the 13th application with all animals showing similar signs by the 16th application. For the 5% group, this was only apparent for 3 days in one of six rats. Both EGCG preparation challenges elicited positive effects in the test groups (Table 2). Control animals showed no response following the first challenge, but one or two of the six control guinea pigs did have slight or well defined erythema after the second challenge with 0.8% or higher EGCG. Although there was a positive dose–response effect for the challenge, this did not clearly correlate to the sensitization doses. More animals in the 5% induction dose group had erythema responses to the challenges than did those in the 30% induction group.

Preliminary studies in the guinea pig maximization test found that the intradermal injection of 0.09% EGCG was the greatest tolerable dose. This dose produced a grade 3 erythema in the animals, but not necrosis. Also, the dermal exposure to EGCG for 48 h did not evoke any reaction in the preliminary test at concentrations up to 50%. All test animals in the final study had grade 3 or 4 erythema

Table 2

Positive dermal reactions at 24 and 48 h after EGCG challenge; tested 4 and 6 weeks following the start of the sensitization period

Sensitization dose	No. Animals with positive reactions (at 24 h/48 h)			
	0% (n = 6)	5% (n = 6)	10% (n = 6)	30% (n = 6)
Challenge dose:	<i>First challenge</i>			
0%	0/0	0/0	0/0	0/0
1%	0/0	1/2	2/0	1/0
3%	0/0	3/5	5/3	1/1
5%	0/0	6/6	6/5	2/1
10%	0/0	6/6	6/6	6/5
	<i>Second challenge</i>			
0%	0/0	0/0	0/0	0/0
0.1%	0/0	1/0	2/0	0/0
0.5%	0/0	2/0	3/0	0/0
1%	1/1	5/1	5/4	0/1
3%	1/1	6/4	6/5	4/2
5%	1/1	6/6	6/6	6/4
10%	2/2	6/6	6/6	6/6

Guinea pigs were sensitized with 0 (vehicle control), 5%, 10%, or 30% EGCG preparation for 4 weeks and challenged on the opposite flank by topical application.

Table 3
Guinea pig maximization test

Animal #	Erythema score				Comments
	First challenge		Second challenge		
	Day 24	Day 25	Day 30	Day 31	
<i>Controls</i>					
1	0	0	0	0	
2	0	0	0	0	
3	0	0	0	0	
4	0	0	0	0	
5	0	0	0	0	
<i>Test</i>					
1	0	0	2	2	Sensitized
2	0	0	2	2	Sensitized
3	0	0	0	0	Not sensitized
4	0	0	2	2	Sensitized
5	1	0	2	2	Sensitized
6	0	1	2	1	Sensitized
7	0	0	2	2	Sensitized
8	0	0	2	2	Sensitized
9	0	0	2	1	Sensitized
10	1	0	2	1	Sensitized

Erythema and sensitization responses in guinea pigs exposed to EGCG preparations.

following the intradermal injection of EGCG and/or Freund's Complete Adjuvant. Three test animals (3/10) and no control animals (0/5) had grade 1 erythema following the first test challenge, however 9 of 10 test animals showed stronger, grade 2, erythema following the second EGCG challenge (Table 3). Based on the absence of a dermal response to EGCG challenge in any of the control animals, all of the skin reactions in the test group were considered to be indicative of sensitization. No mortalities or symptoms of systemic toxicity were observed in any of the guinea pigs and body weights for test animals were in the same range as controls during the study period.

3.3. Eye irritation study

The instillation of 0.1 g EGCG preparation (0.093 g EGCG) into the eye of a single rabbit resulted in moderate to severe signs of irritation which included reddening of the conjunctivae and sclera, discharge and chemosis. A slight to moderate corneal opacity affecting the whole area of the cornea was observed during the first 72 h following the application of the EGCG. These effects were reversible and were no longer apparent 17 days following treatment. There were no abnormal observations of the iris, and no corrosion or staining of the eye by EGCG was evident at any point through this study. Due to the severity of the ocular irritation in this one animal, the study was not repeated in other rabbits.

3.4. Acute oral toxicity

Following the gavage administration of 2000 mg EGCG preparation/kg to rats, the animals showed signs of leth-

argy, calm behavior, hunched posture, labored respiration, piloerection and/or ptosis. All of the female and two of three male rats either succumbed to the high-dose treatment within 72 h, or were sacrificed *in extremis*. The third male recovered from the symptoms by day 7 and survived to day 15. No clinical signs were noted in the animals treated with 200 mg EGCG preparation/kg.

Macroscopic post-mortem examination of the animals that were either found dead or euthanized during the study, revealed dark red foci in the glandular mucosa of the stomach with red/brown stomach contents. Hemorrhagic fluid was observed in the small intestines of these animals and isolated dark red foci were noted on the thymus of some animals. No macroscopic abnormalities were found in any of the surviving animals, including the sole surviving high-dose male rat. From this study, the oral LD₅₀ of the EGCG preparation in Wistar rats was determined to be between 200 and 2000 mg/kg body weight (186.8 and 1868 mg EGCG/kg, respectively).

3.5. Repeated dose study in rats

In the 13-week rat feeding study there were no treatment-related deaths or signs of systemic toxicity during the treatment and recovery periods. Mean body weights (Fig. 1) were similar between all groups throughout the study, and there were no statistically significant differences in mean total body weight gain at the end of the 13 week feeding period between groups administered EGCG and the control group for either sex. However, mean body weight gain was significantly increased in high-dose (500 mg EGCG preparation/kg/day) female rats when compared to the control group at week 9 (10 ± 5.1 g vs 5 ± 6.1 g, respectively; $p \leq 0.05$), while mean food consumption was significantly increased in mid-dose males at weeks 5 and 12 ($p \leq 0.05$), and in high-dose females at weeks 1 and 12 ($p \leq 0.05$) and following recovery at week 16 ($p \leq 0.05$). Since these changes were all transient, they were not considered an adverse effect of EGCG

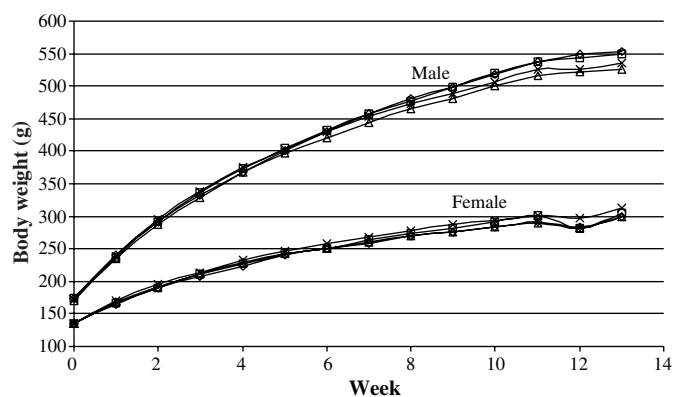


Fig. 1. Mean body weights for male and female rats fed EGCG in diets to deliver 0 (diamonds), 50 (squares), 150 (triangles) or 500 (crosses) mg EGCG preparation/kg/day for 13 weeks.

administration. These animals were inadvertently fed a different rodent chow during week 12 of the study, but returned to their regular feed for the duration of the study. During that period, female rats in all groups, including controls, lost weight (Fig. 1), while group mean body weight gain in male rats was decreased in all groups when compared to previous weeks. In female rats, the body weight loss was associated with significantly decreased food consumption in all groups, including control animals, at week 12 when compared to previous weeks. Following resumption of feeding of the standard diet at week 13, mean food consumption in female rats was increased in all groups from that at week 12, resulting in weight gain in these animals. Therefore, the effects on body weights and food consumption seen in both males and females at weeks 12 and 13 were related to the different diet fed to the animals during week 12, and is not considered an effect of EGCG administration.

Clinical signs observed during the treatment period included redness around the eyes and/or nose, alopecia, ocular opacity, broken/misaligned teeth, chromodacryorrhea and ulcerated or scabbed lesions. These were observed in one to three rats in one or more groups, but since these signs were low in incidence, were not dose-related, and were seen in control animals, they were not considered as related to EGCG administration. No ocular lesions or treatment-related ophthalmic abnormalities were found at the end of the study. Clinical pathology parameters and individual organ weights were within range of control animals. The most common gross findings seen in animals sacrificed at the end of the 13-week treatment period were red or mottled pigmentation changes of the thymus, and/or enlargement of primary mandibular lymph nodes with similar pigmentation effects. Since these findings occurred in low incidence and were present in both the control and EGCG-treated groups, they were not considered treatment-related. The incidence of histopathologic lesions seen in animals in the EGCG-treated groups was comparable to that seen in the control group.

No treatment-related effects on hematology (Table 4) or clinical chemistry parameters (Table 5) were seen in either male or female rats at week 13 or following a 4-week recovery period. At the end of the 13-week treatment period, low-dose male animals (50 mg EGCG preparation/kg/day) had statistically significant decreases in red blood cell counts, hemoglobin, hematocrit and sodium, and a statistically significant increase in the relative reticulocyte count. High-dose male rats (500 mg EGCG preparation/kg/day) had statistically significant decreases in calcium, total protein and albumin at the end of the 13-week treatment period and decreases in fibrinogen, sodium, total protein, creatinine and albumin at the end of the recovery period when compared to control animals. The only statistically significant change seen in female rats was an increase in total bilirubin in the high-dose group at the end of the recovery period. Low- and mid-dose females showed no significant changes in any of their clinical chemistry or hematology

data. Despite the statistical significance of these changes, they generally amounted to less than 5% deviation from control values and remained within normal range for this species. Also, since these changes were only seen in the low-dose male animals and were not seen at the end of the treatment period or in the low-dose female rats, they were not considered to be related to EGCG administration.

No EGCG-related effects on organ weights were seen in rats of either sex at the end of the treatment period and no statistically significant changes in the absolute or relative weight of any organ was seen at the end of the recovery period. Gross pathological observations in animals sacrificed at the end of the 13-week treatment period showed pigmentation changes and/or enlargement of the lymph nodes and thymus. Noted changes were enlargement, redness, dark, and/or mottling of these organs, principally the mandibular lymph node. These findings generally occurred with low incidence in both male and female animals, were present in all treatment groups, including controls, and were not associated with any histopathological abnormalities. There were no other lesions found in any organ that could be related to the administration of EGCG.

Unconjugated EGCG levels in the plasma from male and female rats dosed with 50 mg/kg/day were below the limits of detection (3.1 ng EGCG/ml) after 1 and 13 weeks treatment. Only 2 males in the 150 mg/kg/day dose had detectable EGCG levels at week 1 (4.4 and 4.7 ng/ml) with none detected at week 13. EGCG was not detected in the plasma from any of the mid-dose female rats. Mean plasma EGCG concentrations in high-dose male rats were 11.5 ng/ml during week 1 and 8.0 ng/ml during week 13 (detected in 9 of 10 animals). High-dose female animals had plasma EGCG concentrations of 8.7 ng/ml during week 1 (detected in 9/10 animals) and 3.8 ng/ml (detected in 5 of 10 animals). However, rat blood samples for EGCG analysis were stored only with EDTA and not in combination with ascorbic acid as is recommended for EGCG stability (Lee et al., 1995).

3.6. Repeated dose study in fasted dogs

The high-dose level of 500 mg EGCG preparation/kg/day caused occasional vomiting after treatment and frequent diarrhea in all dogs of this group throughout the study. Similar, but less frequent and less severe signs were observed in the intermediate-dose level group given 150 mg/kg/day. Three dogs from the high-dose and two from the intermediate-dose level died, or were sacrificed in a moribund condition, prior to the end of the 13-week study. One high-dose male and a high-dose female were found dead on days 51 and 70, respectively. Another high-dose female and two intermediate-dose females were sacrificed moribund between days 71 and 79. All of these dogs had marked body weight loss and were generally anorexic over the 3 weeks preceding death. The high-dose male was found to have severe proximal tubular necrosis in the kidneys. The high-dose female sacrificed in a

Table 4
Clinical hematology and coagulation data from rats dosed with EGCG preparations for 13 weeks

EGCG (mg/kg/day)	13 weeks				Recovery	
	0	50	150	500	0	500
<i>Males</i>						
WBC ($10^3/\mu\text{l}$)	12.6 ± 2.27	12.9 ± 2.55	12.7 ± 1.62	13.8 ± 2.85	12.2 ± 2.11	12.0 ± 3.44
RBC ($10^6/\mu\text{l}$)	8.04 ± 0.493	7.58 ± 0.304*	7.99 ± 0.355	7.82 ± 0.427	8.53 ± 0.562	8.31 ± 0.338
Hgb (g/dL)	15.4 ± 0.64	14.5 ± 0.72**	15.4 ± 0.56	15.3 ± 0.74	16.2 ± 0.63	16.1 ± 0.41
Hct (%)	44.3 ± 2.40	41.5 ± 1.88**	43.7 ± 1.04	43.9 ± 2.39	16.2 ± 2.38	16.1 ± 0.41
MCV (fL)	55.2 ± 2.27	54.7 ± 1.83	54.8 ± 2.06	56.2 ± 1.65	55.7 ± 2.41	56.1 ± 1.93
MCH (pg)	19.1 ± 0.76	19.2 ± 0.73	19.3 ± 1.13	19.6 ± 0.80	19.0 ± 0.84	19.4 ± 0.68
MCHC (%)	34.7 ± 0.79	35.0 ± 0.81	35.2 ± 1.07	34.8 ± 1.13	34.2 ± 0.82	34.7 ± 0.63
PLT ($10^3/\mu\text{l}$)	1060 ± 78	1127 ± 114	1127 ± 113	1020 ± 84	1002 ± 81	977 ± 59
Retic (%)	3.6 ± 0.67	4.7 ± 1.33**	3.6 ± 0.82	3.7 ± 0.92	2.6 ± 0.38	2.6 ± 0.64
Abs Ret ($10^3/\mu\text{l}$)	287.2 ± 57.7	352.5 ± 102.7	285.5 ± 68	284.5 ± 66.4	225.1 ± 39.3	212.3 ± 47.0
Neut ($10^3/\mu\text{l}$)	2.6 ± 0.64	2.6 ± 0.55	2.8 ± 1.00	2.7 ± 0.59	1.9 ± 0.48	1.9 ± 0.58
Lymph ($10^3/\mu\text{l}$)	9.6 ± 1.72	9.7 ± 1.97	9.5 ± 1.53	10.5 ± 2.42	9.9 ± 1.84	9.8 ± 2.93
Mono ($10^3/\mu\text{l}$)	0.3 ± 0.23	0.5 ± 0.41	0.5 ± 0.16	0.4 ± 0.22	0.3 ± 0.19	0.4 ± 0.17
Eosin ($10^3/\mu\text{l}$)	0.1 ± 0.09	0.1 ± 0.09	0.1 ± 0.07	0.1 ± 0.15	0.1 ± 0.10	0.0 ± 0.06
Baso ($10^3/\mu\text{l}$)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
PT (s)	15.8 ± 0.28	15.7 ± 0.45	15.8 ± 0.41	16.0 ± 0.38	15.5 ± 0.30	15.8 ± 0.48
APTT (s)	18.9 ± 1.66	20.1 ± 2.05	19.8 ± 1.85	19.5 ± 1.52	20.1 ± 2.07	21.4 ± 1.80
Fib (mg/dL)	241 ± 25.2	236 ± 11.4	240 ± 30.2	222 ± 12.5	236 ± 8.5	217 ± 11.1**
<i>Females</i>						
WBC ($10^3/\mu\text{l}$)	9.1 ± 1.55	9.1 ± 2.64	10.2 ± 2.19	8.4 ± 1.33	8.9 ± 1.55	8.6 ± 2.37
RBC ($10^6/\mu\text{l}$)	7.62 ± 0.468	7.66 ± 0.282	7.36 ± 0.226	7.63 ± 0.467	7.96 ± 0.375	8.04 ± 0.375
Hgb (g/dL)	15.2 ± 0.92	15.2 ± 0.77	14.6 ± 0.25	15.3 ± 0.70	16.0 ± 0.45	15.8 ± 0.93
Hct (%)	44.6 ± 2.90	44.1 ± 2.33	42.9 ± 0.83	44.8 ± 2.81	47.6 ± 1.61	47.2 ± 2.67
MCV (fL)	58.5 ± 2.26	57.5 ± 2.46	58.3 ± 1.51	58.8 ± 2.09	59.8 ± 2.02	58.6 ± 1.81
MCH (pg)	19.9 ± 0.70	19.9 ± 0.93	19.8 ± 0.68	20.0 ± 0.86	20.1 ± 1.04	19.6 ± 0.94
MCHC (%)	34.0 ± 0.81	34.5 ± 0.86	34.0 ± 0.45	34.1 ± 1.01	33.6 ± 0.89	33.4 ± 0.69
PLT ($10^3/\mu\text{l}$)	1086 ± 104	1091 ± 78	1120 ± 135	1049 ± 66	1061 ± 84	1042 ± 75
Retic (%)	4.2 ± 1.14	4.7 ± 0.78	5.0 ± 0.75	4.3 ± 1.53	3.2 ± 0.67	2.9 ± 0.39
Abs Ret ($10^3/\mu\text{l}$)	313.5 ± 71.7	357.8 ± 48.8	365.0 ± 61.0	323.3 ± 96.7	250.4 ± 53.0	233.4 ± 36.4
Neut ($10^3/\mu\text{l}$)	1.6 ± 0.46	1.7 ± 0.81	2.1 ± 0.52	1.5 ± 0.39	1.1 ± 0.27	1.2 ± 0.51
Lymph ($10^3/\mu\text{l}$)	7.1 ± 1.20	6.9 ± 1.89	7.7 ± 1.64	6.7 ± 1.11	7.5 ± 1.23	7.1 ± 1.87
Mono ($10^3/\mu\text{l}$)	0.3 ± 0.14	0.3 ± 0.13	0.3 ± 0.10	0.2 ± 0.12	0.3 ± 0.14	0.2 ± 0.09
Eosin ($10^3/\mu\text{l}$)	0.1 ± 0.09	0.1 ± 0.09	0.1 ± 0.09	0.0 ± 0.00	0.1 ± 0.11	0.1 ± 0.12
Baso ($10^3/\mu\text{l}$)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
PT (s)	15.0 ± 0.49	15.1 ± 0.52	15.1 ± 0.64	15.6 ± 0.61	15.0 ± 0.36	14.9 ± 0.37
APTT (s)	16.0 ± 1.47	16.7 ± 1.66	16.4 ± 1.69	16.8 ± 1.26	15.2 ± 0.90	15.7 ± 1.45
Fib (mg/dL)	168 ± 9.2	171 ± 18.6	175 ± 8.5	168 ± 11.3	181 ± 13.0	184 ± 29.1

A sub-set of control and high-dose rats were allowed to recover from dosing for an additional 4 weeks.

Abbreviations: Abs Ret = absolute reticulocyte count; APTT = activated partial thromboplastin time; Baso = basophil; Eosin = eosinophils; Fib = fibrinogen; Hct = hematocrit; Hgb = hemoglobin; Lymph = lymphocytes; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; Mono = monocytes; Neut = neutrophils; PLT = platelet count; PT = prothrombin time; RBC = red blood cells; Retic = relative reticulocyte count; WBC = white blood cells. Significant difference from control.

* $p < 0.05$.

** $p < 0.01$.

moribund condition had hemolytic anemia, liver necrosis, moderate erosion in the stomach and basophilic degeneration of some tubules in the kidneys. A decedent intermediate-dose female also had liver necrosis, moderate erosion in the stomach and myocardial necrosis. The cause of death could not be determined for the remaining two decedent females. All dogs in the low-dose (50 mg EGCG preparation/kg/day) and control groups survived to the end of the study without serious clinical incident.

One of the three surviving males and one of the two surviving females given the high-dose level had periodic episodes of anorexia and transient severe weight loss. The

two surviving females in the group given 150 mg EGCG preparation/kg/day showed a slightly reduced weight gain throughout the treatment period. Body weight of the males given the intermediate-dose level, and all dogs given the low dose, were not adversely influenced by treatment. At the end of the treatment period there was evidence of a trend towards slight regenerative anemia in the males and females administered 150 and 500 mg/kg/day by comparison with the control group. The low-dose group was not affected by treatment. Arterial blood pressure, heart rate and cardiac conduction (as assessed by electrocardiography) were not influenced by treatment.

Table 5
Clinical chemistry data from rats dosed with EGCG preparations for 13 weeks

EGCG (mg/kg/day)	13 weeks				Recovery	
	0	50	150	500	0	500
<i>Males</i>						
Na (mmol/L)	149 ± 1.7	147 ± 1.8*	148 ± 1.3	148 ± 1.4	150 ± 1.0	148 ± 2.1**
K (mmol/L)	5.9 ± 0.55	6.1 ± 0.75	6.2 ± 0.83	6.3 ± 0.75	6.0 ± 0.40	6.3 ± 0.36
Cl (mmol/L)	102 ± 1.7	102 ± 1.5	101 ± 2.5	102 ± 1.8	101 ± 1.7	101 ± 2.7
Ca (mg/dL)	11.0 ± 0.31	10.9 ± 0.38	11.0 ± 0.44	10.7 ± 0.30*	10.8 ± 0.26	10.6 ± 0.22
PO ₄ (mg/dL)	7.7 ± 1.13	7.7 ± 0.99	8.3 ± 1.02	8.0 ± 0.95	7.4 ± 0.91	7.6 ± 0.80
Prot (g/dL)	6.8 ± 0.21	6.8 ± 0.35	6.9 ± 0.27	6.5 ± 0.32**	6.8 ± 0.23	6.5 ± 0.31*
Alb (g/dL)	4.5 ± 0.21	4.5 ± 0.29	4.6 ± 0.23	4.3 ± 0.25*	4.4 ± 0.21	4.2 ± 0.21*
Glob (g/dL)	2.3 ± 0.16	2.3 ± 0.19	2.3 ± 0.19	2.2 ± 0.15	2.4 ± 0.13	2.3 ± 0.17
Glu (mg/dL)	116 ± 25.7	111 ± 10.1	126 ± 41.4	107 ± 11.7	117 ± 19.9	111 ± 13.7
Chol (mg/dL)	41 ± 9.3	48 ± 13.7	45 ± 12.0	39 ± 5.7	39 ± 10.0	43 ± 4.9
TG (mg/dL)	82 ± 38.5	89 ± 36.0	59 ± 21.5	70 ± 24.5	91 ± 35.4	106 ± 36.5
HDL (mg/dL)	44 ± 9.5	50 ± 13.3	50 ± 11.1	41 ± 6.4	42 ± 11.0	43 ± 4.5
A.P. (IU/L)	102 ± 26.6	85 ± 15.7	91 ± 24.9	91 ± 21.9	84 ± 17.8	95 ± 14.4
ALT (IU/L)	30 ± 5.7	29 ± 3.5	33 ± 6.5	32 ± 5.0	29 ± 5.6	38 ± 22.7
AST (IU/L)	74 ± 8.5	72 ± 5.0	75 ± 10.3	75 ± 7.9	81 ± 8.7	109 ± 47.0
GGT (IU/L)	3 ± 1.0	2 ± 1.1	3 ± 1.2	2 ± 1.0	3 ± 1.1	3 ± 0.6
Bil. (mg/dl)	0.52 ± 0.09	0.51 ± 0.07	0.53 ± 0.07	0.56 ± 0.10	0.45 ± 0.06	0.54 ± 0.08*
BUN (mg/dL)	14 ± 2.0	15 ± 1.5	14 ± 1.9	14 ± 1.4	14 ± 1.5	15 ± 2.2
Crea (mg/dL)	0.5 ± 0.08	0.5 ± 0.07	0.5 ± 0.08	0.6 ± 0.11	0.7 ± 0.10	0.6 ± 0.07*
<i>Females</i>						
Na (mmol/L)	148 ± 2.0	148 ± 1.5	147 ± 0.8	148 ± 1.5	147 ± 1.3	147 ± 1.5
K (mmol/L)	5.7 ± 0.46	5.5 ± 0.28	5.7 ± 0.36	5.8 ± 0.60	5.7 ± 0.34	5.7 ± 0.70
Cl (mmol/L)	103 ± 2.9	103 ± 2.7	104 ± 3.0	104 ± 2.2	103 ± 1.0	103 ± 2.8
Ca (mg/dL)	11.3 ± 0.53	11.1 ± 0.30	11.3 ± 0.27	11.2 ± 0.40	11.5 ± 0.35	11.2 ± 0.42
PO ₄ (mg/dL)	6.1 ± 0.78	5.4 ± 1.27	6.0 ± 0.66	6.1 ± 0.80	5.2 ± 0.49	5.7 ± 1.05
Prot (g/dL)	7.6 ± 0.52	7.4 ± 0.46	7.5 ± 0.36	7.4 ± 0.54	8.2 ± 0.45	7.9 ± 0.51
Alb (g/dL)	5.7 ± 0.52	5.6 ± 0.48	5.6 ± 0.40	5.5 ± 0.50	6.0 ± 0.46	5.7 ± 0.55
Glob (g/dL)	1.9 ± 0.24	1.8 ± 0.23	1.9 ± 0.16	1.8 ± 0.19	2.2 ± 0.17	2.1 ± 0.24
Glu (mg/dL)	104 ± 11.1	109 ± 15.6	107 ± 15.9	108 ± 16.2	103 ± 9.7	101 ± 10.8
Chol (mg/dL)	48 ± 10.1	50 ± 9.2	52 ± 7.6	46 ± 8.8	47 ± 9.7	54 ± 10.9
TG (mg/dL)	55 ± 26.4	51 ± 13.4	64 ± 27.4	56 ± 24.5	109 ± 45.5	96 ± 61.9
HDL (mg/dL)	58 ± 9.9	58 ± 9.5	60 ± 6.5	56 ± 10.3	58 ± 9.9	66 ± 11.3
A.P. (IU/L)	56 ± 20.6	60 ± 12.9	49 ± 19.5	59 ± 18.5	50 ± 19.9	50 ± 10.8
ALT (IU/L)	31 ± 7.1	40 ± 18.5	48 ± 52.3	42 ± 27.9	37 ± 12.0	36 ± 8.5
AST (IU/L)	82 ± 12.8	88 ± 14.0	92 ± 39.6	90 ± 19.7	88 ± 12.8	93 ± 16.6
GGT (IU/L)	3 ± 1.2	3 ± 1.0	2 ± 1.3	3 ± 1.4	3 ± 1.1	2 ± 1.1
Bil. (mg/dl)	0.51 ± 0.11	0.51 ± 0.05	0.45 ± 0.15	0.56 ± 0.06	0.46 ± 0.09	0.56 ± 0.08*
BUN (mg/dL)	16 ± 2.9	16 ± 2.6	16 ± 1.5	15 ± 1.9	17 ± 1.7	17 ± 2.9
Crea (mg/dL)	0.6 ± 0.09	0.6 ± 0.10	0.6 ± 0.00	0.6 ± 0.08	0.7 ± 0.10	0.6 ± 0.08

A sub-set of control and high-dose rats were allowed to recover from dosing for an additional 4 weeks.

Abbreviations: Alb = albumin; ALT = alanine aminotransferase; AP = alkaline phosphatase; AST = aspartate aminotransferase; Bil = bilirubin; BUN = blood urea nitrogen; Ca = calcium; Chol = cholesterol; Cl = chloride; Crea = creatinine; GGT = γ -glutamyl transpeptidase; Glob = globulin; HDL = high-density lipoprotein; K = potassium; Na = sodium; PO₄ = inorganic phosphorus; Prot = total protein; TG = triglycerides.

Significant difference from control.

* $p < 0.05$.

** $p < 0.01$.

On day 9 of treatment serum bilirubin levels were elevated in all high-dose dogs (results not shown). This was particularly marked in one male animal which had lost weight and was associated with increased aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ -glutamyl transferase (γ -GT). Marked increases in ALT values were also noted in one of these male and three female dogs, but only the male had a high AST value. All other AST, ALT, and γ -GT values were within normal range or showed only a small elevation within this dose group.

At the end of the study, hematology revealed that white blood cell (WBC) counts were elevated in the high-dose males and females as compared to the control dogs (Table 6). This increase in total WBC number could be accounted for principally by a significant rise in neutrophil count. Due to the low number of high-dose females surviving through the study ($n = 2$), statistical comparison was not available for this group. Serum bilirubin was increased in all remaining high-dose animals (Table 7), but these values were similar, or reduced, when compared to their individual values observed on day 9. One male and one female

Table 6
Clinical hematology data on Beagle dogs administered EGCG preparations by capsules 4 h prior to feeding for 13 weeks

	EGCG (mg/kg/day)			
	0	50	150	500
Males	<i>n</i> = 4	<i>n</i> = 4	<i>n</i> = 4	<i>n</i> = 3
WBC (10 ³ /μl)	13.48 ± 1.01	13.00 ± 1.80	12.53 ± 3.95	25.59 ± 2.13***
RBC (10 ⁶ /μl)	6.64 ± 0.31	6.72 ± 0.31	5.37 ± 1.38	4.65 ± 0.92*
Hgb (g/dL)	15.0 ± 0.9	15.3 ± 0.9	12.4 ± 3.3	10.2 ± 1.8
MCV (fL)	65.4 ± 1.5	66.1 ± 2.5	67.8 ± 1.3	65.8 ± 3.3
MCH (pg)	22.5 ± 0.5	22.8 ± 0.9	23.0 ± 0.8	21.9 ± 0.5
MCHC (%)	34.4 ± 0.3	34.4 ± 0.8	33.9 ± 1.7	33.3 ± 1.0
PLT (10 ³ /μl)	438 ± 89	396 ± 7	292 ± 112	629 ± 150
Retic (%)	0.5 ± 0.2	0.9 ± 0.4	1.9 ± 1.8	3.2 ± 0.6*
Neut (10 ³ /μl)	6.7 ± 0.2	7.0 ± 1.1	6.1 ± 2.5	13.6 ± 3.2**
Lymph (10 ³ /μl)	5.4 ± 0.5	4.9 ± 0.5	5.1 ± 1.4	7.1 ± 2.0
Mono (10 ³ /μl)	0.8 ± 0.2	0.7 ± 0.2	0.8 ± 0.3	1.6 ± 1.1
Eosin (10 ³ /μl)	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.3	3.1 ± 3.6
Baso (10 ³ /μl)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
PT (s)	6.2 ± 0.1	6.3 ± 0.1	6.0 ± 0.1	6.5 ± 0.5
APTT (s)	10.9 ± 0.2	10.9 ± 0.7	11.9 ± 1.4	11.9 ± 1.4
Females	<i>n</i> = 4	<i>n</i> = 4	<i>n</i> = 2	<i>n</i> = 2
WBC (10 ³ /μl)	13.69 ± 2.46	11.32 ± 0.84	(8.41, 14.62)	(22.30, 17.89)
RBC (10 ⁶ /μl)	7.45 ± 0.48	7.4 ± 0.17	(6.10, 4.89)	(5.62, 5.48)
Hgb (g/dL)	16.6 ± 0.9	16.7 ± 0.3	(14.0, 11.9)	(12.6, 12.7)
MCV (fL)	66.0 ± 1.3	66.8 ± 0.9	(67.2, 76.7)	(65.8, 70.2)
MCH (pg)	22.2 ± 0.6	22.6 ± 0.4	(22.9, 24.3)	(22.5, 23.1)
MCHC (%)	33.7 ± 0.3	33.8 ± 0.3	(34.1, 31.8)	(34.2, 32.9)
PLT (10 ³ /μl)	344 ± 37	387 ± 39	(473, 442)	(603, 557)
Retic (%)	0.8 ± 0.4	0.9 ± 0.5	(0.2, 6.3)	(1.6, 2.2)
Neut (10 ³ /μl)	7.7 ± 1.7	5.7 ± 0.4	(3.0, 7.4)	(12.0, 10.5)
Lymph (10 ³ /μl)	4.8 ± 1.1	4.6 ± 1.0	(3.9, 5.5)	(8.3, 6.2)
Mono (10 ³ /μl)	0.8 ± 0.1	0.7 ± 0.1	(1.2, 1.1)	(1.0, 0.6)
Eosin (10 ³ /μl)	0.3 ± 0.2	0.2 ± 0.1	(0.2, 0.5)	(0.8, 0.5)
Baso (10 ³ /μl)	0.1 ± 0.0	0.0 ± 0.0	(0.0, 0.1)	(0.1, 0.1)
PT (s)	6.2 ± 0.2	6.1 ± 0.1	(5.9, 5.9)	(6.1, 6.0)
APTT (s)	11.3 ± 0.3	10.8 ± 0.7	(12.1, 11.1)	(10.6, 11.1)

Values are the average ± standard deviation, or the individual values when *n* = 2.

Abbreviations: APTT = activated partial thromboplastin time; Baso = basophil; Eosin = eosinophils; Hgb = hemoglobin; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; Mono = monocytes; Neut = neutrophils; PLT = platelet count; PT = prothrombin time; RBC = red blood cells; Retic = relative reticulocyte count; WBC = white blood cells.

Significant difference from control.

* *p* < 0.05.

** *p* < 0.01

*** *p* < 0.001.

intermediate-dose dog also had elevated bilirubin at the end of the study, which was accompanied by a small increase in AST in the male and by a large elevation in AST and ALT in the female (Table 7). γ -GT of all dosed dogs remained within range of control animals. Two of the high-dose male dogs had greatly elevated AST levels, of which one was accompanied by a high ALT. For the third male these values were within range of control animals. The two remaining high-dose female dogs also had AST and ALT values similar to those of the controls. Serum albumin was slightly reduced and serum globulin slightly increased in the high-dose male dogs. Although these reached statistical significance when compared to the control group, the values remained within range of historical controls. The two high-dose female dogs also showed reduced serum albumin as compared to the control group, but this, too, was in range of historical controls. The urine collected from high-dose males at the end of the

study had a dark aspect. Protein was present in the urine of one high-dose male. Two other high-dose dogs had traces of blood in the urine.

Proximal tubular necrosis in the kidneys, similar to that seen in the decedent males, was also noted in one surviving high-dose male. Degenerative tubules, similar to those found in a decedent female, were also present in the kidneys of a surviving high-dose female. Lymphoid atrophy of the thymus, usually associated with a reduced number of secondary follicles in one or more lymph nodes, was found in seven out of eight high-dose dogs and five out of eight intermediate-dose dogs. There was no apparent liver necrosis (as was seen in two decedent females) in any of the surviving dogs. No obvious treatment-related lesions were detected in the low-dose group.

Plasma concentration time profiles revealed that the area under the curve (AUC) increased linearly with dose

Table 7
Clinical chemistry data on Beagle dogs administered EGCG preparations by capsules 4 h prior to feeding for 13 weeks

	EGCG (mg/kg/day)			
	0	50	150	500
Males	<i>n</i> = 4	<i>n</i> = 4	<i>n</i> = 4	<i>n</i> = 3
Na (mmol/L)	148 ± 1	148 ± 1	148 ± 1	147 ± 2
K (mmol/L)	5.0 ± 0.2	4.9 ± 0.3	5.0 ± 0.5	4.9 ± 0.5
Cl (mmol/L)	115 ± 1	115 ± 2	116 ± 3	117 ± 2
Ca (mg/L)	110 ± 4	110 ± 2	109 ± 6	103 ± 4
PO ₄ (mg/L)	69 ± 9	69 ± 9	66 ± 5	56 ± 10
Gluc. (g/L)	1.04 ± 0.03	1.10 ± 0.10	1.10 ± 0.10	0.97 ± 0.07
Urea (g/L)	0.36 ± 0.04	0.35 ± 0.09	0.36 ± 0.05	0.28 ± 0.04
Chol. (g/L)	1.15 ± 0.19	1.26 ± 0.10	1.20 ± 0.11	0.92 ± 0.32
Trigs (g/L)	0.49 ± 0.08	0.42 ± 0.11	0.39 ± 0.13	0.43 ± 0.13
Ph. Lip. (g/L)	2.43 ± 0.49	2.68 ± 0.32	2.39 ± 0.25	1.96 ± 0.63
Tot. Bil. (mg/L)	1.0 ± 0.2	1.2 ± 0.1	1.7 ± 0.7*	4.5 ± 1.6*
Tot. Prot. (g/L)	56 ± 4	57 ± 2	56 ± 2	54 ± 2
Alb. (g/L)	36 ± 1	35 ± 2	34 ± 1	29 ± 1***
Glob. (g/L)	20 ± 3	22 ± 1	22 ± 1	25 ± 2**
Creat. (mg/L)	9.6 ± 0.3	8.7 ± 1.4	9.0 ± 1.5	6.3 ± 0.5
Alk. Phos (IU/L)	186 ± 44	206 ± 29	183 ± 67	221 ± 49
AST (IU/L)	38 ± 5	34 ± 2	33 ± 11	123 ± 87
ALT (IU/L)	37 ± 7	42 ± 10	39 ± 17	88 ± 94
CK (IU/L)	167 ± 42	184 ± 36	263 ± 234	181 ± 128
GGT (IU/L)	2 ± 1	3 ± 1	3 ± 1	2 ± 1
LDH (IU/L)	219 ± 123	211 ± 56	496 ± 753	206 ± 64
Females	<i>n</i> = 4	<i>n</i> = 4	<i>n</i> = 2	<i>n</i> = 2
Na (mmol/L)	147 ± 1	147 ± 1	(145, 148)	(145, 144)
K (mmol/L)	5.0 ± 0.3	4.9 ± 0.1	(5.1, 4.8)	(5.1, 5.2)
Cl (mmol/L)	115 ± 3	115 ± 1	(115, 114)	(114, 115)
Ca (mg/L)	116 ± 5	113 ± 4	(116, 121)	(105, 111)
PO ₄ (mg/L)	72 ± 4	72 ± 4	(76, 69)	(54, 76)
Gluc. (g/L)	1.16 ± 0.10	1.13 ± 0.10	(1.07, 1.15)	(1.00, 1.14)
Urea (g/L)	0.58 ± 0.08	0.53 ± 0.13	(0.45, 0.30)	(0.39, 0.60)
Chol. (g/L)	1.12 ± 0.10	1.32 ± 0.34	(1.39, 1.75)	(1.05, 1.15)
Trigs (g/L)	0.31 ± 0.05	0.37 ± 0.05	(0.29, 0.69)	(0.43, 0.36)
Ph. Lip. (g/L)	2.26 ± 0.10	2.69 ± 0.74	(2.81, 3.51)	(2.00, 2.42)
Tot. Bil. (mg/L)	1.1 ± 0.1	1.4 ± 0.1*	(1.3, 2.8)	(2.7, 2.1)
Tot. Prot. (g/L)	58 ± 1	58 ± 3	(64, 66)	(54, 55)
Alb. (g/L)	37 ± 1	39 ± 0*	(35, 39)	(34, 34)
Glob. (g/L)	20 ± 0	20 ± 3	(30, 27)	(20, 21)
Creat. (mg/L)	10.0 ± 1.5	9.7 ± 0.8	(8.2, 7.2)	(7.9, 10.5)
Alk. Phos (IU/L)	167 ± 22	212 ± 40	(216, 249)	(150, 227)
AST (IU/L)	32 ± 6	32 ± 6	(31, 254)	(36, 26)
ALT (IU/L)	41 ± 10	43 ± 15	(33, 119)	(24, 47)
CK (IU/L)	132 ± 18	125 ± 21	(104, 160)	(723, 116)
GGT (IU/L)	3 ± 1	4 ± 1	(1, 2)	(1, 2)
LDH (IU/L)	155 ± 42	128 ± 23	(59, 149)	(64, 83)

Values are the average ± standard deviation, or the individual values when *n* = 2.

Abbreviations: Alb. = albumin; ALT = alanine aminotransferase; Alk.Phos. = alkaline phosphatase; AST = aspartate aminotransferase; Bil = bilirubin; BUN = blood urea nitrogen; Ca = calcium; Chol = cholesterol; Cl = chloride; Creat. = creatinine; CK = creatinine kinase; GGT = γ -glutamyl transpeptidase; Glob = globulin; Gluc. = glucose; K = potassium; LDH = lactate dehydrogenase; Na = sodium; Ph. Lip. = phospholipids; PO₄ = inorganic phosphorus; Tot. Bil. = total bilirubin; Tot. Prot. = total protein; Trigs = triglycerides.

Significant difference from control.

* *p* < 0.05.

** *p* < 0.01.

*** *p* < 0.001.

at both time points (Table 8). Also, the AUC in week 12 was greater than the corresponding AUC of day 1, especially for the high-dose group. However, inter-individual variation in plasma concentrations was large, as is apparent by the high standard deviation values. Maximal plasma concentration of EGCG showed a dose-related, but over-

proportional, increase on day 1 and in week 12. All maximum plasma concentration values occurred between 1 and 2 h after treatment with male dogs showing higher levels than female dogs. In week 12, the values after 24 h returned to the levels near pretreatment values, indicating steady state was reached.

Table 8
Area under curve (AUC; ng h/ml) dose response and maximum plasma concentration (C_{\max}) for free EGCG in dogs orally administered EGCG preparations for 13 weeks

Dose (mg/kg/day)		50	150	500
	Day		Male, fasted dogs	
AUC (ng h/ml)	1	$n = 4$ 6475 ± 2281	$n = 4$ 22,942 ± 15,919	$n = 4$ 76,267 ± 112,341
C_{\max} (ng/ml)	1	1047 ± 498	5781 ± 3674	25,350 ± 11,066
AUC (ng h/ml)	81	$n = 4$ 12,059 ± 9465	$n = 4$ 88,081 ± 135,141	$n = 3$ 372,121 ± 146,690
C_{\max} (ng/ml)	81	3832 ± 5748	13,370 ± 21,310	55,560 ± 170,56
			Female, fasted dogs	
AUC (ng h/ml)	1	$n = 4$ 5736 ± 1348	$n = 4$ 16,867 ± 10,894	$n = 4$ 58,504 ± 40,375
C_{\max} (ng/ml)	1	788 ± 284	4649 ± 4600	14,180 ± 11,511
AUC (ng h/ml)	81	$n = 4$ 9190 ± 3678	$n = 2$ 26,166 ± 9551	$n = 2$ 177,604 ± 79,846
C_{\max} (ng/ml)	81	1810 ± 1267	3086 ± 1250	35,116 ± 5945
Dose (mg/kg/day)		50 (2 × 25)	300 (2 × 150)	500 (2 × 250)
	Day		Male, pre-fed dogs	
AUC (ng h/ml)	1	$n = 6$ 3241 ± 1011	$n = 6$ 16,980 ± 7234	$n = 6$ 62,477 ± 37,489
C_{\max} (ng/ml)	1	655 ± 468	1729 ± 1560	7561 ± 7085
AUC (ng h/ml)	78	$n = 6$ 2440 ± 856	$n = 5$ 34,024 ± 33,527	$n = 6$ 88,278 ± 51,983
C_{\max} (ng/ml)	78	193 ± 137	14,253 ± 1336	7320 ± 6695
			Female, pre-fed dogs	
AUC (ng h/ml)	1	$n = 6$ 4608 ± 2646	$n = 6$ 35,215 ± 14,791	$n = 6$ 48,193 ± 27,638
C_{\max} (ng/ml)	1	287 ± 177	3138 ± 2894	4972 ± 4017
AUC (ng h/ml)	78	$n = 6$ 1799 ± 1048	$n = 4$ 30,527 ± 25,352	$n = 5$ 39,855 ± 35,768
C_{\max} (ng/ml)	78	145 ± 238	4129 ± 6374	5752 ± 6918

Dosing occurred 4 h prior to feeding of animals (fasted) or in divided doses, each provided 1 h after feeding (pre-fed). Results are means ± standard deviations.

3.7. Tolerance study in fasted and pre-fed dogs

None of the animals died during the study and there were no clinical or physical signs indicative of systemic toxicity to EGCG in any of the groups. The most common clinical observations were vomiting and diarrhea, which were observed with greater frequency in the fasted dogs than in the pre-fed dogs. There were no effects on body weight or clinical pathology parameters, and no treatment-related lesions were observed at necropsy in any of the groups. Histology revealed no treatment-related effects of EGCG administration.

Plasma area under the curve (AUC) for EGCG was significantly higher in fasted ($205,753 \pm 91,180$ ng h/ml) than pre-fed ($19,824 \pm 7403$ ng h/ml) animals after 2 weeks dosing at 300 mg/kg/day. However, the profile of the plasma concentration curves was not influenced by feeding (Fig. 2). The AUC in pre-fed animals was comparable in the 300 and 500 mg/kg/day EGCG dose groups. After 28 days of dosing the AUC of the pre-fed 500 mg/kg/day group increased 1.6 times as compared to day 14

($25,966 \pm 11,605$ vs $41,038 \pm 25,309$ ng h/ml); however, this increase did not reach statistical significance due to the large inter-animal variation in plasma levels.

3.8. Repeated dose study in pre-fed dogs

In the second repeated-dose study in Beagles, 4 dogs died or were euthanized for humane purposes during the treatment phase of the study. Three animals (2 females and 1 male) were from the mid-dose group (300 mg EGCG preparation/kg/day), and another female was from the high-dose group (500 mg/kg/day). Prior to death, all affected dogs showed severe turgid swelling of the face and head, one or both ears, and one of both front limbs. One female displayed such swelling in and around the vulva, and one male had similar swelling in the penis, sheath, and scrotum. Microscopy of the abnormal tissues from these dogs revealed the presence of a Gram-positive bacterial infection. Due to the lack of a clear dose-response relation with these 4 dogs and the presence of the infection it was determined that these deaths were not related to EGCG administration.

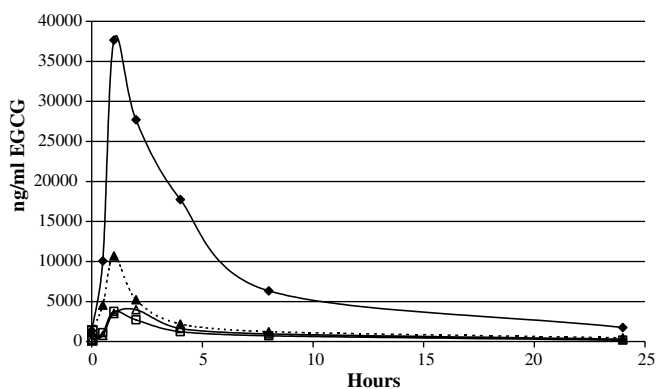


Fig. 2. Plasma EGCG concentrations in female Beagle dogs. Diamonds = fasted dogs, 300 mg/kg/day \times 14 days; Open square = pre-fed dogs, 300 mg/kg/day \times 14 days; Open triangles = pre-fed dogs, 500 mg/kg/day \times 14 days; Solid triangles/dashed line = pre-fed dogs, 500 mg/kg/day \times 28 days.

Vomiting was the most significant clinical observation in the remaining dogs. The frequency of vomiting was dose-dependent and the highest incidences were seen in the early weeks of the study. At the end of the 13-week study period, vomiting was still noted but had declined substantially. Transient conjunctivitis sicca (dry, red eyes) was also prevalent in the mid- and high-dose animals and was seen during the early weeks of the study; however, the cause was unknown. Hematology and clinical chemistry were unremarkable (results not shown) with slight decreases in red blood cell (RBC) count, hemoglobin and hematocrit in the high-dose group. Although these decreases reached statistical significance, they remained within normal range for Beagle dogs. Blood pressure, heart rate and electrocardiographic profile were normal for all dogs.

No treatment-related effects on body weight or food consumption were seen for either sex during the 13-week dosing period. There were also no treatment-related effects on clinical pathology parameters, nor was there any evidence of immunotoxicity or neurotoxicity. The only histopathological finding directly attributed to EGCG administration was the presence of pigmentation in the villus tips of the duodenum of treated animals, and the presence of a similar pigment found in the liver Kupffer cells in one high-dose female. This brown pigment was thought to be due to the absorption, or phagocytosis, of EGCG and was not considered to be a toxicologically relevant finding as it was not associated with any lesions or histopathological abnormalities.

There were large differences in the plasma concentrations of EGCG in all dose groups, with extreme values in the mid- and high-dose groups. These differences were, at least in part, due to differences in food intake between individual animals. The AUC increased in a dose-related, but not dose-proportional, manner, with higher values on day 78 of treatment (Table 8). Correlation of individual AUC values for day 1 and day 78 showed very high scatter. Further, there appeared to be no evidence for EGCG accu-

mulation with continuing exposure. Maximal plasma concentration of free EGCG was higher after administration of the second daily dose. This was seen for both day 1 and day 78, and correlated in most cases with reduced feed intake before and after the second dose. Plasma concentration of EGCG on day 78 pre-dose and 24 h after the first dose showed a dose-dependency, but with low values, further indicating the absence of EGCG accumulation.

4. Discussion

Recently, research has begun to illustrate the variety of mechanisms by which green tea components, and most notably EGCG, can potentially control neoplastic processes, promote cardiac health, reduce lipid levels and offer antioxidant effects (Higdon and Frei, 2003; Ioannides and Yoxall, 2003). Green tea is, by its very nature as a plant extract, a chemically complex beverage and despite the favorable epidemiological profile of green tea consumption or its long history of safe consumption, individual compounds from green tea can potentially have toxic effects. The recent interest in the healthful benefits of EGCG has prompted the manufacture of green tea extracts enriched for this polyphenol. The toxicity and safety profile described in this report support the performance of human clinical studies with the EGCG preparation from the same manufacturing source (Ullmann et al., 2004) and, in conjunction with the human data, provides a basis for establishing a safe upper level for daily and longer-term human exposure.

The results presented here suggest that the topical exposure to EGCG for 24 h can produce minor irritation responses; whereas, repeated exposures could lead to dermal sensitivity. However, when comparing these results to other studies, it appears that dermal responses to EGCG are influenced by the circumstances of exposure and can vary widely depending upon the animal model used. Stratton et al. (2000) reported severe erythema and dermal ulceration to the topical application of 10% EGCG on BALB/c mice, which was dependent on the use of a chemical topical depilatory agent; whereas, Katiyar et al. (1992) found no such response with Sencar mice exposed to 2.3 mg EGCG/0.2 ml acetone. Also, Skh1 hairless mice showed no response to the topical daily application of 10% EGCG for 30 days (Stratton et al., 2000), and UV-irradiated mice had no reported adverse effect to the application of 6.5 μ mol EGCG for 18 weeks (Lu et al., 2002). It is also important to note that dermal irritation only occurred in our study following the topical exposure under a semi-occluded patch for 24 h. Rabbits, which were exposed to higher doses than rats, showed no dermal response to EGCG after a 4 h application. A likely mechanism of dermal irritation from EGCG is due to its relatively low pH and subsequent caustic effects. This would also account for the serious ocular irritation response noted in the rabbit.

Despite these data, human dermal hypersensitivity responses to EGCG have only been reported among tea

factory workers who had developed asthma following their exposure to green tea powder (Shirai et al., 1994, 1997, 2003). Ours is the first known study indicating that EGCG may qualify as a sensitizing agent following dermal exposure, but there is no such indication of hypersensitivity following its dietary intake. In light of the growing interest in topical EGCG as a protectant against UV-induced skin damage and carcinogenesis (Katiyar et al., 1999; Lu et al., 2002) further examination of these results would seem warranted, although the results would likely be more relevant to extreme situations, such as occurs during occupational exposure.

Few studies exist which report on the oral toxicity of purified EGCG or of preparations containing high concentrations of this polyphenol. Our data compare closely to the low acute oral lethality of Polyphenon E (which contains 51% EGCG) in Wistar and B6C3F₁ transgenic mice (Chang et al., 2003). In the acute study reported here the primary target of toxicity appears to be the gastrointestinal tract. Two conference abstracts similarly report GI toxicity in sub-chronic (90 day) studies involving rats administered EGCG (McCormick et al., 1999) or Polyphenon E (Johnson et al., 1999) by oral gavage. Both of these abstracts reported intestinal dilatation in animals exposed to 300 mg EGCG/kg/day or 600 mg Polyphenon E/kg/day. Chang et al. (2003) did not report any adverse effects of Polyphenon E administration on the intestines of mice, but considered the principal histopathological effect to be cardiac myofiber degeneration. However, this cardiotoxicity may be related to the very high doses (2000 mg Polyphenon E/kg/day; approximately 1000 mg EGCG/kg/day) and unique to the transgenic mouse, or may be related to other substances in Polyphenon E. We found no indication of cardiac toxicity in either rats or dogs following the oral administration of EGCG for 13 weeks. Similarly, McCormick et al. (1999) and Johnson et al. (1999) also reported no adverse effect of 300 mg EGCG/kg/day or 600 mg Polyphenon E/kg/day, respectively, on the electrocardiogram profiles of Beagle dogs following their oral administration for 90 days.

Hepatic necrosis has been reported as a toxic response to EGCG and Polyphenon E in mice (Chang et al., 2003; Goodin and Rosengren, 2003) and rats (Johnson et al., 1999; McCormick et al., 1999). The consumption of Exolise[®] (Arkopharma Laboratories), a green tea product derived from strong hydro-alcoholic extraction techniques, has also been implicated in recent cases of hepatic disease in France and Spain (Seddik et al., 2001; Vial et al., 2003). The mechanism of hepatotoxicity has not been established *in vitro* (Schmidt et al., 2005) or in animal models, but in these case reports it appears to be specific to this product and is likely related to the extraction methods used in the manufacturing process. Since the emergence of these cases the French and Spanish governments have suspended the marketing of Exolise[®], but not of other green tea extracts or green tea derived polyphenols extracted by aqueous methods (www.who.int/medicines/library/pnews-let/3news2003.pdf).

Although we found no hepatotoxic effects of EGCG in our acute study or following the sub-chronic dosing of rats for 13 weeks at levels delivering 500 mg EGCG/kg/day, a few fasted dogs did succumb to repeated high dose EGCG administration and showed hepatic necrosis upon examination. However, the majority of high-dose dogs had no evidence of hepatic insult. The hepatic changes that were seen occurred in association with emesis and general loss of condition, and no liver effect was observed when the animals were pre-fed. Similarly, McCormick et al. (1999) and Johnson et al. (1999) reported no hepatic toxicity in dogs dosed orally with EGCG (300 mg/kg/day) or Polyphenon E (600 mg/kg/day) for 90 days. It appears that non-specific toxicity in dogs occurs predominantly in cases where animals are fasted prior to dosing and very high systemic concentrations of EGCG are attained. In our study the surviving high-dose, fasted animals had maximum plasma EGCG concentrations in the range of 35–55.5 µg/ml in female and male dogs, respectively. Results from the tolerance study show that maximum serum EGCG concentrations were approximately 10 times greater in fasted than pre-fed dogs. Also, the plasma EGCG levels in fasted dogs were 12–20 times greater than achieved in human plasma after the daily oral dosing with 800 mg/day (approximately 13 mg/kg/day) for 10 days (Ullmann et al., 2003, 2004). Repeated daily oral administration of 400 mg EGCG/day (approximately 6.6 mg/kg/day) to humans produced maximum plasma concentrations in the range of 155–695 ng/ml (Chow et al., 2003; Ullmann et al., 2003, 2004). Although this data illustrates a greater bioavailability of EGCG in humans than in dogs, the consumption levels by humans is expected to be far smaller than those dose levels producing toxicity in dogs.

Interpretation of the repeated dose studies in dogs are further complicated by the influence of the experimental protocol on the tolerability of EGCG. The 14-day study indicated that fasted animals were less tolerant of EGCG than fed dogs, although this was assessed only on the basis of increased incidence of vomiting. Emesis, which was the principal effect observed in the dog studies, is a non-specific and commonly encountered reaction to bolus doses of irritant materials in dogs and cannot, per se, be considered a toxic reaction. However, in this study there was treatment-related mortality and systemic pathology, which occurred only in fasted dogs dosed with 500 mg/kg/day EGCG. As we have also shown here, dogs have a far greater bioavailability of EGCG following its oral administration than do rats, and fasted dogs have an increased bioavailability as compared to fed animals.

In general, published studies involving the administration of EGCG to mice or rats have reported few adverse effects. Although not examining toxicity per se, anti-carcinogenicity studies involving orally-administered EGCG preparations have been relatively free from mentioning adverse reactions when the compound was provided in water or feed for periods ranging from 3 to 58 weeks (Hirose et al., 1997; Kikuoka et al., 1994; Muto et al.,

1999; Nishida et al., 1994; Yamane et al., 1995). Studies in rats have shown the oral bioavailability of EGCG is only 0.003–0.45% of the ingested dose (Chen et al., 1997; Nakagawa and Miyazawa, 1997). Therefore, these findings of reduced oral toxicity are not surprising given this poor oral bioavailability in conjunction with a rapid first-pass hepatic metabolism in rats (Cai et al., 2002) and mice (Lambert et al., 2003). Although the plasma EGCG concentrations in our repeated dose study in rats are likely underrepresented due to the absence of ascorbic acid as a stabilizing agent in the collected blood, relatively low levels would be expected due to the continual, rather than bolus, administration of EGCG via the feed.

Based on the absence of any treatment-related toxicological or histopathological effects, the no-observed adverse effect level (NOAEL) for EGCG in rats was the highest dose tested, delivering 500 mg/kg/day. This value is the same as the NOAEL established in pre-fed dogs (500 mg EGCG/kg/day). Although the NOAEL for fasted dogs was 40 mg EGCG/kg/day, and this dose did produce plasma concentrations comparable to those seen in humans administered an oral dose of 800 mg, the protocol presented an unrealistic comparison to the most likely human scenario. From these results, and using a safety/uncertainty factor of 100, a dose of 5 mg EGCG/kg/day would seem an acceptable daily intake (ADI) for humans. For a 60 kg adult, this would be equivalent to 300 mg EGCG/day. It has been reported that humans can tolerate, without adverse effects, the consumption of 800 mg/day Teavigo™ for 10 days (Ullmann et al., 2004), suggesting that the proposed ADI derived from the rat and dog data is conservative. However, because the human study was conducted in a controlled clinical environment, and because it is reasonable to expect longer-term consumption of EGCG by the public, a conservative ADI is posited for general human use.

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