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Appendix K

English translations of Japanese Citations

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Appendix L

Studies on the Mechanisms of Action of Green Tea Catechins

Studies Investigating the Mechanisms of Action for Green Tea Catechins

Citation	Study Design	Results	Relevance to Kao GRAS Determination
He, Z., et al. 2008. Fyn is a novel target of (-)-epigallocatechin gallate in the inhibition of JB6 and CI14 cell transformation. *Mol. Carcinogen.* 47(3):172-83.	*In vitro* study of the role of Fyn in the regulation of cell transformation using knockout siRNA plasmid. siRNA was stably co-transfected into JB6 CI14 cells. Studies: • Cytotoxic effects of EGCG on JB6 Cells • Anchorage-independent transformation assay • *In vitro* pull-down assay • Purification of GST-Fyn-SH2 and GST-Fyn-SH3 fusion proteins • Determination of GST-Fyn binding affinity with EGCG • EGCG effects on Fyn kinase activity *in vitro* • Immunoprecipitation and kinase assays • Determination of AP-1, STAT1, and CREB DNA binding	• EGCG inhibits EGF-induced JB6 CI14 cell transformation in a dose-dependent manner • EGCG inhibits EGF-induced Fyn phosphorylation in a dose-dependent manner • EGCG specifically binds with the Fyn SH2 domain • EGF-induced phosphorylation of p38 MAP kinase (THr180/Tyr182), ATF-2 (Thr71), and STAT1 (Thr727) is inhibited in siRNA-Fyn-JB6 CI14 cells • siRNA-Fyn inhibits EGF-induced cell transformation • EGF-induced DNA binding ability of AP-1, CREB, and STAT1 is decreased in siRNA-Fyn-JB6 CI14 cells	This is a novel and important mechanism that may be involved in EGCG-induced inhibition of cell transformation
Devika, P.T., Stanley Mainzen Prince, P. 2008. (-) Epigallocatechin gallate (EGCG) prevents	Male albino Wistar rats weighing 140-160 g were pre-treated with EGCG (30 mg/kg) by gavage daily for a period of 21 days. ISO was then subcutaneously injected into rats at intervals of 24 hours for 2 days	• Pretreatment with EGCG significantly decreased the levels of TBARS and lipid hydroperoxides and increased the activities of SOD and catalase in the heart mitochondria • Pretreatment with EGCG significantly	Study confirms preventative effects of EGCG on isoproterenol-induced mitochondrial damage in experimentally

Citation	Study Design	Results	Relevance to Kao GRAS Determination
mitochondrial damage in isoproterenol-induced cardiac toxicity in albino Wistar rats: A transmission electron microscope and *in vitro* study. *Pharmacol. Res.* 57(5):351-357.	Studies: • Isolation of heart mitochondria • Estimation of lipid peroxidation products, antioxidants, mitochondrial enzymes, and protein content in heart mitochondria • TEM studies • *In vitro* studies on the effects of EGCG on scavenging, superoxide anion, and hydroxyl radicals.	increased the activities of GPx, GRx, GST, and GSH in the heart mitochondria • Pretreatment with EGCG significantly increased activities of ICDH, SDH, MDH, α-KGDH, NADH-dehydrogenase and cytochrome-x-oxidase • Pretreatment with EGCG resulted in mild separation of cristae without swelling and vacuolation • EGCG scavenges radicals *in vitro* in a concentration-dependent manner	induced myocardial infarction in Wistar rats
Jia, Y., Alayash, A.I.. 2008. Effects of (-)-epigallo-catechin gallate on the redox reactions of human hemoglobin. *Free Radic. Biol. Med.* [epub ahead of print]	*In vitro* study investigating the effects of EGCG on auto-oxidation, H_2O_2-induced oxidation and subsequent ferryl heme reduction kinetics in chemically cross-linked human Hb (DBBF), a diaspirin cross-linked human Hb that has been intensively investigated. Studies: • Hb auto-oxidation experiments • Ferryl hemoglobin formation and reduction • Sulf hemoglobin measurement • Kinetics of ferryl hemoglobin reduction	• EGCG appears to exhibit strong pro-oxidant effects on DBBF and unmodified HbA_0 • Considerable reduction in the rates of oxidation of DBBF in the presence of EGCG and SOD were seen • In the presence of EGCG, rates of oxidation of DBBF were increased by approximately 56 times, showing an accelerated oxidation process triggered by EGCG • EGCG is a highly potent reductant of ferryl Hb when compared with some iron chelators	DBBF (ferrous) is rapidly oxidized to the ferric form in the presence of EGCG relative to normal spontaneous oxidation of Hb. A balance between the pro and antioxidant properties of EGCG should be taken into account if EGCG is used in combination therapy with redox active

Citation	Study Design	Results	Relevance to Kao GRAS Determination
		• The level of the sulHb, thus the level of detectable ferryl Hb in the reaction, was significantly reduced as EGCG medium increased • It is physiologically relevant to measure the ferryl Hb reduction by EGCG at low pH to evaluate its protective capability against the pro-oxidant activity of modified and unmodified Hbs, especially when used as cell free blood substitute.	acellular Hbs.
Beretta, G., et al. 2008. Quenching of α,β-unsaturated aldehydes by green tea polyphenols: HPLC-ESI-MS/MS studies. *J. Pharm. Biomed. Anal.* [epub ahead of print]	*In vitro* study in human colon tumor cells exposed to butyrate, investigating whether EGCG was able to eliminate the formation of HNE (4-hydroxyl nonenol), an α,β-unsaturated aldehyde, by a quenching mechanism. Studies: • Consumption of HNE by catechins and green tea • Measurement of H_2O_2 • HPLC – DAD – ESI – MS Analysis • ESI – MS/MS • NMR analysis	• Under the physiological conditions of the lower intestinal tract, EGCG dose- and time-dependently quenched 50 μM HNE • HNE-trapping activity of EGCG is strictly pH-dependent • The reaction mixture of HNE and EGCG after 24-hr incubation did not show epoHNE or the formation of an adduction product between EGCG and epo-HEN, but only a dehydrated adduct between EGCG and HNE • The formation of a EGCG-HNE covalent adduct was established for HPLC-ESI-MS • EGCG can quench different types of	These results suggest that EGCG and green tea extract, besides the proposed mechanisms of chemoprevention that target multiple cell-signaling pathways that control cell proliferation and apoptosis in cancer cells, can also prevent protein carbonylation in the tumor tissue environment, depending on the pH of the medium

Citation	Study Design	Results	Relevance to Kao GRAS Determination
		α,β–unsaturated aldehydes • EGCG is the most active of the green tea catechins in HNE entrapping; only adducts between HNE and EGCG were detected in the green tea chromatogram, indicating that EGCG plays a primary role in HNE sequestering • EGCG's ability to quench HNE through formation of a compound with a highly stable C-C bond, a mechanism different from HNE quenching by histidine-containing di-peptides, which involves the C-N bond • In an aqueous environment, EGCG is present as a tautomeric pair. This equilibrium involves the carbanionic form of dissociated EGCG, with the negative charge equally distributed between positions C-6 and C-8	surrounding the tissue, the type of tumor, the stage of dysregulation of lipid peroxidation, and finally, the stage of carcinoma development.
Ehrnhoefer, D.E., et al. 2008. EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers.	*In vitro* study in molecular chaperone His-tagged αS protein demonstrating the modulation of αS and Aβ fibril formation pathway by EGCG using biochemical, biophysical, and cell biological methods. Studies: • Analyzed the effect of EGCG on αS	• EGCG stimulates αS oligomer assembly • EGCG binds directly to natively unfolded αS • EGCG directly binds to αS polypeptide main chains • EGCG prevents αS β–sheet formation	• EGCG efficiently inhibits fibrillogenesis of both α-synuclein and amyloid-β by directly binding to the natively

Citation	Study Design	Results	Relevance to Kao GRAS Determination
Nat. Struc. Mol. Biol. 15(6): 558-566.	aggregation using a Thioflavin T (Tht) binding assay • Investigated the effect of EGCG on αS fibrillogenesis by negative-stain EM • Analyzed the effect of EGCG on αS aggregation by size-exclusion chromatography • Determined whether EGCG directly associates with unfolded αS using a nitroblue tetrazolium (NBT) staining assay • Investigated whether EGCG also binds directly to both SDS-stable αS oligomers, examining time-resolved aggregation reactions using the NBT assays • Since αS and BSA proteins are both recognized by EGCG, determined individual binding using NMR techniques • Recorded two-dimensional ^{1}H and ^{15}N spectra of αS with different molar ratios of EGCG • Performed CD spectroscopy experiments with EGCG-treated and untreated αS protein • Carried out *in vitro* seeding experiments	• EGCG stimulates assembly of non-toxic, off-pathway oligomers • EGCG prevents amyloid-β fibrollogenesis	unfolded polypeptides and preventing their conversion into toxic, on-pathway aggregating intermediates • This suggests a generic effect on aggregation pathways in neurodegenerative diseases

Citation	Study Design	Results	Relevance to Kao GRAS Determination
	with performed αS fibrils and EGCG-stabilized oligomers • Tested whether amyloidogenic αS oligomers can be specifically detected with the conformation-specific antibody using time-resolved dot blot assays • Investigated EGCG-generated oligomers for mammalian cell toxicity using standardized 3-2,5-diphenyltetrazolium bromide reduction assays with PC12 cells • Incubated different [EGCG] with unpolymerized $A\beta_{42}$ polypeptide and monitored the formation of amyloid using the ThT assay		
Bandele, O.J., Osheroff, N. 2008. (-)-Epigallocatechin gallate, a major constituent of green tea, poisons human type II topoisomerases. *Chem. Res. Toxicol.* 21(4): 936-943.	*In vitro* study in recombinant wt- human topoisomerase IIα and β expressed in *Saccharomyces cerevisiae* and purified to determine the effects of green tea extract and EGCG on DNA cleavage and ligation mediated by human topoisomerase IIα and β. <u>Studies</u>: • Ligation of cleaved plasmid DNA by human Topoisomerase II • DNA cleavage site utilization	• Enhancement of Topoisomerase II-mediated DNA cleavage by green tea extract • Enhancement of Topoisomerase II-mediated DNA cleavage by EGCG • EGCG is a redox-dependent Topoisomerase II poison	Results provide strong evidence that EGCG is a redox-dependent Topoisomerase II poison and utilizes a mechanism similar to that of 1,4-benzoquinone

Citation	Study Design	Results	Relevance to Kao GRAS Determination
Siu, A.W., et al. 2008. Glutamate-induced retinal lipid and protein damage: the protective effects of catechin. *Neurosci. Lett.* 432(3): 193-197.	*In vitro* study investigating the effects of catechin on the glutamate-treated retina. Porcine retinal homogenates were incubated with glutamate (20 nmol) at 37° for 60 min. Catechin was co-incubated with the glutamate-treated retina in the same condition. Studies: • MDA and protein levels were determined using the LPO586 and Bradford assays • Various amounts of catechin and the effect of trolox were compared • Retinal homogenates were incubated with glutamate or TRIS • After glutamate treatment, protein spots with significant changes were marked and excised from the two-dimensional gels	• Mean MDA increased significantly after the glutamate incubation in a dose-dependent manner • A significant difference exists between catechin and trolox • Bonferroni's multiple comparisons showed that all catechin concentrations reduced MDA significantly • Glutamate modifies the lipid and expressions of seven proteins in a homogenized receptor-free retinal system • Catechin protects the retinal tissue from glutamate-induced LPO and protein damage • Catechin significantly reverses the glutamate-mediated LPO and changes of pyruvate dehydrogenasem, 5HT receptor, thioredoxin peroxidase, and peroxiredoxin 6 expressions	Study shows that (a) retinal glutamate toxicity is mediated by LPO and protein modification, and (b) catechin ameliorates the toxicity
Kawai, Y., et al. 2008. Galloylated catechins as potent inhibitors of hypochlorous acid-induced DNA	*In vitro* study screening HCL-60 cells with various phenolic compounds for their scavenging activity for chlorinating intermediates including HOCl and chloramines to determine if tea catechins are good inhibitors of DNA base	• Natural phenolic antioxidants for scavenging and/or reducing chlorinating intermediates *in vitro* - flavonoids including tea catechins and quercetin exhibit a strong inhibitory effect, especially ECg and EGCg	Results showed that green tea catechins, especially 3-galloylated catechins, may be the plausible candidates for the

Citation	Study Design	Results	Relevance to Kao GRAS Determination
damage. *Chem. Res. Toxicol.* 21(7):1407-1414.	chlorination. Studies: • Performed inhibition assay for the formation of chlorinated dC residues • Performed analysis of chlorinated catechin derivates • Performed analysis of ECg and chlorinated ECg in HL-60 cells • Performed HPLC-MS/MS analysis • Immunocytometric analysis of HOCl-modified DNA	• Four catechins inhibited the formation of 5-CldC in DNA in a dose-dependent manner. The inhibitory effect f ECg and EGCg was much stronger than that of the non-galloylated catechins • ECg and EGCg more efficiently inhibit the formation of the Cl_2dC residues in DNA • Catechins dose-dependently reduced N-CldC with the appearance of unmodified dC • ECg inhibits HOCl-derived cellular DNA damage • Pretreatment with different concentrations of ECg for 2 hours dependently inhibit the DNA chlorination in HL-60 cells	prevention of inflammation- derived DNA damage and perhaps carcinogenesis
Sukhthankar, M., et al. 2008. A green tea component suppresses posttranslational expression of basic fibroblast growth factor in colorectal cancer.	*In vitro* study examining posttranslational regulation of bFGF by EGCG in human colorectal cancer cells. bGFG in intestinal tumor formation of $APC^{Min/+}$ mice with and without catechin treatment was also examined. Studies: • RT-PCR and Western blot analysis on cell cultures	• Both EGCG and ECG completely suppressed bFGF protein expression • EGCG suppresses bFGF expression in LoVo cells in a time-dependent manner • All cells showed a response to EGCG, except MCF-7 breast cancer cells, suggesting that bFGF expression occurred in most of the cancer cells	Catechin compounds have fewer adverse affects than chemotherapeutic agents and hence can be used in cancer therapeutics to suppress growth and metastasis by

Citation	Study Design	Results	Relevance to Kao GRAS Determination
Gastroenterology. 134(7): 1972-1980.	• vFGF expression vector cloned, transfected, performed histidine pull-down experiment • Performed enzyme-linked immunosorbent assay for bFGF • Performed ubiquitin and 20S proteasome assay • Performed animal study (mice receive EGCG or ECG in drinking water for 2 months) • Immunostain for factor VIII	and that EGCG may reduce bFGF expression • Proteasome inhibition restores the suppression of bFGF by EGCG • Only lactacystin containing trypsin-like inhibitory property affects bFGF suppression by EGCG • Exogenous bFGF proteins respond to cycloheximide • EGCG may suppress intestinal tumorigenesis *in vivo* by reducing bFGF expression and angiogenesis	targeting proteins such as bFGF
Spina, M., et al. 2008. Mechanism of inhibition of wt-dihydrofolate reductase from *E. coli* by tea epigallocatechin-gallate. *Proteins*. 72(1):240-251.	*In vitro* study using wild-type DHFR from AG-1 strain of *E. coli.* This article reports a detailed kinetic and equilibrium study on the inhibition of DHFR by green tea EGCG, taking into account inhibitory effects. Studies: • DHFR assays and kinetic data analysis • Inhibition of dihydrofolate reductase by tea catechin • Biocensor studies • Molecular modeling of the DHFR/EGCG complex	• EGCG strongly inhibits the activity of wt-DHFR from *E. coli* • EGCG acts as a bisubstrate inhibitor: in fact, according to one of the tested mechanisms, it is possible for inhibitor (I) to bind to free enzyme, E-DHF complex (NADPH site) and/or E-NADPH complex (DHF site) • Biphasic association and dissociation time courses of EGCG binding to immobilized DHFR are reported: this behavior is ascribed to the possible ability of EGCG to bind both to folate and NADPH sites • EGCG association rate to DHFR at	Data confirmed the selectivity of antifolate compounds with respect to the different source of enzyme (bacterial or mammalian DHFR) and the possible role of tea catechins as chemopreventative agents.

Citation	Study Design	Results	Relevance to Kao GRAS Determination
		DHF site was about 30-fold higher in comparison with the association at NADPH site	
Coyle, C.H., et al. 2008. Antioxidant effects of green tea and its polyphenols on bladder cells. *Life Sci.* 83 (1-2):12-8.	*In vitro* study utilizing normal/malignant (high/low grade) human bladder uroepithelial cells to investigate the antioxidant properties of green tea extract (GTE), polyphenon-60 (PP-60; 60% pure catechins), and two GTE components (EGC and EGCG) following oxidative stress with H_2O_2. Studies: • Assessment of cell viability using the Sigma *In Vitro* toxicology assay kit • Measure apoptosis using the Annexin V detection kit • Measure intracellular reactive oxygen species (ROS)	• PP-60, ECG and EGCG catechins protect human bladder cells from H_2O_2-induced oxidative stress • PP-60, EGC, and EGCG significantly improved the viability of UROtsa cells compared to H_2O_2-treated cells • ECG and EGCG catechins protect human bladder cells from H_2O_2-induced apoptosis • Catechins modulate apoptosis in normal human bladder cells via alteration of reactive oxygen species (ROS) signaling • H_2O_2 stimulates ROS production in normal human bladder cells	These findings demonstrate that urothelium cell death via H_2O_2-induced oxidative stress is mediated, in part, through superoxide (O_2-), and potentially, direct H_2O_2 mechanisms, suggesting that green tea polyphenols can protect against oxidative stress/damage and bladder cell death
Nishikawa, H., Kitani S. 2008. Tea catechins have dual effect on mast cell degranulation induced by compound 48/80. *Int.*	*In vitro* study to investigate catechin's effect on intracellular ROS generation and degranulation in mast cell activation. Studies: • Measure the intracellular free Ca^{2+} concentration • Measure degranulation by monitoring release of β–hexosaminidase from cells	• The compound 48/80 activates CM-MC mast cell • C48/80 increased intracellular DCF oxidation dose and time dependently • The intracellular ROS generation by NADPH oxidase is related with C48/80-induced degranulation from CM-MC.	There is an optimal level of ROS for degranulation, and catechins have a dual function by controlling the ROS levels.

Citation	Study Design	Results	Relevance to Kao GRAS Determination
Immunopharmacol. 8(9):1207-1215.	into the supernatant • Measure intracellular ROS generation using a fluorometric assay	• Catalase above 10 units/ml removed inhibitory effects by low ROS or the degranulation by high exogenous ROS and that there is a presence of optimal amount of ROS for mast cell activation	
Lee, S.I., et al. 2008. Effect of green tea and (-)-epigallocatechin gallate on ethanol-induced toxicity in HepG2 cells. *Phytother. Res.* 22(5):669-674.	*In vitro* study in HepG2 cells to compare EGCG, L-theanine, and caffeine for their protective effects against ethanol-induced cytotoxicity, using GGT as a marker. Studies: • GGT activity was assayed calorimetrically • Cell viability was assayed using MMT • Determined intracellular GSH content	• EGCG was found to inhibit the ethanol-induced cell death more efficiently than did green tea extract itself • GTE and EGCG inhibited the GGT increase dose-dependently while L-theanine had no significant effect and caffeine enhanced GGT release even more • EGCG, 4-MP, GSH, NAC, SAM, and acivicin failed to preserve intracellular GSH upon exposure to ethanol • EGCG and 4-MP lowered the GSH significantly, even in the absence of ethanol treatments	• The cyto-protective effects of green tea could be attributed to the inhibition of GGT activity by EGCG. • Study suggests that GGT inhibitors including EGCG may provide a novel strategy for attenuating ethanol-induced liver damage.
Oh, C.J., et al. 2008. Epigallocatechin gallate, a constituent of green tea, regulates high glucose-induced	*In vitro* study with U9 37 cells to investigate the effect of EGCG on high glucose-induced apoptosis. Cells were in two separate groups; pre-treated with 1 μM EGCG for 2 hours or not. They were then exposed to 35 mM glucose for 2 days	• ROS plays a pivotal role in apoptosis and reductants can block or delay this process • Cells pre-treated with 1 μM EGCG for 2 hours were significantly more resistant than un-treated cells to high	EGCG pre-treated cells showed significant suppression of apoptotic features such as DNA

000587

Citation	Study Design	Results	Relevance to Kao GRAS Determination
apoptosis. *Arch. Pharm. Res.* 31(1):31-40.	Studies: • Immunoblot analysis • DAPI staining • DNA fragmentation using the diphenylamine assay • PI staining to determine the portion of apoptotic cells • Determine cellular reduction status using hydrogen peroxide • Oxidative DNA damage estimated using a fluorescent binding assay • Mitochondrial damage determined by mitochondrial membrane potential transition (MPT)	glucose-induced apoptosis • When cells were exposed to glucose, apoptotic cells were increased markedly in untreated cells as compared to EGCG-treated cells • The pre-treatment of EGCG resulted in a significantly lower intracellular level of H_2O_2 as compared to control • The decrease in the efficiency of GSH recycling may be responsible for the higher concentration of intracellular peroxides in control cells treated with high glucose • EGCG appears to protect cells from oxidative DNA damage caused by high glucose • High glucose-induced cleavage of procaspase-3 into the active form of caspase-3 and caspase-3 induces degradation of PARP or lamin B. The results indicate that EGCG exhibits a protective effect on the high glucose-induced apoptosis	fragmentation, damage to mitochondrial function, and modulation of apoptotic marker proteins upon exposure to high glucose. Study indicates that EGCG may play an important role in regulating the apoptosis induced by high glucose presumably through scavenging of reactive oxygen species

000588

Citation	Study Design	Results	Relevance to Kao GRAS Determination
Erguder, I.B., et al. 2008. Effects of aqueous green tea extract on activities of DNA turn-over enzymes in cancerous and non-cancerous human gastric and colon tissues. *Altern. Ther. Health Med.* 14(3):30-33.	*In vitro* study to investigate possible effects of green tea extract on the activities of DNA turn-over enzymes, namely adenosine deaminase (ADA) and xanthine oxidase (XO) in gastric and colon tissues from patients with stomach and colon cancer. Studies: • Six cancerous and 6 non-cancerous adjacent human gastric tissues, and 7 cancerous and 7 non-cancerous adjacent colon tissues obtained surgically were treated with aqueous green tea extract at three different concentrations (0.05%, 0.5%, and 1.25%) for 1 hour and then ADA and XO levels were measured	• In both cancerous and non-cancerous tissue, XO activities were found to elevate in correlation with increased extract concentrations in both cancer types. ADA activity was found to decrease in the cancerous part of the stomach tissue and to increase in the non-cancerous part.	Green tea may support the medical treatment of stomach and colon cancer
Mukai, D., et al. 2008. Potential anthelmintics: polyphenols from the tea plant *Camellia sinensis L.* are lethally toxic to *Caenorhabditis elegans*. *Nat. Med. (Tokyo).* 62(2):155-159.	*In vitro* study to report the isolation and identification of a new tannin gallate, with known polyphenols, from the tea plant *Camellia sinensis* L. and their toxicities to *C. elegans*, with reference to novel anthelmintic relevancies. Studies: • Identify plant material, extraction, and isolation • Enzymatic hydrolysis of compound 8 • Prepare egg-bearing adults of *C. elegans* and determine LC_{50}	• Compound 8 consists of two flavan-3-ol units and one galloyl group • A diagnostic HMBC correlation between H-3' and carbonylic C-7'' was indicative of the presence of a C-3'-O-C-7'' linkage in 8 • Green tea polyphenols are toxic to *C. elegans*	These data shows that many green tea polyphenols may be potential anthelmintics

Citation	Study Design	Results	Relevance to Kao GRAS Determination
Hisamura, F., et al. 2008. Synergistic effect of green tea polyphenols on their protection against FK-506 inducted cytotoxicity in renal cells. *Am. J. Chin. Med.* 36(3): 615-624.	*In vitro* study to evaluate the synergistic interactions of tea polyphenols on FK506-induced cell death and ROS levels in LLC-PK1 renal cells Studies: • Cytotoxicity of FK506 measured by the MTT assay • Measured ROS formation using hydrogen peroxide • Measured caspase activity using CPP32/Caspase-3 colorimetric protease assay kit	• The effect of tea polyphenols on FK 506-induced cytotoxicity in LL-PK1 cells shows a dose-dependent protective effect for EGCG and EGC • CO-treatments with EGCG and EGC, EGCG or ECG, and EGC and ECG have strong synergistic effects on the protection of FK 506-induced cell death • EGCG, EGC, and the combined treatment of EGCG and EGC show significant suppression of the increased levels of intracellular ROS. Time-dependent effects • EGCG, EGC, and combined treatment of EGCG and EGC suppressed the level of caspase-3 activity in cells treated with FK 506	Synergistic effects of the constituents of green tea extract suggest that its protective effects may reside in more than just one of its constituents
Devika, P.T., Mainzen Prince, P.S. 2008. (-)-Epigallocatechin gallate EGCG prevents isoprenaline-induced cardiac marker enzymes and	*In vivo* study using male albino Wistar rats weighing 140-160 g. Different doses of EGCG (5, 10, 20, and 30 mg/kg) were dissolved in DMSO and administered by gavage at 7, 14, 21, and 28 days before isoprenaline administration to determine dose-dependence and duration of treatment against ISO-induced myocardial infarction.	• Pretreatment with EGCG significantly reduced the increase in heart weight • Pretreatment with EGCG significantly decreased the activities of enzymes in serum CK, CK-MB, LDH, AST, and ALT • Pretreatment of EGCG significantly increased activities of enzymes (CK, LDH, AST, and ALT) in the heart	EGCG exhibits beneficial effects on enzymes and electrolytes in the heart due to antioxidant and membrane-stabilizing effects

Citation	Study Design	Results	Relevance to Kao GRAS Determination
membrane-bound ATPases. *J. Pharm. Pharmacol.* 60(1):125-133.	Studies: • Assay of marker enzymes • Assay of Na+/K+ATPase • Assay of Ca2+ ATPase • Assay of Mg2+ ATPase • Estimation of Na+, K+, Ca2+ and protein concentration • Separation and quantification of LDH isoenzymes	• EGCG pretreatment decreased the intensity of LDH 1 and LDH 2 • Pretreatment with EGCG significantly increased the activity of Ca2+ and Mg2+-ATPases • Pretreatment with EGCG significantly decreased the levels of sodium and calcium and increased the levels of potassium in the heart	
Renno, W.M., et al. 2008. Effect of green tea on kidney tubules of diabetic rats. *Br. J. Nutr.* 6:1-8.	*In vivo* study examining the long-term effect of green tea extract on streptozoticin-induced diabetic nephropathy and on the glycogen accumulation in the kidney tubules. Male Sprague-Dawley rats were randomly assigned to normal control groups (2, 6, 8, and 12 weeks) and five diabetic groups (N=10) of comparable age. A GT diabetic group received 16% concentration of GT for 12 weeks post-diabetes induction as their sole source of drinking water. Studies: • Blood glucose levels determined • Blood samples taken by cardiac puncture and used for determination of blood urea nitrogen • Serum glycosylated protein level measured using method of McFarland et	• Body weights of diabetic rats treated with GT were significantly lower than the normal control group • Diabetic rats treated with GT showed significant reduction in both serum glucose and glycosylated protein • Blood urea nitrogen level was significantly lower • GT-treated group showed significant higher Ccr • Urinary urea nitrogen excretion was significantly lower • Creatine, glucose. and protein excretion declined • The number of glycogen-filled tubules decreased almost completely in the GT-treated kidneys	Results indicate that in STZ diabetes, kidney function appears to be improved with GT consumption which also prevents glycogen accumulation in the renal tubules, probably by lowering blood levels of glucose. Therefore, GT could be beneficial therapy in the management of diabetic nephropathy

Citation	Study Design	Results	Relevance to Kao GRAS Determination
	al. • Performed all experimental protocols and group treatments • Performed specimen collection and histological staining • Quantification of glycogen-filled proximal tubules		
Call, J.A., et al. 2008. Endurance capacity in maturing mdx mice is markedly enhanced by combined voluntary wheel running and green tea extract. *J. Appl. Physiol.* [epub ahead of print].	*In vivo* study using male mdx mice testing endurance exercise and GTE (0.5% added into diet) separately and in combination to increase endurance and antioxidant activities. Three hypothesis were tested: 3 weeks of endurance exercise initiated immediately post-weaning age (21 days) will improve skeletal function and serum antioxidant capacity, and decrease muscle lipid peroxidation; GTE supplementation without running will demonstrate similar effects; GTE supplementation in combination with endurance exercise will be more beneficial than either alone. Studies: • Conducted voluntary wheel running experiment • Tested excised muscles for isometric contractile and mechanical properties • Determine contractile protein contents	• GTE mice ran a total distance of 128% greater than controls • Absolute myosin content, total muscle protein relative to muscle mass, and total contractile protein relative to total muscle protein were all increased in GTE mice • 22% greater antioxidant capacity • Demonstrated 64% less lipid peroxidation in the gastrocnemius, and 29% less lipid peroxidation in the heart • Had 95% greater CS activity in the quadriceps muscles • 35% great CS activity in the heart muscle • β-oxidation activity was 36% greater in the quadriceps and 35% greater in the heart muscle	Data suggest that both endurance exercise and GTE may be beneficial therapeutic strategies to improve muscle function

Citation	Study Design	Results	Relevance to Kao GRAS Determination
	and myosin heavy chain (MyHC) isoform distribution • Determine creatine kinase activity and antioxidant capacity • Determine lipid peroxidation • Determine citrate synthase activity • Determine beta-hydroxyl acyl-CoA dehydrogenase (BHAD) activity		
Leong, H., et al. 2008. Inhibition of mammary tumorigenesis in the C3(1)/SV40 mouse model by green tea. *Breast Cancer Res. Treat.* 107 (3): 359-369.	*In vivo* study using C3(1)/SV40 mice in 3 groups receiving either 0.1%, 0.3%, or 0.5% Poly E in tap water every Monday, Wednesday, and Friday beginning at 6 weeks of age as sole source of drinking water (equivalent to daily consumption of six cups of green tea by the average adult human). Animals from each group were killed at 8, 12, 15, and 20 weeks of age. Studies: • Measured chemoprevention with administration of green tea polyphenols • Analyzed mammary glands • Performed an enzyme-linked immunosorbant assay	• Green tea alters the natural history and histopathology of mammary tumor function • Green tea inhibits mammary ductal growth • Green tea has anti-angiogenic effects	Data strongly supports potential use of green tea as a breast cancer chemopreventative agent

Citation	Study Design	Results	Relevance to Kao GRAS Determination
Hsu, C.H., et al. 2008. Effect of green tea extract on obese women: a randomized double-blind, placebo-controlled clinical trial. *Clin. Nutr.* 27(3): 363-370.	A randomized, double-blind, placebo-controlled, clinical trial from July 2006 to June 2007 in Taipei Hospital, Taiwan. Seventy-eight of 100 obese women aged between 16 and 60 years with BMI> 27 kg/m2 and who had not received any other weight control agent within the previous 3 months of the study. Subjects were randomly divided into Groups A and B. • Group A (n=41) received GTE • Group B (n= 37) took cellulose as a placebo • Took one capsule (400 mg) three times a day for 12 weeks • BW, BMI, and WC were measured at the beginning of the study and after 12 weeks of treatment • A hormone peptides analysis was also conducted	• There was no statistical difference in percent reduction in BW, BMI, and WC between the GTE and placebo groups after 12 weeks of treatment • After treatment, the GTE group revealed significant reduction in WC, and levels of LDL-cholesterol and triglyceride, and marked increase in the levels of HDL-cholesterol, adiponectin and ghrelin • The placebo group showed significant reduction in HC and triglyceride only, and marked increase in the level of ghrelin alone	This study shows no statistical difference in percent reduction in body weight, BMI, or waist circumference between the GTE and placebo groups after 12 weeks of treatment.
Gawande, S., et al. 2008. Effect of nutrient mixture and black grapes on the pharmacokinetics of orally administered (-)-epigallocatechin-3-galate from green	*In vivo* study to determine bioavailability of EGCG. Five human volunteers, were each given a single oral dose of GTE (A), nutrient mixture (NM) containing GTE (B), and formulation B along with black grapes 250 mg C0. Blood samples were drawn at 0, 2, 4, 6, and 8 hours.	• EGCG mean plasma levels at hour 2 show significant differences between Group A and Groups B & C. C_{max} for EGCG was reached in hour 4 of each treatment. At hour 6, EGCG levels were only significant for Group C • Mean values for AUC at 8 hours show significantly higher values for C	Supplementation of nutrient mixture normally prescribed to cancer patients containing ascorbic acid, selenium, N-acetyl cysteine and other nutrients

Citation	Study Design	Results	Relevance to Kao GRAS Determination
tea extract: a human study. *Phytother. Res.* 22 (6):802-808.	Studies: • Samples collected for initial processing • Extraction and separation of EGCG from plasma • Extraction and analysis of quercetin equivalents in plasma	compared to groups A and B • The percent increase in the availability of EGCG for B and C groups was 14% and 27% respectively • For plasma levels of quercetin equivalents, the AUC of group C was significantly higher than the AUC values of groups B and A. The percent increase in the availability of quercetin for groups B and C was 9% and 12% respectively.	(formulation B) resulted in an increase of the systemic availability of EGCG by 14%, with C giving an additional 13%.
Mnich, C.D., et al. 2008. Green tea extract reduces induction of p53 and apoptosis in UVB-irradiated human skin independent of posttranscriptional controls. *Exp. Dermatol.* [epub ahead of print].	*In vivo* study to investigate the photochemopreventative effect of low-dose GTE under everyday conditions, addressing questions like GTP stability and tachyphylaxis. The study irradiated up to 100 ml/cm^2 of UVB light skin patches that were pretreated with either OM24 – containing lotion or a placebo lotion (application was applied 3 x daily for 24 days; irradiated on days 5 and 33 from 10 mJ/cm^2 to 100 mJ/cm^2). Biopsies taken from both irradiated and un-irradiated skin for both immunohistochemistry and DNA microarray analysis	• The number of UV-induced p53-positive keratinocytes was significantly reduced in OM24 compared to placebo-pretreated samples by 31.9% on say 6, and 36.3% on day 34 • The level of UV-induced apoptosis was also reduced by OM24 pretreatment on days 6 and 34 • 213 were identified genes that undergo at least a 2-fold expression change upon UV-exposure after either OM24 or placebo-pretreatment • Both analytical approaches show that OM24 pretreatment does not result in any gene expression changes that are	Study suggests GTE as a suitable everyday photochemo-preventative agent.

Citation	Study Design	Results	Relevance to Kao GRAS Determination
	Studies: • Erythema quantification • Immunohistochemistry and quantification of biopsies • Histochemical quantification • Gene expression analysis	significantly different from placebo-pretreatment	
Lee, H.J., et al. 2008. Modification of gamma-radiation response in mice by green tea polyphenols. *Phytother Res*. [epub ahead of print].	*In vivo* study using 7-week old female ICR mice for the jejunal crypt survival and apoptosis assays; 7-week male ICR mice for the endogenous spleen colony formation experiment. Animals were irradiated with 12 Gy for the jejunal crypt survival assay, 6.5 Gy for endogenous spleen colony, and 2 Gy for experiment on apoptosis Studies: • Jejunal crypt: 66 female mice (6 per group) divided into a) untreated controls b) irradiated control c) GT/ GTPs/ catechins/ DDC administration in combination with radiation. Animals were sacrificed 3.5 days after irradiation • Endogenous spleen: 81 male mice (9 per group) divided into a) irradiated control, b) GT/ GTPs/ catechins / DDC administration in combination with radiation. Mice were sacrificed 9 days after irradiation. Spleens were then	• Only the pretreatment with GT, DDC, and ECG resulted in a significant increase in the number of surviving crypts • The number of spleen colonies was higher in the mice that received the GT, GTPs, and EC • Pretreatment of GT, DDC, or polyphenols was associated with decreases in the number of cells with nuclei positively stained for apoptosis compared with control group • GT administration prior to irradiation protected the jejunal crypt, increased the formation of endogenous colonies, and reduced the frequency of radiation-induced apoptosis	Each of the catechins was a much less effective radioprotector, suggesting that total extract or a mixture of GTPS may be more effective than individual catechins

000596

Citation	Study Design	Results	Relevance to Kao GRAS Determination
	removed • Apoptosis experiment: 44 females (4 per group) divided into a) untreated control, b) irradiated control, c) GT/ GTPs/ catechins/ DDC administration in combination with radiation. Mice were sacrificed 6 hours after irradiation		

Legend:

5-CldC: Cationic lipid-DNA (non-coding) complexes are activators of the innate immune response that increase survival of rodents with some acute viral infections and cancers

α-KDGH: α- ketoglutarate dehydrogenase is a multienzymatic complex made up of three different types of enzymes, responsible for the conversion of α -ketoglutarate in to succinyl CoA. This is an important step in the citric acid cycle as it can be used to regulate energy production

ALT: Alanine Aminotransferase is found mainly in the liver, but also in smaller amounts in the kidneys, heart, muscles, and pancreas. ALT is measured to determine if the liver is damaged or diseased

AP-1: Activator protein 1; is a transcription factor which is a heterodimeric protein composed of proteins belonging to the c-Fos, c-Jun, ATF and JDP families. AP-1 upregulates transcription of genes containing the TPA response element

AST: Aspartate Aminotransferase is an enzyme in the blood. AST is normally found in red blood cells, liver, heart, muscle tissue, pancreas, and kidneys. When body tissue or an organ such as the heart or liver is diseased or damaged, additional AST is released into the bloodstream

BSA: Bovine serum albumin (BSA) is one of the most commonly used carriers for conjugation in antibody production. It belongs to the class of serum proteins called albumins, which make up about half of the protein in plasma and are the most stable and soluble proteins in plasma

Ccr: Cinnamoyl-coa reductase

CD: Circular dichroism (CD) spectroscopy measures differences in the absorption of left-handed polarized light versus right-handed polarized light which arise due to structural asymmetry

CK: Creatine kinase catalyses the conversion of creatine to phosphocreatine, consuming adenosine triphosphate (ATP) and generating adenosine diphosphate (ADP)

CK-MB: the CK enzyme consists of two subunits, either brain or muscle type. CK-MB is one of the three isoenzymes. The expression of genes for these subunits are located on different chromosomes: *B* on 14q32 and *M* on 19q13

CREB: cAMP response element-binding: proteins are transcription factors which bind to certain DNA sequences called *cAMP response elements* (CRE) and thereby increase or decrease the transcription of certain genes

CS: Chitin synthesis

DAPI: 4',6-diamidino-2-phenylindole is a fluorescent stain that binds strongly to DNA

DCF: dichlorofluorescin oxidation is used to measure the production of reactive oxygen species (ROS) within cells

DHFR: Dihydrofolate reductase reduces dihydrofolic acid to tetrahydrofolic acid, using NADPH as an electron donor

DMSO: Dimethyl sulfoxide is a colorless liquid that is an important polar aprotic solvent that dissolves both polar and nonpolar compounds

000598

EGF: Epidermal growth factor

EM: Electron microscope

ESI – MS/MS: Electrospray ionization tandem mass spectrometry

GGT: Gamma-glutamyl transferase

GST: Glutathione S-transferase gene

Gy: the SI unit of absorbed radiation dose- One gray is the absorption of one joule of radiation energy by one kilogram of matter

HPLC – DAD – ESI – MS: High-performance liquid chromatography-diode array detector (HPLC-DAD) coupled with electron-spray mass spectrometry (ESI-MS) is used to detect flavonoid profiles

ICDH: Iso-Citrate DeHydrogenase; enzyme which participates in the citric acid cycle by catalyzing the third step

ICR: Imprinting Control Region mouse

LDH: Lactate dehydrogenase is an enzyme that catalyses the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+

LPO: Lipid peroxidation refers to the oxidative degradation of lipids; free radicals remove electrons from the lipids in cell membranes, resulting in cell damage

MDA: Malondialdehyde is a reactive species occurs naturally and is a marker for oxidative stress

000599

MDH: Malate dehydrogenase is an enzyme in the citric acid cycle that catalyzes the conversion of malate into oxaloacetate (using NAD^+) and vice versa (this is a reversible reaction)

MMT: Methylcyclopentadienyl manganese tricarbonyl

NAC: N-acetylcysteine

OM24: a Swiss phytochemical extract; is a powerful Anti-Oxidant

RT-PCR: Reverse transcriptase Polymerase Chain Reaction

SAM: *S*-Adenosyl-L-homocysteine is an amino acid derivative used in several metabolic pathways in the organism *E. coli.*

SDH: Succinate-coenzyme Q reductase is an enzyme complex bound to the inner mitochondrial membrane. It is the only enzyme that participates in both the citric acid cycle and the mitochondrial electron transport chain

SDS: Sodium Dodecyl Sulfate

SOD: Enzyme superoxide dismutase catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide

STAT1: Member of the Signal Transducers and Activators of Transcription family of transcription factors; STAT1 is involved in upregulating genes due to a signal by either type I or type II interferons

TBARS: Thiobarbituric Acid Reactive Substances Assay

TEM: Transmission electron microscope

TRIS: Tris (hydroxymethyl) aminomethane- used as a buffer solution

000600

PARP: Poly (ADP-ribose) polymerase is a protein involved in a number of cellular processes involving mainly DNA repair and programmed cell death

HMBC: heteronuclear multiple bond coherence experiment detects long range coupling between proton and carbon (two or three bonds away) with great sensitivity

SUBMISSION END

Reference List for Industry Submission, GRN 000259

Pages	*Author*	*Title*	*Publish Date*	*Publisher*	*BIB_Info*
000270 - 000285	Sarma, Dandapantula N.; Barrett, Marilyn L.; Chavez, Mary L.; Gardiner, Paula; Ko, Richard; Mahady, Gail B.; Marles, Robin J.; Pellicore, Linda S.; Giancaspro, Gabriel I.; Low Dog, Tieraona	Safety of Green Tea Extracts: A Systematic Review by the US Pharmacopeia	2008	Drug Safety	Volume 31, Number 6, pgs 469-484
000348 - 000376	Kataoka, Kiyoshi; Takashima, Shinichiro; Shibata, Eiichiro; Hoshino, Eiichi	Body fat reduction by the long term intake of catechins and the effects of phyical activity	2004	Progress in Medicine	Volume 24, Number 12, pgs 3358-3370
000377 - 000401	Kozuma, Kazuya; Chikama, Akiro; Hoshino, Eiichi; Kataoka, Kiyoshi; Mori, Kenta; Hase, Tadashi; Katsuragi, Yoshihisa; Tokimitsu, Ichiro; Nakamura, Haruo	Effect of Intake of a Beverage Containing 540 mg Catechins on the Body Compostion of Obese Women and Men	2005	Progress in Medicine	Volume 25, Number 7, pgs 185-197
000402 - 000409	Matsumoto, Natsuki; Yamakawa, Mana; Hara, Yukihiko	Single Dose Oral Toxicity, and Oral Muscous Irritation Study of Green Tea Extract	1999	Jpn Pharmacol Ther	Volume 27, Number 9
000410 - 000422	Miyajima, E., et. al.	Study on the usefulness of combined-food intake for specified health uses - Combination of soybean protein and high-concentration catechins	2005	NA	NA
000423 - 000446	Miyazawa, Teruo; Nakagawa, Kiyotaka; Asai, Akira	Absorption and Metabolism of Natural Antioxidants	2000	Kagaku to Seibutu	Volume 38, Number 2, pgs 104-114
000447	Muramatsu, K.	NA	1991	Science of Tea	pg 153

NA- Not applicable

Pages	Author	Title	Publish Date	Publisher	BIB_Info
000448 - 000485	Nagao, Tomonori; Meguro, Shinichi; Soga, Satoko; Otsuka, Atsuko; Tomonobu, Kazuichi; Fumoto, Shinichi; Chikama, Akiro; Mori, Kenta; Yuzawa, Masayuki; Watanabe, Hiroyuki; Hase, Tadashi; Tanaka, Yukitaka; Tokimitsu, Ichiro; Shimasaki, Hiroyuki; Itakura, Hiroshige	Tea Catechins Suppress Accumulation of Body Fat in Humans	2001	Journal of Oleo Science	Volume 50, Number 9, pgs 717-728
000486 - 000517	Otsuka, Kazuhiro; Uchida, Hiroko; Yuzawa, Masayuki; Fumoto, Shinichi; Tomonobu, Kazuiti; Chikama, Akiro; Hase, Tadashi; Watanabe, Hiroyuki; Tokimitsu, Ichiro; Itakura, Hiroshige	Effects of tea catechins on body fat metabolism in women	2001	Japanese Journal of Nutritional Assessment	Volume 19, Number 3, pgs 365-376
000518 - 000542	Saitou	Study on Tea Drinks: Contents of Catechins (5 types), Caffeine, and Total Amino Acid	1998	NA	NA
000543	NA	NA	2002	Progress in Medicine	Volume 22, pgs 2189-2203
000544 - 000574	Tsuchida, Takashi; Itakura, Hiroshige; Nakamura, Haruo	Reduction of Body Fat in Humans by Long-term Ingestion of Catechins	2002	Progress in Medicine	Volume 26, Number 11, pgs 1459-1464

NA- Not applicable

Harry, Molly *

From: Mahinka, Stephen Paul [smahinka@morganlewis.com]
Sent: Tuesday, December 16, 2008 1:14 PM
To: Harry, Molly *
Cc: Gaynor, Paulette M
Subject: RE: Clarifications for GRN 000259 (Catechins from Green Tea Extract)

Ms. Harry -- thanks very much for your reply and continuing review of our client's Notice.

We have mentioned on several occasions orally, to Dr. Belay and at the meeting we had with numerous FDA official concerning this filing at FDA on July 15, 2008, our client's urgent concern that the Agency might be in a position to provide an indication as to clearance by year-end, in view of planning constraints and decisions that need to be made at the beginning of a new fiscal year for product introduction. It is our hope that, with the Agency having seen this Notice for the third time, its review can be considerably expedited from the 180-days for a filing never previously seen or reviewed in detail by the Agency.

We will providing responses later this week to the clarification questions raised by your email.

Best regards.

Steve Mahinka

Stephen Paul Mahinka
Morgan, Lewis & Bockius LLP
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From: Harry, Molly * [mailto:Molly.Harry@fda.hhs.gov]
Sent: Monday, December 08, 2008 1:15 PM
To: Mahinka, Stephen Paul
Cc: Gaynor, Paulette M
Subject: RE: Clarifications for GRN 000259 (Catechins from Green Tea Extract)

Dear Mr. Mahinka,

Thank you for acknowledging receipt of our e-mail to you seeking clarifications for GRN 000259 (catechins from green tea extract).

It appears from your e-mail that in prior communication with FDA, you expressed an urgent need to receive the response letter from FDA by the end of this calendar year. Neither I, nor several of my colleagues in the division, are aware of such prior communication. Our review of GRN 000259 is on going and we will do everything possible to complete our review within 180 days from the date the notice was filed.

If we have further questions, we will direct it to you, as you indicated in your e-mail.

Sincerely,

Stephen Paul Mahinka
Partner
202.739.5005
smahinka@MorganLewis.com

December 18, 2008

BY HAND DELIVERY

Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Attn: Ms. Molly Harry

Re: Clarifications for GRN 000259 (Catechins from Green Tea Extract)

Dear Ms. Harry:

On behalf of our client Kao Corporation ("Kao"), of Tokyo, Japan, this letter provides responses to the requests for clarification conveyed in your e-mail to Kathleen Sanzo dated December 5, 2008 concerning the GRAS Notice for Kao's Catechins from Green Tea Extract ("CGT"). For ease of review, each of your questions is provided below in bold, italicized text, followed by Kao's response. A revised version of the GRAS Notice, reflecting the corrections described below in track changes mode, as well as a clean copy of the corrected GRAS Notice, are provided at Attachment 1 to this letter (please note that attachments that have not been revised from the August 14, 2008 version of the GRAS Notice are not included in the corrected GRAS Notice).

1. ***Page ii, paragraph 5: Please clarify what the abbreviation "GCT" stands for. We also observed that the abbreviation "CTG" has been used in several instances throughout the notice (for example, in table on page 46/47). Please clarify.***

   The use of "GCT" on page ii, paragraph 5, and the use of "CTG" in a few instances should read "CGT" for Catechins from Green Tea Extract. The substance that is the subject of this GRAS Notice, catechins from green tea, is abbreviated "CGT" throughout the notice, and these references have been changed in the corrected GRAS Notice provided with this letter.

2. ***Page iii, paragraph 4: The last sentence reads: The NOAEL for 14-day oral exposure in rats has been reported to range from 500-20,000 mg/kg-bw/day, with decreased body weight the only treatment-related effect observed." We are unable to locate the study in the submission that used a dose of 20,000 mg/kg bw. Please clarify.***

   The correct upper dose level for the NOAELs from the 14-day studies is 2,000 mg/kg-bw (*see* Table 7, Yamaguchi et al. studies summarized on pages 48 and 52-53), and the reference has been changed in the corrected GRAS Notice attached.

3. ***Section III.A, Page 9-10: Please clarify the starting material for Kao's manufacturing process. Is it green tea leaves or marketed crude catechins? Page 9, Process 1, says "Extraction of crude catechins from tea leaves. Kao uses marketed crude catechins as a primary ingredient." However, Figure 1 on page 10 indicates Process 1 begins with tea leaves. The manufacturing process diagram for Type-2 also indicates that tannase treatment occurs prior to the production of crude catechins.***

   Kao uses "crude catechins" as the starting material for both the Type-1 and Type-2 CGT. Figure 1 in the GRAS Notice shows the complete manufacturing process, but, as noted in Section III.A, the only manufacturing process that is used by Kao is Process 2 in Figure 1 (reproduced below for ease of review).

   Process 1: Extraction of crude catechins from tea leaves. Crude catechins are the primary ingredient in Kao's CGT. Kao uses marketed crude catechins as a primary ingredient.

   Process 2: Manufacturing process of CGT. *This manufacturing process corresponds to the manufacturing process of the GRAS substances in Kao's factory* [emphasis added].

   Process 3: Manufacturing process of final product (beverage containing CGT).

   Tannase treatment occurs at the crude catechins manufacturer's plant.

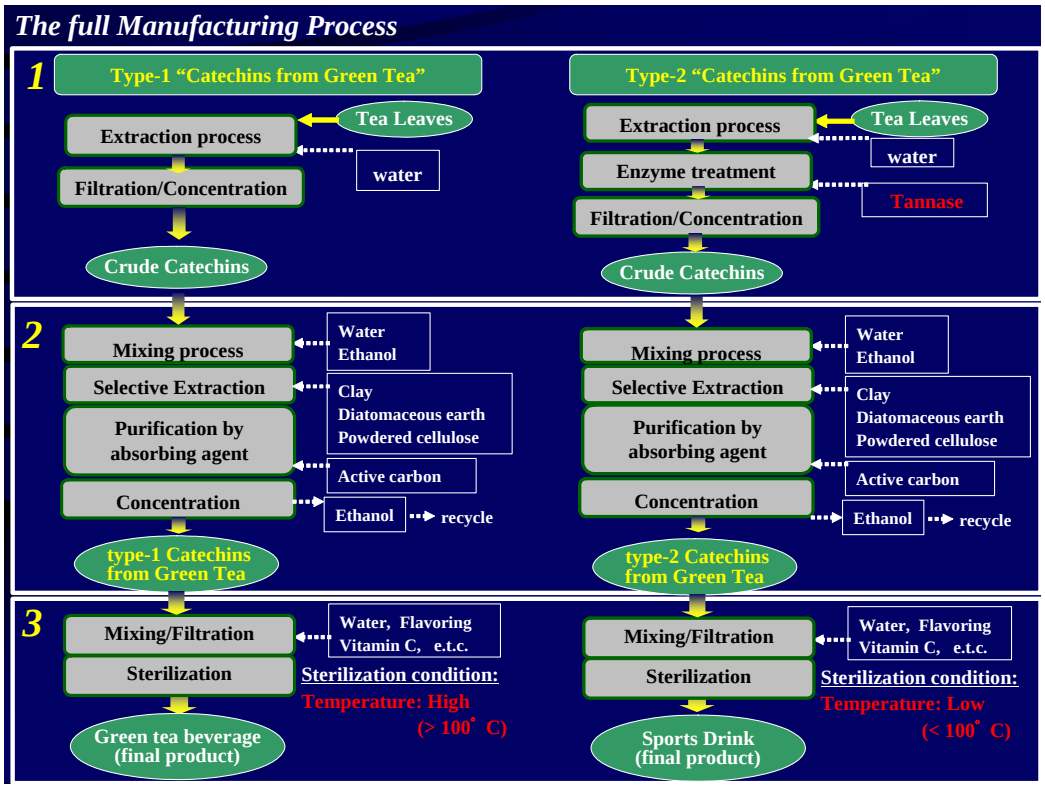

Figure 1.

4. ***Section III.C, Page 13: Heavy metal, specification: Lead <30 ppm. What is the typical lead level in the manufacture of catechins from green tea extract (the specification is <30 ppm, but you report a method LOD of 0.4 ppm). Appendix I does not have heavy metal specs included for the three batches of CGT lots.***

The heavy metal specifications provided in Section III.C, Page 13 (see below) are for the *finished beverage*, as indicated in the footnote to the specifications table.

Heavy metal specification:

Heavy metal*	**Specification (percentage by weight)**
Arsenic	below detection limit (<0.2 ppm)
Sn	not more than 150 ppm
Cadmium	below detection limit (<0.1 ppm)
Lead	below detection limit (<0.4 ppm)

*Microbiological and heavy metal specifications are applied to the beverage form, after manufacture. [Note: This specification is based on Japanese soft drink law.]

As shown on Page 13, following are the specifications used for the CGT preparations, *prior to the beverage manufacturing process*. As the concentration of CGT in the bottled tea and sports drink is 0.1%-0.2% respectively, the specification for lead in CGT is higher than for finished beverages.

Heavy metal	**Specification (percentage by weight)**
Arsenic	<2 ppm
Lead	<30 ppm

Typical heavy metal levels in three lots each of both the finished beverage and CGT are provided below:

	Lot Numbers		
Heavy metal (Green tea beverage using Type-1 CGT)	**M01-06**	**M01-08**	**GSP22**
Arsenic	Not detected	Not detected	Not detected
Sn	Not detected	Not detected	Not detected
Cadmium	Not detected	Not detected	Not detected
Lead	Not detected	Not detected	Not detected
Heavy metal (Sports drink using Type-2 CGT)	**SAS05TA1**	**SJS07TA1**	**SWS04YA1**
Arsenic	Not detected	Not detected	Not detected
Sn	Not detected	Not detected	Not detected
Cadmium	Not detected	Not detected	Not detected
Lead	Not detected	Not detected	Not detected
Heavy metal (Type-1 CGT)	**0307151T**	**040413T**	**040603K**
Arsenic	Not detected	Not detected	0.2 ppm
Lead	2.3 ppm	1.9 ppm	1.7 ppm
Heavy metal (Type-2 CGT)	**0606181C**	**0701311C**	**0804051C**
Arsenic	Not detected	Not detected	Not detected
Lead	Not detected	Not detected	Not detected

5. ***Section III.E, Page 15: In the table, titled, "Typical Product Characteristics," Column 3 (Tannase-treated Catechins from Green Tea Ingredient (w/w%)) has the following entries:***

   Total catechin monomers = 67/4%

   Polyphenols (67.4%)

   Polyphenols (except catechins) = 5.1%

These numbers do not add up. Please clarify.

The total catechin monomers content is 62.3%, as indicated in Tables 9 and 16 of the GRAS Notice. The "Typical Product Characteristics" table in Section III.E, Page 15, has been changed in the corrected GRAS Notice attached to reflect this value.

6. ***Section IV.D, page 24: Using the food codes provided by Kao, and levels of 540 mg per serving (350 ml), the mean and 90th percentile consumption values that we calculated are 50% higher than the values provided by Kao. Please clarify the levels used by Kao to arrive at the reported potential intakes of 602 mg/day (mean) and 1217 mg/day (90th percentile) for Scenario #4.***

The process and assumptions used to calculate the estimated daily intakes ("EDI") by Exponent, an outside consulting firm, for the various consumption patterns are provided at Attachment 2. The discrepancy between Kao's estimates and FDA's estimates appears to be the result of FDA assuming a 350 mL volume for all beverages (bottled teas are 350 mL and sports drinks are 500 mL), and making no adjustments to reflect that 1 mL of beverage is not necessarily equivalent to 1 gram of beverage. Both of these assumptions would result in an over estimation of the estimated daily intake. To confirm whether these differences in assumptions could explain the apparent discrepancies between Kao's and FDA's calculations, Kao requested that Exponent run its analyses using several versions of assumptions. When a 350 mL volume and 1 mL = 1 gram is used for all beverages, the EDIs are approximately 49% higher than the original estimates presented in the GRAS Notice (see Table 9 in Attachment 2), similar to the levels calculated by FDA.

Based on current use and market patterns, it is more accurate to base the EDIs on assumptions used by Exponent and the EDIs derived using these assumptions in "Analysis 2," as detailed in Table 6 of Attachment 2, reflect the most likely exposure scenario for Kao's CGT. Table 4 ("Summary of the Assessment of Potential Intake of CGT") of the corrected GRAS Notice has thus been modified to reflect these calculations. We note that the 90th percentile intake for bottled teas, sports drinks, juices, and carbonated beverages ("Scenario 4") presented in the revised Table 4 is still orders of magnitude lower than the NOAEL derived from the pivotal 90-day rat toxicity study, and supported by the clinical studies discussed in the GRAS Notice.

7. ***Possible typing errors: Appendix H, second page Section I: "Identify and Regulatory Status," Section I.C The title of the article in part reads: Safety evaluatino . . .; third page section II.A last sentence in part reads: Kao is considering the introductino . . ." section II.B. last bullet in part reads: The tannase straing (Kikkoman Corporation) was originall . . .; fifth page: section IV. last sentence in part reads: GThese results, together . . .; please clarify.***

   The corrected GRAS Notice attached has been changed to incorporate these revisions.

* * * * *

We trust that these clarifications respond to your questions and will enable FDA to complete its review of Kao's GRAS Notice expeditiously. In view of the few remaining questions, we request that the Agency provide an indication as to clearance by calendar year-end, because of planning constraints and decisions that need to be made at the beginning of a new year to enable product introduction.

Sincerely,

Stephen Paul Mahinka, Esq.
Counsel for Kao Corporation

Attachments

cc: Mitsuhiro Katashima (Kao Corporation)
Yoshihisa Katsuragi (Kao Corporation)
Taisuke Itoh (Kao Corporation)
Koichi Yasunaga (Kao Corporation)

Kathleen M. Sanzo, Esq.
Morgan, Lewis & Bockius LLP

Sharon A. Segal, Ph.D.
Director of Regulatory Science
Morgan, Lewis & Bockius LLP

ATTACHMENT 1

GENERALLY RECOGNIZED AS SAFE STATUS OF CATECHINS FROM GREEN TEA

Prepared by

Kao Corporation

August 14, 2008
Revised December 18, 2008

EXECUTIVE SUMMARY

The Kao Corporation of Tokyo, Japan has submitted extensive non-clinical and clinical data, including published studies, which demonstrate that its beverage product, Catechins from Green Tea (CGT), is Generally-Recognized-As-Safe (GRAS) for use in bottled teas, sports drinks, carbonated soft drinks, and juice. An independent and well-recognized panel of U.S. experts qualified to review and assess such data has also concluded that CGT is GRAS. CGT has already been reviewed by, and received the approval of, the Ministry of Health and Welfare of Japan for use in teas and sports drinks.

In addition to the consumption of green tea for thousands of years in Asia without any adverse health issues, it has become one of the most popular beverages in the world. Of all of the tea consumed in the world, approximately 20% is green tea, with global green tea production in 2010 predicted to be 900,000 tons. Increasing consumption of green tea has also occurred in the U.S., where green tea accounts for about 12.5% of the 2.25 billion gallons of tea consumed in 2005.

Based on standard USDA databases, Kao has estimated that U.S. consumption of catechin monomers from green tea consumption is 200 mg/day (mean) and 393 mg/day (90th percentile). The consumption of CGT from all intended sources by the U.S. population is estimated to be 859 mg/day or 14.9 mg/kg-bw/day (mean) and 1737 mg/day, or 30.7 mg/kg-bw/day (90th percentile).

CGT is a natural extract of green tea comprised of a mixture of eight catechin monomers. Tea extract, including green tea extract, is already recognized by FDA as GRAS. See 21 C.F.R. §182.20. As described in Section III.A, CGT is manufactured by traditional extraction methods using common additives and processing aids.

The safety of CGT is supported by a number of published studies on Kao's CGT and on substantially equivalent CGT extracts, as well as by a large body of unpublished data on Kao's product, other CGT extracts, and green tea. Pharmacokinetic data from humans and animals demonstrate that CGT and equivalent products have low oral bioavailability, are widely and almost equally distributed into various tissues, do not accumulate in the body with increasing and/or repeated doses, and are absorbed to a greater extent, but still are safe, in the fasting condition. Multiple acute, subchronic, and chronic animal studies consistently report a lack of systemic toxicity until exceedingly high doses are reached. Human studies with Kao's CGT and equivalent products, as well as green tea, corroborate the lack of toxicity seen in animal studies. These data in the aggregate strongly corroborate the safety findings in the published studies. In fact, there are not likely many food substances that have been studied as thoroughly and extensively as CGT.

Pharmacokinetic Studies

Human Studies

The pharmacokinetic parameters of CGT have been investigated in several studies, including randomized, double-blind, placebo-controlled studies, which have demonstrated the safety

and tolerability of CGT in healthy subjects, even when consuming a single oral dose of up to 1600 mg or repeated doses of up to 800 mg/day for 4 weeks. The bioavailability of the component catechins varies slightly but is low (under 9%) for all components due to pre-systemic elimination processes, including intestinal metabolism that poses a major barrier to bioavailability. The remainder of the CGT is excreted. Consequently, data from other studies using other routes of CGT administration, especially parenteral, which bypass the gastrointestinal system, are not likely relevant for the evaluation of bioavailability and systemic effects of orally consumed CGT. Although fasting does appear to affect the bioavailability of CGT, human studies demonstrate that high single or repeated doses are still well tolerated and cause no serious or any other clinically relevant adverse health events. Notwithstanding this issue, as Kao's product will be consumed as a beverage, it will not be consumed under fasting conditions.

Animal Studies

Multiple rat, rat fetus, and dog studies fully support the findings of the human studies demonstrating the low bioavailability of CGT when orally consumed. The studies show that, after oral administration, catechins are widely distributed to tissues and excreted in feces. The liver does not appear to accumulate more CGT than other organs. Because of the importance of the low oral bioavailability of CGT, it must be considered when extrapolating results obtained *in vitro* or when using parenteral (non-oral) forms of administration.

Toxicology Studies: Animal Studies

In a 90-day pivotal study of a substance substantially similar to CGT (Sunphenon 100S), fed to rats in the daily diet in concentrations up to 5%, no test article-related or clinical observations or deaths were found during the study. Other than reduced body weight gains in the high-dose male rats, there were no treatment-related effects on food consumption in the male rats and only a week one decrease in female rats. Although there were signs of weak hepatotoxicity based on increased liver enzymes observed in the high-dose rats, there were no clinically significant histopathological lesions of the liver observed in these animals. A NOAEL of 1.25 % (corresponding to doses of 763.9 mg/kg-bw/day for males and 820.1 mg/kg-bw/day for females) was established for this study. It is also important to note that the effects described above were observed at dietary concentrations (corresponding to doses of over 3500 mg/kg-bw/day) that far exceed any likely exposure to daily CGT in beverages.

Short-term toxicity data on Kao's CGT, as well as other equivalent CGT extracts, provide consistent evidence of the safety of single does of Kao's CGT, as well as repeated exposures (up to 14 days). The acute oral LD_{50} is > 2000 mg/kg body weight in rats, with no signs of treatment–related toxicity. The NOAEL for 14-day oral exposure in rats has been reported to range from 500-2000 mg/kg-bw/day, with decreased body weight the only treatment-related effect observed.

To assess longer-term toxicity, Kao's CGT was tested in two 28-day and one 6-month toxicity studies in rats and produced no relevant test-article related clinical effects. In a developmental toxicity study of catechins from green tea in rats, there was no maternal

toxicity or test article-related macroscopic findings, and no fetal malformations or developmental variations were observed. In genotoxicity studies on the same substance, although there were positive responses in two of the three *in vitro* tests (the CHL/IU and MLA tests), the *in vivo* test did not support a genotoxic effect.

In addition to the safety data on the mixture of catechins in the Kao product, there are also substantial data supporting the safety of the most abundant catechin (EGCG) in Kao's product. These data show that EGCG is not genotoxic, the acute and subchronic oral safety profile of EGCG is similar to that observed for CGT, and EGCG was not toxic to dams or fetuses and did not affect reproduction, fertility, or fetal development.

Human Studies:

There are a number of short and long-term published and unpublished clinical studies on the tolerability and nutritional effects in humans of Kao's product and substantially similar products. In all cases, the studies have demonstrated that CGT are well tolerated and produce no toxicological adverse effects as a result of consumption.

In particular, Kao sponsored an as yet unpublished study (Maki et al.) on the effects of daily consumption of a sports drink containing Kao's catechins from green tea on exercise-induced changes in body composition and fat distribution in a 12-week study. There were no serious adverse events in the catechin group and the incidence of adverse events, which were mild or moderate and unlikely to be related to the study product, were comparable in both groups. There was no evidence of adverse effects on liver enzymes and in fact, mean ALT and AST levels decreased in the catechin group.

In another as yet unpublished 12-week randomized, double-blind placebo controlled study (Sakata et al.) to evaluate the effects of green tea containing 1080 mg/day of CGT on non-alcoholic fatty liver disease, there were no serious adverse events in the CGT group, and there was a reduction in inflammation and fatty accumulation in the liver in the CGT group with CT evaluation.

All published studies for Kao's CGT and substantially similar substances are summarized in Table 14.

Liver Toxicity of CGT

The U.S. Pharmacopeia's (USP) Dietary Supplements Expert Committee (USP Committee), as part of its general review of dietary supplements containing powdered decaffeinated green tea extract, proposed a caution statement for product labeling, based on 34 case reports of liver toxicity in people taking these dietary supplements. The case reports had substantial inadequacies that severely limited their utility in assessing the health risk of green tea extract. Further, the weight of the evidence from published animal studies does not support an association between the green tea extract and clinically significant liver toxicity. Specifically, the *in vivo* studies that examined the effects of the repeated ingestion of green tea extract on liver function in general and *in vivo* studies that examine the liver function of

animals with manipulation-induced liver toxicity treated with catechins from green tea demonstrate no toxic effects, or protective or ameliorative effects on the induced liver damage. All of these studies are summarized in Table 11.

USP has announced that it concluded that the final monograph for green tea extract, which will be effective in December 2008, will not include any proposed caution statement.

Conclusion

Based on the 90-day toxicity study in rats by Takami et al. (2008), corroborated by Kao's 6-month study in rats, and supported by Kao's animal and human toxicity data as well as the published animal and human data, Kao has determined, and an Expert Panel has confirmed, that its CGT product is GRAS for its intended use.

TABLE OF CONTENTS

Page

EXECUTIVE SUMMARY ii

I. GENERALLY RECOGNIZED AS SAFE ("GRAS") CLAIM OF EXEMPTION FROM THE REQUIREMENT FOR PREMARKET APPROVAL PURSUANT TO PROPOSED 21 C.F.R. § 170.36(c)(1) 1

A. Name and Address of Notifier 2

B. Name of GRAS Substance 2

C. Conditions of Use 2

D. Basis for GRAS Determination 2

E. Uses of Green Tea and Green Tea Extract 3

1. Prior and Current Use of Green Tea 3

2. Prior Use of Green Tea Extract 5

3. Prior Use of Beverages Containing Catechins from Green Tea (CGT) 6

F. Availability of Information 6

II. IDENTITY 7

A. Identity 7

B. Chemical Abstract Service (CAS) Registry Number 7

C. Structural Formula 7

D. Empirical Formula and Molecular Weight 8

III. MANUFACTURING PROCESS 9

A. Method of Manufacture 9

B. Regulatory Status of Constituents Used in the Manufacturing Process of Catechins from Green Tea (CGT) 11

C. Specification for Catechins from Green Tea (CGT) 12

D. Analytical Methods 14

E. Typical Product Characteristics 15

F. Stability of CGT 18

IV. SELF-LIMITING LEVELS OF USE 20

V. ESTIMATED CONSUMPTION OF TEA CATECHIN 20

A. Daily Consumption of Green Tea in Japan 20

B. Catechin Monomer Content of Green Tea and Other Tea Beverages 20

C. Daily Consumption of Catechins from Green Tea (CGT) in the United States 21

1. Green Tea Consumption 21

2. Catechin Monomer Content Levels 21

D. Estimated Daily Intake of Catechins from Green Tea (CGT) in the United States 23

VI. BASIS FOR GRAS DETERMINATION 25

A. Absorption, Distribution, Metabolism and Excretion (ADME) 26

1. Human Studies 26

2. Animal Studies 36

3. Summary and Conclusions 45

B. Toxicology Studies 46

1. Short-term Toxicity Studies of Catechins from Green Tea (CGT) 47

2. 28-day Toxicity Study of Catechins from Green Tea (CGT) in Rats (Chengeles et al. 2008) 55

3. 6-Month Toxicity Study of Catechins from Green Tea in Rats 59

4. Developmental Toxicity Study of Catechins from Green Tea in Rats 61

5. Genotoxicity Studies of Catechins from Green Tea 62

6. Toxicology Studies on Substances Substantially Equivalent or Similar in Composition to Catechins from Green Tea 66

7. Toxicology Studies on the Main Catechin Isomer in Catechins from Green Tea 71

8. Liver Toxicity of Catechins from Green Tea 73

C. Human Clinical Studies 83

1. Published Studies Assessing Safety-related Parameters After Consumption of Kao's CGT and Substances Highly Similar to Kao's CGT 83

2. Supportive Unpublished Studies Assessing Safety-related Parameters After Consumption of Kao's CGT 84

D. Potential Interactions Between Catechins from Green Tea and Metabolic Enzymes 97

E. Other Studies Investigating the Mechanisms of Action for Green Tea Catechins 98

F. Reports that May Appear Inconsistent 99

1. Marketing Experience 99

2. USP Dietary Supplements Information Expert Committee Proposed Caution Statement 99

3. The Anecdotal Case Reports that Served as the Sole Basis for the Proposed Caution Statement Are Inadequate and Fraught with Limitations. 100

4. The Causality Assessment Algorithm ("Naranjo") Used by the USP Committee Is Not Generally Accepted. 101

5. The USP Committee's Proposed Toxicological Mechanism for the Observed Hepatotoxicity of Green Tea Extracts is Faulty and Unsupported. 101

6. Predominant Adverse Cases Were Reported Following Consumption of Only Two Specific Products 103

7. The USP Committee's Safety Conclusions and Proposed Caution Statement Were Intended Only for Products Labeled as Containing USP Powdered Green Tea Extract Used as Dietary Supplements. 104

VII. SAFETY SUMMARY AND CONCLUSIONS 105

VIII. LIST OF REFERENCES 109

List of Appendices

Appendix A: Analytical Methods
Appendix B: Bottled Tea
Appendix C: Sports Drinks
Appendix D: Carbonated Beverages and Juice Drinks
Appendix E: The Generally Recognized as Safe Status of Catechins from Green Tea -- Opinion of an Expert Panel
Appendix F: *Curricula Vitae* of Expert Panel Members
Appendix G: Japan Ministry of Health, Labor and Welfare, *Permit for Labeling a Product as a Food for Specified Health Use* (Green Tea, Oolong Tea, and Sports Drink)
Appendix H: Safety Information on Tannase
Appendix I: Representative Batch Analysis Data for Three Lots of CGT
Appendix J: USP Statement Concerning Final Monograph on Decaffeinated Green Tea Extract; USP Final Monograph; Comments to USP Committee Submitted December 28, 2007
Appendix K: English Translations of Japanese Citations
Appendix L: Studies on the Mechanisms of Action of Green Tea Catechins

I. GENERALLY RECOGNIZED AS SAFE ("GRAS") CLAIM OF EXEMPTION FROM THE REQUIREMENT FOR PREMARKET APPROVAL PURSUANT TO PROPOSED 21 C.F.R. § 170.36(c)(1)

Kao Corporation has determined that Catechins from Green Tea (*Camellia sinensis*) is generally recognized as safe ("GRAS") for the intended use, and therefore, exempt from the requirement for premarket approval. As set forth in the following sections, this determination is based on scientific procedures, under the conditions of its intended use in certain beverages, among experts qualified by scientific training and expertise. Therefore, the use of Catechins from Green Tea in certain beverages as described below is exempt from the requirement of premarket approval.

Signed, on behalf of Kao Corporation

_____

Ichirou Tokimitsu
Vice President, Global R&D - Health Care
Kao Corporation

A. Name and Address of Notifier

Kao Corporation
2-1-3, Bunka Sumida-ku
TOKYO 131-8501 JAPAN

Contact: Ichirou Tokimitsu
Vice President, Global R&D - Health Care
Telephone: +81-3-5630-7263
Facsimile: +81-3-5630-7524
E-mail: tokimitsu.ichirou@kao.co.jp

B. Name of GRAS Substance

The beverage ingredient proposed by Kao Corporation for classification as Generally Recognized as Safe ("GRAS") is **Catechins from Green Tea** (*Camellia sinensis*). Catechins from Green Tea (hereafter referred to as "CGT" for purpose of this document only) is a mixture of catechin monomers that is extracted from crude green tea (*Camellia sinensis*) extract.

C. Conditions of Use

Catechins from Green Tea is intended for use as a natural extract in beverages, including bottled teas, sports drinks, carbonated soft drinks, and juice. These beverages are identified in the attached appendices according to their respective food codes as listed in the U.S. Department of Agriculture ("USDA") 1994-1996, 98 Continuing Survey of Food Intakes by Individuals ("CSFII 94-96, 98"), and these codes were used to calculate the Estimated Daily Intake ("EDI") values for CGT consumption.[1] The proposed food uses and the level of use of CGT are summarized below:

Food Category	Proposed Food Use	Use Level for CGT
Beverage	Bottle tea, sports drink, carbonated soft drink, juice	540 mg per bottle

D. Basis for GRAS Determination

Pursuant to 21 C.F.R. § 170.30, Kao Corporation has determined that CGT is GRAS based upon scientific procedures and by the supportive opinion of an outside panel of experts who are qualified by scientific training and experience to evaluate the safety of CGT as a food ingredient ("the Panel").[2] The safety of CGT is supported by a number of published studies

[1] *See* Appendix B, Appendix C, and Appendix D.

[2] *See* The Generally Recognized as Safe Status of Catechins from Green Tea -- Opinion of an Expert (Continued . . .)

on Kao's CGT and on substances that are substantially equivalent to Kao's CGT, including acute, subacute, subchronic, and chronic studies in rats, *in vitro* mutagenicity studies, and clinical studies of the human tolerance and nutritional effects of CGT. These published studies are supported by Kao's data, including a recent 6-month toxicity study on Kao's CGT. Further, the safety and stability of CGT was favorably evaluated by the Ministry of Health and Welfare of Japan and approved for use in foods for specified health use ("FOSHU") in a green tea beverage, an oolong tea beverage, and a sports drink in Japan.[3] The green tea beverage has been marketed in Japan since May 2003, the oolong tea beverage since March 2005, and the sports drink since May 2006.

E. Uses of Green Tea and Green Tea Extract

Set forth below is a summary of information and reports of prior uses of green tea and green tea extract, as well as the history of use of beverages containing green tea catechins.

1. Prior and Current Use of Green Tea

Asian countries, especially Japan and China, have a long-standing custom of drinking green tea, a dietary habit that began several centuries B.C. in China. Green tea was introduced from China to Japan in the early 8th century and had been used as a medicine. In the 15th century, green tea was used as a beverage among the people of "high society." The custom of drinking tea extended widely in civil society around the late 16th century. At present, the tea plant is cultivated in more than 20 counties of Asia, Africa, and South America as one of the most favorite horticultural plants, analogous to coffee and cocoa (Yamamoto et al. 1997).

According to the World Health Organization 2006 World Health Report (WHO 2006), Japanese people exhibit the world's greatest longevity. It is believed that one of the main reasons for this longevity is the Japanese habit of drinking green tea. A few large size epidemiologic investigations are being conducted to confirm the health benefit of green tea in Japan. The current results of these studies are briefly summarized below.

The incidence of prostate cancer is much lower in Asian than Western populations. Given that environmental factors such as dietary habits may play a major role in the causation of prostate cancer, and the high consumption of green tea in Asian populations, this low incidence may be partly due to the effects of green tea. The JPHC Study (Japan Public Health Center-based Prospective Study) was established in 1990 for cohort I and in 1993 for cohort II. Cohort I consisted of five Public Health Center (PCH) areas (Iwate, Akita, Nagano, Okinawa, and Tokyo), and cohort II consisted of six PHC areas (Ibaraki, Niigata, Kochi,

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Panel. (Appendix F). The expert panel consists of: Joseph Borzelleca, Ph.D; James W. Anderson, Ph.D; Michael W. Pariza, Ph.D. *See* Appendix F: *Curricula Vitae* of Expert Panel Members.

[3] Ministry of Health, Labor and Welfare, *Permit for Labeling a product as a Food for Specified Health Use* (2003), green tea; Ministry of Health, Labor and Welfare, *Permit for Labeling a product as a Food for Specified Health Use* (2003), oolong tea; Ministry of Health, Labor and Welfare, *Permit for Labeling a product as a Food for Specified Health Use* (2003), sports drink. Attached at Appendix G are both the original and English versions. The approved specified health use states "[d]ue to a high content of Tea Catechin, this green tea is suitable for people who are concerned about body fat."

Nagasaki, Okinawa and Osaka) across Japan. The subjects were 49,920 men aged 40-69 years who completed a questionnaire that included their green tea consumption habit at baseline and were followed until the end of 2004. The overall conclusion of the study was that green tea may be associated with a decreased risk of advanced prostate cancer (Kurahashi et al. 2008).

The Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC study) started between 1988 and 1990. A total 110,792 individuals (46,465 men and 64,327 women) who were 40 to 79 years of age and living in 45 communities across Japan participated in municipal health screening examinations and completed self-administered questionnaires regarding lifestyle and medical history of cardiovascular disease and cancer. A total of 17,413 individuals (6,727 men and 10,686 women) completed the 5-year follow-up questionnaire and provided valid responses on tea or coffee consumption and history of type 2 diabetes. The data from these 17,413 individuals were used for analyses to examine the relationship between consumption of these beverages and risk for diabetes. At baseline, consumption of tea and coffee was assessed by using a self-administered dietary questionnaire. Participants were asked to state their average consumption of green tea, black tea, oolong tea, and coffee during the previous year. This cohort study showed an inverse association between green tea, coffee, and caffeine consumption and risk for diabetes in women and in overweight men (Iso et al. 2006).

The Osaki National Health Insurance (NHI) Cohort Study, a population-based, prospective cohort study initiated in 1994 among 40,530 Japanese adults aged 40 to 79 years without history of stroke, coronary heart disease, or cancer at baseline. All participants live in the catchment area of Osaki Public Health Center, Miyagi Prefecture, in northeastern Japan. Participants were followed up for up to 11 years (1995-2005) for all-cause mortality and for up to 7 years (1995-2001) for cause-specific mortality. Consumption of green tea and coffee was assessed by using a self-administered dietary questionnaire. This cohort study provided evidence that green tea consumption is associated with reduced mortality due to all causes and due to cardiovascular disease but not with reduced mortality due to cancer (Kuriyama et al. 2006).

Green tea is now considered one of the most popular drinks in the world (Yamamoto et al. 1997). Of the total amount of tea consumed in the world, approximately 20% is green tea (Mukhtar and Ahmad 2000), and both production and consumption are increasing. World green tea production has been forecast to increase from 680,700 tons in 2000 to 900,000 tons in 2010, reflecting an annual average growth rate of 2.6%.[4] Green tea output has expanded faster at 2.5 percent over the past decade compared to 1 percent for black tea.[5]

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[4] Medium-Term Outlook for Tea, Committee on Commodity Problems, Fourteenth Session of the Intergovernmental Group on Tea, New Delhi, India, 10-11 October, 2001. (http://www.fao.org/DOCREP/MEETING/003/Y1419E.HTM; accessed June 8, 2006) * tonne: metric ton = 1000 kg.

[5] Current Market Situation and Outlook, Committee on Commodity Problems, Intergovernmental Group on Tea, Fifteenth Session, Colombo, Sri Lanka, 18-20 August 2003. (http://www.fao.org/es/esc/common/ecg/27747_en_03_2_situation.doc; accessed June 8, 2006)

In the U.S., Americans consumed well over 50 billion servings of tea, or over 2.25 billion gallons in 2005; approximately 12.5% of the tea consumed was green tea.[6] In Canada, there has also been an upward trend; total sales of hot and iced tea in Canada were estimated at $267.3 million in 2000 a significant increase since 1999, with green tea reportedly fueling the growth.[7]

In Europe, consumption of green tea has also increased in recent years. Consumption of green tea products in England increased by 87% between 2000 and 2003.[8] In France, green tea accounts for 10.72% of the total sales volume.[9] In Italy, "black teas are still preferred but green teas are becoming more and more successful."[10] In Switzerland, sales of specialty teas have been rising over the past 13 years – the growth is mainly in the green tea sector, "attributed to a desire for a healthy life style and a calming beverage."[11] In Austria, a major tea seller, Demmer Teas, observed that green tea consumption grew from 5% to 26% of total sales from the mid 1990's to the early part of this century.[12]

2. Prior Use of Green Tea Extract

Green tea extract is comprised of polyphenols (catechins, flavonoids, and tannins), caffeine, and other minor constituents. Catechin monomers are the primary component of low molecular weight preparations of green tea extract.

Tea extract (which includes green tea extract), which is obtained from green tea leaves, is approved by the U.S. Food and Drug Administration ("FDA") for use in food as a GRAS substance (*i.e.*, natural extractives) (21 C.F.R. § 182.20; Katsuo 1987). Green tea extract is approved for use as a food additive in many countries (*e.g.*, Japan, South Korea) and has been consumed for many years in the context of a variety of applications, including, but not limited to, beverages and dietary supplements.

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6 http://www.teausa.com/general/501g.cfm - Tea Fact Sheet; accessed June 6, 2006.

7 Market Trends – Green and herbal tea fuel growth of tea sales in Canada, May 15, 2001. Tea Council of Canada. (http://www.tea.ca/press-trends-green.asp?section=media; accessed June 8, 2006).

8 "Positive research driving green tea consumption", nutraingredients.com, 01/03/2005 (http://www.nutraingredients.com/news/ng.asp?n=58411-positive-research-driving; accessed June 7, 2006).

9 "Tea Time in Europe," Tea and Coffee, Volume 177, No. 1 Jan./Feb. 2003 (http://www.teaandcoffee.net/0103/tea.htm; accessed June 6, 2006).

10 *Id*.

11 *Id*.

12 *Id*.

3. Prior Use of Beverages Containing Catechins from Green Tea (CGT)

Kao Corporation has manufactured beverage products containing CGT in its production facilities in Japan for almost five years. In the period between its release in May 2003 and July 2008, Kao has sold 1,014 million bottles of beverages containing green tea catechins in Japan, and no adverse effects have been reported.

F. Availability of Information

The data and information that serve as a basis for this GRAS determination are available for the FDA's review and copying at a reasonable time at the office of:

Morgan, Lewis and Bockius LLP
1111 Pennsylvania Avenue, N.W.
Washington, DC 20004

Contact: Stephen Paul Mahinka, Esq.
Phone: 202.739.5005
Fax: 202.739.3001
e-mail: smahinka@morganlewis.com

Alternatively, copies of data and information can be provided upon request by contacting Stephen Paul Mahinka, Esq.

II. IDENTITY

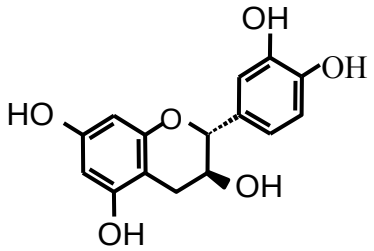
A. Identity

CGT is a natural extract from green tea leaves. It is a brown aqueous solution with a bitter, astringent taste. CGT is a complex mixture of eight catechin monomers, including: catechin ("C"); epicatechin ("EC"); gallocatechin ("GC"); epigallocatechin ("EGC"); catechin gallate ("CG"); epicatechin gallate ("ECG"); gallocatechin gallate ("GCG"); epigallocatechin gallate ("EGCG"). CGT also includes caffeine, organic acids, protein and amino acids, saccharide, and ash.

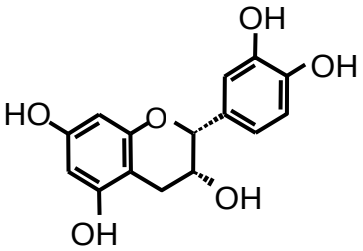
B. Chemical Abstract Service (CAS) Registry Number

CGT has not been assigned a CAS number.

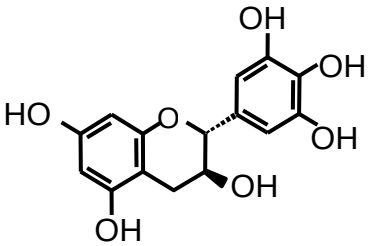
C. Structural Formula



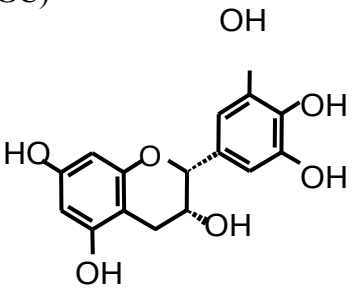
Catechin (C)



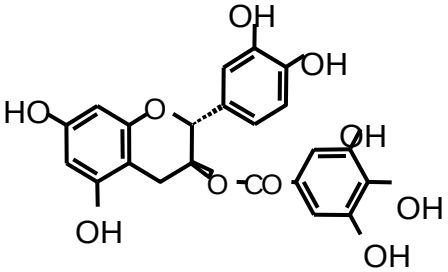
Epicatechin (EC)



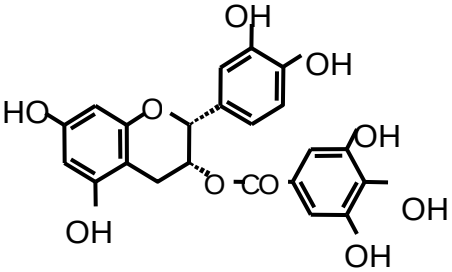
Gallocatechin (GC)



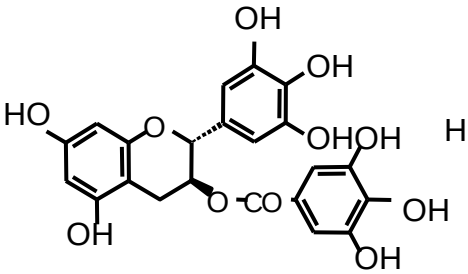
Epigallocatechin (EGC)



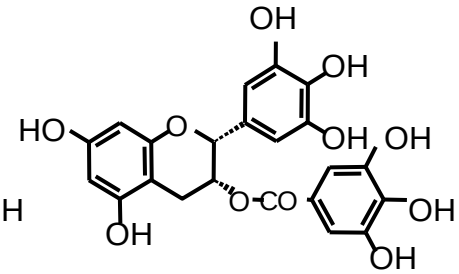
Catechin gallate (CG)



Epicatechin gallate (ECG)



Gallocatechin gallate (GCG)



Epigallocatechin gallate (EGCG)

D. Empirical Formula and Molecular Weight

The following are the empirical formulae and molecular weights of the primary components of CGT:

Catechin (C)	($C_{15}H_{14}O_6$)	290.27
Epicatechin (EC)	($C_{15}H_{14}O_6$)	290.27
Gallocatechin (GC)	($C_{15}H_{14}O_7$)	306.27
Epigallocatechin (EGC)	($C_{15}H_{14}O_7$)	306.27
Catechin gallate (CG)	($C_{22}H_{18}O_{10}$)	442.37
Epicatechin gallate (ECG)	($C_{22}H_{18}O_{10}$)	442.37
Gallocatechin gallate (GCG)	($C_{22}H_{18}O_{11}$)	458.37
Epigallocatechin gallate (EGCG)	($C_{22}H_{18}O_{11}$)	458.37

III. MANUFACTURING PROCESS

A. Method of Manufacture

Green tea extract (the primary ingredient) is mixed with a water and ethanol solution to dissolve the catechin monomers present in green tea extract. Following dissolution of the catechin monomers, the ethanol and water solution containing the extracted catechin monomers is subjected to centrifugal filtration to remove suspended solids and the catechin monomers are purified by adsorption treatment.

The full manufacturing process from extraction of crude catechins to the final beverage product is presented in Figure 1. There are two GRAS CGT substances ("Type-1" and "Type-2"). Type-1 CGT is intended for use in green tea beverages. Type-2 CGT is intended for use in sports drinks. The manufacturing process shown in Figure 1 consists of three processes:

Process 1: Extraction of crude catechins from tea leaves. Crude catechins are the primary ingredient in Kao's CGT. Kao uses marketed crude catechins as a primary ingredient.

Process 2: Manufacturing process of CGT. This manufacturing process corresponds to the manufacturing process of the GRAS substances in Kao's factory.

Process 3: Manufacturing process of final product (beverage containing CGT)

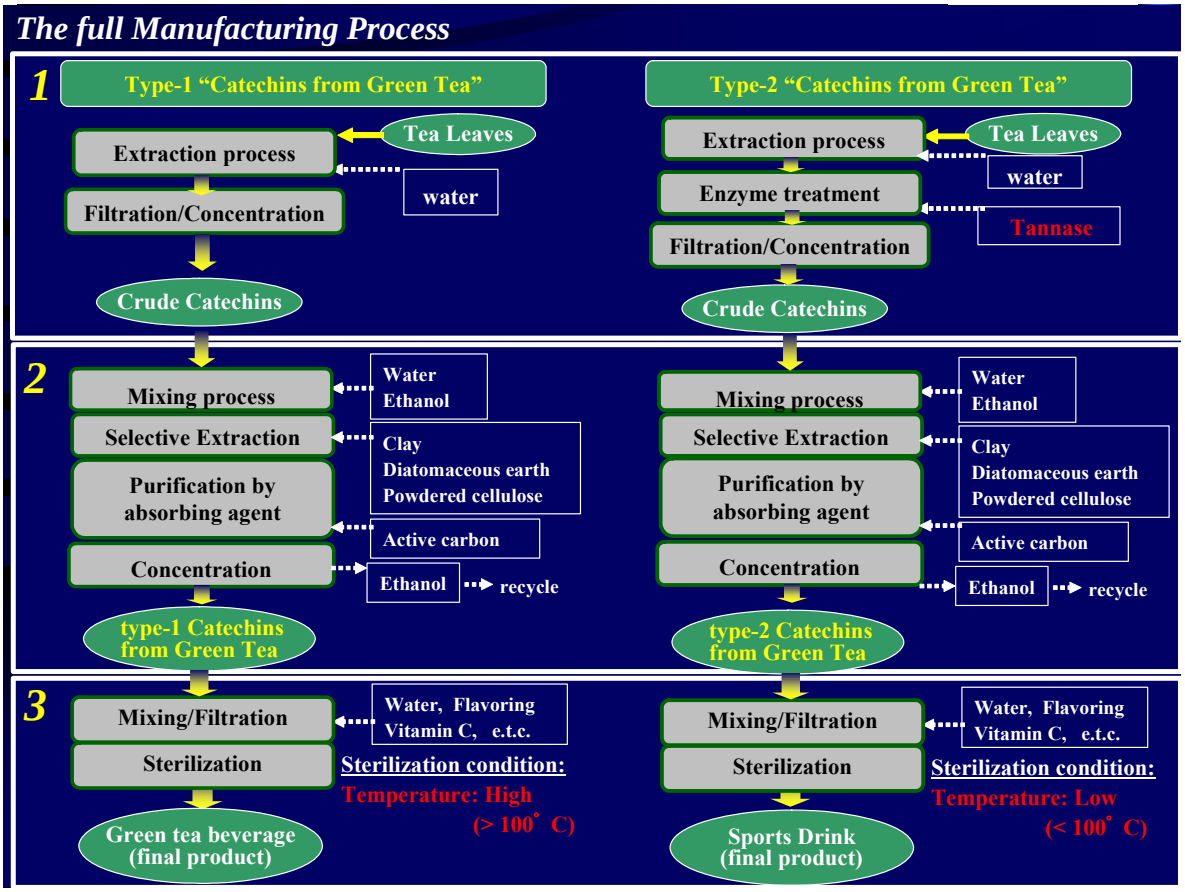


Figure 1

For the purpose of altering the taste for some Type-2 beverages,[13] a tannase-treated version of the CGT is employed in the manufacturing process (referred to as "Tannase-treated CGT"). Tannase treatment (9% tannase) removes gallic acid and gallate from those four catechins with a gallate moiety. The resulting alteration in relative catechin monomer levels is described in Section III.E below.[14]

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13 The differences in the typical product characteristics for Type-1 and Type-2 beverages are described in Section III.E below.

14 The 28-day, 6-month, and developmental toxicity studies for CGT -- described in Section VI.B -- were conducted with CGT that was not treated with tannase. Although Kao expects that all tannase will be removed during the manufacturing process, any residual amount remaining will be less than 20 units per bottled beverage (500 mL). While the product characteristics and specifications of tannase-treated CGT differ from the non-treated CGT, the change is not significant from a safety perspective because the estimated levels of gallate/gallic acid to be ingested by consumption of tannase-treated CGT-containing sports beverages are well below any toxicological levels of concern. *See* Appendix H for additional information on the safety of tannase.

B. Regulatory Status of Constituents Used in the Manufacturing Process of Catechins from Green Tea (CGT)

Constituent	Regulatory Status
Green tea extract (primary ingredient)	21 C.F.R. § 182.20 (GRAS status for tea extract)
Clay	21 C.F.R. § 186.1256 (affirmed as GRAS)
Active carbon	Food Chemicals Codex 5th edition, pp. 94 - 96. CAS No. 7440-44-0 Active carbon is approved for use as a food additive in Japan and is widely used in the manufacturing industry worldwide to refine beverages.
Diatomaceous earth	Food Chemicals Codex 5th edition, pp.137-138. Natural power and calined powder, CAS No. 61790-53-2 Flux-calined powder, CAS # 68855-54-9 Filtration media (Diatomaceous earth and perlite) is GRAS (GRN000087, 2001). Diatomaceous earth is approved for use as a food additive in Japan and is widely used in the manufacturing industry worldwide to refine beverages.
Powdered cellulose	Food Chemicals Codex 5th edition, pp.109 – 110. CAS No.: 9004-34-6 Powdered cellulose is approved for use as a food additive in Japan. For nearly 85 years, powdered cellulose has served as a food ingredient in United States.
Ethanol	Food Chemicals Codex 5th edition, pp. 157-158. CAS No.: 64-17-5 GRAS for use as a direct food substance (21 C.F.R. § 184.1293

Constituent	Regulatory Status
Tannase (only in "Tannase-treated") [15]	Tannase is listed in the CODEX Inventory of Processing Aids Enzyme Commission No.: EC 3.1.1.20 Tannase is approved for use as a food additive in Japan.

C. Specification for Catechins from Green Tea (CGT)

Main ingredient	Specification (percentage by weight)
Total catechin monomers	34% - 72%
Type-1 CGT:	34% - 50%
Type-2 CGT:	50% - 72%
EGCG	Less than 40%
Polyphenols	34% - 80%
Type-1 CGT:	34% - 60%
Type-2 CGT:	50% - 80%

Other Components	Specification (percentage by weight)
Caffeine	Less than 7 %
Organic acids	Not more than 10 %
Protein and Amino Acids	Not more than 10 %
Saccharide	Not more than 12 %
Fiber	Not more than 1 %
Fat	Not more than 1 %
Ash	Not more than 5%

[15] Kao has determined that tannase is GRAS for use in manufacturing its CGT product. The published pivotal study supporting the general recognition of safety of tannase is: Lane, RW. 1997. Safety evaluation of tannase enzyme preparation derived from *Aspergillus oryzae*. *Food Chem. Toxicol*. 35:207-212. *See* Appendix H.

Microbiological*	**Specification (percentage by weight)**
Total plate count	undetectable
Colon *bacillus*	undetectable

Heavy metal*	**Specification (percentage by weight)**
Arsenic	below detection limit (<0.2 ppm)
Sn	not more than 150 ppm
Cadmium	below detection limit (<0.1 ppm)
Lead	below detection limit (<0.4 ppm)

*Microbiological and heavy metal specifications are applied to the beverage form, after manufacture. Following are the specifications for CGT used prior to the beverage manufacturing process:

Microbiological	**Specification (percentage by weight)**
Total plate count	5×10^2 colony forming units (cfu)/g
Colon *bacillus*	undetectable

Heavy metal	**Specification (percentage by weight)**
Arsenic	<2 ppm
Lead	<30 ppm

Analyses for three representative batches of CGT are provided in Appendix I.

D. Analytical Methods

The analytical methods used for these measurements are listed below.[16]

Analyte	Method
Total Catechin monomers	HPLC Method (Kao method)
Polyphenols	Tartaric Acid-Method (Kao method)
Caffeine	HPLC Method (analyzed by Japan Food Research Laboratories)
Organic acids	HPLC Method (analyzed by Japan Food Research Laboratories)
Protein and Amino Acids	Kjeldahl Method (analyzed by Japan Food Research Laboratories)
Saccharide	HPLC Method (analyzed by Japan Food Research Laboratories)
Fiber Food	Enzymatic Gravimetric Method (analyzed by Japan Research Laboratories)
Fat	Acid hydrolysis (analyzed by Japan Food Research Laboratories)
Ash	Ignition at 550° (analyzed by Japan Food Research Laboratories)
Total plate count	JFSL method[17]
Colon *bacillus*	JFSL method[18]
Arsenic	JFSL method[19]
Sn	JFSL method[20]
Cadmium	JFSL method[21]
Lead	JFSL method[22]

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16 A description of the analytical methods listed is provided in Appendix A.

17 These measurements were derived based on the methods set forth under Japanese Law. Food Sanitation Law (2002) (Japan), pp. 879-81.

18 These measurements were derived based on the methods set forth under Japanese Law. Food Sanitation Law (2002) (Japan), pp. 881-882.

19 This atomic absorption spectrometry method was derived based on the methods set forth under Japanese Law. Food Sanitation Law (2002) (Japan), pp. 844-45.

20 *Id*.

21 *Id*.

E. Typical Product Characteristics

Catechins and Polyphenol	Catechins from Green Tea Ingredient (W/W%)	Tannase-treated Catechins from Green Tea Ingredient (W/W%)
Total Catechin Monomers	44.49%	62.3%
Polyphenols Polyphenols (except catechins)[23]	52.9 % 8.5%	67.4 % 5.1%
Caffeine	2.6%	1.7%
Catechin Profile		
Gallocatechin (GC)	2.4 %	5.9%
Epigallocatechin (EGC)	13.2 %	20.5%
Catechin (C)	0.9 %	1.9%
Epicatechin (EC)	3.0 %	6.2%
Epicatechin gallate (ECG)	4.6 %	5.0%
Epigallocatechin gallate (EGCG)	19.4 %	19.8%
Gallocatechin gallate (GCG)	0.5 %	2.5%
Catechin gallate (CG)	0.4 %	0.6%

HPLC Graph of CGT:

The following HPLC graphs show the chemical profile of green tea extract (raw material), Heat-treated CGT (or "Type-1 CGT"), a non-heat treated version of CGT ("UT-CGT" or "Type-2 CGT") and a standard sample of 8 catechin monomers. As shown in the graphs below, the peak pattern of other polyphenols are the same for all of these preparations. Accordingly, other polyphenols in CGT are passed from green tea extract (raw material) to Type-1 CGT and Type-2 CGT. Peak values of Type-1 CGT and Type-2 CGT are lower compared to green tea extract. The content level of other polyphenols is decreased by the

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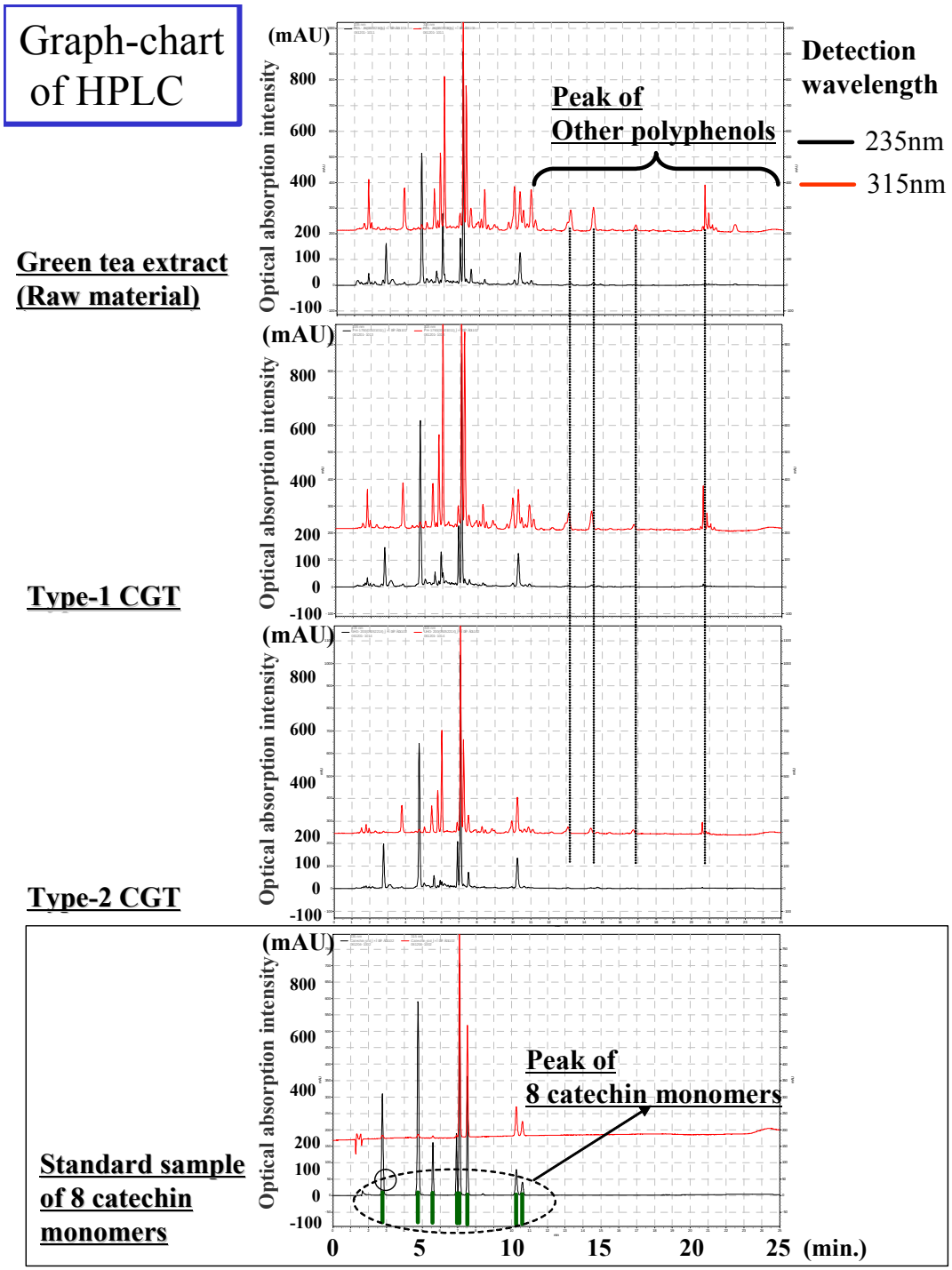
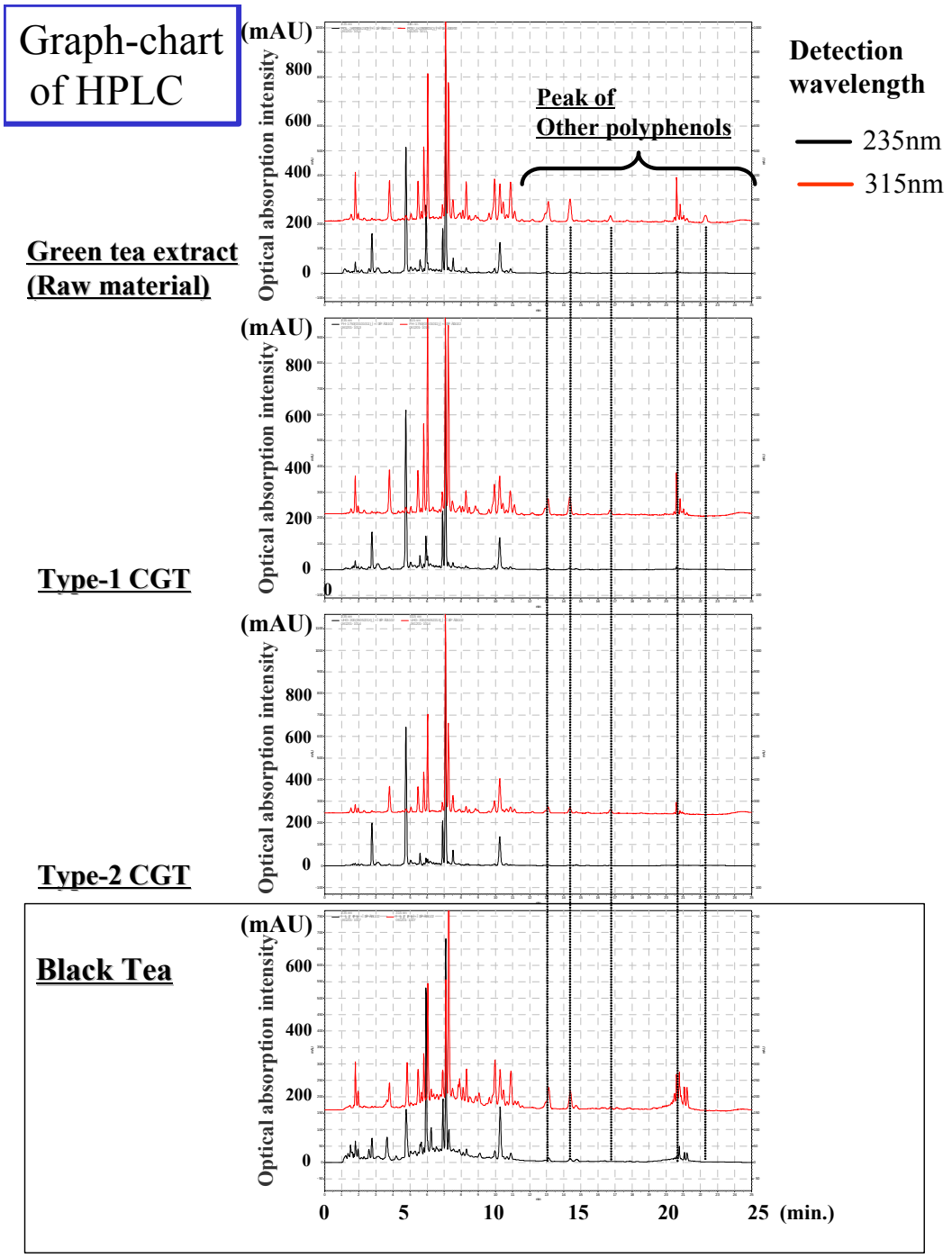
22 This Na_2C calorimetric method was derived based on the methods set forth under Japanese Law. Food Sanitation Law (2002) (Japan), pp. 849-50.

23 The additional polyphenols in CGT are primarily polymeric flavan-3-ols (proanthocyanidins). Many different varieties of proanthocyanidins are found in green tea and green tea extract, including: procyanidin B-2; procyanidin B-2 3,3'-di-*O*-gallate; prodelphinidin B-2 3,3'-di-*O*-gallate; procyanidin B-3; procyanidin B-4; prodelphinidin B-4 3'-di-*O*-gallate; and procyanidin C-1. (Ina K., et al. 2003 Chemical composition and function of TEA. Kogaku Publishing Co., (Tokyo) p. 11). Individual proanthocyanidins are present in CGT at levels too low for quantification.

purification process. Other polyphenols are quantified using the tartaric acid method (see Appendix A).



The following graphs are HPLC-graphs of green tea extract, Type-1CGT, Type-2 CGT, and black tea. Peak patterns of other polyphenols are nearly identical; thus, it is clear that the other polyphenols present in CGT are derived from tea leaves.


Graph-chart of HPLC
Green tea extract (Raw material)
Type-1 CGT
Type-2 CGT
Black Tea
(mAU)
Optical absorption intensity
800
600
400
200
0
Peak of Other polyphenols
Detection wavelength
235nm
315nm
0
5
10
15
20
25
(min.)

F. Stability of CGT

The stability of CGT was assessed with CGT that was stored in three layers of laminated packaging at -20° C and normal atmospheric pressure (Kao's standard storage condition) for more than one year. The substance was then evaluated and compared to the specifications of CGT analyzed immediately after the manufacturing process. Total catechin monomers and polyphenols remained almost unchanged under the standard storage conditions.

Stability Data for CGT (CGT, Lot No. 0310242; Run 1)

	0 months	14 months	18 months
Total catechin monomers	44.2 %	44.1%	42.9%
Polyphenols	51.3 %	51.8 %	51.9 %

Stability data for CGT (CGT, Lot No.0310242; Run 2)

	0 months	14 months	18 months
Total catechin monomers	42.8 %	43.8%	42.6%
Polyphenols	53.5 %	52.8 %	54.2 %

Stability data for Heat-treated CGT (Heat-treated CGT, Lot No.0502271)

	0 months	12 months	18 months
Total catechin monomers	62.0 %	62.0 %	63.2%
Polyphenols	66.0 %	66.0 %	65.6 %

Stability of CGT (total catechin monomers) in Beverages:

As discussed above, CGT is intended for use as a natural extract in beverages, including bottled teas, sports drinks, carbonated soft drinks, and juice. Each beverage contains approximately 540 mg of total catechin monomers (*i.e.*, the main ingredient of CGT). The following stability data for total catechin monomers were collected following storage at 25° C and 37° C in prepared beverage form. These data demonstrate that, in a closed bottle (or can) filled with beverage containing CGT, total catechin monomers are stable for 6 months at 25° C and 3 months at 37° C.

Stability data of Type-1 CGT (total catechin monomers) in Oolong tea beverage
Type-1 CGT
Storage condition A -- Temperature: 25° C, 6-month storage

	0 months	**1 month**	**3 months**	**5 months**	**6 months**
Total catechin monomers (PET Bottle /350 mL)	599 mg (100%)	595 mg (99%)	588 mg (98%)	581 mg (97%)	581 mg (97%)
Total catechin monomers (Steel Can /340 mL)	581 mg (100%)	575 mg (99%)	569 mg (98%)	571 mg (98%)	57 1 mg (98%)

Storage condition B -- Temperature: 37°, 3-month storage

	0 month	**1 month**	**2 months**	**3 months**
Total catechin monomers Type-1 CGT	606 mg (100%)	590 mg (97%)	575 mg (95%)	565 mg (93%)

Stability data of CGT (total catechin monomers) in sports drink
CGT: Type-2 CGT
Storage condition A -- Temperature: 25°, 6-month storage

	0 months	**1 month**	**3 months**	**5 months**	**6 months**
Total catechin monomers (PET Bottle /500 mL)	625 mg (100%)	606 mg (97%)	594 mg (95%)	581 mg (93%)	569 mg (91%)
Total catechin monomers (Steel Can /480 mL)	600 mg (100%)	600 mg (100%)	582 mg (97%)	570 mg (95%)	570 mg (95%)

Storage condition B -- Temperature: 37°, 3-month storage

	0 months	**1 month**	**2 months**	**3 months**
Total catechin monomers Type-2 CGT	641 mg (100%)	628 mg (98%)	602 mg (94%)	590 mg 92%

IV. SELF-LIMITING LEVELS OF USE

Although no limits on the use of CGT have been established, because of the bitter, astringent taste likely, it is to be included in beverages at a level of 540 mg per 350 mL or 500 mL bottle (typical beverage use). Use levels will be in accordance with Good Manufacturing Practices.

V. ESTIMATED CONSUMPTION OF TEA CATECHIN

A. Daily Consumption of Green Tea in Japan

Drinking green tea is an integral part of Japanese life-style, where it is consumed in many aspects of daily life. Typically, Japanese people drink 1-2 cups of green tea daily. Local residents of some areas (*e.g.*, Shizuoka, Saitama, and Miyagi) drink as many as 10 cups of green tea daily (1000–1500 mg of tea catechin/person/day) (Muramatsu 1991; Suga 1998; Sato 1989).

Daily consumption of green tea in the U.S. is discussed in Section V.C.1 below.

B. Catechin Monomer Content of Green Tea and Other Tea Beverages

Catechin monomers are the largest component of the low molecular weight fraction of green tea. The relative composition of catechin monomer content differs between green tea and fermented tea (*i.e.*, black tea and oolong tea), and also due to a variety of manufacturing process conditions (*e.g.*, extraction, filtration, and fermentation) (Saito 1998; Muramatsu 1991). The catechin monomer levels of green tea and other tea beverages are provided in Table 1.

Table 1. Catechin Monomer Levels of Green Tea and Other Tea Beverages

		Total Catechin Monomer Content (mg/100 mL)
Saitou, 1998	Brewed green tea (10 leaf samples)	111.17
	Green tea beverage products (21 products)	6.89
	Brewed oolong tea (10 leaf samples)	15.52
	Oolong tea beverage products (15 products)	10.57

C. Daily Consumption of Catechins from Green Tea (CGT) in the United States

1. Green Tea Consumption

Green tea consumption estimates for the U.S. population were derived from USDA's 1994-96 and 1998 Continuing Survey of Food Intakes by Individuals ("CSF II 94-96, 98"), which is a nationally representative survey that collected 2-day food intake data, in addition to demographic information, for approximately 22,000 individuals. Only individuals with complete and reliable 2-day dietary records were included in the analyses (N=20,607). The 2-day average estimated green tea consumption in the U.S. is provided in Table 2.

Table 2. 2-Day Average Estimated Green Tea Consumption in the U.S.

Population	% Consumers	Unweighted number of consumers[24]	Weighted per-user 2-day consumption (g/day)	
			Mean	90th Percentile
US population (2+ years)	12.73%	1733	363.3	715.9
Children 2-6	2.25%	141	129.6	237.3†
Children 7-12	3.63%	51	177.9	240†
Males 13-19	3.25%	22	430.2	711.7†
Females 13-19	7.03%	45	246.7	470.7†
Males 20+	12.79%	622	418.1	826
Females 20+	19.15%	852	348	716.2

† Indicates estimates are not statistically valid. The unweighted number of consumers does not meet the minimum sample size required to estimate intake at the 90th percentile.

2. Catechin Monomer Content Levels

The levels of catechin monomer used in this analysis were obtained from the USDA Database for the Flavanoid Content of Selected Foods (USDA, 2003).[25] Catechin monomer

[24] The estimates in the tables in this section rely on consumption data extracted from USDA's 1994-96 & 1998 CSFII. The CSFII surveys oversample children, low income individuals, and certain ethnic and racial subpopulations. Thus, the data generated by these surveys cannot be used as is, without adjusting for the differential probability of selection. USDA includes in its database "sampling" weights that should be used in order for the summary estimates (*e.g.*, means and percentiles) derived from the data to be representative of the U.S. population. The summary estimates that are provided in the tables in this section are derived using these weights. However, one needs to examine the actual number of subjects in the subpopulation of interest, or the "UNWEIGHTED NUMBER," because if that number is small, then the estimate derived from the consumption data for this subpopulation would be unreliable. (*See* page 42 of the document at: http://www.ars.usda.gov/SP2UserFiles/Place/12355000/pdf/scs_all.PDF for more details and examples).

[25] This publicly available database can be accessed at

(Continued . . .)

intakes were derived by combining the mean catechin concentration in green tea beverages with the tea consumption levels reported in Table 3. Resulting estimates are summarized in Table 3. The mean and 90th percentile of the distribution of catechin monomer intakes associated with green tea consumption were **200 mg/day** and **393 mg/day**, respectively, for consumers of green tea among the total U.S. population.

Table 3. 2-Day Average Estimated Catechin Intake Associated with the Consumption of Green Tea

Population	Catechin	% Consumers	Unweighted number of consumers	Per-user 2-day consumption (mg/day)	
				Mean	90th Percentile
US population (2+ years)	Total Catechins	12.73%	1733	200	393
	(+)-Catechin			10	20
	(-)-Epigallocatechin			34	67
	(-)-Epicatechin			16	32
	(-)-Epicatechin 3-gallate			44	86
	(-)-Epigallocatechin 3-gallate			96	189
	(+)-Gallocatechin			0	0
Children 2-6	Total Catechins	2.25%	141	71	130
	(+)-Catechin			4	7
	(-)-Epigallocatechin			12	22
	(-)-Epicatechin			6	11
	(-)-Epicatechin 3-gallate			16	28
	(-)-Epigallocatechin 3-gallate			34	63
	(+)-Gallocatechin			0	0
Children 7-12	Total Catechins	3.63%	51	98	132
	(+)-Catechin			5	7
	(-)-Epigallocatechin			17	22
	(-)-Epicatechin			8	11
	(-)-Epicatechin 3-gallate			21	29
	(-)-Epigallocatechin 3-gallate			47	63
	(+)-Gallocatechin			0	0
Males 13-19	Total Catechins	3.25%	22	236	391
	(+)-Catechin			12	20
	(-)-Epigallocatechin			40	66
	(-)-Epicatechin			19	32
	(-)-Epicatechin 3-gallate			52	85
	(-)-Epigallocatechin 3-gallate			113	188
	(+)-Gallocatechin			0	0
Females 13-19	Total Catechins	7.03%	45	136	259
	(+)-Catechin			7	13
	(-)-Epigallocatechin			23	44
	(-)-Epicatechin			11	21

http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/flav.html.

Population	Catechin	% Consumers	Unweighted number of consumers	Per-user 2-day consumption (mg/day)	
				Mean	90th Percentile
	(-)-Epicatechin 3-gallate			30	56
	(-)-Epigallocatechin 3-gallate			65	124
	(+)-Gallocatechin			0	0
Males 20+	Total Catechins	12.79%	622	230	454
	(+)-Catechin			12	23
	(-)-Epigallocatechin			39	77
	(-)-Epicatechin			19	37
	(-)-Epicatechin 3-gallate			50	99
	(-)-Epigallocatechin 3-gallate			110	218
	(+)-Gallocatechin			0	0
Females 20+	Total Catechins	19.15%	852	191	393
	(+)-Catechin			10	20
	(-)-Epigallocatechin			32	67
	(-)-Epicatechin			16	32
	(-)-Epicatechin 3-gallate			42	86
	(-)-Epigallocatechin 3-gallate			92	189
	(+)-Gallocatechin			0	0

D. Estimated Daily Intake of Catechins from Green Tea (CGT) in the United States

The consumption of CGT from all proposed uses was estimated using USDA CSFII 1994-1996 and the USDA CSFII 1998. The following scenarios were used to assess the daily intake of CGT:

Scenario 1: Consumers of all bottled tea in the U.S. will switch to proposed beverage

Scenario 2: Consumers of all sports drinks in the U.S. will switch to proposed beverage

Scenario 3: Consumers of all carbonated beverages and juices in the U.S. will switch to proposed beverage

Scenario 4: Consumers of combined bottled tea, sports drinks, carbonated beverages, and juices in the U.S. will switch to proposed beverage

The potential intake of CGT from each of these scenarios is presented in Table 4.

Table 4. Summary of the Assessment of Potential Intake of CGT

Scenario	Potential Intake of CGT (Per User)		
	Mean (90th Percentile) mg/day	**Mean (90th Percentile) mg/kg-bw/day**	**Unweighted Number of Users (% Users)**
#1. Bottled Tea			
US Population (2+ years)	**424 (779)**	**6.15 (11.1)**	**991 (7.39)**
Children (2 to 6 years)	211 (352)	11.3 (17.1)	106† (1.91)
Children (7 to 12 years)	267 (366)	7.65 (10.6)	40† (3.39)
Males (13 to 19 years)	462 (785)	6.75 (11.8)	55† (8.87)
Females (13 to 19 years)	359(702)	6.33 (10.3)	47† (8.07)
Males (20+ years)	477 (800)	5.84 (11.11)	414 (9.58)
Females (20+ years)	393 (707)	5.95 (10.8)	329 (7.19)
#2. Sports Drinks			
US Population (2+ years)	**372 (761)**	**6.5 (13.6)**	**422 (2.2)**
Children (2 to 6 years)	190 (321)	10.8 (18.8)	178† (2.95)
Children (7 to 12 years)	252 (514)	7.5 (14.5)	51† (3.64)
Males (13 to 19 years)	484 (1016)	6.9 (15.7)	43† (7.50)
Females (13 to 19 years)	319 (873)	5.9 (15.9)	14† (1.83)
Males (20+ years)	451 (1014)	5.6 (11.8)	106† (2.55)
Females (20+ years)	268 (510)	4.4 (7.9)	30† (0.69)
#3. Carbonated Beverages and Juice			
US Population (2+ years)	**835 (1673)**	**14.6 (30.3)**	**15292 (84.06)**
Children (2 to 6 years)	536 (996)	30.3 (56.7)	5454 (90.27)
Children (7 to 12 years)	698 (1283)	20.0 (36.2)	1358 (88.64)
Males (13 to 19 years)	1360 (2534)	20.5 (37.5)	575 (94.80)
Females (13 to 19 years)	926 (1815)	16.4 (31.7)	584 (94.02)
Males (20+ years)	962 (1900)	11.7 (23.3)	3766 (83.3)
Females (20+ years)	719 (1447)	10.8 (22.2)	3555 (79.53)
#4. Combined Bottled Tea, Sports Drinks, Carbonated Beverages and Juices			
US Population (2+ years)	**859 (1737)**	**14.9 (30.7)**	15,476 (85.27)
Children (2 to 6 years)	539 (1002)	30.5 (56.9)	5,470 (90.47)
Children (7 to 12 years)	707 (1311)	20.2(36.6)	1,360 (88.76)
Males (13 to 19 years)	1396 (2559)	21.0 (38.5)	579 (95.32)
Females (13 to 19 years)	950 (1825)	16.9 (31.9)	587 (94.61)
Males (20+ years)	1001 (1959)	12.16 (24.4)	3,843 (84.67)
Females (20+ years)	739 (1479)	11.13 (22.9)	3,637 (81.25)

† Indicates estimates are not statistically valid. The unweighted number of consumers does not meet the minimum sample size required to estimate intake at the 90th percentile.

VI. BASIS FOR GRAS DETERMINATION

A comprehensive search of the literature for articles on the potential toxicity of green tea and its catechin components was conducted by Morgan Lewis through July 2008. To identify such articles, Medline, Toxline, and The International Bibliographic Information on Dietary Supplements (IBIDS) were searched. Broad search terms were used for the initial searches, which resulted in the identification of over 3,500 abstracts. These abstracts were reviewed for their relevance and validity with respect to pharmacokinetics and safety issues for tea catechins. Kao has made every effort to identify all published literature that raises a safety concern for CGT and related substances.

Articles falling into the following categories generally were not included: (1) studies evaluating efficacy (unless safety data are included); (2) clinical studies in populations of diseased patients; (3) preclinical studies in genetically-altered animals; and (4) studies conducted in a manner inconsistent with FDA's general standards.

A 90-day toxicity study of a substantially equivalent CGT extract is submitted as the pivotal published study supporting the GRAS determination for Kao's CGT extract (Takami et al. 2008). In addition, studies on other CGT products, green tea, or those using only one of the catechins found in Kao's CGT extract are provided as corroborative evidence of the safety of Kao's CGT extract.

It is generally accepted within the scientific community that case reports should not be used to establish causality, and consequently were reviewed here only in the context of responding to potential safety issues initially identified by the United States Pharmacopeia's ("USP") Dietary Supplements Information Expert Committee. The USP Committee's questions were based on a limited number of case reports. Upon further review, public meetings, and the submission of comments, the USP decided not to include any cautionary statement based on case reports in issuing its final monograph on decaffeinated green tea extract for dietary supplement uses (*see* Section VI.F below).

The data discussed below, which include a number of toxicological and metabolic studies, provide a well-documented basis from which to evaluate and document the safety of CGT. Published studies on Kao's CGT and substantially equivalent CGT extracts, as well as unpublished studies on Kao's CGT extract, in both animals and humans, provide strong evidence in support of the safe use of CGT as an additive to beverages for human consumption.

Kao's approach to assessing the safety/toxicity of a food ingredient as set out in this Notice is consistent with the leading U.S. and international food standards organizations. In properly evaluating data on the safety/toxicity of a food ingredient, there are certain principles that are critical. To establish causality (*i.e.*, that treatment with the ingredient caused the adverse effects noted), it is important to establish that any apparent adverse and treatment-related effect is consistently seen in all treated groups, males and females [consistency of effect], that the intensity and/or frequency of the effect is dose-dependent [dose-dependency], the effect is statistically significantly different from the concurrent control group(s) [statistically significant difference], the effect is outside the historic control range for this strain, sex, and

age of animal [historic control difference], there are histopathological correlates for the effect [structure-function correlation], and the effect is consistent with current scientific thinking [biological plausibility]. Unless these criteria are met, the effect is not properly considered treatment related. This approach is based on scientific/toxicological principles and the policies of the FDA, FAO/WHO, JECFA, FAO/WHO JMPR, and other regulatory or scientific entities.

A. Absorption, Distribution, Metabolism and Excretion (ADME)

Epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), EC-3-gallate (ECG), and epicatechin (EC) are the major catechins from green tea (CGT). EGCG comprises 50-60% of green tea catechins (Miyazawa et al. 2000).

1. Human Studies

Absorption of CGT into human plasma was investigated by chemiluminescence (CL)-HPLC in healthy subjects (n=3, two male, 1 female; 21-23 years of age; non-smokers) who orally ingested 3, 5, or 7 capsules of green tea extract corresponding to 225, 375, and 525 mg EGCG and 7.5, 12.5, and 17.5 mg EGC, respectively after 12 hours of fasting (Nakagawa et al. 1997). Generally, one tea cup of green tea contains about 100 mg of catechins. Therefore, the total amount of EGCG and EGC provided by 3, 5, and 7 capsules of green tea extract was roughly comparable to that of tea catechins in 2, 4, and 6 cups of green tea, respectively. Ninety minutes after a single oral dose, the plasma EGCG concentrations were significantly increased to 300, 1970, and 2020 ng/mL (657, 4300, and 4410 pmol/mL) in subjects who received 3, 5, and 7 capsules of green tea extract, respectively. The plasma EGC concentrations also were significantly increased to 10, 44, and 78 ng/mL (35, 144, and 255 pmol/mL) in subjects who received 3, 5, and 7 capsules of green tea extract, respectively. Plasma EGCG and EGC levels before administration of the green tea extract were below the detection limit of <2 pmol/mL plasma. These results suggested a dose-dependent absorption of EGCG and EGC in their free forms into human plasma. No difference was observed for calculated percentages of absorbed amount against dosage between EGCG (0.2-2%) and EGC (0.2-1.3%). These data may imply that there is no difference in the absorption kinetics of EGCG and EGC.

In another study, EGCG was detected by HPLC-electrochemical detector (ECD) system in the serum of four healthy volunteers (gender and age not specified) who had ingested green tea (Unno et al. 1996). Sixty minutes after single oral intake of green tea extract containing 105 mg EGCG, EGCG concentrations in human serum were 30-80 ng/mL (65-175 pmol/mL). The concentration of EGCG in the serum reached the highest level approximately 2 hours after ingestion of green tea, and then decreased. The highest serum concentrations of EGCG ranged from 63 ng/mL to 142 ng/mL. Similarly, mean peak levels of EGCG in plasma samples from 12 fasted healthy male volunteers (22±2 years of age) were measured 2 hours after ingestion of single doses of CGT in free or phospholipid complex form (equivalent to 400 mg EGCG), as determined by HPLC with coulometric detector (Pietta et al. 1998). The subjects had a habitual low intake of flavonoid-rich foods, were not taking antioxidant supplements, and abstained from beverages containing flavonoids for 3 days before the study. The mean peak levels of EGCG were 1.84±0.29 µg/mL (4±0.6 µM) and

0.9±0.1 μg/mL (2±0.2 μM) for phospholipid complex and free catechins, respectively, and decreased slowly to approximately 0.4±0.1 μg/mL at 6 hours. Thus, catechins were absorbed in higher amounts when administered as a phospholipid complex, although the percent absorption of EGCG did not exceed 2% of the given dose.

In a study using an HPLC-coulochem electrode array detection (CEAS) method, the limits of detection for EGCG, EGC, ECG, and EC ranged from 0.5 ng/mL to 1.5 ng/mL of plasma or urine (Lee 1995). One hour after ingestion of 1.2 g of decaffeinated green tea (containing 88, 82, 32, and 33 mg of EGCG, EGC, EC, and ECG, respectively), the plasma samples from four healthy male volunteers (aged 40 to 50 years weighing 50-85 kg and not having ingested tea or tea-related beverages for at least 2 days before the experiment) contained 46-268 ng/mL (100-585 pmol/mL) EGCG, 82-206 ng/mL EGC, and 48-80 ng/mL EC. ECG was not detected in human plasma samples. Plasma EGC was mostly in the glucuronide form (57-71%), followed by the sulfate (23-36%), and the free (3-13%) forms. Plasma EC was exclusively in the conjugated form, with approximately two-thirds sulfate and one-third glucuronide. Plasma EGCG was mostly in the sulfate form (58-72%) followed by the free (12-28%) and glucuronide (8-19%) forms. The maximum urinary excretion of EGC and EC occurred at 3-6 hours after dosing. Most of the EGC and EC was excreted in the first 9 hours. The cumulative urinary excretions in the first 24 hours were 2.8-3.2 mg EGC and 1.6-2.3 mg EC. EGCG and ECG were not detected in the urine samples. The sulfate and glucuronide forms of both EGC and EC were present at approximately a 2:1 ratio. Free EGC was present at <1% of the conjugated form, but free EC was not detected. The ratio of different forms of metabolites did not change in the early and late urine samples. Since plasma EGC was mostly in the glucuronide form and urinary excretion of EGC and EC was mainly in the sulfate forms, it is likely that EGC glucuronide is excreted in the bile, released into the intestine, and excreted in the feces. The total amount of excreted EGC and EC was approximately 5 mg, accounting for 2% of the total polyphenol ingested. These data suggest that the major elimination pathways of tea polyphenols are via glucuronidation and sulfation.

The pharmacokinetic parameters of CGT have been investigated in several human clinical studies, including randomized, double-blind, placebo-controlled studies demonstrating safety and good tolerability of CGT in healthy volunteers. In one study, 18 healthy volunteers (six subjects per dose level, gender and age not specified) were given 1.5, 3, and 4.5 g of decaffeinated green tea solids dissolved in 500 mL of water (Yang et al. 1998). The subjects refrained from drinking tea for two days prior to the experiment. The plasma concentrations of EGCG, EGC, and EC reached peak levels between 1.5 and 2.5 hours and declined to undetectable levels after 24 hours, as determined by the HPLC-CEAS method. When the dose of decaffeinated green tea solids was increased from 1.5 g to 3 g, the mean maximum concentration (C_{max}) values increased 2.7-fold for EGCG (from 120 to 326 ng/mL), 3.4-fold for EGC (from 148 to 508 ng/mL), and 3.4-fold for EC (from 55 to 189 ng/mL). The higher C_{max} value for EGC than EGCG suggests that EGCG has a lower absorbability or a larger volume of distribution (Vd) than EGC. The mean area under the concentration-time curve (AUC) values, which reflect the extent of absorption, increased 2.5-fold for EGCG (from 897 to 2223 ng/hour/mL), 4-fold for EGC (from 617 to 2493 ng/hour/mL), and 3.8-fold for EC (from 279 to 1059 ng/hour/mL). However, increasing the dose from 3 g to 4.5 g did not further increase C_{max} or AUC values for any of the three catechins, suggesting a saturation

phenomenon. The mean time to peak concentration (T_{max}) for EGCG (1.6-2.7 hours) was longer than that for EGC or EC (1.3-1.8 hours). T_{max} values were not affected by the dose increase, suggesting that the dose may not affect the absorption and access of these tea catechins to the bloodstream. The mean half-life ($t_{½}$) values were approximately 5 hours and 2.7 hours for EGCG and EGC, respectively, and were not affected by the dose increase. For EC, the mean $t_{½}$ values decreased at higher doses (from 5.7 to 3.2 hours). Consistent with the data described above, most of the catechins in plasma were in the sulfate- and glucuronide-conjugated forms. Over 90% of the total urinary EGC and EC was excreted within 8 hours. After 24 hours, EGC and EC levels in the urine were below the detection limit. Consistent with previous observations, EGCG was not excreted in the urine.

In another human study, the systemic availability of CGT was determined after single oral dose administration of EGCG and Polyphenon E (decaffeinated mixture of EGCG, EGC, EC, and other tea polyphenols) by the HPLC-CEAS method (Chow et al. 2001). Twenty healthy volunteers (4 males and 16 females with mean ages of 44.6 to 49.2 years and mean weights of 145.2 to 166.6 pounds, five subjects per dose level) with normal liver and kidney function were randomly assigned to 200, 400, 600, and 800 mg (based on EGCG content) dose levels. All subjects were randomly crossed-over to receive the two catechin formulations at the same dose level. The subjects were instructed to refrain from the ingestion of tea or tea products for 7 days prior to the study through to the end. Following oral administration immediately after or during breakfast (one to two bagels), plasma EGCG levels increased toward a peak and declined rapidly, with very low/undetectable levels at 24 hours after dosing. After EGCG *versus* Polyphenon E administration, the mean AUC values of unchanged EGCG at the 200, 400, 600, and 800 mg dose levels were 22.5 *versus* 21.9, 35.4 *versus* 52.2, 101.9 *versus* 79.7, and 167.1 *versus* 161.4 min×μg/mL, respectively. The mean C_{max} values of EGCG were 73.7 ± 25.3, 111.8 ± 98.6, 169.1 ± 139.6, and 438.5 ± 284.4 ng/mL after a 200, 400, 600, and 800 mg dose of EGCG, respectively. Plasma EGCG levels observed after Polyphenon E administration were similar to those seen after EGCG administration, with the mean C_{max} values of 72.7 ± 66.4, 125.3 ± 50.4, 165.7 ± 126.9, and 377.6 ± 149.8 ng/ml at the 200, 400, 600, and 800 mg dose levels, respectively. There were no significant differences in the pharmacokinetic characteristics of EGCG between the two study treatments. The AUC and C_{max} values of EGCG after the 800 mg dose of EGCG were significantly higher than those after the 200 mg and 400 mg doses ($p<0.05$). The AUC and C_{max} values of EGCG after the 800 mg dose of Polyphenon E were significantly higher than those after 200, 400, and 600 mg doses ($p<0.05$). EGC and EC were not detected in plasma after EGCG administration and were present at low/undetectable levels after Polyphenon E administration. High concentrations of EGC and EC glucuronide/sulfate conjugates were found in plasma and urine samples after Polyphenon E administration. EGCG was not detectable in the urine. The investigators attributed the low oral bioavailability to a marked pre-systemic elimination process. The systemic availability of EGCG increased at higher doses, possibly due to saturable presystemic elimination of orally administered green tea polyphenols. Ullmann and colleagues suggested that another important reason for the observed low oral bioavailability is that the study subjects took EGCG together with food (Ullmann et al. 2003).

In a third study, the pharmacokinetic parameters of CGT were analyzed by the HPLC-CEAS method after administration of a single oral dose of green tea or decaffeinated green tea (20

mg tea solids/kg corresponding to 195 mg EGCG, 154 mg EGC, and 45 mg EC for an individual with a body weight of 70 kg) or EGCG (2 mg/kg) to eight healthy subjects (5 males and 3 females, 25-35 years of age weighing 45 to 85 kg) (Lee et al. 2002). The study subjects were fasted overnight before the administration and did not ingest tea or tea-related beverages for at least 2 days before the study and during the urine collection period. The peak plasma concentrations of EGCG, EGC, and EC were reached in 1-2 hours in all subjects, and gradually reduced to undetectable levels in 24 hours. The mean C_{max} values of EGCG, EGC, and EC after a single dose of green tea were 77.9 ± 22.2, 223.4 ± 35.2, and 124.03 ± 7.86 ng/mL, respectively, and the corresponding mean AUC values were 508.2 ± 227, 945.4 ± 438.4, and 529.5 ± 244.4 ng×hour×mL^{-1}, respectively. The T_{max} values were in the range of 1.3-1.6 hours. The elimination $t_{½}$ values of EGCG, EGC, and EC were 3.4 ± 0.3, 1.7 ± 0.4, and 2.0 ± 0.4 hours, respectively. At the time point T_{max}, 0.16%, 0.58%, and 1.1% of the ingested doses of EGCG, EGC, and EC from green tea administration were present in the circulating plasma. In the case of decaffeinated green tea administration, the values in the blood were 0.13%, 0.53%, and 0.71%, respectively. When pure EGCG was administered, only 0.1% of the ingested dose appeared in the blood at $T_{max.}$ These results indicate that a lower fraction of EGCG than EGC and EC appears in the blood, but the EGCG has a longer $t_{½}$. Considerable interindividual differences and variations in the pharmacokinetic parameters were noted between repeated experiments with green tea, but not when EGCG was given in decaffeinated green tea or in pure form. Consistent with the previous reports, EGCG was mostly present in the free form in the plasma, whereas EGC and EC were mostly in the conjugated form. Most of the conjugated catechins were in the glucuronide form. Over 90% of the total urinary EGC and EC, almost all in the conjugated forms, were excreted during the first 8 hours. EGCG was present in trace or undetectable amounts in the urine samples. Substantial amounts of 4'-*O*-methyl EGC metabolite, at levels higher than EGC, were detected in the urine and plasma. The plasma level of 4'-*O*-methyl EGC peaked at 1.7 ± 0.5 hours with a $t_{½}$ of 4.4 ± 1.1 hours. It remains to be determined whether the level of 4'-*O*-methyl EGC is influenced by the polymorphism of catechol-*O*-methyl transferase, an enzyme that catalyzes the methylation of EGC (Weinshilboum et al. 1999; Zhu et al. 2000a). Two ring-fission metabolites, (-)-5-(3',4',5'-trihydroxyphenyl)-γ-valerolactone (M4) and (-)-5-(3',4'-dihydroxyphenyl)-valerolactone (M6), appeared in significant amounts after 3 hours and peaked at 8-15 hours in the urine as well as in the plasma (Lee et al. 2002). The levels of urinary M6 (5 ± 2 mg) accounted for 11.2% of the ingested EC. The amount of excreted M4 corresponded to 1.4% of the ingested EGC, the direct precursor of M4.

A comparison of the pharmacokinetic parameters of EGCG from the three studies discussed above is shown in Table 5 below. These human studies demonstrated that only a small percentage (*e.g.*, typically less than 1%) of the orally-ingested catechins appears in the blood.

EGC and EC appear to be more bioavailable, but the fractions of these compounds that appeared in the plasma also are low, and only 3.3% and 8.9% of the ingested EGC and EC were excreted in the urine (Lee et al. 2002). The presence of substantial amounts of 4'-*O*-methyl EGC, M4, and M6 in the plasma and urine account for some of the ingested catechins.

Table 5. Comparison of the EGCG pharmacokinetics

	Lee et al. 2002[a]	Yang 1998[b]			Chow et al. 2001[c]			
Dose of EGCG (ng)	195	110	220	330	200	400	600	800
T_{max} (hour)	1.6	1.6	2.4	2.7	2.1	1.8	3	4
$t_{½}$ (hour)	3.4	5.5	5	4.9	2	2.7	3.1	1.9
C_{max} (ng/mL)	78	119	326	321	74	112	169	438
AUC (ng×hour/mL)	508	897	2223	2462	367	623	2214	2779

[a] Green tea was used; the data are for EGCG and its conjugates (analyzed together).
[b] Decaffeinated green tea was used; the data are for EGCG and its conjugates (analyzed together).
[c] EGCG tea was used; the data are for EGCG.

The effects of dosing conditions, *i.e.*, consumption in the absence or presence of food, on the oral bioavailability of CGT were evaluated after single-dose administration of Polyphenon E in 30 healthy, non-smoking volunteers with normal liver and kidney function (7 males and 23 females with mean ages of 39 to 45 years and mean weights of 157 to 165 pounds) (Chow et al. 2005). The study subjects were randomly assigned to one of the following doses of Polyphenon E (a decaffeinated and defined green tea catechin mixture): 400, 800, or 1200 mg, based on the EGCG content (10 subjects per dose group). After an overnight fast, study participants took a single dose of Polyphenon E with or without a light breakfast, which consisted of one or two 4-oz muffins and a glass of water. Following a 1-week wash-out period, subjects were crossed over to take the same dose of Polyphenon E under the opposite fasting/fed condition. Tea catechin concentrations in plasma and urine samples collected after dosing were determined by HPLC analysis. As in the previous studies, there was a large subject-to-subject variability in the pharmacokinetics of CGT. Consistent with previous reports, EGCG and ECG were present in plasma mostly as the free form, whereas EC and EGC were mostly present as the glucuronide and sulfate conjugates. After dosing, plasma EGCG levels increased toward a peak and declined rapidly as a function of time. There was >3.5-fold increase in the average C_{max} of free EGCG when Polyphenon E was taken in the fasting condition than when taken with food. The EGCG AUC obtained without food was significantly higher than that obtained with food for all three doses (126.96 ± 47.02 *versus* 36.70 ± 24.66 min×μg/mL at 400 mg; 254.48 ± 214.21 *versus* 90.89 ± 37.39 min×μg/mL at 800 mg; 685.53 ± 394.33 *versus* 299.40 ± 285.86 min×μg/mL at 1200 mg; $p<0.0001$ when data were pooled from all three doses). Similarly, the EGCG C_{max} obtained without food was significantly higher than that obtained with food (798.7 ± 573.1 *versus* 141.8 ± 89.1 ng/mL at 400 mg; 1522.4 ± 1357.8 *versus* 294.0 ± 113.5 ng/mL at 800 mg; 3371.6 ± 1651.2 *versus* 923.6 ± 755.3 ng/mL at 1,200 mg; $p<0.0001$ when data were pooled from all three doses). The dosing condition also had a significant effect on the systemic clearance adjusted for oral bioavailability (CL/F) and volume of distribution adjusted for bioavailability (Vd/F), mostly due to an increase in the oral bioavailability of EGCG in the fasting condition. Consistent with the previous studies, free EC levels were very low or undetectable in plasma samples. Similar to changes observed for plasma free EGCG, plasma free EGC and ECG C_{max} levels

in the fasting condition were significantly higher than those in the fed condition. Comparing the total and free catechin C_{max} data suggested that EGC and EC were mostly present in plasma as the conjugated forms and EGCG and ECG were mostly present as the free form. The fasting effect on the C_{max} of total EGCG and ECG was consistent with that of free EGCG and ECG. Taking Polyphenon E in the fasting state did not have a significant effect on the plasma levels of total (free and conjugated) EGC, but resulted in lower plasma levels of total EC ($p<0.005$). Urinary EGCG and ECG levels were very low or undetectable following Polyphenon E administration with either dosing condition. Taking Polyphenon E under the fasting state resulted in a significant decrease in the urinary recovery of total EGC and EC. The investigators suggested that less EGC and EC undergo presystemic glucuronidation and/or sulfation reactions in the fasting condition, resulting in more free catechins escaping the presystemic loss and being available in the systemic blood. Luminal catechin degradation may be an additional factor that could affect the oral bioavailability of CGT. The investigators noted that it is possible that CGT may be more stable in a fasted stomach (*i.e.*, acidic conditions or lower pH values) than a fed stomach (*i.e.*, at higher pH levels), which could contribute to the enhanced oral bioavailability observed in the fasting condition. Additional factors, such as food decreasing the intestinal absorption and dissolution rate of CGT, and interaction between CGT and bile acids secreted following food ingestion, could also contribute to the increase in the oral bioavailability of CGT observed in the fasting condition. The investigators concluded that greater oral bioavailability of free catechins can be achieved by taking the Polyphenon E capsules on an empty stomach after an overnight fast, and that Polyphenon E up to a dose that contains 800 mg EGCG is well-tolerated when taken under the fasting condition.

The plasma-kinetic profile of EGCG was assessed in two randomized, double-blind, placebo-controlled studies in healthy volunteers. The first study assessed the pharmacokinetics of single oral doses of 94% pure crystalline bulk EGCG under fasting conditions in 60 healthy Caucasian male volunteers aged 18-45 years with no clinically relevant abnormal physical or clinical chemistry findings and height and weight within 15% of the reference value of the Lorentz index (Ullmann U et al. 2003). In each group of 10 subjects, eight received oral EGCG in single doses of 50 mg, 100 mg, 200 mg, 400 mg, 800 mg, or 1600 mg, and two received placebo. The pharmacokinetic analysis was performed on those randomized subjects who received the study drug and completed the study (48 subjects, eight per dose). Blood samples were taken pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 5, 8, 12, 16, 22, and 26 hours after oral administration of the EGCG capsules. In each dosage group, the kinetic profile revealed rapid absorption with a one-peak plasma concentration *versus* time course, followed by a multiphasic decrease consisting of a distribution phase and an elimination phase. EGCG was absorbed rapidly, with individual T_{max} values ranging between 1 and 3 hours for free EGCG and between 1 and 5 hours for total EGCG. The mean $AUC_{0-\infty}$ of total EGCG varied between 442 ng×hour/mL in the lowest dose group and 10,368 ng×hour/mL in the highest dose group. The mean $AUC_{0-\infty}$ of free EGCG varied between 382 ng×hour/mL in the lowest dose group and 9628 ng×hour/mL in the highest dose group. The mean C_{max} values of total EGCG ranged from 130 ng/mL in the lowest dose group to 3392 ng/mL in the highest dose group and were observed after 1.3-2.2 hours. The mean C_{max} values of free EGCG ranged from 120 ng/mL in the lowest dose group to 2911 ng/mL in the highest dose group. The mean apparent terminal $t_{½}$ values ranged between 1.8 and 4.9 hours for free EGCG and

between 1.9 and 4.6 hours for total EGCG. The investigators attributed an approximately three-fold increase in systemic availability of EGCG in this study compared to the previous study by Chow et al. (2001) to the fact that in the present study the EGCG capsules were ingested under fasting conditions and subjects continued to fast for 2 hours after administration, while the Chow *et* al. study volunteers took EGCG with food. Additionally, the investigators noted that the increased, compared to the Chow *et al.* study, T_{max} values suggest that food slows the EGCG absorption process. However, the short half-lives were comparable between the studies suggesting that there is no risk of accumulation with multiple doses of EGCG. As expected and previously reported by Chow *et al*., the inter-individual variability in this parallel-group comparison was high. The investigators concluded that single oral doses of EGCG up to 1600 mg were safe and very well tolerated, with no adverse events reported during the study, even though the highest dose tested was equivalent to eight times the highest reported daily intake from tea.

In the second randomized, double-blind, placebo-controlled study, the same investigators assessed the pharmacokinetics of 94% pure crystalline EGCG after 10-day repeated oral dosing under fasting conditions in 36 healthy Caucasian male volunteers aged 18-45 years with Lorentz Indices within 15% of the reference value (height and weight), and no clinically relevant deviations from normal (Ullmann et al. 2003). Each of the three treatment groups consisted of 12 subjects, nine of them received oral EGCG in one dose of 200, 400, or 800 mg daily, and three received a placebo. Blood samples for plasma-kinetic EGCG characterization were taken on Day 1 and Day 10. Orally administered EGCG was rapidly absorbed from the gut. Average times to reach C_{max} (T_{max}) were between 1.39 and 2 hours, independent of dose, time, or EGCG form (free or total). The constancy of T_{max} values across the different dosage groups and study days suggested that no relevant changes in the absorption process occurred. Despite a very high observed inter-individual variability of EGCG plasma concentrations and, consequently, highly variable plasma-kinetic parameters, the investigators concluded that EGCG as a single dose administration (Day 1) was dose-proportional between 200 mg, 400 mg, and 800 mg in rate and extent, without significant changes in the elimination half-lives. The mean C_{max} on Day 1 was 376.9 ng/mL in the 200 mg group, 525.2 ng/mL in the 400 mg group, and 1682.1 ng/mL in the 800 mg group. The mean $AUC_{0-\infty}$ on Day 1 was 1415.9, 2111.4, and 5254.9 ng×hour/mL in the 200, 400, and 800 mg group, respectively. After repeated EGCG dosing (Day 10), dose linearity was applied between the 200 mg and 400 mg groups only. Dose escalation to 800 mg was more than dose-proportional in rate and extent, and statistically different from the 200 mg and 400 mg groups. The mean C_{max} on Day 10 was 267.9 ng/mL in the 200 mg group, 695.8 ng/mL in the 400 mg group, and 2431.4 ng/mL in the 800 mg group. The mean $AUC_{0-\infty}$ on Day 10 was 1128.8, 2668.7, and 8542.9 ng×hour/mL in the 200, 400, and 800 mg group, respectively. The mean apparent terminal $t_{½}$ for total EGCG were 2.7 hours in the 200 mg group, 3 hours in the 400 mg group, and 3.4 hours in the 800 mg group on Day 1. On Day 10, the mean apparent terminal $t_{½}$ for total EGCG were 2.3 hours in the 200 mg group, 4.2 hours in the 400 mg group, and 5.2 hours in the 800 mg group. The investigators noted that it appears that after repeated dosing with 200 mg EGCG, a certain induction of elimination pathways occurred (metabolization and/or excretion), resulting in a lower extent of systemic availability, and reduced C_{max} and elimination half-life of EGCG. The mean Accumulation Factor (R) values were 0.8 in the 200 mg group, 1.4 in the 400 mg group, and 1.9 in the 800

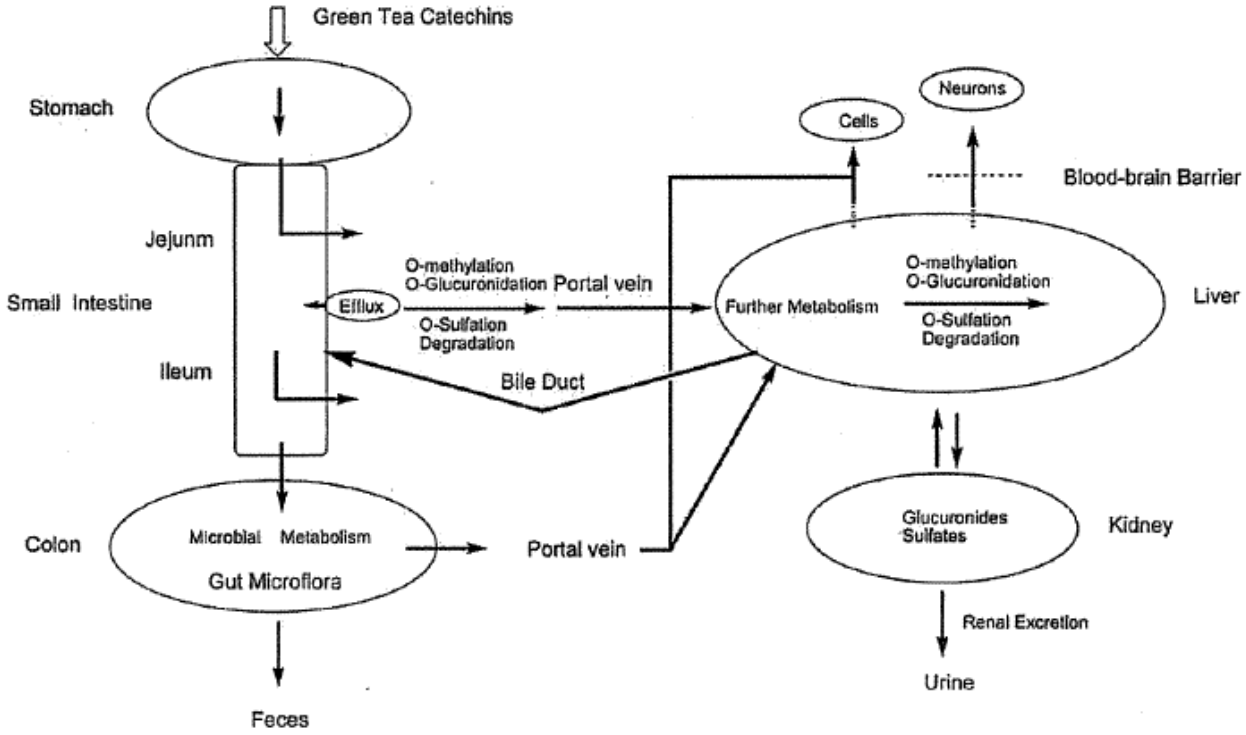
mg group. However, the R value in each group was not statistically different from one (R=1), meaning no accumulation has occurred with the increased dosage. Although concentration values were zero at all time points in the 200 mg group and nearly all in the 400 mg group, with detectable EGCG concentrations only after 800 mg dosing, where minimal concentrations reflected the late stage of elimination after 24 hours (approximately 0.5% of corresponding C_{max} values). A continuous steady state in the pharmacological sense was not reached. The investigators concluded that repeated dosing of 200, 400, or 800 mg EGCG once daily was well tolerated, with no serious or any other clinically-relevant adverse events reported, even though the highest dose tested exceeded the average catechin intake of tea-drinking populations. Moreover, minor changes in different physiological parameters, including blood pressure, ECG, blood lipids, liver enzymes, *etc*., were all without clinical relevance and no consistent EGCG or dose-related effect could be demonstrated. Importantly, because R values for total EGCG did not significantly differ from one, an accumulation of EGCG in the body is not to be expected for the average population.

Another repeat-dosing study assessed pharmacokinetics of EGCG and Polyphenon E after 4-week repeated oral dosing in 40 healthy male and female volunteers with normal liver and kidney function and Fitzpatrick skin type II or III (14 males and 26 females with mean ages of 39.4 to 39.5 years and mean weights of 150 to 169 pounds) (Chow et al. 2003). Study subjects were randomly assigned to receive one of the five treatments for 4 weeks, with a standardized light breakfast: 800 mg EGCG once/day, 400 mg EGCG twice/day, 800 mg EGCG as Polyphenon E once/day, 400 mg EGCG as Polyphenon E twice/day, or a placebo once/day (eight subjects/group). EGCG was present mostly in the free form in the systemic circulation (>92% as the free form, based on the AUC ratio of free *versus* total (free and conjugated) EGCG). Before repeated tea polyphenol treatment, pharmacokinetic parameters of EGCG were similar among the different treatment groups. The AUC and C_{max} of EGCG after the administration of 800 mg EGCG or Polyphenon E were higher, but not statistically significantly different, than those obtained after 400 mg dose of the respective formulation. After repeated tea polyphenol administration, the AUC of EGCG obtained after the 800 mg dose of Polyphenon E was significantly higher than that obtained from the 400 mg dose of either product. The AUC of EGCG obtained after the 800 mg dose of EGCG was significantly higher than that after the 400 mg dose of EGCG. Four weeks of repeated tea polyphenol administration at 800 mg once daily resulted in significant changes in the AUC of free EGCG, whereas repeated administration at 400 mg twice daily did not result in significant changes in the pharmacokinetics of free EGCG. The AUC of free EGCG increased from 95.6 ± 46.8 to 145.6 ± 85.1 min×μg/mL ($p<0.05$) and from 98.1 ± 46.5 to 158.4 ± 89.8 min×μg/mL ($p<0.05$) for the 800 mg once daily EGCG and Polyphenon E treatment, respectively. A decreasing trend was observed in the systemic clearance adjusted for oral bioavailability (CL/F) and volume of distribution adjusted for bioavailability (Vd/F) of EGCG after repeated treatment at 800 mg once daily, however, the changes did not reach statistical significance. The large CL/F and Vβ/F values observed in this study indicate that the oral bioavailability of CGT in humans is low. Repeated administration of green tea polyphenols did not result in significant changes in C_{max}, T_{max}, and $t_{½}$ of EGCG. On the basis of the observed plasma half-lives of EGCG, EGCG is not expected to accumulate in the body after repeated dosing at a once daily schedule (the accumulation ratio <1.05). After repeated dosing at a twice daily schedule, measurable concentrations of EGCG were present in most of

the predose samples collected on the last treatment day, with average predose EGCG levels of 29.9 ng/mL and 54.8 ng/mL after repeated dosing of EGCG and Polyphenon E, respectively. However, the fact that some of the subjects had a short elapsed time from the time the predose sample was collected to the time the previous dose was taken could contribute to the predose EGCG levels. The concentrations of free EGC and EC were below the limit of quantification in most plasma samples (0–4% present as the free form, based on the AUC ratio of free *versus* total (free and conjugated) catechins). Four weeks of tea polyphenol treatment with either dosing schedule did not result in significant changes in the levels of conjugated EGC and EC. The investigators concluded that it is safe for healthy individuals to take green tea polyphenol products in amounts equivalent to the EGCG content in 8–16 cups (one cup = 120 mL) of green tea once a day or in divided doses twice a day for 4 weeks.

There is some discrepancy between the results from human studies and cultured cell and animal models (Feng 2006). Two reasons for CGT's limited *in vivo* activity have been considered: metabolism and bioavailability. The fate of CGT in animals and humans depends on intestinal metabolism, microbial metabolism, hepatic metabolism, transporters, and chemical stability. Figure 2 below provides graphical representations of the proposed metabolism of CGT in humans. Methylation, glucuronidation and sulfation of four catechins have been observed in *in vitro* cellular fractions and cells and in animals and in humans. Several presystemic processes contribute to the low oral bioavailability of CGT, including chemical degradation, intestinal metabolism, microbial metabolism, hepatic metabolism, poor membrane permeability, and efflux transporter-mediated intestinal excretion. Eighteen metabolites from (-)-EC, at least six metabolites from ECG, 19 metabolites from EGC, and 22 metabolites from EGCG have been identified in humans. In humans, major metabolites of (-)-EC appear to be conjugates of (-)-EC and 3-*O*-methyl-(-)-EC, and 5-(3',4'-dihydroxyphenyl)-γ-valerolactone. Metabolites of ECG have not been detected in human plasma and urine. Major metabolites of EGC appear to be 4'-*O*-methyl-EGC, 4'-*O*-methyl-EGC-glucuronide, 4'-*O*-methyl-EGC-sulfate, 5-(3',4'-dihydroxyphenyl)-γ-valerolactone, 5-(3',5'-dihydroxyphenyl)-γ-valerolactone, and (-)-5-(3',4',5'-trihydroxyphenyl)-γ-valerolactone. Major metabolites of EGCG appear to be 4',4"-di-*O*-methyl-EGCG, 5-(3',4'-dihydroxyphenyl)-γ-valerolactone, 5-(3',5'-dihydroxyphenyl)-γ-valerolactone, and (-)-5-(3',4',5'-trihydroxyphenyl)-γ-valerolactone, and their sulfates and glucuronides.

Figure 2. Proposed Metabolism of CGT in Humans



Source: Feng 2006.

Because of low oral bioavailability, plasma tea catechin concentrations determined in humans after oral administration of green tea extract or CGT were 5 to 50 times less than the concentrations shown to exert biological activities in *in vitro* systems (Chow et al. 2005). Lee and colleagues (2002) noted that after drinking the equivalent of approximately two cups of tea, the mean peak plasma EGCG level in humans was 0.17 µM, while most of the published studies in cell culture systems used 10-100 µM of EGCG (Lee et al. 2002). The investigators further commented that the rather poor bioavailability of tea catechins needs to be considered when extrapolating results obtained *in vitro* to situations *in vivo*.

2. Animal Studies

Animal studies of CGT support findings of human studies demonstrating the low bioavailability of CGT. The absorption characteristics and oral bioavailability of three tea catechins, EC, ECG and EGCG, were assessed in male Sprague-Dawley rats administered either an intravenous (i.v. 50 mg/kg) or oral (5000 mg/kg) dose of decaffeinated catechin-fraction containing EC (5%), EGCG (50%), and ECG (13%) (Zhu et al. 2000b). The C_{max} for the catechins (15-112 μg/mL) were achieved at 2 hours post-oral dosing and the apparent volume of distribution (Vd/F) ranged from 30 to 63 L/kg. Absolute bioavailability (F, a parameter estimated from the dose-corrected i.v. to oral $AUC_{0-\infty}$ ratio) of EC, EGCG, and ECG was assessed to be 0.39, 0.14, and 0.06, respectively. Estimates of terminal elimination $t_{½}$ of the catechins after oral dosing were 451-479 minutes, 1.4-10-fold longer than those observed for the i.v. dosing. The discrepancy in terminal elimination and the low rate and extent of absorption indicated the possibility of flip-flop kinetics. Respective urinary recoveries were 0.17-4.72% and 2.11-14.2% after oral and i.v. dosing. The investigators concluded that the observed low systemic availability of CGT could be a result of slow absorption, high first-pass effect, and wide tissue distribution. The contribution of first-pass hepatic elimination to the low oral bioavailability of CGT was investigated in male Sprague-Dawley rats administered CGT mixture by i.v. (6000 μg EGCG, 1101 μg EGC and 930 μg EC) or intraportal infusion (6041 μg EGCG, 1285 μg EGC and 1048 μg EC), five animals per group (Cai et al. 2002). Blood samples were collected after dosing and analyzed using HPLC with the coulometric electrode array detection system. The systemic clearance of EGCG, EGC, and EC was 8.9, 6.3, and 9.4 mL/min, respectively. The steady state volume of distribution (Vdss) of EGCG, EGC, and EC was 432, 220, and 187 mL, respectively. Following intraportal infusion, a high percentage of CGT escaped first-pass hepatic elimination, with 87.0, 108.3, and 94.9% of EGCG, EGC, and EC, respectively, available in the systemic blood. These results suggest that first-pass hepatic elimination of CGT does not play a significant role in the presystemic elimination of orally administered catechins. Instead, factors within the gastrointestinal tract such as limited membrane permeability, transporter mediated intestinal secretion, or gut wall metabolism may contribute more significantly to the low oral bioavailability of CGT.

The pharmacokinetic study of EGCG in conscious and freely moving male Sprague-Dawley rats indicated that the oral bioavailability of EGCG was about 4.95% (Lin et al. 2007). EGCG was measured in rat plasma by a liquid chromatography technique coupled with tandem mass spectrometry (LC-MS/MS) electrospray ionization and using an automated blood sampling device. The protein binding of EGCG in rat plasma was 92.4 ± 2.5%. The EGCG concentrations in the various brain regions were around the lower limit of quantification, 5 ng/mL. The brain distribution result indicated that EGCG may potentially penetrate through the blood-brain barrier at a lower rate. The disposition of EGCG in the rat blood was fitted well by the two-compartmental model after 10 mg/kg i.v. administration. The elimination $t_{½}$ of EGCG was 62 ± 11 and 48 ± 13 minutes for 10 mg/kg i.v. and 100 mg/kg oral administration, respectively.

Most of the ingested EGCG (*i.e.*, >99%) apparently does not get absorbed into the blood, and the absorbed EGCG (0.1%) is preferentially excreted through the bile to the colon in rats (Chen, L., et al., 1997). After intravenous administration of decaffeinated green tea solution

(25 mg/kg in saline) to rats, the highest concentration of EGCG was found in the intestine samples, and the intestinal EGCG level declined slowly with an estimated $t_{½}$ of 173 minutes as determined by the HPLC-CEAS method. The highest levels of EGC and EC were observed in the kidney, and the levels declined rapidly with $t_{½}$ of 29 minutes and 28 minutes, respectively. Consistent with the observations in the plasma, the $t_{½}$ for EGCG was longest among the three tea polyphenols in the tissues examined. The AUC values of EGCG were 1.7, 0.3, and 0.4 mg×min/g in the intestine, kidney, and lung, respectively. The AUC values of EGC were 1.4, 1.2, and 0.9 mg×min/g in the kidney, lung, and intestine, respectively. The AUC values of EC were 0.3, 0.1, and 0.4 mg×min/g in the kidney, lung, and intestine, respectively. Thus, the AUC value of EGCG in the intestine was 4-fold higher than that in the kidney, but the AUC values of EGC and EC in the intestine were similar to those in the kidney. These results suggest that EGCG is excreted mainly through the bile, while EGC and EC are excreted through both the bile and urine. These results are in agreement with the observations in humans, when EGC and EC, but not EGCG, were detected in human urine samples. Low levels of EGCG, EGC, and EC were also detected in the liver. The liver and lung levels of EGCG, EGC, and EC were generally lower than those in the intestine and the kidney.

When EGCG (500 mg/kg body weight in distilled water) was administered orally to rats via gavage, the highest distribution of EGCG was detected by CL-HPLC in the gastrointestinal mucosa, mainly in the small intestine (565 nmol/g), followed by the colon mucosa (68.6 nmol/g), liver (48.4 nmol/g), and plasma (12.3 nmol/g) (Nakagawa and Miyazawa, 1997). A very low concentration of EGCG (0.5 nmol/g) was detected in the brain. The direct incorporation of EGCG into the intestinal mucosal cells of a rat that had ingested EGCG was confirmed by CL-HPLC (Miyazawa and Nakagawa 1998). A specific maximum emission wavelength of 630 nm was detected in small intestinal mucosal cells in rats administered oral EGCG (23 mg/100 g body weight in distilled water). EGCG, EGC, ECG, and EC, administered orally to rats (100 mg each in distilled water), were absorbed into portal blood via the intestinal tract as determined by the HPLC-UV method (Okushio et al. 1996).

Several *in vitro* studies showed that the rat liver cytosol has a greater ability than mouse or human liver cytosol to methylate and sulfate EGCG, whereas mouse and human liver cytosol have a greater ability to glucuronidate EGCG than rat liver cytosol (Lu et al. 2003a, 2003b; Lambert et al. 2003). These *in vitro* results suggest that humans and mice might be more similar to each other in terms of EGCG biotransformation than either species is to rats. However, these *in vitro* studies provide no information about the interplay between biotransformation pathways *in vivo*, the expression patterns of Phase II enzymes in whole organisms and the possible role of other proteins such as efflux transporters. These factors may lead to relevant differences between humans and mice in terms of EGCG metabolism (Li et al. 2001).

When tritium-labeled EGCG ([^{3}H]EGCG) was administered to mice, the radioactivity was detected in various tissues including the gastrointestinal tract, liver, brain, lung, heart, kidney, bone, and skin (Miyazawa et al. 200). In another study, the [^{3}H]EGCG solution was directly administered into the stomachs of CD-1 female or male mice (Suganuma et al. 1998). Significant radioactivity was found in the previously reported target organs of EGCG and

green tea extract (digestive tract, liver, lung, pancreas, mammary gland and skin), as well as other organs (brain, kidney, uterus, heart, spleen, bladder, bone, and ovary and testes) in both sexes. Incorporation of radioactivity in the cells was confirmed by microautoradiography. One hour after administration, the average percent of total administered radioactivity in the digestive tract, including dietary content, was 30.7%, 40.6%, and 3.9% for the stomach, small intestine, and colon, respectively, in female mice, and 56.9%, 25.8%, and 1% for the stomach, small intestine, and colon, respectively, in male mice. Twenty-four hours after administration, radioactivity in the digestive track (stomach, small intestine and colon) had decreased, but 14.5% of total administered radioactivity for female mice and 18.2% for male mice remained. The 24-hour radioactivity counts in the stomach, small intestine, and colon were the highest counts among various organs measured in both sexes. Twenty-four hours after administration, radioactivity was equally distributed among almost all organs in both sexes. Total radioactivity incorporated was 0.89% for liver, 0.32% for brain, 0.28% for kidney, and 0.16% for lung in female mice, and 0.67% for liver, 0.33% for brain, 0.43% for kidney, and 0.16% for lung in male mice. The investigators commented that the percentages are those of total administered radioactivity, and differences, obviously, reflect the sizes of the organs. Approximately 2% of total administered radioactivity had been incorporated into total blood in female and male mice 24 hours after intubation. This radioactivity was not separated into free EGCG, its metabolites or the protein-bound forms of EGCG; it was calculated as 1.2 μg/mL EGCG under the assumption that all radioactivity was [^{3}H]EGCG. Within 24 hours, 6.6% (females) and 6.4% (males) of total administered radioactivity was excreted in the urine, and 37.7% (females) and 33.1% (males) in feces. HPLC analysis of urine from both sexes revealed that 0.03-0.59% of administered [^{3}H]EGCG, along with at least five metabolites, was excreted. A second, equal administration to female mice after a 6-hour interval enhanced tissue levels of radioactivity in blood, brain, liver, pancreas, bladder, and bone 4-6 times above those observed after a single administration. The radioactivity of the digestive tract, including dietary content, increased 1.3-2-fold, and radioactivity of urine increased about 6-fold.

The plasma and tissue pharmacokinetic parameters of EGCG in mice were determined after either i.v. or intragastric administration of a single dose of pure EGCG (Lambert et al. 2003). After administration of EGCG intravenously at 21.8 μmol/kg or intragastrically at 163.8 μmol/kg, the peak plasma levels of EGCG in male CF-1 mice were 2.7 ± 0.7 and 0.28 ± 0.08 μmol/L, respectively. EGCG was present mainly (50–90%) as the glucuronide. The plasma bioavailability of EGCG after intragastric administration was higher than previously reported in rats (26.5 ± 7.5% *versus* 1.6 ± 0.6%). The conjugated EGCG displayed a shorter $t_{½}$ (82.8–211.5 *versus* 804.9–1102.3 minutes) than unconjugated EGCG (p<0.01, Student's t test). After intravenous administration, EGCG was present in the unconjugated form in all tissues analyzed. The C_{max} values were 3.56 ± 0.80, 2.66 ± 1.0, 2.40 ± 1.1 nmol/g, and 2.12 ± 0.6 nmol/g in the liver, lung, small intestine, and kidney, respectively. Although the colon had a lower C_{max} (1.20 ± 0.30 μmol/g), it had a higher exposure ($AUC_{0–720\ min}$ = 325.3 ± 88.7 nmol×min)/g) and a longer $t_{½}$ (224 ± 61.1 minutes). Levels in the prostate and the spleen were 0.31–0.83 nmol/g. Although intragastric administration resulted in lower levels in most tissues compared with intravenous administration (*e.g.*, 0.006 ± 0.004 *versus* 2.66 ± 1.0 nmol/g in the lung), the levels in the small intestine and colon were high at 45.2 ± 13.5 and 7.86 ± 2.4 nmol/g, respectively. After intragastric administration, prostate, liver, and lung

levels were 0.002–0.15 nmol/g, but were not sufficiently high to accurately determine tissue kinetics. After i.v. or intragastric administration of EGCG, the levels of EGCG in feces were greater than those in urine. In urine, >90% of the EGCG was in the conjugated form, whereas in feces, nearly all was in the unconjugated form. The investigators concluded that the study results point to the potential role of glucuronidation in affecting EGCG bioavailability and demonstrate important species differences among humans, rats and mice. This study confirmed the results of previous studies suggesting that the intestine represents a major barrier to the bioavailability of EGCG, and that fecal excretion is the major route of elimination (Cai et al. 2002).

In addition to the studies in adult animals, pharmacokinetic parameters of CGT were determined in rat fetuses. The distribution of CGT in fetal rat organs was investigated after maternal exposure to green tea extract (550 mg/kg) or water administered orally at 15.5 days of gestation (Chu et al. 2007). Catechins and catechin gallates were determined by HPLC after solid-phase extraction. In the green tea extract-administered group, low levels of catechins were detected in most of the fetal organs studied, including the brain, eyes, heart, lungs, kidneys and liver, but not in the control group. T_{max} values were about 0.5-1 hours. C_{max} of catechins in the fetal eye were about 2-10 times higher than in the other organs, ranging from 249 pmol/g for EC to 831 pmol/g for EGCG. Catechin gallates were generally more readily taken up by fetal organs than catechins. EGCG had the highest level of uptake according to AUC plots and the highest C_{max} in all organs, suggesting the absorption and deposition could be related to structure. Maternal plasma concentrations of CGT were about 10 times higher than in placenta and 50-100 times higher than in the rat fetus (Chu et al. 2006). AUC and C_{max} levels of EC were the highest in plasma, while the levels of EGCG were the highest in the placenta and the fetus. The exposure level of catechin derivatives was higher than the gallate derivatives in maternal plasma after normalization but reversed in the placenta and fetus. The absorption of epi-isomers in plasma was found to be more favorable than their non epi-isomer counterparts. EGCG had the highest level of exposure (AUC) and the highest C_{max} in the fetus. The levels of catechin gallate were too low for accurate determination.

The results of CGT studies in dogs are generally in agreement with previous studies in rodents, and indicate that, after oral administration, EGCG (as parent compound and metabolites) is widely distributed to tissues (Swezey et al. 2003). Four male beagle dogs were administered doses of [^{3}H]EGCG by i.v. and oral routes. Food was withdrawn until 4 hours after dose administration. Following i.v. administration of 25 mg/kg EGCG, radioactivity in the bloodstream resided predominantly in the plasma. Distribution occurred during the first hour, and the plasma levels of total radioactivity declined with a mean $t_{½}$ of approximately 7 hours. The apparent Vd (0.65 L/kg) indicated wide distribution, and the total body clearance was 1.01 mL/min/kg. A subsequent single oral dose (250 mg/kg) was rapidly absorbed, with peak plasma levels at about 1 hour after administration, followed by elimination with a mean $t_{½}$ of 8.61 hours. Following oral or i.v. administration, the major route of elimination of radioactivity was via the feces rather than the urine. This finding supports previous studies conducted in rodents. Tissue distribution was determined 1 hour after an i.v. dose (25 mg/kg) administered after 27 days of oral treatment with EGCG (250 mg/kg/day) to mimic chronic consumption of tea. Radioactivity was distributed to a variety

of epithelial tissues including the liver, stomach, esophagus, small intestine, colon, bladder, prostate gland, lungs, and kidneys. Repeat dose oral administration of EGCG resulted in significantly lower blood radioactivity compared to the concentration following a single dose ($p<0.5$). The investigators commented that, apparently, chronic exposure to tea polyphenols alters the disposition of EGCG, and adaptive responses may differ with species.

Another study in beagle dogs assessed, for the first time in beagles, the major flavanol forms in plasma and urine after oral administration of a green tea extract (Mata-Bilbao et al. 2008). Ten female beagle dogs were administered 173 mg (12.35 mg/kg body weight) of catechins as a green tea extract, in capsules. The dogs were deprived of food overnight before the experiment. Two catechins with a galloyl moiety (EGCG and ECG) and three conjugated metabolites were detected in plasma. Most of the detected forms in plasma reached their C_{max} at around 1 hour. Median C_{max} for EGCG, ECG, EGC-glucuronide, EC-glucuronide, and EC-sulfate were 0.3 (range 0.1-1.9), 0.1 (range 0-0.4), 0.8 (range 0.2-3.9), 0.2 (range 0.1-1.7), and 1 (range 0.3-3.4) µmol/L, respectively. The AUC_{0-24} were 427 (range 102-1185) µmol/L×min for EGC-glucuronide, 112 (range 53-919) µmol/L×min for EC-sulfate, 71 (range 26-306) µmol/L×min for EGCG, 40 (range 12-258) µmol/L×min for EC-glucuronide, and 14 (range 0.1-124) µmol/L×min for ECG. The values of mean residence time (MRT_{0-24}) were 5 (range 2-16), 2 (range 1-11), 10 (range 2-13), 3 (range 2-16), and 2.4 (range 1-18) hours for EGCG, ECG, EGC-glucuronide, EC-glucuronide, and EC-sulfate, respectively. In urine, EC and EGC were present as conjugated forms, suggesting bile excretion of EGCG and ECG. These results were in agreement with the human data that indicated that after ingestion of EGCG and Polyphenon E (a tea polyphenol preparation) by human volunteers, plasma EGCG was mainly present in the free (unconjugated) form and may be eliminated in feces by biliary excretion (Chow et al. 2001). In addition, T_{max} and AUC values for EGCG compared well with data generated in previous studies with human subjects, while C_{max} values differed, suggesting that the bioavailability of CGT is variable and could be due to differences in species, and quantity and form of catechin administration. The investigators also commented that several previous pharmacokinetic studies (Lee et al. 2002; Chow et al. 2001; Yang et al. 1998; Unno et al. 1996; Pietta et al. 1998; Lee et al. 2995) of CGT evaluated only unchanged catechins, suggesting that the calculated bioavailability in these studies was probably overestimated. The investigators concluded that CGT are absorbed from the intestine as their intact form or as conjugates following oral administration, and EGC-glucuronide is the metabolic form that remains in the organism for a longer period of time, probably due to an enterohepatic cycle (Mata-Bilbao et al. 2008; Miyazawa et al. 2000).

In clinical samples from humans after drinking green tea, EGCG was distributed in plasma, portal blood, bile, stomach mucosa, and large intestinal mucosa as shown in Table 6 below (Miyazawa et al. 2000).

Table 6. Free EGCG concentration after drinking EGCG (250 mg) in humans

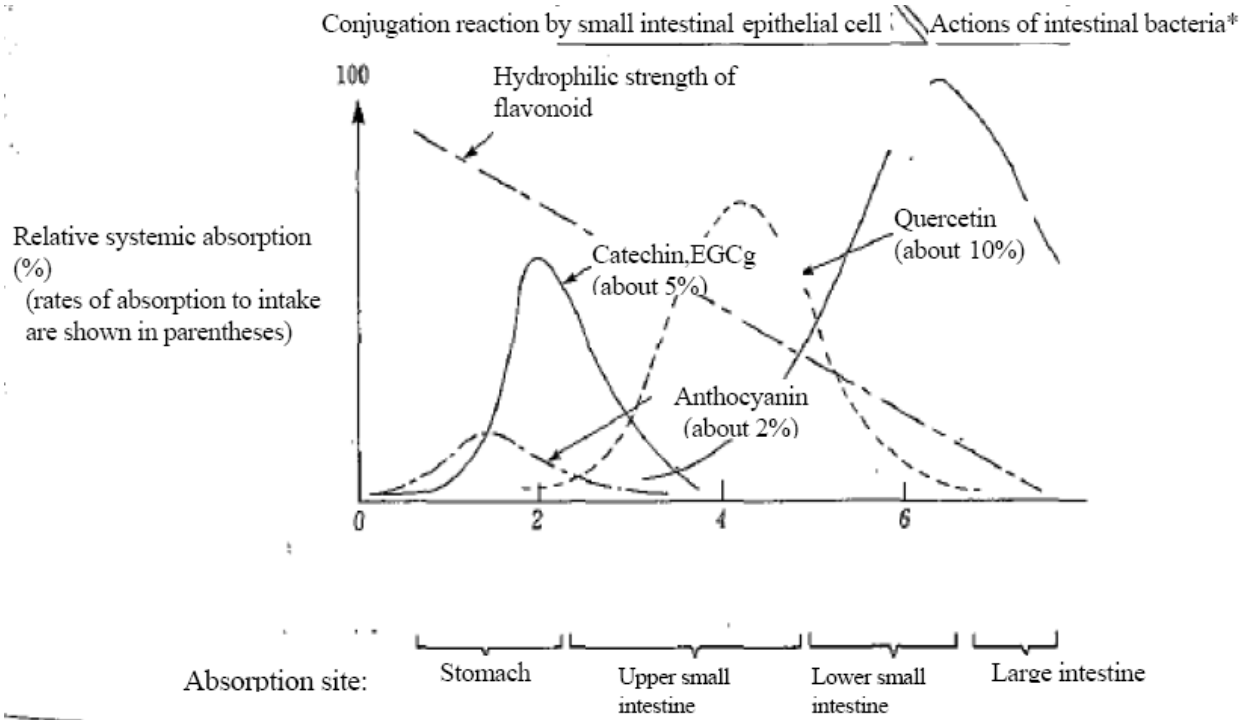
Tissue	3 hours after ingestion	12 hours after ingestion
Blood plasma (ng/mL)	491±502 (115-1229, n=4)	129±90 (28-268, n=11)
Portal blood (ng/mL)*	527±200 (330-730, n=3)	104±65 (61-201, n=4)
Bile (ng/mL)	68±80 (6-158, n=3)	61±6 (56-65, n=2)
Gastric mucosa (ng/g)	343±127 (167-464, n=4)	111±70 (50-207, n=4)
Large intestinal mucosa (ng/g)	Not analyzed	531±366 (51-1015, n=6)

* Venous blood from the right gastroepiploic vein that connects the stomach and portal vein.

However, very large individual variation was noted in the distribution levels. Since the portal blood sample analyzed was venous blood from the right gastroepiploic vein that connects the stomach and portal vein, the detection of EGCG in this sample suggested that a portion of catechins was absorbed from the stomach, in addition to the upper small intestine. This is one of the reasons for the rapid maximum absorption time (time required to reach the maximum blood level after ingestion).

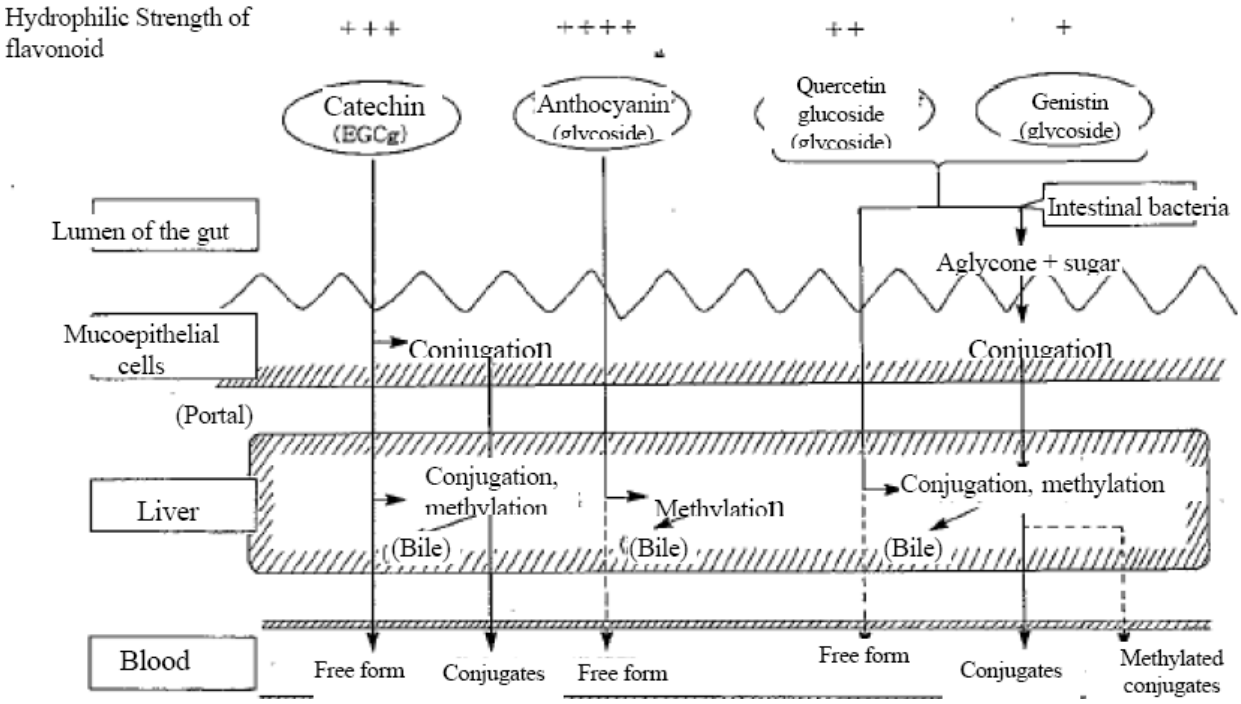
In summary, ingested CGTs in humans are mostly distributed in the gastrointestinal mucosa, and excreted in feces upon detachment from the mucosa (Miyazawa et al. 2000). Five to 8% of ingested CGT is absorbed within a relatively short time (probably from the stomach and upper small intestine), and partially conjugated with glucuronide and sulfate in mucoepithelial cells upon absorption. Figures 3 and 4 below provide graphical representations of the absorption and metabolic pathways for various flavonoids, including catechins, respectively.

Figure 3. Absorption Site, Amount, and Maximum Absorption Time of Major Dietary Flavonoids



*Aglycone production, ring fission, and dehydration reaction by intestinal bacteria.
Source: Miyazawa et al. 2000.

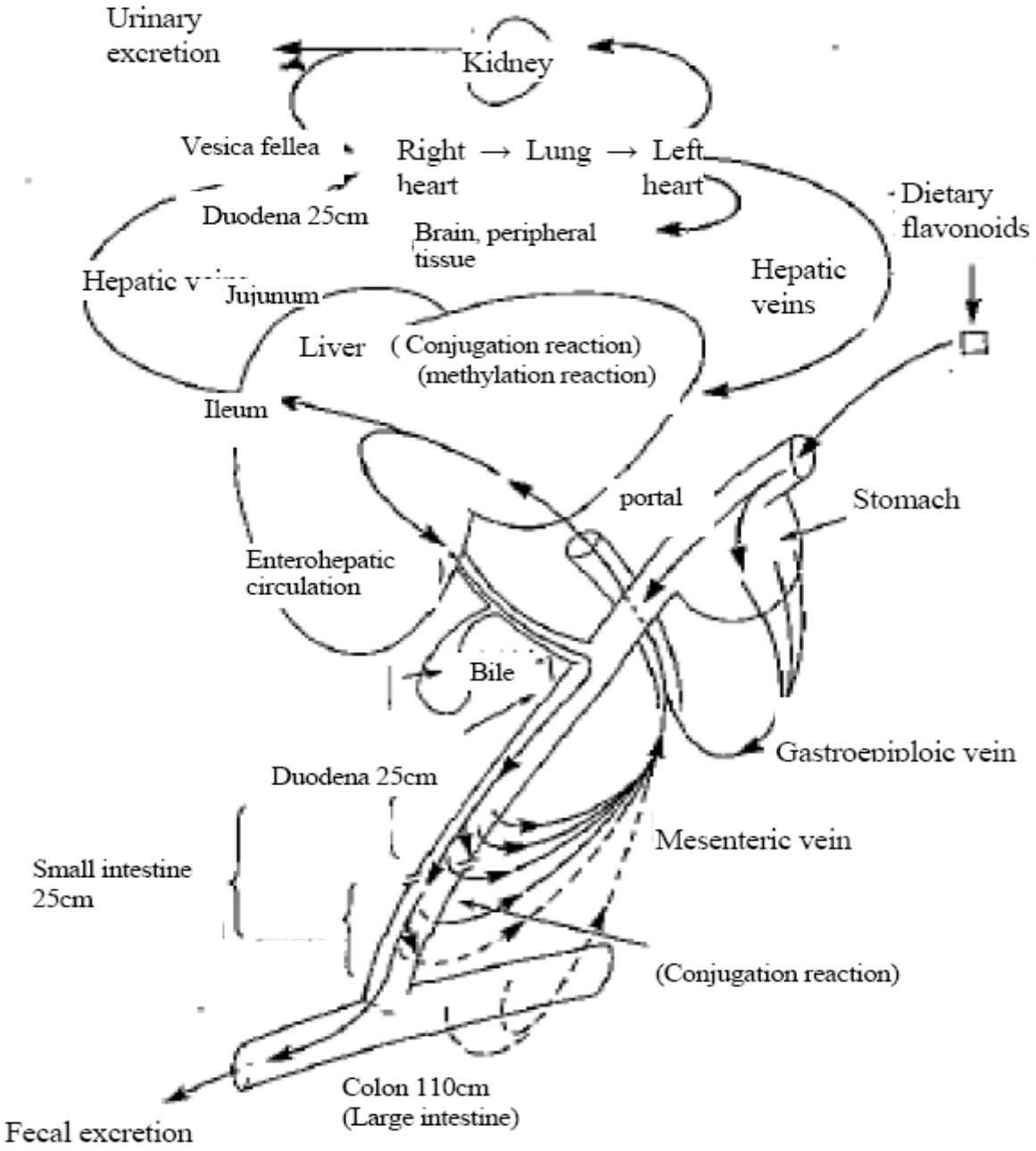
Figure 4. Differences in Conjugation and Methylation due to Dietary Flavonoid Structure



*As glucuronide and sulfate
Source: Miyazawa et al. 2000.

Following absorption, CGT is transported to the liver via the portal system, resulting in the formation of glucuronide, sulfate and/or methyl conjugates. A portion enters blood flow as the free form or conjugates, and is transported to peripheral tissues. Some of the free form and conjugates may be contained in bile secreted by the liver and enter the enterohepatic circulation. Figure 5 below provides a graphic representation of the absorption and metabolism of flavanoids, including catechins.

Figure 5. Absorption and Metabolic Pathway of Dietary Flavanoids



Source: Miyazawa et al. (2000).

Blood catechins are considered to be finally excreted in urine through the kidney. The ratio of the free form:sulfate:glucuronide in human blood changes depending on the catechin intake, is basically determined by the sulfate pool in the body, and is normally 2-3:2-3:1.

Compared to other catechins, EGCG and EGC are less likely to be methylated by liver catechol *O*-methyltransferase, which uses catechol structures as the substrate, because they possess three hydroxyl groups (pyrogallol structure) in the B ring.

3. Summary and Conclusions

In summary, the extensive available data from human and animal studies indicate that:

1) CGT have the low oral bioavailability that has been attributed to several marked pre-systemic elimination processes, including chemical degradation, intestinal metabolism, microbial metabolism, hepatic metabolism, poor membrane permeability, and efflux transporter-mediated intestinal excretion (Chow et al. 2001, 2005; Ullmann et al. 2003; Feng 2006). The available data suggest that the intestine represents a major barrier to the bioavailability of CGT. Accordingly, the data from studies utilizing other routes of CGT administration, *e.g.*, intravenous, which bypasses the gastrointestinal system, are not relevant for evaluation of bioavailability and systemic effects of orally consumed CGT.

2) After oral administration, CGT are widely and almost equally distributed into various epithelial tissues, including the gastrointestinal tract, liver, kidney, lung, brain, pancreas, bladder, heart, prostate gland, skin, bone, *etc*., as shown using a radioactively-labeled CGT (Miyazawa et al. 2000; Suganuma et al. 1998). The human data indicated that CGT in humans are mostly distributed in the gastrointestinal mucosa, and excreted in feces upon detachment from the mucosa (Miyazawa et al. 2000). Importantly, the liver does not appear to accumulate more CGT than other organs.

3) Based on the experimental evidence from the repeat-dose studies, CGT do not appear to accumulate in the body after increased dosage and repeated exposures in the average population (Ullmann et al. 2004; Chow et al. 2003).

4) Dosing conditions, *i.e*., administration with or without food, appear to affect the oral bioavailability of CGT. Specifically, the oral bioavailability of CGT appears to be lower in the fed condition *versus* the fasting condition (Chow et al. 2005). The suggested factors that could contribute to the increase in the oral bioavailability of CGT observed in the fasting condition include decreased presystemic glucuronidation and/or sulfation of certain CGT metabolites in the fasting condition, resulting in more free catechins escaping the presystemic loss and being available in the systemic blood; increased stability of CGT in a fasted stomach (*i.e*., acidic conditions or lower pH values) than a fed stomach (*i.e*., at higher pH levels); decreased intestinal absorption and dissolution rate of CGT in fed condition; and interaction between CGT and bile acids secreted following food ingestion. This suggests that CGT may have an even safer toxicity profile when consumed with food. Importantly, human clinical trials indicate that even when consumed in the fasting condition, high single dose and repeat doses of CGT are well tolerated by healthy individuals, with no serious or any other clinically-relevant adverse events reported, even though the highest dose tested exceeded the average catechin intake of tea-drinking populations (Chow et al. 2005; Ullmann et al. 2003, 2004). Finally, Kao's CGT will be consumed in beverage form; therefore, fasting conditions are not relevant to the intended use for Kao's product.

5) The results of toxicity studies in dogs, especially under fasting conditions, and their comparison to lethal concentrations in *in vitro* systems cannot be directly applied to evaluation of CGT safety in humans due to problems with extrapolating results obtained *in vitro* to situations *in vivo* and extrapolating animal results to humans. Several reports noted discrepancies between the results from human studies and cultured cell and animal models, which can be attributed to the low oral bioavailability of CGT in humans and metabolism of CGT in humans and animals (Feng 2006). The authors of a safety study in fasted dogs administered EGCG as a single bolus dose stated that "this model was considered an unrealistic comparison to the human condition" (Isbrucker et al. 2006b). Because of low oral bioavailability, plasma CGT concentrations after oral administration in humans were 5 to 50 times less than the concentrations shown to exert biological activities in *in vitro* systems (Chow et al. 2005). After drinking the equivalent of approximately two cups of tea, the mean peak plasma EGCG level in humans was 0.17 μM, while most of the published studies in cell culture systems used 10-100 μM of EGCG (Lee et al. 2002). Accordingly, the low oral bioavailability of CGT needs to be considered when extrapolating results obtained *in vitro* to situations *in vivo*.

B. Toxicology Studies

For purposes of the following discussion, provided below is a summary of the nomenclature used to describe the various forms of CGT evaluated in Kao's toxicology studies.

Type-1 CGT	Catechins from green tea for bottled tea beverage
Type-2 CGT	Catechins from green tea for sports drink, carbonated soft drink, and juice
Heat-treated Type-1 CGT ("HT-CGT")	**Test preparation of Type-1 catechins from green tea for toxicity studies:** The sterilization temperature in the green tea beverage production process is higher compared to the sports drink production process. The catechin profile changes at high sterilization temperatures. Therefore, the Type-1 CGT used in the toxicity studies is heat-treated to ensure the equivalence of the catechin profile in the test article to the finished product.
Non-heat-treated Type-1 CGT ("UT-CGT")	A non-heat-treated version of Type-1 CGT also was evaluated in the toxicity tests as a control.
Non-heat-treated Type-2 CGT ("Type-2")	The sterilization temperature in the sports drink production process is lower than that used in the green tea beverage production process. Therefore, the catechin profile does not change at lower sterilization temperatures. The Type-2 CGT used in the toxicity studies is non-heat-treated.

Decaffeinated Heat-treated Type-1 CGT ("D-HT-CGT")	**Decaffeinated test preparation of Type-1 catechins from green tea for toxicity studies:** D-HT-CGT is a control test preparation used to study the potential effects of the caffeine component of CGT.

1. Short-term Toxicity Studies of Catechins from Green Tea (CGT)

As noted in the chart above, because of the heat-sterilization step used in the manufacture of one type of green tea beverage, many of these studies were conducted in parallel with both a heat-treated ("HT-CGT") and non-heat-treated version of Catechins from Green Tea ("UT-CGT"). A number of short-term toxicity studies were conducted on Kao's HT-CGT and UT-CGT. These unpublished studies are summarized in Table 7.

Table 7. Summary of CGT Short-Term Toxicity Studies

Citation	Design/Duration	Test Substance	Test Animal	Endpoints	Results	Comments
Yamaguchi, M., et al. Kao Study 03-158-2. A Single Dose Oral Toxicity Study of KH-1 (FD) in Rats.	A single dose (2000 mg/kg) administered orally by gavage after 18 hours fasting Observations were made following administration continuously until 1 hr, frequently up until 6 hr, twice a day on Day 2 and daily thereafter. All animals sacrificed at Day 15 and necropsied.	Heat-treated CGT (HT-CGT)	5 male (mean body weight 121 ± 7 g) and 5 female (mean body weight 106 ± 3 g) 5-week-old Crj:CD(SD)IGS rats (SPF)	• Death • Gross clinical abnormalities • Food consumption and body weight • Macroscopic evaluation	• No deaths occurred, and no clinical abnormalities were observed. • Body weight continued to increase and food consumption was unaffected. • No abnormalities were observed at macroscopic evaluation. • Acute oral LD_{50} > 2000 mg/kg bw	• No indication as to whether this test was performed under GLP conditions.
Yamaguchi, M. et al. Kao Study 03-159-2. 14-Day Repeated Oral Toxicity Test with KH-1 (FD).	Doses of 500 mg/kg, 1000 mg/kg/day, and 2000 mg/kg/day of heat-treated Type 1 tea catechins were administered orally by gavage to respective groups of 5 male and 5 female rats for 14 days. In addition, 5 male and 5 female rats administered 20 mL/kg of water served as controls.	HT-CGT	20 male (mean body weight 171 ±6 g) and 20 female (mean body weight 148 ± 5 g) 5-week-old Crj:CD(SD)IGS rats (SPF).	• Daily general clinical observation • Hematology (RBC, Hb, Ht, MCV, MCH, MCHC, Reticulocyte, PLT, PT, APTT, WBC, Diff leuk) • Blood Chemistry (GOT, GPT, γGTP, ALP, bilirubin, urea nitrogen, creatinine, glucose, cholesterol, LDH triglycerides, total protein, albumin, Ca2+, inorganic phosphate, Na, K,	• No clinical abnormalities or mortalities were observed. • A statistically significant decrease in body weight and body weight gain was observed in the male rates only at 1000 and 2000 mg/kg/day ($p<0.05$ for 1000 mg/kg/day; $p<0.01$ for 2000 mg/kg/day). • While a dose-	• This testing was performed under OECD GLP. conditions.

Citation	Design/Duration	Test Substance	Test Animal	Endpoints	Results	Comments
				Cl, A/G ratio) • Urinalysis (pH, protein, glucose, ketone bodies, bilirubin, occult blood, urobilinogen) • Ophthalmoscopy • Sensory/reflex • Body weight and food consumption • Macroscopic evaluation • Absolute and relative organ weight (brain, liver kidneys, spleen, heart, thymus, thyroid, pituitary gland, adrenal glands and testes, prostate, seminal vesicle, epididymides, ovaries, and uterus) • Histopathology (brain, pituitary, eyes, thyroid with parathyroid, heart, trachea and lung, liver, kidneys, thymus, spleen, adrenal glands, stomach (fore and glandular), small intestine, large	dependent "decreasing tendency" in food consumption was observed in treated males after 2 weeks, the difference was not statistically significant. • No changes attributable to treatment were observed in any hematological, clinical chemistry, or urinalysis parameter. • No abnormalities were observed during ophthalmoscopy or macroscopic evaluation. • No significant changes in the absolute and relative weights of each organ. • NOAEL is 2000 mg/kg bw, the highest dose tested.	

Citation	Design/Duration	Test Substance	Test Animal	Endpoints	Results	Comments
				intestine, pancreas, gonads (testes or ovaries), accessory reproductive organs, (epididymides, prostate and uterus), urinary bladder, lymph nodes (cervical and mesenteric), bone and bone marrow (femur))		
Hurley, J. WIL Research Lab Study WIL-101096. Acute Oral Toxicity Study of KH-1 (HFD) in Albino Rats	Heat-treated Tea Catechins (CGT HS-type-1) was initially administered once orally by gavage at a dose level of 5000 mg/kg/day to one male and one female. Based on the results of this administration, additional testing was performed on six male and six female rates. Rats were observed at 15 minutes, 1 hr, 2 hr, and 4 hrs after dosing; thereafter, the rats were observed daily.	HT-CGT	Selected rats for the study were approximately 8-weeks-old for males (mean body weight 237 ± ±20.5g), and 9-weeks-old for females mean body weight 192 ± 2.3 g).	• Death • Gross clinical abnormalities • Food consumption and body weight • Macroscopic evaluation	• One animal died on Day 1 and one animal died on Day 2 following the first administration of 5000 mg/kg. • For the 2000 mg/kg/day dose, 2/6 females died on Days 2 and 3 and no male rats died. • The acute oral LD_{50} is >2000 mg/kg but <5000 mg/kg	• Study was performed according to U.S. GLP conditions.

Citation	Design/Duration	Test Substance	Test Animal	Endpoints	Results	Comments
Kirkpatrick, J., et al. WIL Research Labs study WIL-101097. 14-Day Repeated Dose Oral Toxicity Test with KH-1 (HFD) in Rats.	Doses of 500 mg/kg, 1000 mg/kg/day, and 1500 mg/kg/day of Heat-treated Tea Catechins (CGT HS-type-1) were administered orally by gavage to respective groups of 5 male and 5 female rats for 14 days. In addition, 5 male and 5 female rats administered 20 mL/kg of water served as controls.	HT-CGT	20 male (mean body weight across dose groups: 227-229 ± 6.1-19.1 g) and 20 female (mean body weight across dose groups: 158-162 ± 5.9-11.5 g) 7-week-old Crj:CD(SD)IGS rats (SPF).	• Twice daily general clinical observation • Hematology (RBC, Hb, Ht, MCV, MCH, MCHC, PLT, PT, APTT, WBC, Diff leuk) • Blood Chemistry (AT, ASAT, γGT, ALP, bilirubin, urea nitrogen, creatinine, glucose, cholesterol, LDH triglycerides, total protein, albumin, Ca^{++}, inorganic phosphate, Na, K, Cl, A/G ratio) • Body weight and food consumption, • Macroscopic evaluation • Absolute and relative organ weight (adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thymus, thyroid)	• No clinical abnormalities or mortalities were observed. • Hyperactivity was noted in some of the male and female rates in the two higher dose groups. • There was a slight, but statistically significant, decrease in the cumulative body weights of the 1000 mg/kg/day males and 1500 mg/kg/day males and females. • Food consumption was unaffected by administration of KH-1 (HFD). • No test article-related changes in hematology or clinical chemistry parameters were observed. Statistically significant changes in various parameters were not considered treatment-related because of	• Due to the very low incidence of hyperactivity and the lack of effects on body weights at 500 mg/kg/day, the No Observed Adverse Effect Level (NOAEL) was determined to be 500 mg/kg/day for rats, based on changes in body weight gain seen at 1000 and 1500 mg/kg/day. • This was a range-finding study for a 6-month study in rats. • This testing was performed under OECD GLP conditions.

Citation	Design/Duration	Test Substance	Test Animal	Endpoints	Results	Comments
					inconsistency between sexes, small magnitude of change, and/or no dose-response relationship. • No abnormalities were observed at the macroscopic evaluation. • No test article-related changes in absolute and relative organ weights were observed.	
Yamaguchi, M., et al. Kao Study 03-158-01. Acute Oral Toxicity with UHG(FD) in Rats.	A single dose (2000 mg/kg) administered orally by gavage after fasting. Observations were made following administration continuously until 1 hr, frequently up until 6 hr, twice a day on Day 2, and daily thereafter. All animals were sacrificed at day 15 and necropsied.	Unheat-treated CGT (UT-CGT)	5 male (mean body weight 119 ±3 g) and 5 female (mean body weight 105 ± 7 g) 5-week-old Crj:CD(SD)IGS rats (SPF)	• Death • Gross clinical abnormalities • Food consumption and body weight • Macroscopic evaluation	• No deaths occurred, and no clinical abnormalities were observed. • Body weight continued to increase and food consumption was unaffected. • No abnormalities were observed from the necropsy. • Acute oral LD_{50} is >2000 mg/kg bw.	• This testing was performed under GLP conditions.
Yamaguchi, M., et al. Kao Study 03-159-1. 14-Day Repeated Oral Toxicity Test with	Doses of 500 mg/kg, 1000 mg/kg/day, and 2000 mg/kg/day of UHG (Type 2) were administered orally	UT-CGT	20 male (mean body weight 178 ±9 g) and 20 female (mean body weight 155 ± 6 g)	• Daily general clinical observation • Hematology (RBC, Hb, Ht, MCV, MCH, MCHC,	• No clinical abnormalities or mortalities were observed. • No statistically	• This testing was performed under OECD GLP conditions.

Citation	Design/Duration	Test Substance	Test Animal	Endpoints	Results	Comments
UHG (Type 2).	by gavage to groups of 5 male and 5 female rats for 14 days. In addition, 5 male and 5 female rats in the control group were administered 10 mL/kg of water.		5-week-old Crj:CD(SD)IGS rats (SPF).	Reticulocyte, PLT, PT, APTT, WBC, Diff leuk) • Blood Chemistry (GOT, GPT, γGTP, ALP, bilirubin, urea nitrogen, creatinine, glucose, cholesterol, LDH triglycerides, total protein, albumin, Ca^{++}, inorganic phosphate, Na, K, Cl, A/G ratio) • Urinalysis (pH, protein, glucose, ketone bodies, bilirubin, occult blood, urobilinogen) • Body weight and food consumption • Macroscopic evaluation • Absolute and relative organ weight (brain, liver kidneys, spleen, heart, thymus, thyroid, pituitary gland, adrenal glands and testes, prostate, seminal vesicle, epididymides, ovaries, and uterus)	significant effects on body weight or food consumption were noted. • The platelet count was significantly increased in the 1000 and 2000 mg/kg/day female groups and the WBC count was significantly elevated as well; however, these differences were within background of historical controls. • No changes attributable to UT-CGT were observed from the blood biochemical or urinalysis studies. • No abnormalities were observed during macro- or microscopic evaluation. • No significant changes were observed in the absolute and relative weights of each organ.	

Citation	Design/Duration	Test Substance	Test Animal	Endpoints	Results	Comments
				• Histopathology (brain, pituitary, eyes, thyroid with parathyroid, heart, trachea and lung, liver, kidneys, thymus, spleen, adrenal glands, stomach (fore and glandular), small intestine, large intestine, pancreas, gonads (testes or ovaries), accessory reproductive organs, (epididymides, prostate and uterus), urinary bladder, lymph nodes (cervical and mesenteric), bone and bone marrow (femur)).	• The NOAEL was 2000 mg/kg bw, the highest dose tested.	

2. 28-day Toxicity Study of Catechins from Green Tea (CGT) in Rats (Chengeles et al. 2008)

The potential toxicity of heat-treated CGT (HT-CGT) was evaluated via daily administration to Crl:CD® (SD) rats of both sexes for 28 days. CGT (which, for the purposes of dose calculations, was considered to be 100% pure) was administered by gavage in deionized water once daily for 28 consecutive days to three groups of rats at levels of 500, 1000, and 2000 mg/kg-bw/day, respectively (5 rats per sex/group). As a control for the potential effects of the caffeine component of CGT, an additional group (5 males and 5 females) was administered decaffeinated heat-treated Type-1 CGT (hereafter referred to as "D-HT-CGT") in deionized water, by gavage once daily for 28 consecutive days at a level of 2000 mg/kg-bw/day. A concurrent control group (5 males and 5 females) received the vehicle (deionized water) according to a comparable regimen. The dosage volume was 10 mL/kg for all groups. Following 28 days of dose administration, all animals were euthanized. This study was conducted in compliance with FDA and OECD Good Laboratory Practices, and was designed in accordance with the OECD Guidelines for Testing of Chemicals, Health Effects Test Guidelines, Section 407 (July 1995).

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed weekly. Individual body weights and food consumption were recorded weekly. Functional observational battery and locomotor activity data were recorded for all animals prior to the initiation of dose administration and during study the last week of dosing. Clinical pathology evaluations (*i.e.*, hematology,[26] serum chemistry,[27] and urinalysis[28]) were performed on all animals prior to the primary necropsy (*i.e.*, study week 4). Complete necropsies were

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[26] Total leukocyte count (white cells); erythrocyte count (red cells); hemoglobin; hematocrit; mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); platelet count (platelet); prothrombin time (ProTime); activated partial thromboplastin time (APTT); reticulocyte count (percent (reticulocyte), absolute (retic absolute)); differential leukocyte count (percent and absolute, neutrophil, lymphocyte, monocyte, eosinophil, basophil, large unstained cell).

[27] Albumin; total protein; globulin [by calculation]; albumin/globulin ratio (A/G ratio) [by calculation]; total bilirubin (total bili); urea nitrogen; creatinine; alkaline phosphatase (alkaline phos'tse); alanine aminotransferase (alanine transfer); aspartate aminotransferase (AspartatTransfer); gamma glutamyltransferase (GlutamylTransfer); glucose; total cholesterol (cholesterol); calcium; chloride; phosphorus; potassium; sodium; triglycerides (triglyceride).

[28] Specific gravity (SG); pH; urobilinogen (URO); total volume (TVOL); color (CLOR); appearance (APP); protein (PRO); glucose (GLU); ketones (KET); bilirubin (BIL); occult blood (BLD); leukocytes (LEU); nitrites (NIT); microscopy of sediment [tabular abbreviations appear on individual tables].

conducted on all animals, and selected organs[29] were weighed at the scheduled necropsy. Selected tissues[30] were examined microscopically from all animals.

Although there was one death in this study, it was not considered related to treatment. The liver findings reported for the dead female rat were seen only in this one animal. No similar adverse liver findings were observed in the animals that survived the full study. The finding was not even uniform across the liver, thus indicating that it was a localized and not a systemic event. The isolated and non-uniform nature of the occurrence supports the conclusion that these liver effects were not related to treatment with the test substance.

No test article-related clinical observations were observed during the study. Further, there were no treatment-related differences between the control and test article-treated groups when physiological observations were evaluated during the pretreatment period. During the functional observational battery testing, no home cage, handling, open field, sensory, or neuromuscular observations were noted. There were no test article-related effects on motor activity, hematology, serum chemistry, or urinalysis parameters. Macroscopic findings and relative organ weights were not altered due to administration of the test article.

Statistically significant test article-related effects on body weight consisted of lower body weights and body weight gains throughout the study in the 1000 and 2000 mg/kg-bw/day HT-CGT group males. These findings correlated with lower food consumption in these groups in the first week of dosing. Kao does not believe that that the decreases in food consumption are related to changes seen in the endocrine glands (*i.e.,* absolute thyroid weight, *see* discussion below). A decrease in thyroid weight (with no change in cellular architecture) would not occur precipitously upon initiation of dosing. It would take several days, if not weeks, to develop, and the decrease in food consumption was seen only during the first week of the study. The usual reason for eating less is palatability. Occasionally, animals eat less in the beginning but then apparently become accustomed to the taste and begin to eat more, but rarely catch up to the controls unless the lower intake was a week or less. In fact, when food efficiency is calculated, it is apparent that food efficiency is decreased for the first two weeks in these two groups of treated males compared to the control animals, but that after week 2, food efficiency was comparable across groups (*see* Figure 6 below). It is a well-established principle in safety studies that a lower food consumption usually results in a lower body weight gain; animals eat less and gain less weight. This has been clearly

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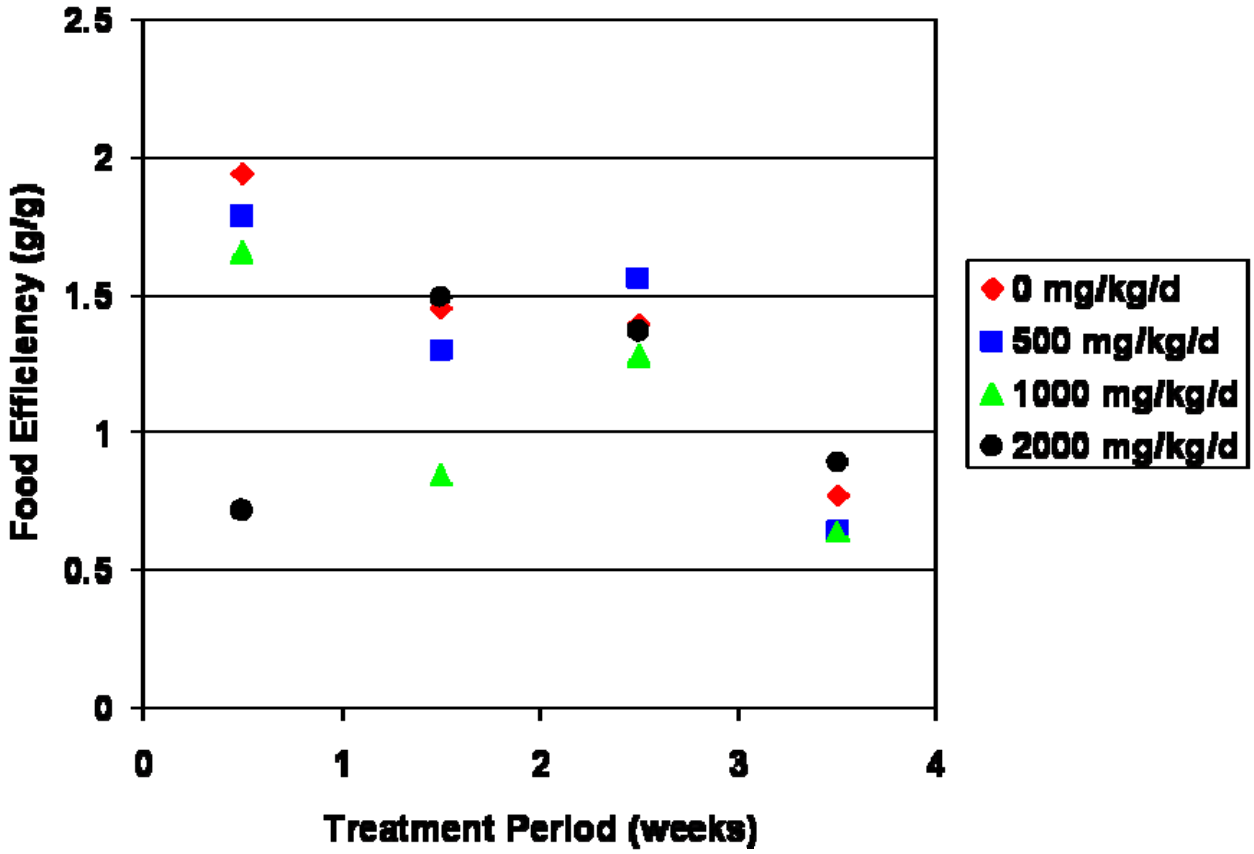
29 Adrenal glands; brain; epididymides; heart; kidneys; liver; ovaries with oviducts; pituitary; spleen; testes; thymus; thyroid with parathyroids; and uterus.

30 Adrenal glands (2); aorta; bone with marrow (femur, sternum); bone marrow smear; brain (cerebrum level 1, cerebrum level 2, cerebellum with medulla/pons); epididymides; gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectrum); heart; kidneys (2); liver (sections of 2 lobes); lungs (including bronchi, fixed by inflation with fixative); lymph nodes (mandibular, mesenteric); mammary gland (females only); ovaries with oviducts (2); pancreas; peripheral nerve (sciatic); pituitary; prostate; seminal vesicles (2); spinal cord (cervical, midthoracic, lumbar); spleen; testes (2)b; thymus; thyroid [with parathyroids, if present (2)]c; trachea; urinary bladder; uterus with cervix; vagina; gross lesions (when possible). (a – Bone marrow smears were obtained at necropsy but not placed in formalin; slides were examined only if scientifically warranted. b – Fixed in Bouin's solution. b – Parathyroids were examined only when in the plane of section.)

enunciated by the World Health Organization's Joint Expert Committee on Food Additives (WHO JECFA 1987). Further, this effect was seen in males only and only at the two highest doses.

Figure 6. Food Efficiency in Male Rats



Body weights and food consumption were not affected in the 2000 mg/kg-bw/day D-HT-CGT groups. Minimal erosions of the glandular stomach were observed for one male and one female each in the 2000 mg/kg-bw/day HT-CGT group. It is believed that these glandular erosions, which were extremely minimal and only occurred in two animals, are the result of localized irritation, possibly from the gastric tube used to deliver the doses of CGT. These erosions were not ulcers, and there was no evidence of blood loss.

Statistically significant decreases in absolute organ weight were observed, but these decreases were not dose-related, and likely can be attributed to the decreases in body weight observed. When relative organ to body weight ratios, which are a more reliable index than absolute organ weight, were calculated (not reported in the published article but available in the final study report), no statistically significant differences were observed. Further, there were no histopathological correlates to suggest any adverse effects in these organs.

Changes observed in absolute thymus weight of the female rats were not dose-dependent, only seen in one sex, and there were no histopathological correlates to suggest any adverse effects in the thymus. Thymus weights are extremely variable and, with the group size used in this study, statistically significant changes are not uncommon. The testing laboratory's historical range for Charles River Sprague Dawley rats of this age and sex is 0.163 to 0.735 grams, with a mean of 0.449 grams.[31] The high-dose findings in this study are, in fact, closer to the historical control mean than is the control group. Thus, a relative (to control) difference of this magnitude that is not accompanied by histopathological changes and/or changes in lymphocyte count is not adverse, and is unlikely to be related to test article treatment. Further, relative organ to body weight ratios exhibited no statistically significant or dose-related differences.

With regard to clinical chemistry findings, an increase in thromboplastin time was observed that was not dose-dependent (17.3, 19.8, 18.8, and 19.9, for the 0, 500, 1000, and 2000 females, respectively) and only was observed in the females. For these reasons, this observation was not considered adverse or related to treatment. Similarly, an increase in glutamyl transferase activity observed in female rats was not considered treatment-related because there were no histopathological correlates in the liver. (A full discussion of the potential hepatotoxicity of CGT is provided in Section VI.B.8. and Section VI.F.)

A no-observed-adverse-effect level (NOAEL) of 1000 mg/kg-bw/day based on the lack of *local toxicity* (*e.g.*, gastric erosions seen in two CGT animals) was derived from this study. The NOAEL for *systemic toxicity* was 2000 mg/kg-bw/day, the highest dose tested. Observations for the 2000 mg/kg/day D-HT-CGT-treated animals were similar to the 2000 mg/kg-bw/day HT-CGT-treated animals with the following exceptions: body weights and body weight gains were similar to the control animals and there were no microscopic findings in any of the tissues examined. It is important to note that the findings summarized above were not observed in the 6-month toxicity study of Kao's CGT, thus corroborating the conclusion that these effects were not treatment-related (see Section IV.B.3 below).

In a second study, the potential oral toxicity of the Type-2 CGT (for sports drink; using UT-CGT) tea catechin preparation was studied in Crl:CD® (SD) rats of both sexes (5 animals/sex/group) for 28 days (Chengeles et al. 2008). The treatment regimen was identical to that described above, *i.e.*, doses of 500, 100, and 2000 mg/ kg bw/day were administered in 10 mL/kg of deionized water once daily by gavage. A control group received 10 mL/kg deionized water. The same in-life and post-sacrifice observations and evaluations were conducted as in the study described above.

No test article-related deaths or clinical observations were observed during the study. One high-dose female was found dead on study day 23. Clinical observations in this animal prior to death consisted of intermittent convulsions, gasping, hypoactivity, hypothermia, labored respiration, and prostrate. Microscopic examination of the liver in this animal revealed focal extended necrotic areas. The cause of death for this animal was not determined, but was not considered to be treatment-related because similar microscopic changes were not observed in

[31] Personal communication with Dr. C. Chengelis from WIL Laboratories dated April 10, 2008.

any other animals that received the test article. In addition, there were no treatment-related differences between the control and test article-treated groups when physiological observations were evaluated during the pretreatment period. During the functional observational battery testing, no home cage, handling, open field, sensory or neuromuscular observations were noted. There were no test article-related effects on motor activity, hematology or urinalysis parameters. Macroscopic findings and organ weights were not altered due to administration of the test article. Lower mean total protein and higher mean chloride were noted in the 2000 mg/kg bw group males. Due to the minimal magnitude of the differences from the control group, these serum chemistry changes were not considered to be toxicologically relevant.

Due to the minimal magnitude of the serum chemistry changes in the 2000 mg/kg bw/day group males, and lack of treatment-related toxicity in all groups, the NOAEL for UT-CGT is 2000 mg/kg bw/day, the highest dose tested.

3. 6-Month Toxicity Study of Catechins from Green Tea in Rats

The potential toxicity of HT-CGT was evaluated via daily administration to Crl:CD® (SD) rats of both sexes for 182 days (Kirkpatrick et al. 2007). HT-CGT was administered by gavage in deionized water once daily for 182 consecutive days to 3 groups (Groups 2-4) of Crl:CD® (SD) rats. D-HT-CGT was administered by gavage in deionized water once daily for 182 consecutive days to 1 group (Groups 5) of Crl:CD® (SD) rats. Dosage levels were 120, 400, 1200, and 1200 mg/kg-bw/day for Groups 2-5, respectively. A concurrent control group (Group 1) received the vehicle on a comparable dosing regimen. The dosage volume was 10 mL/kg for all groups. Each group consisted of 10 animals/sex. Following 26 weeks of dose administration, all animals were euthanized. This study was conducted in compliance with FDA and OECD Good Laboratory Practices, and was designed in accordance with the OECD Guidelines for Testing of Chemicals, Health Effects Test Guidelines, Section 407 (July 1995).

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed weekly. Individual body weights and food consumption were recorded weekly. Functional observational battery and locomotor activity data were recorded for all animals prior to the initiation of dose administration and during study the last week of dosing. Clinical pathology evaluations (*i.e.*, hematology,[32] serum chemistry,[33] and urinalysis[34]) were performed on

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[32] Total leukocyte count (white cells); erythrocyte count (red cells); hemoglobin; hematocrit; mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); platelet count (platelet); prothrombin time (ProTime); activated partial thromboplastin time (APTT); reticulocyte count (percent (reticulocyte), absolute (retic absolute)); differential leukocyte count (percent and absolute, neutrophil, lymphocyte, monocyte, eosinophil, basophil, large unstained cell), platelet estimate, red cell morphology.

[33] Albumin; total protein; globulin [by calculation]; albumin/globulin ratio (A/G ratio) [by calculation]; total bilirubin (total bili); urea nitrogen; creatinine; alkaline phosphatase (alkaline phos'tse); alanine aminotransferase (alanine transfer); aspartate aminotransferase (AspartatTransfer); gamma glutamyltransferase (GlutamylTransfer); glucose; total cholesterol (cholesterol); calcium; chloride; phosphorus; potassium; sodium; triglycerides (triglyceride).

5 animals/sex/group at study week 12 and on all animals prior to the primary necropsy (*i.e.*, study week 26). Complete necropsies were conducted on all animals, and selected organs[35] were weighed at the scheduled necropsy. Selected tissues[36] were examined microscopically from all animals.

There were no test article-related effects on food consumption or clinical pathology parameters. There were no ophthalmic or macroscopic pathology findings related to treatment. Various group mean differences in hematology, serum chemistry, and urinalysis parameters were observed, but were not considered to be test article-related because the values did not show a dose- or time-related response, were due to obvious outliers or spontaneous lesions in individual animals, or the parameter was altered in an unexpected and unlikely direction. There were no test article-related deaths, or gross or microscopic pathology findings. While brown pigment was noted in the splenic red pulp of all females examined microscopically, this is considered a common, spontaneous finding in the spleen of aging rats. Other histopathologic changes were considered to be incidental findings, manifestations of spontaneous diseases, or related to some aspect of experimental manipulation other than administration of the test article. There were no test article-related alterations in the incidence, severity, or histologic character of those incidental and spontaneous tissue alterations.

Lower body weight gains and lower overall body weights that were not accompanied by decreases in food consumption were noted in the 1200 mg/kg-bw/day HT-CGT and 1200 mg/kg-bw/day D-HT-CGT group females. Several organ weight changes were statistically significant when compared to the control group at the scheduled necropsy, but were considered to be a result of the test article-related effect on final body weight. No microscopic correlates were observed for the higher kidney weight ratios noted, and they were attributed to minor physiologic adaptations in conjunction with lower total body weight. Physiological adaptation may have included minor compensatory hypertrophy that was not appreciated at the light microscopic level. A clinical observation possibly related to treatment with the test article of intermittent increased activity was noted in both sexes of the 400 and 1200 mg/kg/day HT-CGT groups. The NOAEL for this study is 400 mg/kg-bw/day, based on reduced weight gain in females at 1200 mg/kg bw/day.

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34 Specific gravity (SG); pH; urobilinogen (URO); total volume (TVOL); color (CLOR); clarity (CLA); protein (PRO); glucose (GLU); ketones (KET); bilirubin (BIL); occult blood (BLD); leukocytes (LEU); nitrites (NIT); microscopy of sediment [tabular abbreviations appear on individual tables].

35 Adrenal glands; brain; epididymides; heart; kidneys; liver; ovaries with oviducts; pituitary; spleen; testes; thymus; thyroid with parathyroids; and uterus.

36 Adrenal glands (2); aorta; bone with marrow (femur, sternum); bone marrow smear; brain (cerebrum level 1, cerebrum level 2, cerebellum with medulla/pons); epididymides; eyes with optic nerve, gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectrum); heart; kidneys (2); liver (sections of 2 lobes); lungs (including bronchi, fixed by inflation with fixative); lymph nodes (mandibular, mesenteric); mammary gland (females only); ovaries with oviducts (2); pancreas; peripheral nerve (sciatic); pituitary; prostate; seminal vesicles (2); spinal cord (cervical, midthoracic, lumbar); spleen; testes (2); thymus; thyroid [with parathyroids, if present (2)]; trachea; urinary bladder; uterus with cervix; vagina; gross lesions (when possible).

4. Developmental Toxicity Study of Catechins from Green Tea in Rats

The effects of HT-CGT on maternal toxicity and embryo/fetal development were studied in rats after maternal exposure during the period of major organogenesis (Knapp et al. 2008). HT-CGT was administered by gavage to 3 groups of 25 bred Crl:CD(SD) rats once daily from gestation days 6 through 17 at dosage levels of 200, 600, and 2000 mg/kg-bw/day in a volume of 10 mL/kg. A concurrent control group consisting of 25 females received the vehicle (deionized water) on a comparable dosing regimen. All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights, and food consumption were recorded at appropriate intervals. On gestation day 20, a laparohysterectomy was performed on each female. The uteri, placentae, and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed, and examined for external, visceral, and skeletal malformations and developmental variations. This study was conducted in compliance with FDA and OECD Good Laboratory Practices, and was designed in accordance with the International Conference on Harmonisation (ICH) Tripartite Guideline on Detection of Toxicity to Reproduction for Medicinal Products, Section 4.1.3 (September, 22, 2004).

All females in the control, 200, 600 and 2,000 mg/kg/day groups survived to the scheduled necropsy on gestation day 20 and there were no test article-related macroscopic findings. There were no test article-related fetal malformations or developmental variations in this study.

Increased incidences of hair loss on various body surfaces were noted in the 2000 mg/kg/day group during the treatment period. There were no test article-related clinical findings observed in the 200 and 600 mg/kg/day groups. Lower maternal mean body weight gains were observed in the 600 and 2000 mg/kg/day groups during gestation days 6-9 and 6-18; mean body weight gain in the 2000 mg/kg/day group was also lower during gestation days 9-12 and 12-18 compared to the control group. Mean food consumption was lower than the control group during gestation days 6-9 in the 600 and 2000 mg/kg/day groups and during gestation days 9-12 in the 2000 mg/kg/day group. The decreased food consumption noted in the 2000 mg/kg/day group was of sufficient magnitude to affect the entire treatment period (gestation days 6-18). In addition, the decrements in mean body weight gains and food consumption resulted in mean body weights in the 600 and 2000 mg/kg/day groups that were up to 5.1% and 7.7% lower, respectively, than the control group values during gestation days 9-20. Lower mean net body weight gains were also observed in these groups, and lower mean net body weight was noted in the 2000 mg/kg/day group. Mean gravid uterine weights in the 200, 600 and 2000 mg/kg/day groups were unaffected by test article administration. Intrauterine growth and survival in the 200, 600 and 2000 mg/kg/day group were not affected by test article administration.

The NOAEL for maternal toxicity was 200 mg/kg-bw/day based on the magnitude of the body weight changes, even though there was a corresponding decrease in food consumption. The NOAEL for embryo-fetal development was 2,000 mg/kg/day, the highest dose tested.

5. Genotoxicity Studies of Catechins from Green Tea

The following genotoxicity tests were conducted on Kao's HT-CGT and/or UT-CGT: bacterial reverse mutation assay (Ames test), chromosomal aberration assay in cultured Chinese hamster lung cells (CHL/IU), mouse lymphoma L5178Y/tk assay (MLA), and a bone marrow micronucleus assay in ICR CD mice (Ogura et al. 2008). No significant increases in the number of revertant colonies were found in the Ames test, but positive responses were observed in the two other *in vitro* tests (chromosomal aberration assay and mouse lymphoma L5178/tk assay). The *in vivo* study (bone micronucleus test), however, demonstrated no significant increase in micronucleated erythrocytes in mouse bone marrow at doses up to 2,000 mg/kg. Despite positive findings in the *in vitro* studies, the *in vivo* study does not support a genotoxic effect of CGT.

The results of these studies are summarized in Table 8. These tests were conducted in accordance with Good Laboratory Practice regulations and OECD Guidelines.

Table 8. Summary of Genotoxicity Studies Conducted with Kao's CGT (Ogura et al. 2008)

Test	CGT/Levels Tested/Design	Results
Bacterial reverse mutation assay (Ames test)	• Heat-treated CGT (HT-CGT): 156-500 µg/plate (in dimethylsulfoxide (DMSO)) • Unheat-treated CGT (UT-CGT): 78.0-5000 µg/plate (in physiological saline) • Tests performed in the presence and absence of metabolic activation (S9 mix) • Controls: Negative (vehicle): (DMSO) Positive: 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2); 2-aminoanthracene (2AA), and sodium azide (NaN_3) • Strains tested: *Salmonella typhimurium* TA98, TA100, TA1533, and TA1537 *Escherichia coli* WP2*uvrA*	No bacterial toxicity was observed. No more than a two-fold increase in the mean number of revertants per plate compared to the negative control value was observed. The positive controls performed as expected.
Chromosomal aberration assay in cultured Chinese hamster lung cells (CHL/IU)	• HT-CGT in physiological saline: 56.3-900 µg/mL • UT-CGT in physiological saline: 35.1-700 µg/mL • Short-term treatment: 6-hour incubation with or without metabolic activation (S9 mix), followed by a washing and incubation in fresh medium for an additional 18 hours • Continuous treatment: 24- or 48 hour-exposure • Controls: Negative: Physiological saline Positive: mitomycin (MMC) (for treatment without metabolic activation) and benzo(a)pyrene (B(a)P) (for treatment with metabolic activation)	• Cytotoxicity: dose-related increases in cytotoxicity were observed for every treatment • Numerical aberrations (polyploidy, endo-reduplication): No increases in the number of cells with numerical aberrations was observed at any dose for either treatment • Structural aberrations (chromatid break, chromatid exchange, chromosome break, chromosome exchange): HT-CGT: An increase of >10% was observed in the absence of metabolic activation in both the short-term and continuous treatments. UT-CGT: An increase of >10% was observed in the presence and absence of metabolic activation in the

Test	CGT/Levels Tested/Design	Results
		short-term treatment. • The positive controls performed as expected. • The results of this study were considered positive for chromosomal aberrations.
Mouse Lymphoma L5178Y/tk assay	• HT-CGT in physiological saline: 52.7-3600 µg/mL • UT-CGT in physiological saline: 21.2-4000 µg/mL • Controls: Negative: physiological saline Positive: MMC and cyclophosphamide monohydrate (CP) • Cell suspensions, with or without metabolic activation (S9 mix), were incubated for either 3 hours (short-term treatment) or 24 hours (continuous treatment). After treatment, the cells were washed and resuspended. An aliquot of each cell suspension was diluted and transferred to well plates and incubated for 10 or 11 days, and then the number of wells containing colonies was counted microscopically for evaluation of cytotoxicity (plating efficiency) and relative survival at Day 0. • Remaining cell suspension was cultured for 2 days to allow for expression of mutant phenotype. After the expression period, the cell suspension in each culture was diluted, plated, and incubated to evaluate plating efficiency and relative survival at Day 2. • Trifluorothymidine was added to the remaining cell suspension at a concentration of 3 µg/mL. The cell suspension was plated and incubated for 11 or 12 days, after which time the number of wells containing colonies was counted macroscopically for calculation of mutant frequency.	• HT-CGT: A dose-related in mutant frequency was observed after short-term treatment in both the presence and absence of metabolic activation. A pronounced drop in cell growth was observed a 400 µg/mL (highest dose tested) in the 24-hour continuous treatment. • UT-CGT: A dose-related increase in mutant frequency was observed in the short-term treatment both with and without metabolic activation, and in the 24-hour continuous treatment. • The results of this study were considered positive for gene mutation.

Test	CGT/Levels Tested/Design	Results
Bone marrow micronucleus assay in mice	• HT-CGT and UT-CGT in physiological saline: 500, 1000, and 2000 mg/kg bw • Administered to 9-week-old male ICR (Crj: CD-1) mice in two gavage doses separated by 24 hours. • Bone marrow smears were prepared from each treatment group 24 hours after the last administration and examined using optical microscopy to detect polychromatic erythrocytes (PCE), normochromatic erythrocytes (NCE), micronucleated polychromatic erythrocytes (MNPCE), and the PCE/NCE ratio. • Controls: Negative: Physiological saline Positive: MMC	• No deaths occurred as a result of test article administration. • There was no significant increase in the incidence of MNPCEs in any test group, as compared to the vehicle controls. • There was no significant difference in PCE/NCE ratio in any test group as compared to the controls. • The results of this study were considered negative for suppression of erythrocyte growth. • The possibility that HT-CGT and UT-CGT were not available following oral administration was considered and rejected based on published and internal pharmacokinetic data demonstrating absorption following oral administration. • The positive controls performed as expected.

6. Toxicology Studies on Substances Substantially Equivalent or Similar in Composition to Catechins from Green Tea

The 90-day toxicity study by Takami et al. (2008), on a substance that is substantially equivalent to Kao's CGT, serves as the pivotal study supporting the GRAS determination for Kao's CGT. In this study, groups of 10 F344 rats per sex each were administered "Sunphenon 100S," a green tea catechin extract manufactured by Taiyo Kagaku Co., Ltd. (*see* Table 9), in the diet at concentrations of 0 (control), 0.3%, 1.25%, and 5.0% for 90 days. The following parameters were evaluated: clinical signs and mortality (at least once daily); individual body weights and food consumption per cage (once a week); hematological endpoints;[37] clinical chemistry endpoints;[38] macroscopic examination; organ weights;[39] and microscopic examination.[40]

No test article-related clinical observations or deaths were found during the study. The high-dose males exhibited dirty tails from week 5 throughout the rest of the study, but this was attributed to the coloration of the feed from the test article. There was a significant decrease in body weight gains in the 5.0% group males from week 1 through the end of the study, but only at week 1 in the 5.0% females, as compared to the control animals. Palatability problems likely explain the transient effect on body weight gain observed in the females. There was no treatment-related effect on food consumption in the male rats in this study, and the female rats exhibited a decrease at week 1 only.

With regard to hematological parameters, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were significantly increased in both sexes that received 5.0% Sunphenon 100S as compared to controls, and the white blood cell count (WBC) was significantly decreased in the 0.3% females. The MCV and MCH findings were not associated with any clinically meaningful change in other erythrocytic parameters, and the effect on WBC seen in the 0.3% females was not considered treatment-related because it was not seen in any other group of treated animals and was therefore, not dose-related.

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37 Red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (Plt) and white blood cell count (WBC)

38 Total protein (TP), albumin (Alb), albumin/globulin ratio (A/G), total bilirubin (T-Bil), total cholesterol (T-Cho), triglyceride (TG), c-glutamyl transpeptidase (c-GTP), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (Cre), calcium (Ca), inorganic phosphorus (IP), sodium (Na), potassium (K) and chloride (Cl)

39 Brain, thymus, lungs, heart, spleen, liver, adrenals, kidneys, and testes

40 Brain, thymus, lungs, heart, spleen, liver, adrenals, kidneys, testes, nasal cavity, trachea, aorta, pituitary, thyroids, parathyroids, salivary glands, tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, urinary bladder, epididymides, prostate, seminal vesicles, bulbourethral glands, ovaries, uterus, vagina, mammary glands, skin, cervical and mesenteric lymph nodes, sternum and femur including bone marrow, trigeminal nerve, sciatic nerve, spinal cord (cervical, thoracic and lumber cord), eyes, Harderian glands, thigh muscle, and gross abnormalities

Statistically significant changes in clinical chemistry parameters included:

- Decreased total cholesterol and creatinine in the 0.3-5.0% group males
- Decreased triglycerides and creatinine in the 5.0% group females
- Decreased triglycerides in the 1.25% and 5.0% group males
- Increased albumin/globulin ratio, alanine transaminase (ALT), and alkaline phosphatase (ALP) in the 5.0% group males
- Increased albumin, albumin/globulin ratio AST, ALT, and ALP in the 5.0% group females
- Increased inorganic phosphorus in the 1.25% and 5.0% group males
- Increased inorganic phosphorus in the 0.3-5.0% group females
- Increased potassium in the 0.3% and 5.0% group females
- Increased chloride in the 1.25% group females

The increase in liver enzymes observed in the high-dose males and females is suggestive of weak hepatotoxicity. Relative liver weights in these groups also were increased (*see* below), but there were no clinically significant histopathological lesions of the liver observed in these animals. The decreases in total cholesterol and triglycerides potentially could be considered beneficial, but are not adverse. The increased albumin and albumin/globulin ratio observed in some groups are not considered to be toxicologically meaningful because of a lack of change in total protein and significant microscopic liver pathology. The creatinine changes were not accompanied by histopathological changes in the kidneys or thigh muscle, and the increased inorganic phosphate was not accompanied by changes in calcium levels or evidence of bone pathology; thus, they are not considered to be treatment-related. None of the other clinical chemistry changes listed above were considered to be related to CGT treatment because they were not dose-related.

Many of the absolute organ weight changes observed in this study were not evident when absolute organ weights were calculated, with the following exceptions:

- Decreased relative thyroid weights in the males (1.25% and 5.0% groups) and increased relative thyroid weights in females (5.0% group)
- Decreased relative adrenal weights (0.3% groups)
- Increased relative liver and kidney weights (5.0% groups)

All of these organ weight changes occurred in the absence of any treatment-related macroscopic or microscopic findings. A recent study by Renno and colleagues (2008), in which rats with streptozoticin-induced diabetic nephropathy were treated with green tea

extract in the drinking water for up to 12 weeks at a concentration of 16% provides evidence that green tea catechins are not nephrotoxic. In fact, in this study, green tea extract was found to protect the kidney against the effects of diabetic nephropathy, and improved kidney function (*e.g.*, lower BUN, urinary urea nitrogen, creatine, glucose, and protein excretion, and lower glycogen accumulation in the renal tubules) observed in the treated diabetic rats as compared to the control animals.

Based on the reduced body weight gains in the 5.0% males, the increase in ALT and ALP in the 5.0% males and females and AST in the 5.0% females, and the increase in relative liver weight in the 5.0% males and females, a NOAEL of 1.25 % (763.9 mg/kg-bw/day for males and 820.1 mg/kg-bw/day for females) was established. It is important to note that the potential weak hepatotoxicity was observed at a dietary concentration that far exceeds any likely exposure scenario for Kao's CGT (5% in the diet, which according to the authors, corresponds to a daily CGT intake of 3524.6 and 3541.6 mg/kg-bw/day for males and females, respectively).

The medical literature contains other toxicity studies performed on substances similar in composition to CGT (*e.g.*, Polyphenon E, Polyphenon 70S, Polyphenon 30, Sunphenon, and AR25). The relative composition of these related substances are presented below in Table 9. While there are some differences in the content of individual monomers between these substances and Kao's CGT, as can be seen in Table 9, the catechin levels in Type-1 CGT and Type-2 CGT are not unique, and are comparable to, and fall within the range of, the catechin content of other CGT preparations. A notable exception is AR25, which is the green tea extract contained in the majority of dietary supplements associated with hepatotoxicity (*see* Sections VI.B.7, VI.C, and VI.F). AR25 is of lower purity, and has a higher EGCG content than Kao's CGT.

The other studies summarized in Table 10 and below are useful in that they corroborate Kao's findings discussed above.

Table 9. Catechin and Polyphenol Composition of Related Tea Catechin Products

	CGT Type 1	CGT Type 2	Sunphenon 100S	Taiyo Kagaku Co., Ltd CGT	Polyphenon 70S	Polyphenon E	Polyphenon 30	Polyphenon 60	AR25 *
Total Catechin Monomer (%) (purity)	44.4	62.3	68.5	83.1	78.7	87.3	34.2	66.2	24.7
Polyphenols (%)	52.9	67.4	80.8	90.1	86.4	96.7	No data	No data	No data
Caffeine (%)	2.6	1.7	0.4	0.27	0.0	0.0	6.9	No data	5.1
Gallocatechin (GC)	2.4	5.9	5.3	3.7	6.9	0	2.1	No data	1.2
Epigallocatechin (EGC)	13.2	20.5	12.7	13.1	20.1	4.2	9.0	7.1	4.5
Catechin (C)	0.4	0.6	1.8	1.5	2.7	0.9	0	No data	0.4
Epicatechin (EC)	3.0	6.2	7.3	6.2	8.5	8.9	2.3	5.6	1.5
Epigallocatechin gallate (EGCG)	19.4	19.8	29.4	42.5	42.9	65.3	17.0	21.3	11.8
Gallocatechin gallate (GCG)	0.5	2.5	2.1	5.1	4.7	3.5	0.7	2.2	0.9
Epicatechin gallate (ECG)	4.6	5.0	9.9	10.4	12.7	4.3	2.7	11.7	3.8

*Note that the absolute percentages of the individual catechin monomers in AR25 are lower than the other tea catechin products because the purity (total monomer content) of AR25 is markedly lower than the other tea catechin products. The relative percentage of EGCG (*i.e.* percent of total catechin monomers) in AR25 is 47.6%.

Table 10. Toxicology Studies on Substances Similar in Composition to Catechins from Green Tea (CGT)

Reference	Results of Toxicity study
Matsumoto et al. 1999 (Polyphenon 70S)	Green tea extract (Polyphenon 70S) was administered orally to rats for 6 months at levels of 500-2000 mg/kg/day. In rats administered 2000 mg/kg/day, the weight of liver increased, and plasma alkaline phosphatase and GPT levels rose concomitant with a slight degeneration of hepatocytes observed upon histopathological examination. The NOAEL for green tea extract (Polyphenon 70S) was 1000 mg/kg/day**.**
Faqui et al. 2001 [abstract] (Polyphenon E)	Pregnant Sprague Dawley rats were administered Polyphenon E green tea extract orally by gavage on gestation on gestation days 6-15 at doses of 0, 125, 250, 500, and 1000 mg/kg bw. No signs of maternal toxicity were observed in any treatment group, and there was no difference in maternal body and organ weights as compared to the vehicle control group. There was no evidence of developmental toxicity: resorption rates, mean litter size, sex ratio, fetal body weights, and fetal morphology were comparable across all groups. The NOAEL was determined to be 1000 mg/kg bw for developmental toxicity (the highest dose tested).
Chang et al. 2003 (Polyphenon E)	The following assays were conducted: bacterial mutagenesis, mouse lymphoma cell thymidine kinase (TK), gene mutation, the Big Blue CII transgenic mutation (both acute and repeated dose), and the mouse micronucleus assay. • No significant colony revertants were observed in the bacterial assay • A significant increase was observed in the TK assay • Genotoxicity was observed at levels of 2,000 mg/kg-bw/day in mice. No significant increases in micronucleated erythrocytes in the bone marrow of Swiss-Webster mice and no significant increases in cII transgene MF in the liver, lung, or spleen were observed at lower doses as compared with controls.
Matsumoto et al. 1999 (Polyphenon 30)	A single-dose oral of green tea extract was administered to mice at levels of 3000-6221 mg/kg (in physiological saline), and the animals were observed for 14 days**.** As a result of the deaths observed at doses of 4320 mg/kg and higher, the oral LD_{50} was estimated to be 4647.29 mg/kg. Irritation of the oral mucosa also was assessed in hamster cheek pouches. Three groups of 9-10 animals each were exposed to a negative control (physiological saline), a positive control (sodium lauryl sulfate), and 50% Polyphenon 30 in water for 30 minutes. Exposure to Polyphenon 30 resulted in no irritation immediately after or 24 hours after treatment.

Another study on a green tea catechin extract (manufactured by Taiyo Kagaku Co., Ltd., *see* Table 9) containing substantially equivalent levels of catechin monomers to Kao's CGT reported that the combined administration of CGT (1%) and sodium nitrite (0.2%) in the diet selectively increased the incidence and multiplicity of neoplastic lesions in the forestomach of F344 rats after initiation with *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG), as well as significant increases in 8-hydroxyguanosine levels in DNA suggesting oxidative damage (Kuroiwa et al. 2007). Administration of this CGT substance alone resulted in suppression of

the development of forestomach papillomas. While the authors concluded that these data suggest that excessive intake of green tea and sodium nitrite might represent a potential human risk, Kao concludes that the results of this study are not relevant to the potential exposures anticipated with its CGT-containing beverages. First, the human does not have a forestomach (which is only found in rodents), and the relevance of these findings to humans is questionable, especially since the authors report that no effects were seen in the glandular stomach, which is similar to the human stomach (*see* Hirose and Ito 1999). Second, the doses of CGT used in this study are far in excess of that expected for Kao's CGT-containing beverages. The animals in this study received 432-580 mg/kg-bw/day of CGT, whereas the highest (*i.e.*, 90th percentile) estimated daily intake of all possible beverages is 21.5 mg/kg-bw/day (*see* Table 4).

F344 rats fed Polyphenon 60 in the diet at concentrations of 1.25, 2.5, and 5.0% for 13 weeks exhibited dose-dependent, statistically significant increases in thyroid weight, in addition to goiter and histopathological evidence of thyroid effects (marked hypertrophy and/or hyperplasia of the follicles, formation of a fibrous capsule at 5.0%) (Sakamoto et al. 2001). Kao concludes that the results of this study are not relevant to the potential exposures anticipated with its CGT-containing beverages. First, the authors note that extrapolation of these results in rats to humans should be made with caution, as rodents have been demonstrated to be highly susceptible to goitrogenic agents in comparison to humans, because rats lack the high-affinity thyroxine-binding globulin that humans have, and the plasma half-life of T4 in rats is much shorter than in humans. Second, the doses of CGT used in this study are far in excess of that expected for Kao's CGT-containing beverages. The animals in this study received 584 mg/kg-bw/day (1.25% group) to 2334 mg/kg-bw/day of CGT (5% group), whereas the highest (*i.e.*, 90th percentile) estimated daily intake of all possible beverages is 21.5 mg/kg-bw/day (*see* Table 4). Third, no increase in thyroid weight or microscopic evidence of hypothyroidism was observed in Kao's 6-month study described in Section VI.B.3. In fact, the study authors note that the absolute thyroid/parathryoid weight was decreased in the 1200 mg/kg-bw/day female rats that were administered decaffeinated CGT. The authors of the study where goiters were observed even concluded that "although we observed goitrogenic effects of catechins by dietary administration at high doses in rats, it would be unlikely that drinking a usual amount of green tea causes hypothyroidism in endocrinologically normal humans."

7. Toxicology Studies on the Main Catechin Isomer in Catechins from Green Tea

While, as noted above, the literature search for this GRAS Notice focused on the mixture of catechins from green tea, and not individual catechin monomers, the database on the most abundant catechin in Kao's CGT (epigallocatechin gallate, EGCG) supports the safety of CGT. While not intended to be inclusive or exhaustive, Kao reviewed a number of recent articles on the safety of EGCG. For example, Isbrucker et al. (2006a, 2006b, and 2006c) conducted a series of safety studies on EGCG, including genotoxicity studies, dermal, acute, and short-term toxicity studies, and teratogenicity and reproductive toxicity studies. They reported that: (1) EGCG was not genotoxic; (2) the acute and subchronic oral toxicity of EGCG was similar to that observed for CGT; and (3) EGCG was not toxic to dams or fetuses and did not affect reproduction, fertility, or fetal development.

In the subchronic toxicity study conducted by Isbrucker and colleagues (2006b), Teavigo (80% EGCG) was administered orally in capsules to fasted beagle dogs for 13 weeks, respectively. Vomiting and diarrhea were observed occasionally throughout the study in dogs administered 150 or 500 mg/kg/day in bolus doses. There were six high-dose animals and four intermediate-dose animals that died or were sacrificed in a moribund condition during the study. Marked body weight loss was observed in all of these animals. Proximal tubular necrosis was observed in one of the dead high-dose males, and one of the dead high-dose females was found to have hemolytic anemia, liver necrosis, moderate erosion in the stomach, and basophilic degeneration of some kidney tubules. Similar liver, stomach, and kidney lesions were observed in a dead intermediate-dose female. Elevated AST and ALT levels were observed in some of the animals, but the values were within the range of historical controls. The authors did not observe any of this toxicity when EGCG was administered to fed dogs at daily doses of up to 500 mg/kg that were divided into twice daily administrations. While hepatic toxicity was observed in the fasted animals administered bolus doses, those animals also were the ones with emesis and general poor condition, and the majority of high-dose fasted animals and the fed animals exhibited no evidence of hepatotoxicity. The authors concluded that the toxicity observed in the fasted animals administered a single bolus dose may have been non-specific to EGCG and was an unrealistic comparison to the human condition, where oral bioavailability is low, because of the very high blood levels achieved in the fasting animals. Additionally, the pharmacokinetics of bolus doses, such as those administered to the fasting dogs, are not equivalent to a more continuous and divided dosing pattern that is expected during the consumption of beverages containing CGT. The purity of the EGCG used in the fasted study was 80%, whereas in the fed study, the purity was 91.8%. Therefore, it cannot be determined whether impurities present in the test substance administered in bolus doses to the fasted dogs contributed to the toxicity observed in some of these animals. Finally, the standard deviations observed in many of the parameters measured are larger than the experimental mean, precluding any meaningful interpretation of these findings.

In the teratogenicity and reproductive toxicity studies conducted by Isbrucker et al. (2006c), pregnant rats were fed diets containing 1,400, 4,200, or 14,000 ppm EGCG (Teavigo) in the diet or 1200, 3600, or 12,000 ppm in the diet for the 2-generation reproductive toxicity study. No teratogenic effects were observed. Delayed sexual maturation was noted in the 12,000 F_1 offspring in the 2-generation reproductive toxicity study, but the authors concluded that this effect likely was due to the reduced weight gain observed in these animals. Subsequent reproductive performance of these offspring was not affected by this delay. A statistically significant increase in pup loss was observed between days 4 and 21 postpartum among the 12,000 ppm groups of both the F_0 and F_1 generations.

Wang et al. (2007) reported that, when developing rat embryos are exposed to individual green tea catechins during early organogenesis (gestation day 9.5 through 11.5) *in vitro*, EGCG was found to be "mildly embryotoxic," whereas the other catechin isomers (DBC, GCG, GC, ECG, EC, CG, and C) were not embryotoxic. The embryotoxic effects observed included caudal retardation and abnormal axial flexion and delayed hind-foot formation. The mean IC_{50} for embryo malformations was 45.80 mg/L for EGCG. Kao believes that this study is not relevant to the potential exposures anticipated with its CGT-containing beverages. First, this is an *in vitro* study where embryos were exposed directly to the tea

catechins. As discussed in Section VI.A, the oral bioavailability of CGT is low; therefore, humans consuming EGCG at levels shown to induce embryo malformations *in vitro* likely would not absorb anything near those levels. Second, also as discussed in Section VI.A, while CGT crosses the placenta, maternal plasma concentrations of CGT were about 10 times higher than in the placenta and 50-100 times higher than in the rat fetus (Chu, K.O., et al., 2006), further reducing the likelihood that embryotoxic levels would reach the developing embryo *in utero*. Finally, in the developmental toxicity study conducted with Kao's CGT (*see* Section VI.B.4), malformations were observed in only 3 fetuses in the control group, 1 fetus in the 200 mg/kg-bw/day group, and no fetuses in the 600 and 2000 mg/kg-bw/day groups. These included external malformations 2 control fetuses and 1 200 mg/kg-bw/day fetus, no visceral malformations, and one fetus with skeletal malformations in the control group. In Kao's view, this *in vivo* study is more relevant to assessing the developmental toxicity potential of Kao's CGT than the *in vitro* study conducted by Wang et al.

8. Liver Toxicity of Catechins from Green Tea

As discussed in Section VI.F below, the U.S. Pharmacopeia's (USP) Dietary Supplements Expert Committee (USP Committee) initially proposed that dietary supplements containing powdered decaffeinated green tea extract contain the following caution statement in their labeling:[41]

> "Caution: Must take with a meal. In rare cases, extracts from green tea have been reported to adversely affect the liver. Discontinue use and consult a healthcare practitioner if you have a liver disorder or develop symptoms of liver trouble such as abdominal pain, dark urine, or jaundice."

This proposed caution statement was based on the review of 34 case reports of liver toxicity in people taking dietary supplements containing green tea extract. These case reports are fraught with inadequacies (*see* discussion in Section VI.F below) that severely limit their utility in assessing the health risk of green tea extract. Further, the weight of evidence from published animal studies does not support an association between green tea extract and clinically significant liver toxicity. Specifically, *in vivo* studies that examined the effects of repeated ingestion of green tea extract on liver function in general and *in vivo* studies that examine the liver function of animals with drug, chemical, alcohol, diet, or other manipulation-induced liver toxicity treated with catechins from green tea demonstrate no toxic effects, or protective or ameliorative effects on the induced liver damage. One recent abstract by Lambert et al. (2008) reported that high doses of EGCG (1500 mg/kg) administered by gavage resulted in hepatotoxicity in mice. The dose of EGCG that elicited hepatotoxicity in this study is extremely high and not likely to be encountered through ingestion of Kao's CGT beverages (*see* Table 4, where the 90th percentile consumer of all possible beverages for all of the U.S. population would only be expected to ingest 21.5 mg/kg/day). The pivotal study supporting the GRAS determination for Kao's CGT (Takami

[41] USP. 2007. 33(6) *Pharmacopeia Forum* (Nov.-Dec. 2007). Proposed In-Process Revision of USP's monograph on powdered decaffeinated green tea extract, published at 33(4) *Pharmacopeia Forum* 693 (July-Aug. 2007).

et al. 2008) reported elevations in liver enzymes and increased relative liver weight in animals fed 5.0% (equivalent to 763.9-820.1 mg/kg-bw/day) of a green tea catechin extract preparation ("Sunphenon 100S"), but there were no accompanying histopathological correlates in the livers of these animals, thus providing suggestive evidence of mild hepatotoxicity, but only at very high doses. *In vitro* studies on hepatic cells in culture show mixed results, and typically require high concentrations that are not expected to be seen following *in* vivo oral exposure to induce cytotoxicity. These studies are summarized in Tables 11**,** 12, and 13 below.

If CGT possesses hepatotoxic activity, then the effect of concomitant exposure to potentially hepatotoxic agents, such as acetaminophen or other dietary supplements, should be considered. Kao conducted a literature search to identify any evidence of possible hepatotoxic interactions between green tea catechins/extract, and none were identified. Two articles by Oz and colleagues, however, provide evidence of a protective effect of green tea polyphenols against hepatotoxicity induced by acetaminophen (Oz et al. 2004; Oz and Chen 2008).

Pursuant to the June 23, 2008 press release from the USP, the USP concluded that the final monograph, which will be published on December 1, 2008 (*Second Supplement to USP 31-NF 26*), will not include any such proposed caution statement.

Table 11. Studies on Liver Function in Animals Ingesting Catechins from Green Tea

Citation	Animal	Test Article	Catechin Dose (mg)	Catechin Treatment Period	Summary of Results
Bun, S.S., et al. 2006. Effect of green tea extracts on liver functions in Wistar rats. *Food Chem. Toxicol.* 44:1108-13	Wister albino rats	Aqueous, Ethanol (AR25) and CH_2Cl_2 extracts	1st study: 2500mg /kg/d 2nd study: 1400, 2000 mg/kg/d	1st study: oral administration for 6 wks 2nd study: oral administration for 12 wks	No sign of evidence of hepatotoxicity (changes in ALT, AST, LDH, ALP, GGT, iron, total bilirubin, and cholesterol) was found in rats treated with a high level of different green tea extracts.
Bruno, R.S. et al. 2008. Green tea extracts protects leptin-deficient, spontaneously obese mice from hepatic steatosis and injury. *J. Nutr.* 138:323-31.	Male leptin-deficient mice (C57BL/6J)	Green tea extract (EGCG 48%)	0, 1 and 2 % in diet	6 wks	GTE protects against NAFLD by limiting hepatic lipid accumulation and injury without affecting hepatic antioxidant status and adiponectin-mediated lipid metabolism. Decreased ALT and AST activities also were observed.
Nakae, Y., et al. 2008. Subcutaneous injection, from birth, of epigallocatechin-3-gallate, a component of green tea, limits the onset of muscular dystrophy in *mdx* mice: a quantitative histological immunohistochemical and electrophysiological study.	*Mdx* mice	EGCG	5 mg/kg s.c.	4 x/week for 8 weeks after birth	While not the aim of the study, no effect on the histology of the livers was observed.
Takami, S., et al. 2008. Evaluation of toxicity of green tea catechins with 90-day dietary administration to F344 rats. *Food Chem. Toxicol.* 46:2224-2229.	F344 rats	Sunphenon 100S	0, 0.3%, 1.25%, and 5% in diet	90 days	A significant increase was observed in ALT and ALP in the 5.0% males and females and AST in the 5.0% females; an increase in relative liver weight was observed in the 5.0% males and females; no histopathological effects were seen in the livers of these animals.

Table 12. Studies on Liver Function in Animals with Induced Liver Injury Ingesting Catechins from Green Tea

Citation	Animal	Pretreatment for Liver Injury	Test Article	Catechin Dose (mg)	Catechin Treatment	Summary of Results
Zhong, Z., et al. 2002. Prevention of hepatic ischemia-reperfusion injury by green tea extract. *Am. J. Physiol. Gastrointest. Liver Physiol*. 283:G957-64.	SD rats	Hepatic warm ischemia and reperfusion	Green tea extract polyphenols (EGCG 47.2%, EGC 11.0%, GC 8.6%, EC 8.4%, ECG 10.8%, C 3.0%, GCG 11.0%)	0-0.3% in diet	5 days before hepatic warm ischemia and reperfusion	Hepatic ischemia-reperfusion resulted in increased transamination release and pathological liver changes including focal necrosis and leukocyte infiltration. Pretreatment with green tea extract polyphenols reduced transaminase release by >85%, and almost complete inhibition of hepatic pathological changes. Green tea extract also prevented the formation of NF-κB activation and proinflammatory cytokine formation.
Fiorini, R.N., et al. 2005. Short-term administration of (-)-epigallocatechin gallate reduced hepatic steatosis and protects against warm hepatic ischemia/reperfusion injury in steatotic mice. *Liver Transpl.* 11(3):298-308.	*ob/ob* mice	Hepatic warm ischemia and reperfusion	EGCG	85 mg/kg in drinking water	Oral for 5 days i.p. at 48, 24, 2 hrs before hepatic warm ischemia and reperfusion	Total baseline hepatic fat content decreased from baseline after EGCG treatment; ALT levels reduced in all EGCG-treated animals compared to control and necrosis decreased by 65% compared to control in EGCG-treated animals after hepatic ischemia-reperfusion. Other changes observed in EGCG animals included an increase in resting hepatic energy stores (ATP), and increase in glutathione level.

Citation	Animal	Pretreatment for Liver Injury	Test Article	Catechin Dose (mg)	Catechin Treatment	Summary of Results
Zhong, Z., et al. 2003. Polyphenols from *Camellia sinenesis* attenuate experimental cholestasis-induced liver fibrosis in rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* 285:G1004-13.	SD rats	Bile duct ligation (BDL) Gadolinium chloride for 3 days (destroy Kupffer cells)	*C. sinenesis* EGCG 50%	0 or 0.1 wt% in diet	3 day before BDL and subsequent 3 wks	Polyphenols feeding blocked or blunted all BDL-dependent changes (ALT increase, focal necrosis, bile duct proliferation, and fibrosis) by 45-73%.
Kuzu, N., et al. 2007. Epigallocatechin gallate attenuates experimental non-alcoholic steatohepatitis induced by high fat diet. *J. Gastroenterolo. Hepatol.* 22(11):2009-14.	SD rats	High fat diet for 6 weeks	EGCG	0 or 1g/L in drinking water	6 weeks with high fat diet	High fat diet induced steatosis, inflammation, ballooning degeneration, and necrosis. EGCG fed animals exhibited a significant decrease in steatosis and inflammation, as well as ALT, triglyceride, insulin, and glucose levels compared to the high fat diet group.
Arteel, G.E., et al. 2002. Green tea extract protects against early alcohol-induced liver injury in rats. *Biol. Chem.* 383:663-70.	Wistar rats	High fat diet with or without ethanol (10-14 g/kg/day)	Sunphenon DCF-1 green tea extract (polyphenolic content >80%)	300 mg/kg/day i.g. in liquid diet	4 weeks	Green tea extract significantly reduced the increase in ALT and increases in hepatic necrosis seen in ethanol-treated animals.
Augustyniak, A., et al. 2005. Preventive action of green tea from changes in the liver antioxidant abilities of different aged rats intoxicated with ethanol. *Nutrition*. 21:925-32.	Wistar rats (2, 12, 4 wks old)	Ethanol Liber-Decarli diet for 5 wks	Liquid diet containing green tea extract (EGCG mg/g, EGC 82 mg/g, EC 90 mg/L, EG 15 mg/g)	7g/L in liquid diet	Liquid diet containing GTE or control for 4-5 wks	The protective effect of green tea was confirmed by the significantly lower activity of biomarkers of liver damage (ALT, AST) in serum of rats that received green tea with ethanol compared with rats from the control ethanol group.

Citation	Animal	Pretreatment for Liver Injury	Test Article	Catechin Dose (mg)	Catechin Treatment	Summary of Results
Zhang, X-G., et al. 2006. Effect of tea polyphenols on cytokine gene expression in rats with alcoholic liver disease. *Hepatobiliary Pancreat. Dis. Int.* 5:268-72.	SD rats	56% ethanol 7g/kg once daily by gastric infusion	Tea polyphenol (composition not specified)	0.25g/kg	once daily by gastric infusion	Tea polyphenol blocked the development of alcoholic liver disease (ALD) by amelioration of inflammatory reaction and oxidative stress (*i.e.*, slight steatosis, liver cellular swelling in small areas, less spot and focal necrosis, no bridging necrosis, less mega-bubble steatosis, less collagen deposition.
Yun, J-W., et al. 2007. Effect of dietary epigallocatechin-3-gallate on cytochrome P450 2E1-dependent alcoholic liver damage: enhancement of fatty acid oxidation. *Biosci. Biotechnol. Biochem.* 71:2999-3006.	Male Wister rats	Ethanol Liber-Decarli liquid diet for 7 wks	Liber-Decarli liquid diet supplemented with EGCG	3g/l	7 wks	EGCG increased the rate of hepatic fatty acid oxidation and reduced alcoholic hepatic damage (fatty liver, reduced AST and ALT), but this effect did not appear to be mediated by the inhibition of CYP2E1. Instead, this effect of EGCG on fatty liver may be due in part to its antioxidant action.
Chen, J-H., et al. 2004. Green tea polyphenols prevent toxin-induced hepatotoxicity in mice by down-regulating inducible nitric oxide-derived prooxidants. *Am. J. Clin. Nutr.* 80:742-51.	ICR mice	i.p. administration of CCl_4 for 3 consecutive days	Tea Polyphenol EGCG	50, 75 mg/kg EGCG	ip administration simultaneously with CCl_4	EGCG administration led to a dose-dependent decrease in all of the histologic (necrosis) and biochemical (liver enzymes) variables of liver injury observed in the CCl_4-treated mice.

Citation	Animal	Pretreatment for Liver Injury	Test Article	Catechin Dose (mg)	Catechin Treatment	Summary of Results
Zhen, M-C., et al. 2007. Green tea polyphenol EGCG inhibits oxidative damage and preventive effects on carbon tetrachloride-induced hepatic fibrosis. *J. Nujtr. Biochem.* 18:795-805.	SD rats	i.p. administration of CCl_4 (1 mg/kg)	EGCG	25 mg/kg	7 wks	EGCG caused significant amelioration of liver injury (reduced hepatic fibrosis and reduced activities of serum ALT and AST).
Sugiyama, K., et al. 1998. Green tea suppresses D-galactosamine-induced liver injury in rats. *Biosci. Biotechnol. Biochem.* 62(3):609-11.	Wistar rats	D-galactosamine or CCl_4	Green tea extract	0.5-3%	14 days in diet prior to D-galactosamine or CCl_4	Green tea extract suppressed the elevation in plasma ALT and AST induced by D-galactosamine, but not by CCl_4, in a dose-dependent manner.
Abe, K., et al. 2005. Green tea with a high catechin content suppresses inflammatory cytokines expression in the galactosamine-injured rat liver. *Biomed. Res.* 26(5):187-92.	SD rats	ip administration (500 mg/kg)	Healthya Green Tea	540 mg/340 mL	Drinking Healthya green tea for 2 weeks before galactosamine intoxication	Oral administration of green tea rich in catechins restored galactosamine-induced hepatic injury as evidenced by increased serum transaminase activities and histological observation to near the control levels.
El-Beshbishy, H.A. 2005. Hepatoprotective effect of green tea (*Camellia sinensis)* extract against tamoxifen-induced liver injury in rats. *J. Biochem. Mol. Biol.* 38(5):563-70.	Albino rats	i.p. administration of tamoxifen (TAM) for 7 days	Green tea in drinking water	1.5% GTE in water	GTE was included in drinking water 4 days before and 14 days after TAM	The oral administration of 1.5% GTE to TAM-intoxicated rats produced significant increments in the antioxidant enzymes and reduced glutathione concomitant with significant decrements in thiobarbituric acid reactive substance (TBARS) and liver serum transaminases GPT, GOT) levels.

Citation	Animal	Pretreatment for Liver Injury	Test Article	Catechin Dose (mg)	Catechin Treatment	Summary of Results
Wang, Y., et al. 2006. (-)-Epigallocatechin-3-gallate protects mice from concanavalin A-induced hepatitis through suppressing immune-mediated liver injury. *Clin. Exper. Immunol.* 145:485-92.	C57BL/6	ConA was injected via tail vein (15 mg/kg)	EGCG	5 mg/kg dissolved in PBS	Administered by gavage twice daily for 10 days before induction of ConA-induced hepatitis	Mice pretreated with EGCG exhibited suppression of increase in ALT levels in plasma, reduced inflammatory infiltration, intralobular necrosis, and hepatocyte apoptosis in liver compared with control mice.
Bursill, C.A., and Roach, P.D. 2007. A green tea catechin extract upregulates the hepatic low-density lipoprotein receptor in rats. *Lipids*. 42:621-7.	SD rats	0.25% cholesterol	Green tea extract (58% total catechins, EGCG 30%, ECG 21%, EGC 4%, and GCG 2%)	0, 0.5, 1.0, or 2.0% in diet	12 days	Cholesterol absorption reduced by 24%, suggesting that the hepatic LDL receptor was upregulated.
Lambert, et al. 2008. Epigallocatechin-3-gallate prevents obesity-related fatty liver but is hepatotoxic at high doses. Abstract No. 1112 presented at the 2008 Society of Toxicology Annual Meeting, March 16-20, 2008.	C57B16/J mice	High fat diet (60% kcal) for 16 weeks	EGCG	0.32% in diet 1500 mg/kg i.g.	Administered in diet to mice fed high fat diets for 16 weeks Single or multiple bolus doses administered i.g.	Control mice fed a high fat diet exhibited fatty liver characterized by periportal microvesicular fat and increased hepatic triglycerides. The control high fat diet mice also exhibited elevated ALT levels compared to control mice fed a normal diet (105.2 vs. 13.2 U/mL). EGCG inhibited the incidence of obesity-related fatty liver disease by 98% compared to the high fat diet control mice; hepatic triglycerides and plast ALT levels

Citation	Animal	Pretreatment for Liver Injury	Test Article	Catechin Dose (mg)	Catechin Treatment	Summary of Results
						were also reduced by 69 and 66%, respectively, compared to the high fat diet controls. A single bolus oral administration of 1500 mg/kg EGCG resulted in increased ALT levels (138-fold) and reduced survival by 85%. Daily dosing increased hepatotoxicity (duration of dosing and liver data not provided).

Table 13. Studies on the Effects of Catechins from Green Tea on Liver Damage *In Vitro*

Title	Cells	Treatment	Summary of Results
Schmidt, M., et al. 2004. Toxicity of green tea extracts and their constituents in rat hepatocytes in primary culture. *Food Chem. Toxicol*. 43:307-14.	Rat hepatocytes	two 80% ethanolic dry green tea extract, one 80% ethanolic dry green tea extract with lipophilic compounds removed; 100-500 µg/mL	High concentrations of green tea extracts can exert acute toxicity (necrosis, increased leakage of LDH activity from cells) in rat liver cells. EGCG may be a key constituent responsible for this effect. The authors concluded that extremely high concentrations were required and argued that the low oral bioavailability of catechins "argues against a causal role of these constituents in the reported liver disorders."
Gelati, G., et al. 2006. Cellular and *in vivo* hepatotoxicity caused by green tea phenolic acids and catechins. *Free Rad. Biol. and Med.* 40:570-80.	Rat hepatocytes	Individual green tea catechins (EC, EGC, ECG, EGCG); various concentrations	Results suggest a cytotoxic mechanism for tea catechins and simple phenolics involving mitochondrial toxicity and reactive oxygen species formation.
Kaviarasan, S., et al. 2007. Epigallocatechin-3-gallate(-) protects Chang liver cells against ethanol-induced cytotoxicity and apoptosis. *Basic Clin. Pharmacol. Toxicol.* 100(3):151-6.	Human Chang liver cells	30 mM ethanol alone or with 25 µM EGCG for 24 hrs	EGCG significantly attenuated the ethanol-induced cell loss and LDH leakage, which was accompanied by a reduction in the generation of reactive oxygen species, as well as lipid peroxidation and maintenance of the GSH/GSSG ratio.
Lee, S.I., et al. 2008. Effect of green tea and (-)-epigallocatechin gallate on ethanol-induced toxicity in HepG2 cells. *Phytother. Res.* 22(5):669-74.	HepG2 cells	ethanol, green tea extract, and EGCG.	A dose-dependent increase of GGT activity in the culture media and loss of cell viability was observed in HepG2 cells treated with ethanol. Pretreatment of the cells with green tea extract significantly attenuated these changes.

C. Human Clinical Studies

A series of short- and long-term clinical investigations examining tolerability and nutritional effects in humans have been conducted with substances highly similar to CGT. In all cases, clinical studies have demonstrated that substances highly similar to CGT are well tolerated, and no toxicological adverse effects were reported as a result of consumption. It is important to note, however, that these studies were primarily conducted to evaluate the potential weight/fat reducing or cancer prevention effects of CGT.

1. Published Studies Assessing Safety-related Parameters After Consumption of Kao's CGT and Substances Highly Similar to Kao's CGT

Many of the studies assessing the safety of CGT or highly similar substances are published in peer-reviewed journals. Summaries of select published studies are provided in Table 14 below. To address the USP Committee's conclusions concerning green tea extract and liver toxicity based on case reports, Table 15 provides the results of liver function assays that were performed in many of the published studies presented in Table 14. As can be seen in Table 15, and discussed further in Section VI.F, when liver function is evaluated in well-controlled clinical trials, there do not appear to be any adverse effects associated with ingestion of catechins from green tea. Further, although green or black tea is not the focus of this GRAS Notice, Jin et al. (2008) recently published a systematic review evaluating the relationship between green tea consumption and liver disease. They identified 10 studies (8 from China, 1 from Japan, and 1 from the U.S.) that met their criteria for inclusion in the analysis (each study considered for inclusion should have an interventional or an observational design; the focus of interest should be on green tea or its main ingredient (tea polyphenol, catechin); and the outcomes should be major liver diseases including liver disorders, hepatic cirrhosis, hepatocellular carcinoma, and fatty liver disease). The outcomes investigated included primary liver cancer (two studies), mortality associated with primary liver cancer (two studies), incidence of liver disorders and cirrhosis (two studies), incidence of chronic liver disease (one study), and incidence plus attenuation of fatty liver disease (three studies). Of these 10 studies, 8 reported statistically significant results demonstrating a protective effect of green tea against liver disease, and two reported a partial protective tendency. Four of the studies reported a positive association between green tea intake and attenuation of liver disease. The authors speculated that the protective effect of green tea against liver disease could be attributed to green tea polyphenols because they have been demonstrated to prevent oxygen free radical-induced hepatocyte lethality, prevent lipopolysaccharide-induced liver injury through inhibition of inducible nitric oxide synthase and tumor necrosis factor-α expression, and inhibit carcinogen or toxin-induced liver oxidative DNA damage, among several purported mechanisms. Importantly, there was no evidence of liver injury associated with green tea consumption in these studies.

Although green or black tea are not the focus of this GRAS Notice, Kapiszewska et al. (2006) reported that women who consumed "high" (the levels were not provided) amounts of black or green tea exhibited lower levels of salivary 17β-estradiol throughout the entire menstrual cycle. The authors suggested that this effect may be the result of inhibiting the enzyme, aromatase, which catalyzes the conversion of androgens to estrogens. In

conclusion, the authors also suggested that tea consumption-mediated reduction in estrogens may reduce hormone-related cancer risk.

2. Supportive Unpublished Studies Assessing Safety-related Parameters After Consumption of Kao's CGT

Kao has conducted and/or sponsored a number of human studies that are awaiting publication. The most recent and relevant of these studies are summarized below.

Maki et al. studied the effect of daily consumption of a sports drink containing Kao's catechins from green tea on exercise-induced changes in body composition and fat distribution in overweight and obese individuals. Using a randomized, double-blind, placebo-controlled design, 132 male and female subjects were enrolled in this 12-week study and received either a placebo or 540 mg/day tea catechins from green tea in a sports drink beverage (500 mL). With regard to safety, the incidence of adverse events was comparable for the catechin (66.7%) and control (71.4%) groups. The most common adverse events were joint pain and respiratory disorders, which were characterized as mild or moderate and unlikely to be related to the study product. No serious adverse events occurred in the catechin group. Abnormal laboratory values occurred at a similar frequency in the catechin and control groups with the exception of ALT and AST. Mean ALT and AST levels decreased in the catechin group (-2.0 IU/L and -1.0 IU/L, respectively), but increased in the control group by 1.0 UL/L each. The results of this study support the safety of Kao's CGT, and provide evidence of a lack of hepatotoxicity associated with ingestion of this product.

Sakata et al. conducted a randomized, double-blind, placebo-controlled study to evaluate the effects of green tea containing 1080 mg/day of CGT in 17 patients with non-alcoholic fatty liver disease. A control group of patients received a placebo beverage containing <200 mg/day of CGT. The patients were treated for 12 weeks, and followed for an additional 16 weeks. With regard to safety, no serious adverse events occurred in the CGT group. A reduction in inflammation and fatty accumulation in the liver was observed in the CGT group with CT evaluation. Consistent with the improvement in liver pathology, a decrease in serum AST also was observed compared to baseline in the CGT group (64.7±7.3% ($p<0.05$) at 4 weeks and 58.6±6.5% ($p<0.05$) at 12 weeks). AST levels remained unchanged in the placebo group. The results of this study suggest that CGT ameliorates the hepatotoxicity associated with non-alcoholic fatty liver disease, and is not hepatotoxic.

Table 14. Summary of Published Studies Assessing Safety-related Parameters for Kao's CGT and Highly Similar Substances[42]

Study	Characteristics of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
Nagao, T., et al. 2007. A green tea extract high in catechins reduces body fat and cardiovascular risks in humans. *Obesity*. 15:1473-83.	Kao's green tea beverage	Double-blind, parallel Control group: 96.3 CGT mg/day (n=135) Test group: 583 CGT mg/day (n=135) 2-week run-in followed by 12-week test period	240 obese males and females (270 enrolled) Mean age: 41.7±9.9 years Mean BMI: 26.8±2.0	• Anthropometry (height, body weight, waist circumference, hip circumference, body fat ratio) • Abdominal fat (CT) • Systolic blood pressure (SBP) • Diastolic blood pressure (DBP) • Pulse rate	At baseline and every 4 weeks Clinical chemistry: TG, T-CHO, HDL-CHO, LDL-CHO, FFA, plasma glucose, GOT, GPT, γ-GTP, LDH, ALP Hematology: WBC, RBC, Hb, Hct, PLT	• Decreases in body weight, body mass index, body fat ratio, waist circumference, hip circumference, body fat mass, visceral fat area, and subcutaneous fat area were significantly greater in the catechin group as compared to the control group. • For subjects with baseline systolic blood pressures of >130 mmHg, a significant decrease in SBP was observed. • LDL-CHO decreased to a significantly greater extent in the catechin group vs. the control group. • There were no differences between the groups in any other clinical chemistry or

42 Abbreviations used in the table: CGT = green tea catechins; TC = tea catechins (type unspecified); TG = triacylglycerol; T-CHO = total cholesterol; HDL-CHO = high-density lipoprotein cholesterol; LDL-CHO = low-density lipoprotein cholesterol; RLP-CHO = remnant-like lipoprotein cholesterol; FFA = free fatty acid; NEFA = non-esterified fatty acid; FBS = fasting blood glucose; HbA_{1C} = hemoglobin A_{1C}; PAI-1 = total plasminogen activator-1; MDA-LDL = malondialdehyde-modified LDL; BUN = blood urea nitrogen; IP = inorganic phosphate; GOT = glutamic oxalacetic transaminase; GPT = glutamic-pyruvic transaminase; γ-GTP = γ-glutamic-pyruvic transaminase; ALB = albumin; ALP = alkaline phosphatase; LDH = lactate dehydrogenase; TP = thymidine phosphorylase; CRE = creatine; CPK = creatinine phosphokinase, TP = total protein; TBIL = total bilirubin; UA = uric acid; WBC = white blood cell count; RBC = red blood cell count; Hb = hemoglobin; Hct = hematocrit; PLT = platelets; PL = phospholipids; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; UIBC = unsaturated iron binding capacity; CPK = creatine phosphokinase; AMY = amylase; AST = aspartate aminotransferase, ALT = alanine aminotransferase, GGT = gamma-glutamyltransferase; Fe = iron; Na = sodium; Mg = magnesium; K = potassium; Ca = calcium.

Study	Characteristics of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
						hematology parameter. • No adverse effects were reported by the subjects in this study.
Nagao, T., et al. 2005. Ingestion of tea rich in catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men. *Am. J. Clin. Nutr.* 81:1-8.	Kao's green tea beverage	Double-blind, parallel 2-week run-in followed by 12-week test period Subjects consumed one 340 mL bottle of control or test beverage per day Control group: 21.7 CGT mg per beverage (oolong tea) (n = 18) Test group: 689.9 mg CGT per beverage (oolong tea) (n = 17)	35 healthy males Mean age: 36.2 years (24-46 years) Mean weight (control group): 73.8 ± 1.3 kg Mean weight (test group): 73.9 ± 1.8 kg	• Anthropometry (height, body weight, waist and hip circumference, body fat ratio) • Abdominal fat (CT)	At baseline and 12 weeks: <u>Clinical chemistry</u>: TG, T-CHO, HDL-CHO, LDL-CHO, RLP-CHO, FFA, total ketone body, FBG, insulin, PAI-1, leptin, Vitamin A, BUN, Vitamin E, MDA-LDL, Ca, Fe, IP, Na, K, Cl	• Body weight, BMI, waist circumference, body fat mass, and subcutaneous fat area were significantly lower in the test group. • Changes in MDA-LDL were positively associated with changes in body fat mass and total fat area in the test group. • No other significant differences in clinical chemistry parameters. • Occupational health physician assessments conducted every four weeks reported "no side effects related to the study."
Tsuchida, T., et al. 2002. Reduction in body fat in humans by long-term ingestion of catechins. *Progress in Medicine.* 26(11):1459-1464	Kao's green tea beverage	Double-blind, parallel 24 weeks (dosed for 12 weeks, wash-out for 12 weeks) Subjects consumed one 340 mL bottle of control or test beverage per day Control group: 126 mg CGT per	Control group: 41 male; 23 postmenopausal females Test group: 39 male, 20 postmenopausal females Mean age (male): 42.1 years (combined	• Diet diary (3 days/ wk at week 0, 12, and 24) • Anthropometry (every 4 weeks): height, body weight, waist circumference, hip circumference, waist/hip ratio, body fat ratio • Abdominal fat (CT) (Weeks 0, 12, and 24)	Weeks 0, 8, 12, 20, and 24 <u>Clinical Chemistry</u>: TG, T-CHO, HDL-CHO, LDL-CHO, FFA, PL, GOT, GPT, γ-GTP, TP, ALB, CRE, BUN, LDH, ALP, FBG, PAI-1, Na, K, Ca, Cl, IP, magnesium, Fe	• Body weight, BMI, body fat ratio, waist circumference, hip circumference, and body fat mass were all significantly lower in the test group than baseline and control; waist/hip ratio was significantly higher in the test group compared to baseline and control. • Total abdominal body fat, visceral body fat, and subcutaneous body fat were all significantly reduced in the test

Study	Characteristics of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
		beverage (green tea-like drink) Test group: 588 mg CGT per beverage (green tea-like drink) Caffeine: 81-83 mg CGT in each beverage	groups) Mean age (female): 54.8 years (combined groups) Mean BMI (male): 26.5 (combined groups) Mean BMI (female): 25.9 (combined groups)		Hematology: WBC, RBC, Hb, Hct, PLT Urinalysis: glucose, protein, urobilinogen, ketone body, and protein; sediments if protein was positive Circulatory Pulse, diastolic blood pressure Daily diary	group compared to baseline and control. • No significant differences between groups were observed for any hematologic, circulatory (pulse and diastolic blood pressure), or urinalysis parameter. • Significant changes in TG, FFA, and ALB were observed between groups at various time points, but were not considered to be test-article-related because the values were not different from respective baselines. • Interviews with subjects "showed no subject complaints of a poor physical condition due to the intake of the test beverage."
Otsuka, K., et al. 2002. Effects of tea catechins on body fat metabolism in women. *Jap. J. Nutritional Assessment*. 19(3):365-76.	Kao's green tea beverage	Double-blind, parallel 8 weeks Daily meals and exercise maintained; alcohol ingestion limited Control group: 9.7 mg/100 mL TC in 340 mL oolong tea beverage (33 mg TC per day) Test group: 163.3 mg/100 mL TC in	40 women (19-21 per group) Age: 22-53 years	At baseline and every 4 weeks: • Anthropometry (body weight, waist circumference, hip circumference) • CT (abdominal fat)	At baseline and 8 weeks: Blood chemistry: TG, T-CHO, HDL-CHO, LDL-CHO, FFA, acetoacetic acid, 3-hydroxybutyric acid, total ketonic acid, AMY, ALP, insulin, FBG, TP, ALB, γ-GTP, GOT, GOT, LDH, BUN, CRE, UA, Fe, Ca, Mg, Na, K, IP, Cl	• No significant differences between groups in any anthropometric measurement. • Statistically significant differences in test group compared to baseline values: waist circumference at 4 and/or 8 weeks, waist/hip ratio, total body fat area, and visceral fat area. • When stratified into subjects with BMI< 22 or ≥22, test group exhibited significantly lower total fat and visceral fat areas when compared to controls.

Study	Characteristics of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
		340 mL oolong tea beverage (562 mg TC/day) Caffeine concentration kept constant			Hematology: WBC, RBC, Hb, Hct, MCV, MCH, MCHC, PLT, UIBC	• No clinically significant changes in blood parameters were observed between groups. • No "study-related poor physical condition" reported.
Nagao, T., et al. 2001. Tea catechins suppress accumulation of body fat in humans. *J. Oleo Sci.* 50(9):717-28.	Kao's green tea beverage	Double-blind; parallel 12 or 20 weeks Subjects consumed one 340 mL bottle of control or test beverage per day Control group: 101.5 mg TC per oolong or green tea beverage 600 mg test group: 555.4 mg TC in oolong tea beverage 900 mg group B: 901.9 mg TC in oolong tea beverage Subjects instructed to "maintain usual food intake and constant exercise;" alcohol ingestion restricted; maintain diet diary	Experiment 1: 27 healthy males (8-10 per group) Age: 26-52 years Mean BMI: 25-25.2 Experiment 2: 17 healthy males (8-9 per group) Age: 25-48 years Mean BMI: 3.5-24.4 Experiment 3: 38 healthy males Age: 24-49 years Mean BMI: 24.3-34.6	At baseline and 12 weeks: • Anthropometry (body weight, waist circumference, body fat ratio) • CT (abdominal fat)	At baseline and 12 weeks: Blood chemistry: TG, T-CHO, PL, FBS, insulin, GOT, GPT, γ-GTP, AMY, BUN, Vitamin A, Vitamin E, Na, K, Ca, Cl, IP, Fe Hematology: WBC, RBC, Hb, Hct, and PLT	• Significant decreases in body weight and BMI observed in 900 mg group compared to control group (1.2±1.0%, vs. 0.0±0.0.6%, p=0.0440). • Significant decrease in skinfold thickness (body fat ratio) compared to control in 900 mg group (15.1±3.3% versus 2.6±4.5%, p=0.015). • Significant decreases compared to control in total fat area (900 mg group: 13.8±3.6% vs. 0.7±3.8%, p=0.0142), visceral fat area (600 mg and 900 mg groups: 10.7±2.3% and 13.0±4.0%, respectively vs. a gain of 12.5±8.5%, p=0.0317 and p=0.0098, respectively); no change in subcutaneous fat area. • No significant changes in any hematologic or clinical chemistry parameters. • No "study-related poor physical condition" reported.

Study	Characteristics of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
Kataoka, K., et al. 2004. Body fat reduction by the long term intake of catechins and the effects of physical activity. *Progress in Medicine*. 24(12):3358-70.	Kao's sports drink	Subjects consumed one 500 mL bottle of control or test beverage per day 4-week run-in followed by 12-week test period Subjects instructed to adjust food intake and physical activity to constant levels and refrain from ingesting catechin or caffeine-containing beverages	192 healthy males: Control: n=71 278 mg: n=25 570 mg: n=71 845 mg: n=25 Mean age: 39.0±0.6 yrs Mean BMI: 24.9±0.2 kg/m^2	At baseline and weeks 4, 8, and 12 • Anthropometry (height, body weight, waist circumference, hip circumference) • CT (abdominal fat) • Survey of exercise habits • Questionnaire re: adverse effects	At baseline and weeks 4, 8, and 12: Blood chemistry: TG, T-CHO, LDL-CHO, HDL-CHO, FFA, FBS, GOT, GPT, γ-GTP, ALB, ALP, LDH, TP, CRE, UA, Fe, Na, Mg, K, Ca, Cl, IP, BUN, FBG. PAI-1 Hematology: WBC, RBC, Hb, Hct, and PLT Urinalysis: Glucose, protein, urobilinogen, ketone body, sediment	• Significant decreases in body weight, BMI, body fat ratio, hip circumference, total fat area, visceral fat area, and subcutaneous fat area were observed in the 570 mg catechin group. • Several significant changes were observed in hematology parameters, but there was no dose-response relationship and the degree of changes was small and within the normal range for Japanese people. • No abnormal findings were observed in either subjective or objective indices of health.
Miyajima, E., et al. 2005. Efficacy of combination of FOSHU products. Combination of soy protein with tea high in catechin. *Progress in Medicine.* 25:831-5.	Kao's green tea beverage	Open (no control) 10g/day soybean protein as soy milk for 3 months alone during months 1 and 3 and in combination with green tea beverage containing 540 mg CGT	Outpatients (4 males, 16 females) Mean age: 62.7 yrs±5.6 years Mean BMI: 23.8	• Body weight, BMI, body fat % • Blood pressure, heart rate • Adverse events	CHO, TG, HDL, LDL, glucose, hs CRP, malondialdehyde-LDL, adiponectin A, AST, ALT, LDH, Cr, UA, CPK Hematology: Leukocytes, erythrocytes Hb, Hct, MCV, MCH, MCHC, and platelets	• No abnormal symptoms or adverse liver, kidney, or hematological effects observed (no significant changes in AST or ALT).

Study	Characteristics of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
Kozuma, K., et al. 2005. Effects of intake of a beverage containing 540 mg catechins on the body composition of obese women and men. *Progress in Medicine*. 25:1945-57.	Kao's sports drink	Multicenter, randomized, double-blind, placebo-controlled, parallel study Subjects consumed one bottle of control or catechin-containing (540 mg in 500 mL) sports drink per day 2-week run-in followed by 12-week treatment	226 healthy males and females Males: 36 treated, 73 control Females: 24 treated, 93 control Mean age: 26.8 yrs Mean BMI: 26.8 kg/m^2	At baseline and every 4 weeks: • Anthropometry (height, body weight, waist circumference, hip circumference, body fat ratio) • Blood pressure and heart rate • CT (abdominal fat) (baseline and weeks 8 and 12) • Questionnaire re: adverse effects	At baseline and every 4 weeks: Blood chemistry: TG, T-CHO, LDL-CHO, HDL-CHO, RLP-CHO, NEFA, FBS, HbA_{1C}, total ketone body; acetoacetic acid; 2-hydroxybutyric acid; AST, ALT, γ-GTP, ALB, ALP, LDH, TP, CRE, UA, Fe, Na, Mg, K, Ca, Cl, IP, BUN Hematology: WBC, RBC, Hb, Hct, and PLT	• Significant decreases in body weight, BMI, body fat ration, hip circumference, total fat area, visceral fat area, and subcutaneous fat area were observed in the 570 mg catechin group. • Several significant changes were observed in clinical chemistry parameters, but no between-group or interaction between group and time were noted. • No adverse events were reported.
Matsuyama, T., et al. 2008. Catechin safety improved higher levels of fatness, blood pressure and cholesterol in children. *Obesity* [ePub ahead of publication]	Kao's green tea beverage	Double-blind, randomized, controlled study 4-week lead-in, 24-week beverage consumption, 12-week follow-up 576 mg Green-tea beverage containing 576 mg CGT or control beverage (75 mg CGT)	40 male and female obese children (21 active) Age range: 6-16 years	• Anthropometry (body weight, waist circumference, hip circumference, lean body mass, body fat mass, body fat ratio • Visceral fat area; subcutaneous fat area (by CT) • Systolic blood pressure, diastolic blood pressure	TG, CHO, LDL, HDL, FFA, total ketone bodies, glucose, PAI-1, Leptin, growth hormone, Hs CRP, ALP, LDH, γ-GTP, AST, ALT, Creatinine, UA, BUN, total protein, albumin, IP, Ca, Fe, Mg, Na, K, Cl Hematology: WBC, RBC, Hb, Hct, platelets	• Significant decrease in ALP in the catechin treated group from week 0 to week 24. AST and ALT also decreased, but the difference was not statistically significant. • Authors concluded that catechins "did not appear to affect the measured safety parameters in children."

Study	Characteristics of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
Chow, H.H., et al. 2003. Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. *Clin. Cancer Res.* 9:3312-19.	Refer to composition of Polyphenon E in Table 9 above	Five groups (8 subjects/group): 800 mg EGCG once daily; 400 mg ECGC twice daily; 1070 mg of Polyphenon E (800 mg ECGC) once daily; 535 mg of Polyphenon E (400 mg ECGC) twice daily; and placebo 2-week placebo run in and 4 weeks of treatment with blood collections at Treatment Day 1 and 4 weeks later	40 healthy subjects (8 subjects/group) Between 2 and 4 males in each group Mean age: 34.5 ± 10.6 years (control); 39.5 ± 10.4 (800 mg EGCG once daily); 32.4 ± 10.1 years (Polyphenon E once daily); 29.4 ± 10.7 years (400 mg EGCG twice daily); and 34.1 ± 11.9 years (Polyphenon E twice daily) Mean weight: 169 ± 36 pounds (control); 165 ± 52 pounds (800 mg EGCG once daily); 167 ± 30 pounds (Polyphenon E once daily); 160 ± 40 pounds (400 mg EGCG twice daily); and 150 ± 19	• Adverse event reports • Pharmacokinetics • Blood chemistry	At weeks 0 and 4: <u>Hematology</u> WBC and RBC <u>Clinical Chemistry</u> TG, T-CHO, HDL-CHO, LDL-CHO, FFA, ALP, total protein, total bilirubin, globulin, FBG, ALB, AST, ALT, GGT, LDH, BUN, CRE, UA, Fe, Ca, Na, K, IP, Cl	• All reported events (including excess gas, upset stomach, nausea, heartburn, stomach ache, abdominal pain, dizziness, headache, and muscle pain) were mild and not different in frequency and kind from placebo. • No significant differences in hematologic or clinical chemistry parameters compared to placebo. • AUC differed significantly in the 800 mg Polyphenon E group compared to the 400 mg Polyphenon E group only after 4 weeks repeated administration. • C_{max}, T_{max}, and $t_{½}$ not significantly different after repeat administration.

Study	Characteristics of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
			pounds (Polyphenon E twice daily)			
Pisters, K.M.W., et al. 2001. Phase I trial of oral green tea extract in adult patients with solid tumors. *J. Clin. Oncol.* 19(60:1830-8.	Capsules containing 26.9 % total catechins; 13.2% EGCG, 8.3% EGC; 3.3% ECG; 2.2% EC; caffeine 6.8%	Phase I study Green tea extract capsules (110, 200, or 270 mg) were administered to at least 3 patients per dose once daily (total dose 0.5-5.05 g/m^2) or three times per day (1.0-2.2 g/m^2) after meals for up to 6 months	Adult cancer patients with solid, incurable tumors (26 males and 12 females) Age range: 27-77 yrs	• Pharmacokinetic indices • Adverse events	None	• Adverse events noted were largely neurologic or gastrointestinal in nature, and were attributed to the caffeine content of the green tea extract. • The maximum-tolerated dose was 4.2 mg/m^2 once daily or 1.0 g/m^2 three times per day.
Kajimoto, O., et al. 2005. Tea catechins with a galloyl moiety reduce body weight and fat. *J. Health Sci.* 51(2):161-171.	Green tea catechins (THEA-FLAN 90S: 86% total catechins 30% EGCG, 14% ECG, 31%, GCG, 10% CG, 0.3% EGC, 0.6%) EC, .04% GC, 0.4% C	Randomized, double-blind, placebo-controlled study 12 weeks 250 mL beverage Control: 3 placebo bottles per day at meals (total intake of catechins: 41.1 mg/day) Low-dose group: 1 bottle of catechin drink at breakfast and dinner, 1 bottle of placebo drink at lunch (total intake of catechins: 444.3	129 healthy adults	• Dietary intake diary for 3 days prior to each follow-up examination (baseline and every 4 weeks) • Anthropometry (height, body weight, waist circumference, hip circumference • CT (abdominal fat) • Adverse events	Hematology: WBC, RBC, Hb, Hct, PLT Clinical Chemistry: TG, T-CHO, LDL-CHO, HDL-CHO, ketone body, ferritin, AMY, cholinesterase, AST, ALT, γ-GTP, LDH, ALP, UA, BUN, CRE, TP, ALB, TBIL, FBS, HbA_{1C}, CPK, insulin, unsaturated iron binding capacity, Fe, IP, Na, K, Cl, Ca, and Mg	• Significant decreases in body weight, BMI, and total and visceral fat area were observed in both dose groups. • Clinical chemistry results demonstrated no abnormal changes in hepatic or renal function or electrolytes (data not shown). • Adverse events reported included 34 cases of cold-like symptoms, 3 cases of eczema, 7 cases of diarrhea and soft stool, and 1 case of lack of appetite; symptoms were mild, temporary, disappeared spontaneously in the course of the study, and reported in all

Study	Characteristics of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
		mg/day) High-dose group: 3 bottles of catechin drink per day (total intake of catechins: 665.9 mg/day) 2-week run-in, 12-week treatment, 8-week withdrawal				groups.
Betuzzi, et al. 2006. Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: A preliminary report form a one-year proof-of-principle study. *Cancer Res.* 66:1234-40.	Green tea catechins (5.5% EGC, 12.2% EC, 51.9% EGCG, 6.1% ECG, total catechins 75.7%, caffeine <1%)	Double-blind, placebo-controlled 1-Year follow-up Total daily doses of 600 mg green tea catechin capsules (3 x 200 mg capsules /day)	60 men with High-Grade Prostate Intraepithelial Neoplasia (HG-PIN)	• Development of prostate cancer • Adverse events	None	No significant side effects or adverse effects were documented.

Study	Characteristics of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
Hill, A.M., et al. 2007. Can EGCG reduce abdominal fat in obese subjects? *J. Amer. Coll. Nutr.* 26(4):396S-402S.	EGCG only (Teavigo)	Total dose 300 mg EGCG/day (two capsules of 150 mg EGCG/day) or placebo 12 weeks Women required to run or walk for 45 minutes 3 times/wk	38 overweight or obese post-menopausal women Age: 45-70 yrs BMI: 25-39.9 kg/m^2	At baseline and weeks 6 and 12: • Resting BP, HR and indices of arterial compliance • Anthropometry (height, body weight, waist and hip circumference) • Total body and abdominal fat composition by DXA and CT	At baseline and weeks 6 and 12: • Insulin, glucose, blood lipids, adipocytokines, hematology, liver function, and serum electrolytes	• Significant reduction in waist circumference, total body fat, abdominal fat, and intraabdominal fat (by CT) seen in both groups as compared to baseline; no difference between groups. • Significant reduction in HR seen in EGCG subjects. • Measures of liver function, hematology, and serum electrolytes remained within healthy ranges (data not shown) and there were no adverse effects reported.

Table 15. Effects of Catechins from Green Tea Ingestion on Liver Function in Clinical Trials[43]

Citation	Changes in Liver Function (IU/L)		
	AST	ALT	γ-GTP
Nagao, T., et al. 2001. Tea catechins suppress accumulation of body fat in humans. *J. Oleo Sci.* 50(9):717-28.	Control: -0.1±1.4 Low: +4.3±2.7 High: -2.9±3.7 Not significant	Control: +3.8±4.0 Low: +5.7±4.4 High: -1.3±6.5 Not significant	Control: +2.3±7.6 Low: +5.8±13.1 High: +4.3±3.9 Not significant
Otsuka, K., et al. 2002. Effects of tea catechins on body fat metabolism in women. *Jap. J. Nutritional Assessment*. 19(3):365-76.	Control: 27 to 36 Active: 20 to 18 Not significant	Control: 18 to 22 Active: 13 to 14 Not significant	Control: 27 to 36 Active: 20 to 18 Not significant
Tsuchida, T., et al. 2002. Reduction in body fat in humans by long-term ingestion of catechins. *Progress in Medicine.* 26(11):1459-1464.	Control: 29.8 to 26.7 Active: 26.4 to 24.5 Not significant	Control: 39.4 to 34.4 Active: 38.3 to 35.5 Not significant	Control: 46.0 to 37.7 Active: 34.5 to 32.5 Not significant
Kataoka, K., et al. 2004. Body fat reduction by the long term intake of catechins and the effects of physical activity. *Progress in Medicine*. 24(12):3358-70.	0 mg: -2.2±0.6 278 mg: 0.4±0.9 570 mg: -2.6±0.6 840 mg: 1.7±1.4 Not significant	0 mg: -3.1±1.5 278 mg: 0.7±0.9 570 mg: -2.9±1.1 840 mg: -0.8±2.8 Not significant	0 mg: -4.2±1.7 278 mg: 0.7±1.1 570 mg: 0.0±3.7 840 mg: -3.7±1.9 Not significant
Nagao, T., et al. 2005. Ingestion of tea rich in catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men. *Am. J. Clin. Nutr.* 81:1-8.	Control: 25.1 to 20.6 Active: 22.7 to 19.9 Not significant	Control: 35.7 to 29.9 Active: 30.5 to 26.5 Not significant	Control: 36.1 to 34.3 Active: 53.4 to 41.1 Not significant

[43] *See* Table 14 for study design and protocol details.

Citation	Changes in Liver Function (IU/L)		
	AST	ALT	γ-GTP
Miyajima, E., et al. 2005. Efficacy of combination of FOSHU products. Combination of soy protein with tea high in catechin. *Progress in Medicine.* 25:831-5.	Control: 22.9 to 25.6 Active: 22.9 to 23.8 Not significant	Control: 31.6 to 34.1 Active: 31.6 to 34.1 Not significant	No information
Kozuma, K., et al. 2005. Effects of intake of a beverage containing 540 mg catechins on the body composition of obese women and men. *Progress in Medicine*. 25:1945-57.	Control: 25.8 to 26.1 Active: 31.0 to 28.2 Not significant	Control: 36.5 to 40.9 Active: 48.3 to 43.6 Not significant	Control: 53.0 to 49.5 Active: 64.8 to 63.5 Not significant
Nagao, T., et al. 2007. A green tea extract high in catechins reduces body fat and cardiovascular risks in humans. *Obesity*. 15:1473-83.	Control: 24.2 to 23.8 Active: 25.0 to 25.6 Not significant	Control: 33.2 to 32.2 Active: 34.3 to 33.6 Not significant	Control: 46.2 to 47.3 Active: 51.9 to 54.1 Not significant
Matsuyama, T., et al. 2008. Catechin safety improved higher levels of fatness, blood pressure and cholesterol in children. *Obesity* [epub ahead of print]	Control: 23.6 to 23.0 Active: 29.0 to 25.3 Not significant	Control: 29.4 to 28.7 Active: 38.5 to 32.2 Not significant	Control: 22.4 to 24.9 Active: 22.5 to 22.7 Not significant

D. Potential Interactions Between Catechins from Green Tea and Metabolic Enzymes

Green tea has been reported to modulate activation and detoxification enzymes. This effect on metabolic enzymes raises the possibility of altering the efficacy or toxicity of marketed drugs, which these enzymes are responsible for metabolizing.

In vitro and animal studies suggest that green tea catechins modulate expression and activation of certain members of the cytochrome P450 ("CYP") family, "Phase I" metabolic enzymes. In contrast, recent studies in humans show that repeated administration of green tea catechins do not influence the activation of clinically significant enzyme family members – that is, those which are known to be responsible for the metabolism of the vast majority of drugs in the marketplace. Accordingly, current evidence supports the conclusion that green tea catechins do not affect clinically significant CYP enzymes.

Multiple *in vitro* studies provide evidence for the induction of CYP enzymes by green tea catechins -- primarily induction of CYP1A1. For example, Yang and colleagues investigated the effect of green tea extract on mRNA expression levels of CYPs 1A1, 1A2, 2C, 2E1, 2D6, and 4F3. Of these, only CYP1A1 and CYP1A2 showed an increase in mRNA expression (Yang and Raner, 1998). Similarly, Williams et al. showed that modulation of CYP1A1 expression is dependent on multiple components of the extract, rather than merely ECGC alone (Williams et al. 2003). Evidence from a recent study by Netsch and colleagues suggests that green tea catechins modulate the expression of drug-metabolizing enzymes, but non-specifically inhibit the function of CYP family members (Netsch et al. 2006).

In addition to these *in vitro* data, other studies have assessed the potential to modulate the expression of CYP enzymes in animals. Maliakal et al. (2001) administered green tea or green tea extract to Wistar rats and observed an increase in both CYP1A1 and CYP1A2 expression. In another study, increased apoprotein levels of CYP1A1 and CYP4A1 were observed following administration of green tea to rats Bu-abbas et al. 1994). Sohn and colleagues showed that consumption of green tea by rats led to significant increases in the liver of rats drinking green or black tea of CYP1A1, 1A2 and 2B1 activities, but no change in CYP2E1 and 3A4 activities (Sohn et al. 1994).

Notwithstanding the *in vitro* and animal evidence suggesting a modulatory effect of green tea catechins on the expression and activity of CYP enzymes, recent studies in humans provide strong evidence against the assertion that co-administration of green tea catechins could alter the metabolism and toxicity of marketed drugs. Chow and colleagues (2006) administered green tea catechins and a cocktail of probe drugs to 42 healthy volunteers to assess the activity of CYP1A2, 2D6, CYP2C9, and CYP3A4. As noted by the authors, these CYP family members were selected because they account for the metabolism of the majority of medications currently on the market and have been the cause of clinically significant interactions with drugs metabolized by these enzymes. Following repeated administration for 4 weeks, the subjects showed no alteration in the activity of CYP1A2, CYP12D6, and CYP12C9, but a small reduction in CYP3A4 activity was observed. Other recent studies in humans are consistent with these results (*see*, *e.g.*, Donovan et. al, 2004).

In vitro studies also suggest that green tea catechins may influence the activity of other enzymes, including "Phase II" metabolic enzymes. For example, Lambert et al. (2007a, 2007b) report that catechol-*O*-methyltransferase (COMT) is inhibited by green tea polyphenols in human liver cytosol. Kao is unaware of any *in* vivo studies that corroborate these findings. Individuals with a polymorphism for COMT resulting in low activity (or inhibited activity, as discussed above), thus, may have increased exposure to unmetabolized catechins. COMT metabolizes ECGC to 4"-*O*-methyl-(-)-EGCG or 4', 4"-*O*-dimethyl-(-)-EGCG (Lambert et al. 2003). However, there are other enzymes that also are involved in the phase II metabolism of EGCG, such as glucuronidase. Glucuronidase is one of the phase II enzymes that transforms EGCG to EGCG-4"-*O*-glucuronide, which is the major metabolite of EGCG formed by human, mouse, and rat microsomes. Therefore, even if individuals with polymorphism and/or inhibition of COMT are adversely affected by tea polyphenols (and there is no evidence to suggest that this occurs), other pathways and enzymes exist to metabolize EGCG.

In a 4-week clinical study where 42 volunteers ingested Polyphenon E, Chow et al. (2007) found that glutathione-*S*-transferases (GST) were differentially affected depending on baseline enzyme activity/level. GST activity and GST-π level increased in subjects with low baseline enzyme activity/level, and it is believed that stimulation of this enzyme may be beneficial in inactivating potential carcinogens.

In summary, the data suggesting an interaction between CYP and other metabolic enzymes and marketed drugs are limited primarily to *in vitro* and animal studies. Recent studies in humans do not support a conclusion that green tea catechins may alter the efficacy and toxicity of marketed drugs. In fact, many of these studies conclude that alteration of activity of these enzymes may be providing a protective effect against metabolism-dependent activation of carcinogens. Consequently, it is unlikely that CGT will present a concern with respect to drug interactions.

E. Other Studies Investigating the Mechanisms of Action for Green Tea Catechins

Recent publications regarding green tea extracts further support the Kao GRAS determination by contributing additional data supporting the safety of green tea catechins. A majority of these studies, all published after January 2008, detail the mechanisms of action of green tea extract constituents. Green tea total extract, a mixture of catechins, and individual catechins themselves have all been shown to produce beneficial health effects in clinical studies. Green tea polyphenols protect against oxidative or stress-related cellular damage. Catechins have a dual function by controlling reactive oxygen species (ROS) levels in the body. The chemopreventative effects of GTE and EGCG contribute a role in the preventative mechanisms that target multiple cell-signaling pathways, and determine that catechin compounds have fewer adverse effects than typical chemopreventative agents. EGCG also efficiently inhibits the generic effect of aggregation pathways in neurodegenerative diseases. Galloylated catechins play a role in the prevention of inflammation-derived DNA damage and carcinogenesis. Synergistic effects of green tea extract, in conjunction with therapeutic strategies, amplify the beneficial health results, suggesting that GTE is an efficient protective agent in humans (*see* Appendix L).

F. Reports that May Appear Inconsistent

1. Marketing Experience

In the period between its release in May 2003 and July 2008, Kao has sold 1,014 million bottles of beverages containing green tea catechins in Japan. Through November 2007, Kao received 1,188 reports of consumer complaints from green tea catechin drink consumers.[44] Most of these reports indicated minor, transient events, such as stomach aches, nausea, diarrhea, constipation, insomnia, and frequent urination. To date, based on medical confirmation, in no case has a connection clearly been established between any reported adverse event and green tea catechin. Apart from these reports, there also has been a small number of reports of hypersensitivity, elevated test results of liver function, or hypoglycemia, in which no causal relationship to green tea catechin could be established. Kao has confirmed recovery or remission in each of these events, and will continue to monitor adverse events reports closely.

2. USP Dietary Supplements Information Expert Committee Proposed Caution Statement

As noted above in Sections VI.B.8 and VI.F, In the November-December 2007 *Pharmacopeial Forum* (33(6)), the USP's Dietary Supplements Information Expert Committee proposed that a caution statement be included in the labeling for USP dietary supplements containing powdered decaffeinated green tea extract. Following further meetings of the USP Committee, and submissions of additional evidence and arguments, issued a press release on June 23, 2008 stating that, on December 1, 2008, its final monograph for powdered decaffeinated green tea extract will be published in the *2nd Supplement to USP 31-NF 26* without any such cautionary labeling statement. The text of the June 23, 2008 statement is provided in Appendix J.

Kao does not agree that there was sufficient evidence to support the proposed cautionary labeling statement for powdered decaffeinated green tea extract, and so informed the USP Committee.

The basis for the proposed caution statement was 34 anecdotal case reports describing liver toxicity following ingestion of dietary supplements containing green tea extract and in Kao's view, ill-founded conclusions based on limited pharmacokinetic data. Kao has chosen not to rely upon case reports for its GRAS determination because, while they may provide information that should be further investigated in well-controlled studies, they typically offer limited evidence in the assessment of causality, especially when there are well-conducted animal studies and controlled human studies available.

Further, and most important, the weight of evidence provided by the well-conducted and controlled animal and human studies described in previous sections demonstrate no

[44] Complaint data for the period since November 2007 are not yet available.

association between CGT (and green tea) and liver toxicity, and these studies properly should have far greater weight in determining the safety of Kao's CGT than case reports. The USP Committee chose to ignore these studies in its initial safety assessments. Based on the preclinical and clinical data discussed above, Kao was strongly of the view that the proposed caution statement was unwarranted and unsupported by relevant clinical and other data. The comments submitted to the USP Committee on December 28, 2007 in response to its proposed caution statement, in which the scientific validity of the case reports relied upon by the USP Committee, as well as the methods used to establish causality, and the supporting pharmacokinetic data are discussed, are provided in Appendix J, and summarized briefly below (the earlier safety evaluation attached to the December 28, 2007 comments to UPS is superseded by the more recent information contained in this GRAS Notice).

3. The Anecdotal Case Reports that Served as the Sole Basis for the Proposed Caution Statement Are Inadequate and Fraught with Limitations.

The USP Committee considered only 34 case reports from six countries regarding the safety of decaffeinated powdered green tea extracts in determining that a caution statement was warranted, as described in their publication (Sarma et al. 2008, *see* Appendix J). These case reports are summarized in Appendix J. The USP Committee thus concluded that green tea extracts, at least probably or possibly (according to the algorithm used to assess causality), caused the liver injury in those cases.

Additional case reports have been published since the USP Committee's review. For example, Federico et al. (2007) report the case of a 51-year-old woman who presented with a 5-year history of elevated liver enzymes, but was asymptomatic. There was no histological evidence of liver injury. Medical history revealed that she had been drinking green tea every day for at least 5 years. She stopped drinking green tea and her liver function tests improved. Upon reintroduction and subsequent discontinuation of green tea, her liver enzymes increased again, and then returned to normal, respectively. While it appears that green tea may have been a causative agent for the observed effects on this patient's liver function tests, the authors note that the case showed features suggestive of "an idiosyncratic, drug-related liver injury," and that "given the relative rarity of the reaction, it seems likely that host genetic factors are important in modulating susceptibility."

The USP Committee nonetheless also concluded that, when powdered decaffeinated green tea extract is used and formulated appropriately, it presents "no significant safety issues" of which the USP Committee is aware.

The limitations associated with the case reports reviewed by the USP Committee and published since the USP Committee's review that preclude the determination of a causal association between powdered decaffeinated green tea extract and hepatotoxicity are as follows:

- Multiple ingredients, in addition to green tea extract, were contained in several of the dietary supplements ingested by the individuals that are the subject of the case reports. Some of these other ingredients have been suggested to possess toxic

activity. For example, Jimenez-Saenz and Martinez-Sanchez (2007) note that, in one case of hepatotoxicity reported by Molinari et al. (2006), the patient also was taking progesterone, and these authors had a patient on progesterone therapy who developed acute hepatitis with jaundice. They note that "in the patient reported by Molinari et al., progesterone and green tea extracts both had to be considered potential hepatotoxic agents and discussed in the context of the conventional diagnostic scores for drug-induced livery injury before a conclusion could be drawn on the role of green tea extracts in the pathogenesis of her liver disease."

- Composition of some of the green tea extract products is unknown;
- Incomplete, and/or weak or missing information such as prior medical history;
- Failure to report use of alcohol and/or other concurrent medications that could have contributed to or caused hepatotoxicity;
- Failure to determine and/or consider preexisting risk factors; and
- Failure to determine and/or consider other confounding variables.

4. The Causality Assessment Algorithm ("Naranjo") Used by the USP Committee Is Not Generally Accepted.

The algorithm that the USP Committee applied for its causality assessment ("Naranjo") in an effort to enhance the weight of the 34 anecdotal case reports is not generally accepted due to its lack of validity and reliability, as well as a lack of critical information concerning the cases. Further, many critics have suggested that the Naranjo scale is inappropriate to assess causality, especially in this instance (*see, e.g.,* Kane-Gill et al. 2005; Horn et al. 2007).

5. The USP Committee's Proposed Toxicological Mechanism for the Observed Hepatotoxicity of Green Tea Extracts is Faulty and Unsupported.

The USP Committee proposed the following possible mechanism to explain the liver toxicity observed in the case reports. Green tea extracts administered under fasting conditions may lead to a higher concentration of the extract components in plasma, which may result in liver damage. However, there was no evidence of liver damage after administration of green tea extracts *in vivo* in the human studies the Committee reviewed, or in the studies described in previous sections. The hepatotoxicity reported in animal studies following ingestion of green tea extracts or individual catechins are not relevant to Kao's product and anticipated ingestion conditions, as discussed above. Although the USP Committee relied on the pharmacokinetic profile of green tea extract components in plasma for its analysis, there is no consensus or convincing rationale for adopting plasma concentration as a parameter in the assessment of liver damage.

The USP Committee cited studies in humans and animals to explain this possible mechanism of liver damage following the consumption of green tea extract. In its manuscript, the Committee outlined the safety information in animal and human studies as follows:

1. The predominant green tea catechin (EGCG) was identified as 10-times more cytotoxic to isolated rat hepatocytes *in vitro* compared to minor catechins (LD_{50} 91.6 µg/mL for EGCG).

2. Studies in beagle dogs indicated the potential for toxic plasma C_{max} concentrations at higher levels of green tea consumption under fasting conditions (comparable to lethal concentration *in vitro*).

3. The biodistribution data revealed significant uptake of EGCG by the liver in diverse animal models.

4. A significant observation in the mouse model was the increase in concentration of EGCG in all tissues (predominantly in the liver) when a second dose of EGCG was administered orally 6 hours after the first dose.

5. Studies on healthy volunteers showed that plasma C_{max} of free EGCG in the fasting condition was more than 5 times that obtained after administering the same dose with food.

6. In keeping with other reports about the effect of food on green tea bioavailability, researchers noted no adverse effects when 500 mg of a spray dried green tea preparation (80% EGCG)/kg/day was administered to pre-fed dogs in divided doses. This dose caused morbidity when administered to fasted dogs as a single bolus.

The USP Committee used these data to support its proposed mechanism that green tea extracts administered under fasting conditions lead to higher concentration in plasma compared to those administered under pre-fed conditions, which may result in liver damage. However, one critical piece of evidence that is lacking is that there are no data indicating the incidence of liver damage after administration of green tea extracts *in vivo* under fasting conditions. For example, the Committee referred to the dog study, which reported morbidity when administered to fasted dogs as a single bolus (*see* Section VI.B.7 above). However, the symptoms of morbidity were vomiting and diarrhea, but nothing related to liver damage, and as discussed in Section VI.B.7 above, there are a number of other considerations that severely limit the extrapolation of the findings in fasted dogs to the intended use scenario for Kao's CGT.

In addition, the Committee primarily relied on the pharmacokinetic profiles for its analysis. However, the plasma concentration of catechins varies significantly across animal species and even between individuals. The sample size for the determination of pharmacokinetic parameters referred to in the manuscript also is limited. Although the Committee recognized the absence of data regarding the tissue distribution of EGCG in humans, there is no consensus or scientific rationale for adopting the plasma levels of green tea extracts as a

surrogate for the assessment of liver damage. Additionally, as summarized in Section VI.A.3, the Committee's arguments are largely based on animal data obtained under conditions that are not extrapolatable to the ingestion scenarios anticipated for Kao's CGT-containing beverages. Specifically,

1. CGT have low oral bioavailibility, so results from studies using other (*i.e.*, parenteral) routes of administration or *in vitro* studies are not relevant.

2. CGT are widely and essentially equally distributed throughout the body following oral exposure. There is no evidence to suggest that the liver accumulates CGT to any greater extent than other organs.

3. Repeat-dose experiments do not provide evidence that CGT accumulates in the body.

4. The toxicity observed upon oral administration in the fasted condition is not relevant to the anticipated exposure to Kao's CGT because Kao's CGT products will be incorporated into a beverage and therefore, the stomach will not be empty. Moreover, no serious or other clinically-relevant adverse events have been reported in fasted subjects who ingest CGT.

5. The doses demonstrated to result in hepatotoxicity in animal studies far exceed the doses expected from ingestion of CGT-containing beverages.

Finally, and most significantly, to the best of our knowledge, no well-designed placebo-controlled clinical studies in humans and animals are available that report the occurrence of liver damage resulting from the consumption of green tea extract (*see* Sections VI.B and VI.C above).

In view of the severe limitations discussed above, therefore, we believe that the toxicological mechanism proposed by the USP Committee is insufficient to explain the incidence of liver damage purportedly associated with consumption of green tea extracts, because the proposed mechanism described in the manuscript is supported only by possibly unrelated and indirect evidence.

6. Predominant Adverse Cases Were Reported Following Consumption of Only Two Specific Products

The 34 case reports relied upon by the USP Committee were categorized as "possible causality" and "probable causality" with regard to green tea extract and liver damage. Of these case reports, 50% involved only two specific products: "Exolise" and "Tealine." However, green tea extract is often used both as a component of food and beverage products and as dietary supplements, and there are thus literally hundreds of products currently on the market containing green tea extract.[45] Because the reported liver toxicity was restricted

[45] Number of dietary supplement products containing green tea extracts: US: 395, UK: 25, CANADA: 61, FRANCE: 59, GERMANY: 12, PAIN: 26 (Mitel database, 1996-2007, keyword: green tea, subcategory: supplement).

primarily to two specific products, we believe that these findings may be associated more with the ingestion of those particular products and not necessarily to the ingestion of green tea extract in other products.

7. The USP Committee's Safety Conclusions and Proposed Caution Statement Were Intended Only for Products Labeled as Containing USP Powdered Green Tea Extract Used as Dietary Supplements.

Kao's CGT is not a dietary supplement and is not intended to be labeled as "USP." As shown in Table 16, green tea extract used in dietary supplements differs from Kao's CGT products because the dietary supplements (as specified in the USP's proposed monograph) are higher in ECGC content. Additionally, the green tea extract used in the dietary supplements that are the subject of the USP's monograph do not contain the other substances (*i.e.,* amino acids and saccharide) that are present in Kao's CGT.

Table 16. Catechin and Polyphenol Composition

	Brewed Green Tea	Crude green tea extract (raw material)	Type-1 CGT	Type-2 CGT	USP spec.
Total Catechin Monomer (%)	**27.6**	**36.6**	**44.4**	**62.3**	
Polyphenols (%)		**30.6**	**52.9**	**67.4**	**60.0**
Caffeine (%)	**8.5**	**4.9**	**2.6**	**1.7**	**0.1**
Catechin Profile (%)					
Gallocatechin(GC)	0.2	2.1	2.4	5.9	
Epigallocatechin(EGC)	9.0	9.9	13.2	20.5	
Catechin (C)	0.0	1.0	0.9	1.9	
Epicatechin (EC)	2.4	2.2	3.0	6.2	
Epigallocatechin gallate(EGCG)	**12.7**	**11.4**	**19.4**	**19.8**	**40.0**
Gallocatechin gallate(GCG)	0.2	0.5	0.5	2.5	
Epicatechin gallate(ECG)	3.1	3.2	4.6	5.0	
Catechin gallate(CG)	0.0	0.4	0.4	0.6	

Further, dietary supplement usage is very dissimilar to foods and beverages with regard to strength, dosage, and route of administration. The USP Committee's proposed caution statement stated that dietary supplements containing powdered decaffeinated green tea extract should be taken with a meal. Kao's CGT is a food additive to be used in beverages, so by definition and intended use, the USP Committee's safety concerns do not apply to Kao's product. As noted above, the USP Committee acknowledged that if used and

formulated appropriately, decaffeinated green tea extract presents "no significant safety issues" (*i.e.*, it is generally recognized as safe for the intended use).

Other than the case reports discussed above, Kao is not aware of any other reports that may appear inconsistent.

VII. SAFETY SUMMARY AND CONCLUSIONS

The data discussed in Section VI above, which include a large number of toxicological and metabolic studies, provide a well-documented basis from which to evaluate the generally recognized safety of CGT. Published animal and humans studies on Kao's CGT and substantially equivalent CGT extracts, as well as unpublished studies on Kao's CGT extract, in both animals and humans, provide strong evidence of the safe use of CGT as an additive to beverages for human consumption.

CGT has low oral bioavailability that has been attributed to several marked presystemic elimination processes. Thus, data from studies utilizing other routes of CGT administration, especially parenteral, which bypass the gastrointestinal system, are likely not relevant for the evaluation of bioavailability and systemic effects of orally consumed CGT because these other routes of administration result in higher blood levels than following oral administration. Further, the oral bioavailability appears to be lower in the fed condition versus the fasted condition, providing evidence that CGT may have an even safer profile when consumed with food. Nonetheless, human clinical studies indicate that, even when consumed in the fasting condition, high single dose and repeated doses of CGT are well tolerated by healthy individuals, with no serious or any other clinically-relevant adverse events noted. CGT are widely and almost equally distributed into various epithelial tissues, with no organ (including the liver) appearing to accumulate more CGT than other organs. Moreover, evidence from repeat-dose studies demonstrates that CGT do not appear to accumulate in the body after increased dosage and repeated exposures in the average population.

Short-term toxicity data on Kao's CGT, as well as other equivalent CGT extracts, provide consistent evidence of the safety of single doses of Kao's CGT, as well as repeated exposures (*i.e.*, up to 14 days). The acute oral LD_{50} is >2000 mg/kg body weight in rats, with no signs of treatment-related toxicity. The NOAEL for 14-day oral exposure in rats has been reported to range from 500-2,000 mg/kg-bw/day, with decreased body weight the only treatment-related effect observed.

With regard to the longer-term toxicity of Kao's CGT, a published 28-day toxicity study in rats identified a NOAEL of 1000 mg/kg-body weight/day based on the lack of *local toxicity* (*i.e.*, gastric erosions seen in two CGT animals) was derived from this study. The NOAEL for *systemic toxicity* was 2000 mg/kg-bw/day, the highest dost tested, because the decrease in body weight observed in the 1000 and 2000 mg/kg-bw/day male animals was accompanied by a decrease in food consumption during the first 2 weeks of the study. Kao does not believe that these body weight gain deficits were related to any endocrinological disturbance or other treatment-related adverse effect because, according to well-established principles regarding safety studies that have been enunciated by the WHO, a decrease in food consumption usually is accompanied by a lower body weight gain because animals that eat

less gain less weight and therefore, the decreased body weight gain observed in this study is properly should not be considered an adverse effect. Other statistically significant differences between treated animals and controls, such as select absolute organ weights and a number of clinical chemistry parameters, were not considered related to treatment because they were either not accompanied by changes in relative organ weight or histopathological correlates, lack of dose-dependency, and/or observation in only one gender.

Any potential safety issues unresolved by the 28-day toxicity study have been addressed by a recently published 90-day study on a substantially equivalent CGT extract (Takami et al. 2008) and Kao's as yet unpublished 6-month oral toxicity study on its CGT in rats. In the 6-month study, there were no test article-related deaths, and no test article-related effects on food consumption, clinical pathology or hematology parameters, or gross or microscopic pathology findings were observed. Various group mean differences in hematology, serum chemistry, and urinalysis parameters were observed, but were not considered to be test article-related because the values did not show a dose- or time-related response, were due to obvious outliers or spontaneous lesions in individual animals, and/or the parameter was altered in an unexpected an unlikely direction. Lower body weight gains and lower overall body weights that were not accompanied by decreases in food consumption were noted in the 1200 mg/kg-by/day animals. A NOAEL of 400 mg/kg-bw/day was estimated, based on the reduced weight gain observed.

The 90-day toxicity study by Takami et al. (2008) serves as the pivotal study supporting the GRAS determination for Kao's CGT. In this study, Sunphenon 100S, which is substantially equivalent to Kao's CGT with regard to total catechin monomer content and the content of individual catechin monomers, was fed to rats in the daily diet at concentrations of up to 5%. No test article-related clinical observations or deaths were found during the study. A significant decrease in body weight gain in the 5.0% males was observed in the absence of food consumption changes (with only transient body weight gain decreases seen in the 5% females). There were a number of hematological, clinical chemistry, and relative organ weight changes observed, but with the few exceptions discussed below, none were considered to be treatment-related because of a lack of dose-dependency or accompanying changes in related parameters or histopathological correlates. An increase in liver enzymes observed in the high-dose animals of both genders, along with an increase in relative liver weight, is suggestive of weak hepatotoxicity; however, there were no clinically significant histopathological lesions of the liver observed in these animals. Also, it is important to note that these effects were observed at a dietary concentration that far exceeds any proposed or likely exposure scenario for Kao's CGT (5% in the diet, which according to the authors, corresponded to a daily CGT intake of 3524.6 and 3541.6 mg/kg-bw/day for males and females, respectively. A NOAEL of 1.25% (corresponding to a CGT intake of 763.0 and 820.1 mg/kg-bw/day for males and females, respectively) was estimated from this study, based on reduced body weight gains in the 5.0% males and increases in liver enzymes and relative liver weight, which also far exceeds any exposure scenario for Kao's CGT.

The published animal toxicity literature on similar CGT extracts or individual components of CGT (*i.e.,* EGCG) reviewed supports the safety of Kao's CGT for its proposed use. While adverse effects have been reported in some studies (*e.g.*, Isbrucker et al. 2006b), these

findings are not considered relevant to the proposed use of Kao's CGT in beverages at levels not to exceed 540 mg/beverage for the following reasons. In some studies, toxicity was observed when the test substance was administered by a parenteral route of administration or in a single bolus dose to fasted animals, resulting in unrealistic and likely non-specific responses when considering the low bioavailability following oral exposure in the fed condition. Also, toxic effects were typically reported at doses of CGT or its component monomers that far exceed, by orders of magnitude, the highest estimated daily intakes for Kao's CGT (21.5 mg/kg-bw/day for the 90th percentile in the most inclusive exposure scenario). The purity of the test substances used in some of the published studies also varied from that of Kao's product. For example, in the Isbrucker study (2006b), the purity of the ECGC used in the fasted portion of the study was 80%. In still other studies, reported adverse effects are not considered relevant to humans (*e.g.,* an increase in forestomach tumors in rats when humans do not have a forestomach; heightened susceptibilities of animals to goitrogenic agents as compared to humans; use of cultured cells *in vitro*).

The developmental and reproductive toxicity of Kao's CGT extract also has been studied and found to result in no adverse effects on reproduction or development of the offspring of treated dams. Adverse effects reported by others (*e.g.*, Isbrucker et al. 2006c; Wang et al. 2007) may have been the result of reduced weight gain or irrelevant exposure conditions (*e.g.*, direct *in vitro* exposure of embryos to massive concentrations).

The genotoxicity of Kao's CGT also has been evaluated in several systems. While positive responses were observed in two *in vitro* tests (chromosomal aberration assay and mouse lymphoma L5178/tk assay), an Ames test and an *in vivo* study (bone micronucleus test), however, demonstrated no genotoxic effects, thus supporting the conclusion that CGT is not genotoxic.

In view of the USP Committee's proposed cautionary label statement concerning the potential liver toxicity of powdered decaffeinated green tea extract, which was based on 34 significantly flawed human case reports, Kao evaluated its own studies and those in the published literature specifically for evidence of CGT-induced liver toxicity. The results of Kao's 28-day and 6-month studies with regard to liver toxicity are discussed above. The pivotal 90-day study as well as others (*e.g.*, Lambert et al. 2008) reported weak hepatotoxicity in rats and mice at massively high dietary concentrations. Other animal studies were limited by their use of extremely high doses, administration to fasted animals in bolus doses, and use of *in vitro* systems. Other than the flawed and limited case reports, clinical data on Kao's product as well as substantially equivalent products reveal no evidence of treatment-related liver toxicity, and in fact, green tea catechins have been reported to protect against liver injury by other agents or conditions.

If CGT possesses hepatotoxic activity, then the effects of CGT on activation and detoxification enzymes in the liver and potential exposure to hepatotoxic agents should be considered. *In vitro* and animal studies suggest that green tea catechins modulate expression and activation of certain members of the cytochrome P450 (CYP) and Phase II metabolic enzymes (COMT). However, these effects were only observed in *in vitro* and some *in vivo* animal studies. Recent studies in humans do not support a conclusion that green tea

catechins may alter the efficacy and toxicity of marketed drugs. Indeed, many of these studies conclude that alteration of activity of these enzymes may be providing a protective effect against metabolism-dependent activation of carcinogens. Further, even if COMT activity was adversely affected by CGT, other pathways exist to metabolize catechin monomers, such as glucuronidase, one of the major Phase II enzymes. With regard to concomitant exposure to other hepatotoxic agents, such as acetaminophen, Kao could find no evidence of hepatotoxic interactions between green tea catechins and acetaminophen or other hepatotoxins. As noted above, green tea catechins have been shown to have a protective effect against hepatotoxins.

In conclusion, based on the Takami et al. (2008) 90-day toxicity study in rats, corroborated by Kao's 6-month toxicity study in rats, and supported by Kao's animal and human toxicity data as well as the published animal and human data, Kao has determined that its CGT products are GRAS for their intended use.

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Appendix A
Analytical Methods

Appendix A: Summary of CGT GRAS Notice Analytical Methods

Total Catechin Monomers: HPLC Method (Kao Method)

1. Definition:

   The sample is measured using a designated method, and the result is displayed as the catechin content and caffeine content in units of mg/100 mL or percentage.

2. Scope of Application

   This test method is applied to beverage ingredients and products

3. Equipment
   High-Performance Liquid Chromatography Unit

4. Operation:
   Adjust the sample to a specified temperature, and injection 10 µl into a high-performance liquid chromatogram. Catechin content / caffeine content are calculated from each peak area that is obtained.

5. Computation:
   Computation of catechin content / caffeine content shall be performed using the formula below.

   Catechin content (mg/100 ml) =
   Peak area of 8 catechin types × slope on calibration curve + section of calibration curve

   Caffeine content (mg/100ml) =
   Peak area of caffeine × slope on calibration curve + section of calibration curve

   Gallocatechin ratio (1) (%) =
   Catechin content of 4 gallo types (EGC+GC+EGCg+GCg)/Catechin content × 100

   Gallocatechin ratio (2) =
   Catechin content in (EGC+GC) / Catechin content in (EGCg+GCg)

   Catechin gallate ratio (%) =
   Catechin content of 4 gallate types (EGCg+GCg+ECg+Cg)/Catechin content ×100

6. Report:
   Catechin content / caffeine content and gallocatechin ratio (1) / catechin gallate ratio were reported to the nearest integer. Gallocatechin ratio (2) was reported to the nearest hundredth.

Polyphenols: Tartaric Acid Method (Kao Method)

1. Definition

The total polyphenol content is displayed as a percentage or mg/100 ml when the content is measured based upon the designated method.

2. Scope of application:

This test method is applied to beverage ingredients and products of green tea.

3. Analysis Conditions:
   (1) Measured wavelength 540 nm (Absorption Spectrometer (U-2010; by Hitachi))
   (2) Reagent preparation is used at room temperature (20° C ± 2° C)

4. Operation
   (1) Create calibration curve

   (2) Sample measurement
      1) Spectrophotometer is zero-compensated using ion-exchange water.
      2) Sample is measured and placed in a flask, tartar reagent is added, phosphoric acid buffer is added, after which absorption is measured.

   (Measure absorption within 40 minutes of coloration).

5. Computation

Computation of total polyphenol content shall be performed in accordance with the formula below:

1) Product: Unit (mg/100 ml)

$$\text{Total polyphenol} = \frac{5\ \text{ml}}{1\ \text{ml}} \times 1.5 \times ((\beta)\times(A)\times(\alpha))$$

(A): Absorbance = Actual measurement – blank value on calibration curve
(β): Slope in calibration curve
(α): Section of calibration curve

2) Ingredient (Crude Catechin, refined catechin preparation, tea leaves): Unit(%)

$$\text{Total polyphenol content} = \frac{5\ \text{ml}}{\text{Sample ml}} \times 1.5 \times ((\beta)\times(A)\times(\alpha)) \times \frac{(\text{Dilution multiple})}{1000}$$

6. Report

Report total polyphenol content to the decimal below.

Product:	Integer
Crude Catechin:	Integer
Refined catechin preparation:	To the nearest tenth
Tea leaves:	Integer

Caffeine: HPLC Method (analyzed by Japan Research Food Laboratories)

Analysis method simplified flow chart:

Sampling of specimen

↓

Extraction (Shaking)

↓ Aliquot of the organic solvent layer

↓ Dehydration filtration

Concentration and exsiccation

↓

Dilution to the volume

↓

High performance liquid chromatography (HPLC)

Protein and Amino Acids: Kjeldahl-Method (analyzed by Japan Food Research Laboratories)

Sampling of the specimen: S (g) of the specimen should be sampled to Kjeltec tube

Catalyst 10 g
Conc. sulfuric acid (15 mL)

↓

Heating Decomposition: 2 hours

↓

Radiation Cooling

Ion exchange water 80 ml

↓

Heat Distillation: Steam distillation (Distillation under excessive alkali after addition of sodium hydroxide solution)

↓

Titration

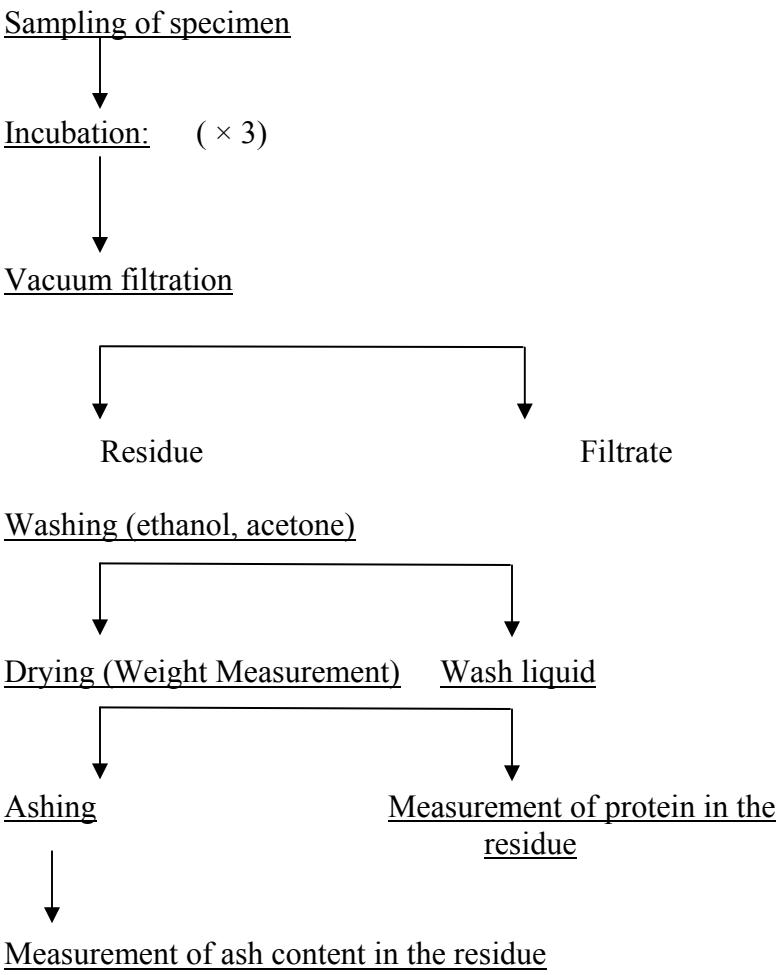
Equipment used
Kjeltec Auto Sampler System Analyzer

*Calculation Formula

Protein (g/100 g) = $\{(V-B) \times F \times 0.0014 \times K\}/S \times 100$

Where;
V: Titration volume in this test (ml)
B: Titration volume in the blank test (ml)
F: 0.05 mol/L titer of sulfuric acid standard solution
0.0014: Nitrogen amount (g) for 1 ml of 0.05 mol/L sulfuric acid standard solution
K: Nitrogen / protein conversion factor
S: Sampling amount of the specimen

Fiber: Enzymatic-gravimetric Method (analyzed by Japan Food Research Laboratories)



Fat: Soxhlet Extraction Method (analyzed by Japan Food Research Laboratories)

Sampling of the specimen: (Extraction thimble) S (g)

↓

Drying

↓

Extraction

↓

Solvent distillation removal

↓

Drying

↓

Radiation cooling:

↓

Weighing: W_2 (g)

*Calculation Formula

Fat (g/100 g) = $(W_2 - W_1) \times 100/S$

W_1 (g):constant weight of the Soxhlet flask(g)

Ash: Direct Ashing Method (analyzed by Japan Food Research Laboratories)

Sampling of the specimen: Constant weigh of the ceramic crucible should be obtained in advance: $W_{1\ (g)}$
Specimen should be taken in the crucible and should be weighed: W_2 (g)

↓

Preliminary ashing

↓

Ashing

↓

Radiation cooling

↓

Weighing: W_3 (g)

*Calculation formula:
Ash content (g/100 g) = $(W_3 - W_1)/(W_2 - W_1) \times 100$

Organic acids: HPLC- Method (analyzed by Japan Food Research Laboratories)

1. Preparation of the test solution

(1) Test substance 1)

The test substance is measured, with perchloric acid and water added before shaking. Water was added to the solution to bring the mixture to a constant volume. The filtrate was then used as a test solution for analysis of citric acid and tartaric acid. The test substance was then accurately measured, with perchloric acid and water before shaking. Water was then added to the solution to bring the mixture to a constant volume.

The filtrate was then used as the test solution for analysis of malic acid and quinic acid. The test substance was accurately measured with perchloric acid and water before shaking. Water was added to the solution to bring the mixture to a constant volume. The filtrate was then used as the test solution for analysis of lactic acid. Finally, the test substance was accurately measured, and water added before shaking for ten minutes. Water was added to the solution to bring the mixture to a constant volume. The filtrate was then used as the test solution for analysis of other organic acids.

(2) Test substance 2) and 3)

The test substance was accurately measured, with perchloric acid added. Water was added to the solution to bring the mixture to a constant volume. The filtrate was then used as the test solution for analysis of citric acid, tartaric acid, malic acid, and quinic acid. The test substance was accurately measured, with perchloric acid added. Water was added to the solution to bring the mixture to a constant volume. The filtrate was then used as the test solution for analysis of lactic acid. The test substance was accurately measured, adding water to bring the mixture to a constant volume. The filtrate was then used as the test solution for analysis of other organic acids.

2. Operating conditions for high speed liquid chromatography

(1) Operating conditions (citric acid, tartaric acid, and lactic acid)

Model: LC-10ADvp [from Shimadzu Corporation]
Detector: Ultraviolet-visible spectrophotometer SPD-10AVvp [from Shimadzu Corporation]
Column: Shodex RSpak KC-811X2, Ø 8 mm × 300 mm [from Showa Denko K. K.]
Column Temperature: 40° C
Mobile Phase: 3 mmol/l perchloric acid
Flow Rate: Mobile phase 1.0 ml/min, reaction solution 1.4 ml/min
Measured Wavelength: 445 nm

(2) Operating conditions (malic acid and quinic acid)

Model: LC-10ADvp [from Shimadzu Corporation]
Detector: Ultraviolet-visible spectrophotometer SPD-20AV [from Shimadzu Corporation]
Column: Shodex RSpak C-811, Ø 8 mm × 500 mm [from Showa Denko K. K.]
Column Temperature: 60° C
Mobile Phase: 3 mmol/l perchloric acid
Flow Rate: Mobile phase 1.0 ml/min, reaction solution 1.4 ml/min
Measured Wavelength: 445 nm

(3) Operating conditions (other organic acids)
Model: LC-10ADvp [from Shimadzu Corporation]
Detector: Ultraviolet-visible spectrophotometer SPD-20AV [from Shimadzu Corporation]
Column: TSKgel 0A-pak, Ø 7.8 mm × 300 mm [from Showa Denko K. K.]
Column Temperature: 40° C
Mobile Phase: 0.75 mmol/l perchloric acid
Flow Rate: Mobile phase 0.8 ml/min, reaction solution 0.8 ml/min
Measured Wavelength: 445 nm

Saccharide: HPLC-Method (analyzed by Japan Food Research Laboratories)

Test substance was accurately measured and added to a 1:1 water and ethanol mixture. After the solution neutralized, the substance was extracted with ultrasonic treatment. The same mixture was added again to bring the mixture to a constant volume, and then filtered with filter paper. After some of the filtrate was condensed and dried, water was added to make a solution again, which was then passed through Sep-Pak plus Accell QMA and Sep-Pak plus C_{18} filters (from Waters Japan), followed by a membrane filter with a pore diameter of 0.45 μm.

Test substance 2) and 3) were accurately measured and then water was added.

After the solution neutralized, water was added to bring the mixture to a constant volume. The solution was then passed through Sep-Pak plus Accell QMA and Sep-Pak C_{18} filters (from Waters Japan), followed by a membrane filter with a pore diameter of 0.45 μm. Analysis was then carried out on the filtrate using the high speed liquid chromatography method to quantitatively determine sugar levels.

Total Plate Count

Prepare decimal dilutions of the sample of 10-1, 10-2, and others as appropriate

↓

Take part of the sample and of the dilutions into separate, duplicate, Petri dishes

↓

Add plate counter agar

↓ Mix and cool to solidify

Incubate

↓

Count the duplicate plates in suitable range (30-300 colonies)

↓

Reports as aerobic plate count per ml or not more than 30 per ml

Colon bacillus

Weighing	Specimen should be aseptically weighed
↓	
Preparation of the specimen solution	After adding sterilized phosphoric acid buffered physiological salt solution, the solution should be adequately mixed.
↓	
Primary culturing	Prepared specimen solution and diluted solution of the specimen solution should be seeded in fermentation tubes, and cultured.
↓	
Subculturing	Specimen from the fermentation tubes where generation of gas is recognized should be transferred to another fermentation tube and should be cultured in a constant temperature bath.
↓	
Separation	Specimen from the fermentation loop where generation of gas is recognized should be streaked onto an agar plate, and should be cultured.
↓	
Identification	IMVic test should be conducted for typical colonies on the agar plate using the following culture media. Specimen should simultaneously be identified as gram negative *asporogenic bacillus* by microscopic examination by transferring on to the agar slant medium • SIM culture medium • Glucose, phosphoric acid peptone agar • Simmons' citrate agar
↓	
Indication	Negative: 2.22 g, Positive 1g Positive: 0.1 g Positive: 0.01 g

Arsenic, lead, cadmium, and tin (Sn) methods

1. Preparation of test solution
   A test solution is prepared by the wet digestion shown in a. or the dry ash method shown in b. The test for arsenic is conducted by the wet digestion showed in a.

   a. Wet digestion method

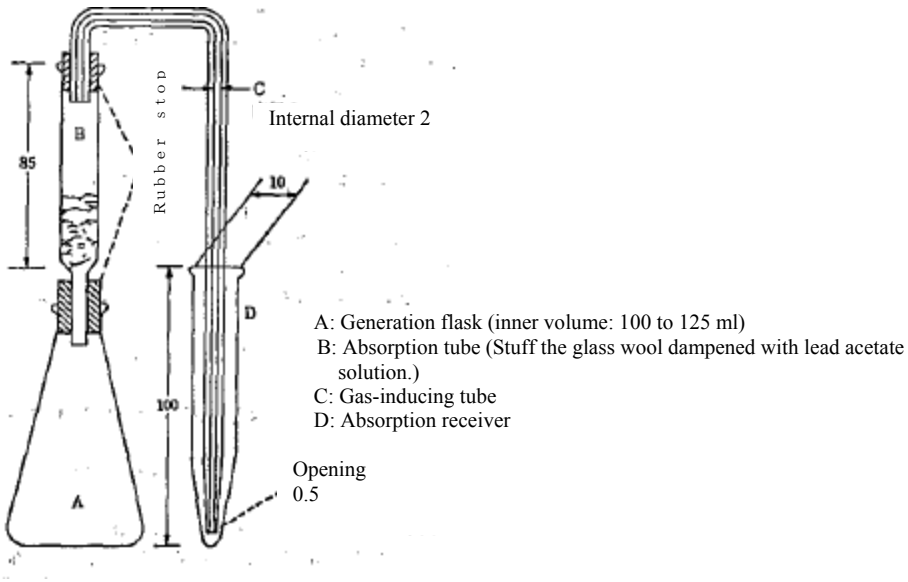
      Take the sample, humidify on a water-bath until it is syrupy after evaporative concentrations. Transfer it into a decomposition flask by using water, add and dissolve sulfuric acid and nitric acid, and then continue heating until the solution becomes almost colorless or pale yellow, adding nitric acid sometimes while heating. After cooling once, add water and the ammonium oxalate solution and hear until white mist appears at the neck part of the flask. After cooling, add water to obtain total volume of test solution. The solution obtained separately by using water in place of the sample and the same procedure as that for the sample us used as a blank test solution.

   b. Dry ash method

      Take the sample, dry it under an infrared ray lamp or in a drying apparatus, and heat until almost a white ash content can be obtained. After cooling, pour gently and dissolve hydrochloric acid and evaporate to dryness on a water-bath. After cooling, dissolve hydrochloric acid to obtain final test solution. The solution obtained separately by using water in place of the sample and using the same procedure as that for the sample is used as a blank test solution.

2. Test method for arsenic
   The test method for arsenic is performed by the silver diethyldithiocarbamate method as described in a.

   a. Silver diethyldithiocarbamate method
      i. Apparatus
         The apparatus is shown in the following diagram (unit: mm).



ii. Reagent/test solution

Reagent/test solution, etc., part 2 are used, except for those shown below.

Silver diethyldithiocarbamate pyrimidine solution: dissolve silver diethyldithiocarbamate with pyrimidine and store in a cool place until light shielding.

Sandy zinc: Soak meshes of arsenic-free zinc in cupric sulfate solution until blackened and dry after washing.

Stannous chloride solution: Dissolve stannous chloride with arsenic-free hydrochloric acid, add water, transferred it in a stoppered bottle, and stopper it tightly for storage.

iii. Test procedure

Take the test solution in the generation flask, add water, hydrochloric acid, potassium iodide solution, and stannous chloride solution, and allow to stand at room temperature. Add sandy zinc in this generation flask, and immediately connect it to the absorption tube and gas-introducing tube. Connect it to the absorption receiver in which the silver diethyldithiocarbamate pyridine solution is filled in advance, and allow to stand. Remove the apparatus and mix the fluid in the gas-introducing tube with absorbed fluid in the absorption receiver. Take this absorbed fluid and measure the absorbance with the silver diethyldithiocarbamate pyridine solution as the control solution. In this case, the absorbance of the test solution should not exceed the absorbance obtained for the solution by adding arsenic standard solution to the blank test solution, adding water, and taking the same procedure as in the case of the test solution.

3. Test method for lead and cadmium

Tests for lead and cadmium are performed by the atomic absorption spectrometry shown in a.

a. Atomic absorption spectrometry

i. Apparatus
Atomic absorption spectrometry
Light source: Lead hollow cathode lamp in the test for lead and cadmium; hollow cathode lamp in the test for cadmium
Fuel: Acetylene gas or hydrogen

ii. Reagent/test solution
Reagent/test solution, etc., Part 2 are used, except for those shown below.
Ammonium citrate solution: Dissolve diammonium hydrogen citrate with water.
Ammonium sulfate solution: Dissolve ammonium sulfate with water.
DDTC solution: Dissolve sodium diethyldithiocarbamate with water.
Lead standard solution: Dissolve lead nitrate with nitric acid. Take part of this solution and add nitric acid.
Cadmium standard solution: Add nitric acid to metallic cadmium, heat and dissolve it, and add nitric acid after cooling. Take part of this solution and add nitric acid.

iii. Test procedure
Take a part of each test solution and blank test solution, and add ammonium citrate solution and bromthymol blue reagent to each of them. Neutralize with ammonia and water until the color of the solution goes from yellow to green, and then add ammonium sulfate solution and water. Add DDTC solution to each, mix, and allow to stand for a few minutes. Remove the layer of methyl isobutyl ketone and measure the absorbance A of the test solution, and Ab of the blank test solution at a measured wavelength for the zinc test and for the cadmium test. Take part of the zinc standard solution or of the cadmium standard solution together with water, and add nitric acid. Measure the absorbance As of the standard solution and Ao of water in the same procedure as the case of the test solution. In this case, the value of A- Ab should not exceed the value of As – Ao.

4. Test for tin (Sn)

The test for tine (Sn) is performed by the salicylidene amino-2- thiophenol method shown in a.

a. Reagent/test solution

i. Reagent/test solution, etc., Part 2 are used, except for those shown below.

SATP solution: Dissolve L-ascorbic acid with a small amount of water and add ethanol. Add salicyllidene amino-2-thiophenol to this solution and dissolve it by heating.

Dinitrophenol solution: Add and dissolve ethanol to 2,4-dinitrophenol.

Lactic acid solution: Add water to lactic acid.

Tin (Sn) standard solution: Add hydrochloric acid to metallic Tin (Sn) and dissolve it by heating on a water bath. After cooling, add hydrogen peroxide water and then add hydrochloric acid. Take part of this solution and add hydrochloric acid.

Sodium hydrate solution: Dissolve sodium hydrate with water.

Sodium thiosulfate solution: Dissolve thiosulfate with water.

ii. Test procedure

Take the test solution and add hydrochloric acid. Take part of this solution and add hydrochloric acid, add dinitrophenol solution, add the sodium hydrate solution for neutralization, and add water. Add and mix the lactic acid solution, the sodium thiosulfate solution, the SATP solution, and allow to stand. Add xylene and shake strongly. Allow to stand. Take the layer of xylene, measure the absorbance of xylene as the control solution and obtain the quantity of tin (Sn) in the test solution from the calibration curve to calculate the concentration of tin (Sn) in the sample.

iii. Preparation of the calibration curve

Take 0, 1.0, 2.0, 3.0, 4.0, and 5.0 of the tin (Sn) standard solution and add part of these solutions to the blank solution separately. Add hydrochloric acid twice to each one, and then add dinitrophenol solution. Perform the same procedure as in the case of the test solution to measure respective absorbance, and prepare the calibration curve.

Appendix B

Bottled Tea

92301000	TEA, NS AS TO TYPE, UNSWEETENED	Bottled Tea
92301060	TEA, NS AS TO TYPE, PRESWEETENED W/ SUGAR	Bottled Tea
92301080	TEA, PRESWEETENED W/ LOW CALORIE SWEETENER	Bottled Tea
92301100	TEA, NS AS TO TYPE, DECAFFEINATED, UNSWEETENED	Bottled Tea
92301130	TEA, NS AS TO TYPE, PRESWEETENED, NS AS TO SWEETENER	Bottled Tea
92301160	TEA, DECAFFEINATED, W/ SUGAR, NFS	Bottled Tea
92301180	TEA, DECAFFEINATED, LOW CALORIE SWEETENER, NFS	Bottled Tea
92301190	TEA, PRESWEETENED, NS SWEETENER, DECAFFEINATED	Bottled Tea

Appendix C

Sports Drinks

92553000	FRUIT-FLAVORED THIRST QUENCHER BEVERAGE, LOW CAL	Sports drink
92560000	FRUIT-FLAVORED THIRST QUENCHER BEVERAGE	Sports drink
92582050	FRUIT-FLAVORED DRINK, VITAMIN & MINERAL FORTIFIED	Sports drink?

Appendix D

Carbonated Beverages and Juice Drinks

11551050	MILK FRUIT DRINK (INCL LICUADO)	Carb bevs/juices
11552200	MILK-BASED FRUIT DRINK (INCL ORANGE JULIUS)	Carb bevs/juices
11553000	FRUIT SMOOTHIE DRINK, W/ FRUIT AND DAIRY PRODUCTS	Carb bevs/juices
11553100	FRUIT SMOOTHIE DRINK, NOT FURTHER SPECIFIED	Carb bevs/juices
61200500	ACEROLA JUICE	Carb bevs/juices
61201000	GRAPEFRUIT JUICE, NFS	Carb bevs/juices
61201020	GRAPEFRUIT JUICE, UNSWEETENED, NS AS TO FORM	Carb bevs/juices
61201220	GRAPEFRUIT JUICE, CANNED, BOTTLED, CARTON, UNSWEET	Carb bevs/juices
61201230	GRAPEFRUIT JUICE, CANNED, BOTTLED, CARTON, W/ SUGAR	Carb bevs/juices
61201240	GRAPEFRUIT JUICE, CANNED/BOTTLE/CARTON, W/ LOW CAL SWEETENER	Carb bevs/juices
61204000	LEMON JUICE, NS AS TO FORM	Carb bevs/juices
61204200	LEMON JUICE, CANNED OR BOTTLED	Carb bevs/juices
61207000	LIME JUICE, NS AS TO FORM	Carb bevs/juices
61207200	LIME JUICE, CANNED OR BOTTLED	Carb bevs/juices
61210000	ORANGE JUICE, NFS	Carb bevs/juices
61210220	ORANGE JUICE, CANNED/BOTTLED/CARTON, UNSWEETENED	Carb bevs/juices
61210230	ORANGE JUICE, CANNED/BOTTLED/CARTON, W/ SUGAR	Carb bevs/juices
61210250	ORANGE JUICE, W/ CALCIUM, CAN/BOTTLE/CARTON, UNSWEETENED	Carb bevs/juices
61213000	TANGERINE JUICE, NFS	Carb bevs/juices
61214000	GRAPE-TANGERINE-LEMON JUICE	Carb bevs/juices
61219000	ORANGE & BANANA JUICE	Carb bevs/juices
61219100	PINEAPPLE-ORANGE-BANANA JUICE	Carb bevs/juices
61219150	ORANGE-WHITE GRAPE-PEACH JUICE	Carb bevs/juices

61219650	APRICOT-ORANGE JUICE	Carb bevs/juices
61222000	PINEAPPLE-GRAPEFRUIT JUICE, NFS	Carb bevs/juices
61225000	PINEAPPLE-ORANGE JUICE, NFS	Carb bevs/juices
61226000	STRAWBERRY-BANANA-ORANGE JUICE	Carb bevs/juices
64100100	FRUIT JUICE, NFS (INCLUDE MIXED FRUIT JUICES)	Carb bevs/juices
64100110	FRUIT JUICE BLEND, 100% JUICE, W/ VITAMIN C	Carb bevs/juices
64100120	AMBROSIA JUICE (INCL KNUDSEN'S)	Carb bevs/juices
64101010	APPLE CIDER (INCLUDE CIDER, NFS)	Carb bevs/juices
64104010	APPLE JUICE	Carb bevs/juices
64104050	APPLE JUICE, W/ ADDED VITAMIN C	Carb bevs/juices
64104090	APPLE JUICE WITH ADDED VITAMIN C AND CALCIUM	Carb bevs/juices
64104150	APPLE-CHERRY JUICE	Carb bevs/juices
64104200	APPLE-PEAR JUICE	Carb bevs/juices
64104450	APPLE-RASPBERRY JUICE	Carb bevs/juices
64104500	APPLE-GRAPE JUICE	Carb bevs/juices
64104550	APPLE-GRAPE-RASPBERRY JUICE	Carb bevs/juices
64104600	BLACKBERRY JUICE (INCL BOYSENBERRY JUICE)	Carb bevs/juices
64105400	CRANBERRY JUICE, UNSWEETENED	Carb bevs/juices
64105500	CRANBERRY-WHITE GRAPE JUICE MIXTURE, UNSWEETENED	Carb bevs/juices
64116010	GRAPE JUICE, NS AS TO ADDED SWEETENER	Carb bevs/juices
64116020	GRAPE JUICE, UNSWEETENED	Carb bevs/juices
64116030	GRAPE JUICE, W/ SUGAR	Carb bevs/juices
64116040	GRAPE JUICE, LOW CALORIE SWEETENER	Carb bevs/juices
64116050	GRAPE JUICE, NS AS TO SWEETENED, W/ ADDED VITAMIN C	Carb bevs/juices
64116100	GRAPE JUICE, UNSWEETENED, W/ ADDED VITAMIN C	Carb bevs/juices
64116150	GRAPE JUICE, W/ SUGAR, W/ ADDED VITAMIN C	Carb bevs/juices
64120010	PAPAYA JUICE	Carb bevs/juices

64121000	PASSION FRUIT JUICE	Carb bevs/juices
64122030	PEACH JUICE, W/ SUGAR	Carb bevs/juices
64123000	PEAR-WHITE-GRAPE-PASSION FRUIT JUICE, W/ADDED VIT C	Carb bevs/juices
64124010	PINEAPPLE JUICE, NS AS TO SWEETENED	Carb bevs/juices
64124020	PINEAPPLE JUICE, UNSWEETENED	Carb bevs/juices
64124030	PINEAPPLE JUICE, W/ SUGAR	Carb bevs/juices
64124060	PINEAPPLE JUICE, UNSWEETENED, W/ VIT C	Carb bevs/juices
64124200	PINEAPPLE-APPLE-GUAVA JUICE, W/ ADDED VITAMIN C	Carb bevs/juices
64125000	PINEAPPLE JUICE-NON-CITRUS JUICE BLEND, UNSWEETENED	Carb bevs/juices
64132010	PRUNE JUICE, NS AS TO ADDED SWEETENER	Carb bevs/juices
64132020	PRUNE JUICE, UNSWEETENED	Carb bevs/juices
64132030	PRUNE JUICE, W/ SUGAR	Carb bevs/juices
64132500	STRAWBERRY JUICE	Carb bevs/juices
64133100	WATERMELON JUICE	Carb bevs/juices
64134000	FRUIT SMOOTHIE DRINK, W/ FRUIT ONLY	Carb bevs/juices
64200100	FRUIT NECTAR, NFS	Carb bevs/juices
64201010	APRICOT NECTAR	Carb bevs/juices
64201500	BANANA NECTAR	Carb bevs/juices
64202010	CANTALOUPE NECTAR	Carb bevs/juices
64203020	GUAVA NECTAR	Carb bevs/juices
64204010	MANGO NECTAR	Carb bevs/juices
64205010	PEACH NECTAR	Carb bevs/juices
64210010	PAPAYA NECTAR	Carb bevs/juices
64213010	PASSION FRUIT NECTAR	Carb bevs/juices
64215010	PEAR NECTAR	Carb bevs/juices
64221010	SOURSOP (GUANABANA) NECTAR	Carb bevs/juices
73105010	CARROT JUICE	Carb bevs/juices

74301100	TOMATO JUICE	Carb bevs/juices
74301150	TOMATO JUICE, LOW SODIUM	Carb bevs/juices
74302000	TOMATO JUICE COCKTAIL	Carb bevs/juices
74303000	TOMATO & VEGETABLE JUICE, MOSTLY TOMATO (INCL V-8)	Carb bevs/juices
74303100	TOMATO & VEGETABLE JUICE, MOSTLY TOMATO, LOW SODIUM	Carb bevs/juices
74304000	TOMATO JUICE W/ CLAM OR BEEF JUICE	Carb bevs/juices
75132000	MIXED VEGETABLE JUICE (OTHER THAN TOMATO)	Carb bevs/juices
75132100	CELERY JUICE	Carb bevs/juices
75200700	ALOE VERA JUICE	Carb bevs/juices
92400000	SOFT DRINK, NFS	Carb bevs/juices
92400100	SOFT DRINK, NFS, SUGAR-FREE	Carb bevs/juices
92410110	CARBONATED WATER, SWEETENED (INCL TONIC, QUININE WATER)	Carb bevs/juices
92410210	CARBONATED WATER, UNSWEETENED (INCL CLUB SODA)	Carb bevs/juices
92410250	CARBONATED WATER, SUGAR-FREE	Carb bevs/juices
92410300	SOFT DRINK, COLA, W/ HIGHER CAFFEINE, SUGAR-FREE (INCL JOLT)	Carb bevs/juices
92410310	SOFT DRINK, COLA-TYPE	Carb bevs/juices
92410320	SOFT DRINK, COLA-TYPE, SUGAR-FREE	Carb bevs/juices
92410330	SOFT DRINK, COLA-TYPE, W/ HIGHER CAFFEINE (INCL JOLT)	Carb bevs/juices
92410340	SOFT DRINK, COLA-TYPE, DECAFFEINATED	Carb bevs/juices
92410350	SOFT DRINK, COLA-TYPE, DECAFFEINATED, SUGAR-FREE	Carb bevs/juices
92410360	SOFT DRINK, PEPPER-TYPE (INCL DR. PEPPER, MR. PIBB)	Carb bevs/juices
92410370	SOFT DRINK, PEPPER-TYPE, SUGAR-FREE	Carb bevs/juices
92410390	SOFT DRINK, PEPPER-TYPE, DECAFFEINATED	Carb bevs/juices
92410400	SOFT DRINK, PEPPER-TYPE, DECAFFEINATED, SUGAR-FREE	Carb bevs/juices
92410410	CREAM SODA	Carb bevs/juices
92410420	CREAM SODA, SUGAR-FREE	Carb bevs/juices
92410510	SOFT DRINK, FRUIT-FLAVORED, CAFFEINE FREE	Carb bevs/juices

92410520	SOFT DRINK, FRUIT-FLAV, SUGAR-FREE, CAFFEINE FREE	Carb bevs/juices
92410550	SOFT DRINK, FRUIT-FLAVORED, W/ CAFFEINE	Carb bevs/juices
92410560	SOFT DRINK, FRUIT-FLAVORED, W/ CAFFEINE, SUGAR-FREE	Carb bevs/juices
92410610	GINGER ALE	Carb bevs/juices
92410620	GINGERALE, SUGAR-FREE	Carb bevs/juices
92410710	ROOT BEER	Carb bevs/juices
92410720	ROOT BEER, SUGAR-FREE	Carb bevs/juices
92410810	CHOCOLATE-FLAVORED SODA	Carb bevs/juices
92410820	CHOCOLATE-FLAVORED SODA, SUGAR-FREE	Carb bevs/juices
92411510	COLA W/ FRUIT OR VANILLA FLAVOR	Carb bevs/juices
92411520	COLA W/ CHOCOLATE FLAVOR	Carb bevs/juices
92411610	COLA W/ FRUIT OR VANILLA FLAVOR, SUGAR-FREE	Carb bevs/juices
92411620	COLA W/ CHOC FLAVOR, SUGAR FREE	Carb bevs/juices
92416010	MAVI DRINK	Carb bevs/juices
92417010	SOFT DRINK, ALE TYPE (INCLUDE ALE-8)	Carb bevs/juices
92431000	CARBONATED JUICE DRINK, NS AS TO TYPE OF JUICE	Carb bevs/juices
92432000	CARBONATED CITRUS JUICE DRINK	Carb bevs/juices
92433000	CARBONATED NONCITRUS JUICE DRINK	Carb bevs/juices
92510110	APPLE DRINK	Carb bevs/juices
92510120	APPLE-CHERRY DRINK	Carb bevs/juices
92510150	APPLE JUICE DRINK	Carb bevs/juices
92510170	APPLE-CRANBERRY-GRAPE JUICE DRINK	Carb bevs/juices
92510200	APPLE-ORANGE-PINEAPPLE JUICE DRINK	Carb bevs/juices
92510220	APRICOT-PINEAPPLE JUICE DRINK	Carb bevs/juices
92510310	BANANA-ORANGE DRINK	Carb bevs/juices
92510410	BLACK CHERRY DRINK	Carb bevs/juices
92510610	FRUIT DRINK (INCLUDE FRUIT PUNCH & FRUIT ADE)	Carb bevs/juices

92510630	FRUIT JUICE DRINK, NFS	Carb bevs/juices
92510650	TAMARIND DRINK, P.R. (REFRESCO DE TAMARINDO)	Carb bevs/juices
92510720	FRUIT PUNCH, MADE W/ FRUIT JUICE & SODA	Carb bevs/juices
92510730	FRUIT PUNCH, MADE W/ SODA, FRUIT JUICE & SHERBET	Carb bevs/juices
92510810	GRAPEADE & GRAPE DRINK	Carb bevs/juices
92510820	GRAPE JUICE DRINK	Carb bevs/juices
92510910	GRAPEFRUIT JUICE DRINK	Carb bevs/juices
92510950	GUAVA JUICE DRINK	Carb bevs/juices
92511010	LEMONADE	Carb bevs/juices
92511020	LEMON-LIMEADE	Carb bevs/juices
92511110	LIMEADE	Carb bevs/juices
92511190	ORANGE JUICE DRINK	Carb bevs/juices
92511200	ORANGE-MANGO JUICE DRINK	Carb bevs/juices
92511220	ORANGE DRINK (INCLUDE ORANGE ADE, YABA DABA DEW)	Carb bevs/juices
92511230	ORANGE-APRICOT JUICE DRINK	Carb bevs/juices
92511240	ORANGE-LEMON DRINK	Carb bevs/juices
92511250	CITRUS FRUIT JUICE DRINK (INCL 5-ALIVE)	Carb bevs/juices
92511260	ORANGE-CRANBERRY JUICE DRINK	Carb bevs/juices
92511270	ORANGE-PEACH JUICE DRINK	Carb bevs/juices
92511280	ORANGE-GRAPE-BANANA JUICE DRINK	Carb bevs/juices
92511290	PAPAYA JUICE DRINK	Carb bevs/juices
92511310	PINEAPPLE-GRAPEFRUIT JUICE DRINK	Carb bevs/juices
92511340	PINEAPPLE-ORANGE JUICE DRINK	Carb bevs/juices
92511350	ORANGE-RASPBERRY JUICE DRINK	Carb bevs/juices
92511400	RASPBERRY-FLAVORED DRINK	Carb bevs/juices
92511510	STRAWBERRY-FLAVORED DRINK	Carb bevs/juices
92512090	PINA COLADA, NONALCOHOLIC	Carb bevs/juices

92512110	WHISKEY SOUR, NONALCOHOLIC (INCL LEMIX)	Carb bevs/juices
92520410	FRUIT DRINK, LOW CALORIE	Carb bevs/juices
92520810	GRAPE DRINK, LOW CALORIE	Carb bevs/juices
92520910	LEMONADE, LOW CALORIE	Carb bevs/juices
92530110	APPLE DRINK W/ VITAMIN C ADDED	Carb bevs/juices
92530210	BLACK CHERRY DRINK W/ VITAMIN C ADDED	Carb bevs/juices
92530310	CHERRY DRINK W/ VITAMIN C ADDED	Carb bevs/juices
92530410	CITRUS DRINK W/ VITAMIN C ADDED	Carb bevs/juices
92530510	CRANBERRY JUICE DRINK W/VIT C ADDED(INCL COCKTAIL)	Carb bevs/juices
92530520	CRANBERRY-APPLE JUICE DRINK W/ VITAMIN C ADDED	Carb bevs/juices
92530610	FRUIT PUNCH/DRINK/ADE W/ VIT C ADDED (INCL HI-C)	Carb bevs/juices
92530710	GRAPE DRINK W/ VITAMIN C ADDED	Carb bevs/juices
92530810	GRAPEFRUIT JUICE DRINK W/ VITAMIN C ADDED	Carb bevs/juices
92530840	GUAVA JUICE DRINK W/ VIT C ADDED	Carb bevs/juices
92530910	LEMONADE W/ VITAMIN C ADDED	Carb bevs/juices
92530950	VEGETABLE & FRUIT JUICE DRINK, W/ VIT C	Carb bevs/juices
92531010	ORANGE DRINK & ORANGEADE W/ VITAMIN C ADDED	Carb bevs/juices
92531030	ORANGE BREAKFAST DRINK	Carb bevs/juices
92531110	PINEAPPLE-GRAPEFRUIT JUICE DRINK W/ VIT C ADDED	Carb bevs/juices
92531120	PINEAPPLE-ORANGE JUICE DRINK W/ VITAMIN C ADDED	Carb bevs/juices
92531150	PINEAPPLE-ORANGE-GRAPEFRUIT JUICE DRINK W/VITAMIN C	Carb bevs/juices
92531210	STRAWBERRY-FLAVORED DRINK W/ VITAMIN C ADDED	Carb bevs/juices
92541010	FRUIT-FLAVORED DRINK, FROM SWEETENED PWDR, FORTIFIED W/ VIT C	Carb bevs/juices
92541100	APPLE CIDER DRINK, FROM MIX, SUGAR & VIT C ADDED	Carb bevs/juices
92541120	APPLE CIDER DRINK, FROM MIX, LOW CAL, VIT C ADDED	Carb bevs/juices
92550050	APPLE-WHITE GRAPE JUICE DRINK, LOW CAL, W/VIT C ADDED	Carb bevs/juices
92550110	CRANBERRY JUICE COCKTAIL, LO CAL, W/ VIT C ADDED	Carb bevs/juices

92550210	CRANBERRY-APPLE JUICE DRINK, LO CAL, VIT C ADDED	Carb bevs/juices
92550300	GRAPEFRUIT JUICE DRINK, LOW CALORIE, W/ VITAMIN C	Carb bevs/juices
92550610	FRUIT-FLAVORED DRINK, LOW CAL, W/ VITAMIN C ADDED	Carb bevs/juices
92551600	CITRUS JUICE DRINK, LOW CALORIE	Carb bevs/juices
92551700	JUICE DRINK, LOW CALORIE	Carb bevs/juices
92552000	FRUIT-FLAV DRINK, FROM MIX, HI VIT C ADDED, LOW CAL	Carb bevs/juices
92552050	ORANGE BREAKFAST DRINK, LOW CALORIE	Carb bevs/juices
92552100	ORANGE-CRANBERRY JUICE DRINK, LOW CAL, W/ VIT C ADDED	Carb bevs/juices
92553000	FRUIT-FLAVORED THIRST QUENCHER BEVERAGE, LOW CAL	Carb bevs/juices
92560000	FRUIT-FLAVORED THIRST QUENCHER BEVERAGE	Carb bevs/juices
92570100	FLUID REPLACEMNT, ELECTROLYTE SOLUTN (INCL PEDIALYTE)	Carb bevs/juices
92570500	FLUID REPLACEMENT, 5% GLUCOSE IN WATER	Carb bevs/juices
92582000	FRUIT-FLAVORED DRINK, LOW CALORIE, CALCIUM-FORTIFIED	Carb bevs/juices
92582050	FRUIT-FLAVORED DRINK, VITAMIN & MINERAL FORTIFIED	Carb bevs/juices
92582100	CITRUS JUICE DRINK, CALCIUM FORTIFIED	Carb bevs/juices
92582110	ORANGE BREAKFAST DRINK, CALCIUM FORTIFIED	Carb bevs/juices

Appendix E

GRAS Expert Panel Critical Evaluation of Tea Catechin

Appendix F

Curricula Vitae of Expert Panel Members

Appendix G

Japan Ministry of Health, Labor and Welfare, *Permit for Labeling a Product as a Food for Specified Health Use* (green tea and oolong tea)

Appendix H

Safety Information on Tannase

I. Identity and Regulatory Status

Tannase is an acylhydrolase enzyme preparation from *Aspergillus oryzae* that can be used as a processing aid for manufacture of cold water-soluble tea beverages.

A. Enzyme Commission Number

Tannase is tannin acylhydrolase, **EC 3.1.1.20**
(http://www.chem.qmul.ac.uk/iubmb/enzyme/index.html)

Common name:	tannase
Reaction:	digallate + H_2O = 2 gallate
Other name(s):	tannase S; tannin acetylhydrolase
Comments:	Also hydrolyzes ester links in other tannins
Links to other databases:	BRENDA, EXPASY, KEGG, ERGO
CAS Registry number:	9025-71-2
References:	Dyckerhoff, H., and Armbruster, R. 1933. Zur Kenntnis der Tannase. *Hoppe-Seyler's Z. Physiol. Chem.* 219:38-56.

B. CODEX

Tannase is listed in **CODEX** Inventory of Processing Aids
/download/standards/11/CSA_003e.pdf

C. U.S. and Japan

- Tannase is approved for food additive use in Japan (Food additive No. 263)
- Self-GRAS in the U.S. (Lipton/Unilever) (This information obtained from tannase manufacturer -- Kikkoman)

  The published pivotal study to support self-GRAS: Lane, R.W. 1997. Safety evaluation of tannase enzyme preparation derived from *Aspergillus oryzae. Food Chem. Toxicol.* 35:207-212.

D. Other Information

Tannase (manufactured by Kikkoman) is approved as Kosher by the Union of Orthodox Jewish Congregations of America

II. Prior Use of Tannase and *Aspergillus oryzae*

A. Prior use of Tannase

Tannase originally was used in Japan to produce commercial quantities of gallic acid from tannic acid. Tannase also has been used to remove gallic acid from the polyphenols in tea, resulting in beverage that is cold water-soluble. Tannase is widely used in the U.S. and other countries.

Following is a list of tannase manufacturers:

- Wakko Laboratory Chemicals (US, Japan) http://www.wakousa.com/bioproducts/index.html
- Kikkoman (US, Japan) http://www.kokkoman-usa.com/
- Julich Fine Chemicals (Germany) http://www.juelich-chemicals.de/public/default.cfm
- Great Western Chemical Co. (US) http://www.dashinet.com/chemicals/Ads/greatwestern/greatwestern.html
- Enzyme Development Corporation (US) http://www.enzyme development.com
- SANKYO LIFETECH COL., LTD. (Japan) http://sankyotokyhin.net/fdi/syokuhin/list.html

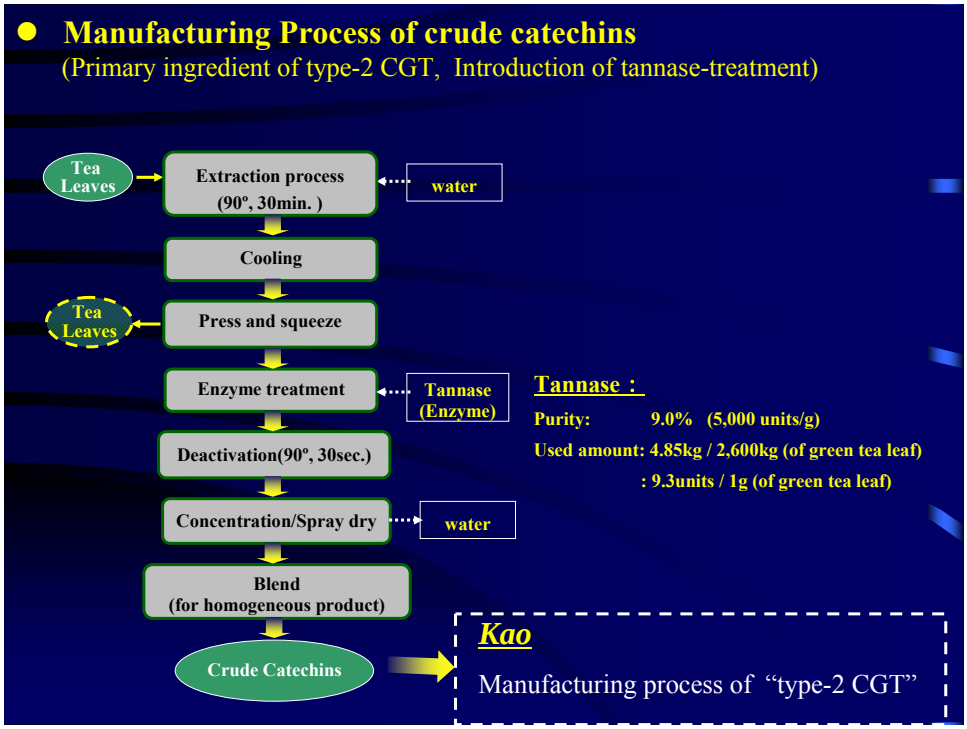
Kao is considering the introduction of tannase manufactured by Kikkoman. Kokkoman has sold tannase in the U.S. for 10 years (several tens of metric tons of tannase per year).

B. Prior Use of *Aspergillus oryzae*

- *Aspergillus oryzaei* is a fungus that has been used extensively in producing foods in Japan and the Far East for perhaps 2000 years.
- *A. oryzae* is used to make α-amylase for flours in the U.S. (21 C.F.R. §§ 137.105, 200).
- *A. oryzae* is used to make enzymes for baking and brewing in Europe.
- Barbesgaard et al. (1992) determined that *A. oryzaei* is not pathogenic or toxigenic to humans.
- The particular strain of *A. oryzaei* used to produce tannase is morphologically equivalent to other strains and is genetically stable.
- The tannase strain (Kikkoman Corporation) was originally obtained from a Japanese Koji manufacturer in 1944 (Sakaguchi and Yamada 1944).

III. Manufacturing Process

Figure 1. Manufacturing Process Using Tannase



IV. Toxicology Study (Pivotal Study to Support Self-GRAS (Lane 1997)

A 91-day oral toxicity study in the rat and gene mutation study in *Salmonella typhimurium* were performed to establish the safety of the enzyme preparation for the consumer. General toxicity was low, and no adverse effects were observed at the highest dose tested (1% in the diet).

Table 1. Daily intake of Tannase in Subchronic Feeding Study

Sex	Conc. tannase in diet (%)	Total food consumption (mean, g)	Total tannase consumption (mean, mg)	Body weight (mean for study, g)	Daily tannase dose (mg/kg/day)	Daily tannase dose (units/kg/day)
M	0	1324	0	232	0	
	0.01	1277	128	228	6.2	
	0.1	1311	1310	231	62.3	
	1.0	1280	12,800	226	622	**31,546** ↓ **(1,577,330 units/day)** **≥50 kg body weight**
F	0	939	0	146	0	
	0.01	921	92	145	7.0	
	0.1	951	951	146	71.6	
	1.0	933	9330	146	702	**35,604**

In Kao's manufacturing process for its catechins from green tea, residual tannase will be removed during the extraction process. If, however, all residual tannase is not removed, the residual amount that could be expected to remain in the finished beverage is 20 units/bottle (calculated by amount of tannase used, and assuming one bottle of sports drink contains 500 mL).

Aspergillus oryzae exhibited no evidence of mutagenic potential with or without metabolic activation. These results, together with knowledge of the production organism and the chemical and microbiological characterization of the enzyme preparation, indicate that tannase can be regarded as safe for its intended use in processing tea.

V. Typical Product Characteristics

Tannase treatment removes gallic acid from gallate type catechins (mainly EGCG). In the current process, the weight percent of EGCG decreased from 26% to 19.8% and the weight percent of gallic acid increased from 0.34% to 2.73% (*see* Table 2 below). This gallic acid level is almost the same level as present in Pu-erh Tea (Chinese Tea, Table 3).

Table 2. Typical Tannase Product Characteristics

Catechin	Type-1 CGT (%)	Type-2 CGT (%)	Type-2 CGT (Tannase Treated) (%)
Total Catechin Monomers	**44.4**	**64.8**	**62.3**
Gallocatechins	2.4	4.8	5.9
Epigallocatechin	13.2	18.7	20.5
Catechin	0.9	2.0	1.9
Epicatechin	3.0	4.9	6.2
Epigallocatechin gallate (EGCG)	19.4	26.0	19.8
Gallocatechin gallate (GCG)	0.5	1.4	2.5
Epicatechin gallate (ECG)	4.6	7.4	5.0
Catechin gallate	0.4	0.6	0.6
Gallic acid	—	0.34	2.73

Table 3. Gallic Acid Content of Tea Products

Reference	Gallic Acid Content of Green Tea (%)	Gallic Acid Content of Black Tea (%)	Gallic Acid Content of Pu-erh Tea (%)
Lin 1998	0.23 (0.1–1.23)	1.83	1.49 (0.78–2.50)
Araki and Matsuda 2002	—	1	3

VI. References Cited

Araki, Y., and Matsuda, M. 2002. *Kocha-no-Jiten*. Page 197 (Japanese).

Barbesgaard, P., et al. 1992. On the safety of *Aspergillus oryzae*: a review. *Appl. Microbiol. Biotechnol.* 36(5):569-572.

Lane, R.W. 1997. Safety evaluation of tannase enzyme preparation derived from *Aspergillus oryzae*. *Food Chem. Toxicol*. 35:207-212.

Lin-J.-K. 1998. Survey of catechins, gallic acid, and methylxanthines in green, oolong, Pu-erh, and black teas. *J. Agric. Food Chem*. 46(9):3635-3642.

Sakaguchi, K., and K. Yamada. 1944. On the morphology and classification of *Aspergilli* (I). Nippon Nogeikagaku Kaishi 20:65–73. (Japanese.)

Appendix I

Representative Batch Analysis Data for Three Lots of CGT

Representative Lots (Type-1 CGT)
Analysis data for 3 lots of Type-1 CGT (Lot: 0404131T, Lot: 0406291T, Lot: 0406031K)

Catechins and Polyphenol	**Type-1 CGT (Lot: 0404131T)**	**Type-1 CGT (Lot: 0406291T)**	**Type-1 CGT (Lot: 0406031K)**
Total Catechin Monomers	42%	39%	43%
Polyphenols	50%	48%	50%
Other components*			
Caffeine	3.8%	3.5%	2.3%
Organic acids	7.1%	7.4%	7.6%
Protein and Amino Acids	5.9%	5.6%	5.9%
Saccharide	9.5%	9.6%	9.7%
Fiber	< 0.1%	< 0.1%	< 0.1 %
Fat	< 0.1%	< 0.1 %	< 0.1 %
Ash	2.4%	3.0%	2.3%
Catechin Profile			
(% of Total Catechin Monomers)			
Gallocatechin (GC)	7.1 %	7.4 %	7.6 %
Epigallocatechin (EGC)	30 %	29.2 %	30.0 %
Catechin (C)	2.1 %	2.3 %	2.8 %
Epicatechin (EC)	8.8 %	7.9 %	7.7 %
Epicatechin gallate (ECG)	12.1 %	11.0 %	10.7 %
Epigallocatechin gallate (EGCG)	39.0 %	39.5 %	37.4 %
Gallocatechin gallate (GCG)	1.9 %	2.3 %	2.1 %
Catechin gallate (CG)	1.0 %	1.0 %	1.0 %

*In addition to the "other components" listed above, CGT may contain the following minor components:

1. Free amino acids: phosphoserine, taurine, phosphoethanolamine, urea, aspartic acid, hydroxproline, threonine, serine, glutamic acid, glutamine, proline, glycine, alanine, citrulline, α-amino-n-butyric acid, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, β-alanine, β-amino-i-butyric acid, γ-amino-n-butyric acid, ethanolamine, hydroxylysine, ornithine, tryptophan, lysine, histidine, anserine, arginine, etc.
2. Minerals: Na, K Ca, Mg, Mn, Fe, AL, Cu, CL, Zn, P, etc.
3. Flavonol components, such as other minor catechins not included in the main 8 catechin monomers listed above).

Representative Lots – Tannase-treated Type-2 CGT
Analysis data for 3 lots of Type-2 CGT
(Lot: 0512231C, Lot: 0601281C, Lot: 606181C)

Catechins and Polyphenol	**Tannase-treated Type-2 CGT (Lot: 0512231C)**	**Tannase-treated Type-2 CGT (Lot: 0601281C)**	**Tannase-treated Type-2 CGT (Lot: 0606181C)**
Total Catechin Monomers	62%	62%	63%
Polyphenols	69%	69%	71%
Other components*			
Caffeine	1.5%	1.5%	1.3%
Organic acids	1.6%	1.8%	2.1%
Protein and Amino Acids	3.7%	4.4%	3.8%
Saccharide	7.3%	7.3%	6.8%
Fiber	< 0.1%	< 0.1%	< 0.1%
Fat	< 0.1%	< 0.1 %	< 0.1%
Ash	1.2%	1.6%	1.7%
Catechin Profile			
(% of Total Catechin Monomers)			
Gallocatechin (GC)	9.5 %	8.2 %	7.6 %
Epigallocatechin (EGC)	33.1 %	33.9 %	33.3 %
Catechin (C)	3.1 %	3.1 %	3.0 %
Epicatechin (EC)	10 %	9.2 %	8.7 %
Epicatechin gallate (ECG)	8.1 %	8.5 %	9.0 %
Epigallocatechin gallate (EGCG)	31.9 %	33.5 %	34.9 %
Gallocatechin gallate (GCG)	4.0 %	2.6 %	1.9%
Catechin gallate (CG)	1.0 %	1.0 %	0.8 %

*In addition to the "other components" listed above, CGT may contain the following minor components:

1. Free amino acids: phosphoserine, taurine, phosphoethanolamine, urea, aspartic acid, hydroxproline, threonine, serine, glutamic acid, glutamine, proline, glycine, alanine, citrulline, α-amino-n-butyric acid, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, β-alanine, β-amino-i-butyric acid, γ-amino-n-butyric acid, ethanolamine, hydroxylysine, ornithine, tryptophan, lysine, histidine, anserine, arginine, etc.
2. Minerals: Na, K Ca, Mg, Mn, Fe, AL, Cu, CL, Zn, P, etc.
3. Flavonol components, such as other minor catechins not included in the main 8 catechin monomers listed above).

Representative Lots – Catechin Content
Analysis data for 3 lots: Type-1 CGT, Type-2 CGT, and Tannase-treated Type-2 CGT
(Lot: 0308291T, Lot: 0502271X, Lot: 0512231C)

Catechins and Polyphenol	**Type-1 CGT (Lot: 0308291T)**	**Type-2 CGT (Non tannase-treated) (Lot: 0502271X)**	**Type-2 CGT (tannase-treated) (Lot: 0512231C)**
Total Catechin Monomers	44.4 %	64.8 %	62.3 %
Gallocatechin (GC)	2.4 %	4.8 %	5.9 %
Epigallocatechin (EGC)	13.2 %	18.7 %	20.5 %
Catechin (C)	0.9 %	2.0 %	1.9 %
Epicatechin (EC)	3.0 %	4.9 %	6.2 %
Epicatechin gallate (ECG)	4.6 %	7.4 %	5.0 %
Epigallocatechin gallate (EGCG)	19.4 %	26.0 %	19.8 %
Gallocatechin gallate (GCG)	0.5 %	1.4 %	2.5 %
Catechin gallate (CG)	0.4 %	0.6 %	0.6 %
Catechin Profile			
(% of Total Catechin Monomers)			
Gallocatechin (GC)	5.4 %	7.4 %	9.5 %
Epigallocatechin (EGC)	29.7 %	28.9 %	32.9 %
Catechin (C)	2.0 %	3.1 %	3.1 %
Epicatechin (EC)	6.8 %	7.6 %	9.9 %
Epicatechin gallate (ECG)	10.4 %	11.4 %	8.0 %
Epigallocatechin gallate (EGCG)	43.7 %	40.1 %	31.8 %
Gallocatechin gallate (GCG)	1.1 %	2.2 %	4.0 %
Catechin gallate (CG)	0.9 %	0.9 %	0.9 %

Appendix J

USP Statement Concerning Final Monograph on Decaffeinated Green Tea Extract; USP Final Monograph; Comments to USP Committee Submitted December 28, 2007

Appendix K

English translations of Japanese Citations

Appendix L

Studies on the Mechanisms of Action of Green Tea Catechins

Studies Investigating the Mechanisms of Action for Green Tea Catechins

Citation	Study Design	Results	Relevance to Kao GRAS Determination
He, Z., et al. 2008. Fyn is a novel target of (-)-epigallocatechin gallate in the inhibition of JB6 and CI14 cell transformation. *Mol. Carcinogen.* 47(3):172-83.	*In vitro* study of the role of Fyn in the regulation of cell transformation using knockout siRNA plasmid. siRNA was stably co-transfected into JB6 CI14 cells. Studies: • Cytotoxic effects of EGCG on JB6 Cells • Anchorage-independent transformation assay • *In vitro* pull-down assay • Purification of GST-Fyn-SH2 and GST-Fyn-SH3 fusion proteins • Determination of GST-Fyn binding affinity with EGCG • EGCG effects on Fyn kinase activity *in vitro* • Immunoprecipitation and kinase assays • Determination of AP-1, STAT1, and CREB DNA binding	• EGCG inhibits EGF-induced JB6 CI14 cell transformation in a dose-dependent manner • EGCG inhibits EGF-induced Fyn phosphorylation in a dose-dependent manner • EGCG specifically binds with the Fyn SH2 domain • EGF-induced phosphorylation of p38 MAP kinase (THr180/Tyr182), ATF-2 (Thr71), and STAT1 (Thr727) is inhibited in siRNA-Fyn-JB6 CI14 cells • siRNA-Fyn inhibits EGF-induced cell transformation • EGF-induced DNA binding ability of AP-1, CREB, and STAT1 is decreased in siRNA-Fyn-JB6 CI14 cells	This is a novel and important mechanism that may be involved in EGCG-induced inhibition of cell transformation
Devika, P.T., Stanley Mainzen Prince, P. 2008. (-) Epigallocatechin gallate (EGCG) prevents	Male albino Wistar rats weighing 140-160 g were pre-treated with EGCG (30 mg/kg) by gavage daily for a period of 21 days. ISO was then subcutaneously injected into rats at intervals of 24 hours for 2 days	• Pretreatment with EGCG significantly decreased the levels of TBARS and lipid hydroperoxides and increased the activities of SOD and catalase in the heart mitochondria • Pretreatment with EGCG significantly	Study confirms preventative effects of EGCG on isoproterenol-induced mitochondrial damage in experimentally

Citation	Study Design	Results	Relevance to Kao GRAS Determination
mitochondrial damage in isoproterenol-induced cardiac toxicity in albino Wistar rats: A transmission electron microscope and *in vitro* study. *Pharmacol. Res.* 57(5):351-357.	Studies: • Isolation of heart mitochondria • Estimation of lipid peroxidation products, antioxidants, mitochondrial enzymes, and protein content in heart mitochondria • TEM studies • *In vitro* studies on the effects of EGCG on scavenging, superoxide anion, and hydroxyl radicals.	increased the activities of GPx, GRx, GST, and GSH in the heart mitochondria • Pretreatment with EGCG significantly increased activities of ICDH, SDH, MDH, α-KGDH, NADH-dehydrogenase and cytochrome-x-oxidase • Pretreatment with EGCG resulted in mild separation of cristae without swelling and vacuolation • EGCG scavenges radicals *in vitro* in a concentration-dependent manner	induced myocardial infarction in Wistar rats
Jia, Y., Alayash, A.I.. 2008. Effects of (-)-epigallo-catechin gallate on the redox reactions of human hemoglobin. *Free Radic. Biol. Med.* [epub ahead of print]	*In vitro* study investigating the effects of EGCG on auto-oxidation, H_2O_2-induced oxidation and subsequent ferryl heme reduction kinetics in chemically cross-linked human Hb (DBBF), a diaspirin cross-linked human Hb that has been intensively investigated. Studies: • Hb auto-oxidation experiments • Ferryl hemoglobin formation and reduction • Sulf hemoglobin measurement • Kinetics of ferryl hemoglobin reduction	• EGCG appears to exhibit strong pro-oxidant effects on DBBF and unmodified HbA_0 • Considerable reduction in the rates of oxidation of DBBF in the presence of EGCG and SOD were seen • In the presence of EGCG, rates of oxidation of DBBF were increased by approximately 56 times, showing an accelerated oxidation process triggered by EGCG • EGCG is a highly potent reductant of ferryl Hb when compared with some iron chelators	DBBF (ferrous) is rapidly oxidized to the ferric form in the presence of EGCG relative to normal spontaneous oxidation of Hb. A balance between the pro and antioxidant properties of EGCG should be taken into account if EGCG is used in combination therapy with redox active

Citation	Study Design	Results	Relevance to Kao GRAS Determination
		• The level of the sulHb, thus the level of detectable ferryl Hb in the reaction, was significantly reduced as EGCG medium increased • It is physiologically relevant to measure the ferryl Hb reduction by EGCG at low pH to evaluate its protective capability against the pro-oxidant activity of modified and unmodified Hbs, especially when used as cell free blood substitute.	acellular Hbs.
Beretta, G., et al. 2008. Quenching of α,β-unsaturated aldehydes by green tea polyphenols: HPLC-ESI-MS/MS studies. *J. Pharm. Biomed. Anal*. [epub ahead of print]	*In vitro* study in human colon tumor cells exposed to butyrate, investigating whether EGCG was able to eliminate the formation of HNE (4-hydroxyl nonenol), an α,β-unsaturated aldehyde, by a quenching mechanism. Studies: • Consumption of HNE by catechins and green tea • Measurement of H_2O_2 • HPLC – DAD – ESI – MS Analysis • ESI – MS/MS • NMR analysis	• Under the physiological conditions of the lower intestinal tract, EGCG dose- and time-dependently quenched 50 μM HNE • HNE-trapping activity of EGCG is strictly pH-dependent • The reaction mixture of HNE and EGCG after 24-hr incubation did not show epoHNE or the formation of an adduction product between EGCG and epo-HEN, but only a dehydrated adduct between EGCG and HNE • The formation of a EGCG-HNE covalent adduct was established for HPLC-ESI-MS • EGCG can quench different types of	These results suggest that EGCG and green tea extract, besides the proposed mechanisms of chemoprevention that target multiple cell-signaling pathways that control cell proliferation and apoptosis in cancer cells, can also prevent protein carbonylation in the tumor tissue environment, depending on the pH of the medium

Citation	Study Design	Results	Relevance to Kao GRAS Determination
		α,β–unsaturated aldehydes • EGCG is the most active of the green tea catechins in HNE entrapping; only adducts between HNE and EGCG were detected in the green tea chromatogram, indicating that EGCG plays a primary role in HNE sequestering • EGCG's ability to quench HNE through formation of a compound with a highly stable C-C bond, a mechanism different from HNE quenching by histidine-containing di-peptides, which involves the C-N bond • In an aqueous environment, EGCG is present as a tautomeric pair. This equilibrium involves the carbanionic form of dissociated EGCG, with the negative charge equally distributed between positions C-6 and C-8	surrounding the tissue, the type of tumor, the stage of dysregulation of lipid peroxidation, and finally, the stage of carcinoma development.
Ehrnhoefer, D.E., et al. 2008. EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers.	*In vitro* study in molecular chaperone His-tagged αS protein demonstrating the modulation of αS and Aβ fibril formation pathway by EGCG using biochemical, biophysical, and cell biological methods. Studies: • Analyzed the effect of EGCG on αS	• EGCG stimulates αS oligomer assembly • EGCG binds directly to natively unfolded αS • EGCG directly binds to αS polypeptide main chains • EGCG prevents αS β–sheet formation	• EGCG efficiently inhibits fibrillogenesis of both α-synuclein and amyloid-β by directly binding to the natively

Citation		Results	Relevance to Kao
Nat. Struc. Mol. Biol. 15(6): 558-566.	aggregation using a Thioflavin T (Tht) binding assay • Investigated the effect of EGCG on αS fibrillogenesis by negative-stain EM • Analyzed the effect of EGCG on αS aggregation by size-exclusion chromatography • Determined whether EGCG directly associates with unfolded αS using a nitroblue tetrazolium (NBT) staining assay • Investigated whether EGCG also binds directly to both SDS-stable αS oligomers, examining time-resolved aggregation reactions using the NBT assays • Since αS and BSA proteins are both recognized by EGCG, determined individual binding using NMR techniques • Recorded two-dimensional ^{1}H and ^{15}N spectra of αS with different molar ratios of EGCG • Performed CD spectroscopy experiments with EGCG-treated and untreated αS protein • Carried out *in vitro* seeding experiments	• EGCG stimulates assembly of non-toxic, off-pathway oligomers • EGCG prevents amyloid-β fibrollogenesis	unfolded polypeptides and preventing their conversion into toxic, on-pathway aggregating intermediates • This suggests a generic effect on aggregation pathways in neurodegenerative diseases

Citation	Study Design	Results	Relevance to Kao GRAS Determination
	with performed αS fibrils and EGCG-stabilized oligomers • Tested whether amyloidogenic αS oligomers can be specifically detected with the conformation-specific antibody using time-resolved dot blot assays • Investigated EGCG-generated oligomers for mammalian cell toxicity using standardized 3-2,5-diphenyltetrazolium bromide reduction assays with PC12 cells • Incubated different [EGCG] with unpolymerized $A\beta_{42}$ polypeptide and monitored the formation of amyloid using the ThT assay		
Bandele, O.J., Osheroff, N. 2008. (-)-Epigallocatechin gallate, a major constituent of green tea, poisons human type II topoisom-erases. *Chem. Res. Toxicol*. 21(4): 936-943.	*In vitro* study in recombinant wt- human topoisomerase IIα and β expressed in *Saccharomyces cerevisiae* and purified to determine the effects of green tea extract and EGCG on DNA cleavage and ligation mediated by human topoisomerase IIα and β. <u>Studies</u>: • Ligation of cleaved plasmid DNA by human Topoisomerase II • DNA cleavage site utilization	• Enhancement of Topoisomerase II-mediated DNA cleavage by green tea extract • Enhancement of Topoisomerase II-mediated DNA cleavage by EGCG • EGCG is a redox-dependent Topoisomerase II poison	Results provide strong evidence that EGCG is a redox-dependent Topoisomerase II poison and utilizes a mechanism similar to that of 1,4-benzoquinone

Citation	Study Design	Results	Relevance to Kao GRAS Determination
Siu, A.W., et al. 2008. Glutamate-induced retinal lipid and protein damage: the protective effects of catechin. *Neurosci. Lett*. 432(3): 193-197.	*In vitro* study investigating the effects of catechin on the glutamate-treated retina. Porcine retinal homogenates were incubated with glutamate (20 nmol) at 37° for 60 min. Catechin was co-incubated with the glutamate-treated retina in the same condition. <u>Studies</u>: • MDA and protein levels were determined using the LPO586 and Bradford assays • Various amounts of catechin and the effect of trolox were compared • Retinal homogenates were incubated with glutamate or TRIS • After glutamate treatment, protein spots with significant changes were marked and excised from the two-dimensional gels	• Mean MDA increased significantly after the glutamate incubation in a dose-dependent manner • A significant difference exists between catechin and trolox • Bonferroni's multiple comparisons showed that all catechin concentrations reduced MDA significantly • Glutamate modifies the lipid and expressions of seven proteins in a homogenized receptor-free retinal system • Catechin protects the retinal tissue from glutamate-induced LPO and protein damage • Catechin significantly reverses the glutamate-mediated LPO and changes of pyruvate dehydrogenase, 5HT receptor, thioredoxin peroxidase, and peroxiredoxin 6 expressions	Study shows that (a) retinal glutamate toxicity is mediated by LPO and protein modification, and (b) catechin ameliorates the toxicity
Kawai, Y., et al. 2008. Galloylated catechins as potent inhibitors of hypochlorous acid-induced DNA	*In vitro* study screening HCL-60 cells with various phenolic compounds for their scavenging activity for chlorinating intermediates including HOCl and chloramines to determine if tea catechins are good inhibitors of DNA base	• Natural phenolic antioxidants for scavenging and/or reducing chlorinating intermediates *in vitro* - flavonoids including tea catechins and quercetin exhibit a strong inhibitory effect, especially ECg and EGCg	Results showed that green tea catechins, especially 3-galloylated catechins, may be the plausible candidates for the

Citation	Study Design	Results	Relevance to Kao GRAS Determination
damage. *Chem. Res. Toxicol*. 21(7):1407-1414.	chlorination. Studies: • Performed inhibition assay for the formation of chlorinated dC residues • Performed analysis of chlorinated catechin derivates • Performed analysis of ECg and chlorinated ECg in HL-60 cells • Performed HPLC-MS/MS analysis • Immunocytometric analysis of HOCl-modified DNA	• Four catechins inhibited the formation of 5-CldC in DNA in a dose-dependent manner. The inhibitory effect f ECg and EGCg was much stronger than that of the non-galloylated catechins • ECg and EGCg more efficiently inhibit the formation of the Cl_2dC residues in DNA • Catechins dose-dependently reduced N-CldC with the appearance of unmodified dC • ECg inhibits HOCl-derived cellular DNA damage • Pretreatment with different concentrations of ECg for 2 hours dependently inhibit the DNA chlorination in HL-60 cells	prevention of inflammation- derived DNA damage and perhaps carcinogenesis
Sukhthankar, M., et al. 2008. A green tea component suppresses posttranslational expression of basic fibroblast growth factor in colorectal cancer.	*In vitro* study examining posttranslational regulation of bFGF by EGCG in human colorectal cancer cells. bGFG in intestinal tumor formation of $APC^{Min/+}$ mice with and without catechin treatment was also examined. Studies: • RT-PCR and Western blot analysis on cell cultures	• Both EGCG and ECG completely suppressed bFGF protein expression • EGCG suppresses bFGF expression in LoVo cells in a time-dependent manner • All cells showed a response to EGCG, except MCF-7 breast cancer cells, suggesting that bFGF expression occurred in most of the cancer cells	Catechin compounds have fewer adverse affects than chemotherapeutic agents and hence can be used in cancer therapeutics to suppress growth and metastasis by

Citation	Study Design	Results	Relevance to Kao GRAS Determination
Gastroenterology. 134(7): 1972-1980.	• vFGF expression vector cloned, transfected, performed histidine pull-down experiment • Performed enzyme-linked immunosorbent assay for bFGF • Performed ubiquitin and 20S proteasome assay • Performed animal study (mice receive EGCG or ECG in drinking water for 2 months) • Immunostain for factor VIII	and that EGCG may reduce bFGF expression • Proteasome inhibition restores the suppression of bFGF by EGCG • Only lactacystin containing trypsin-like inhibitory property affects bFGF suppression by EGCG • Exogenous bFGF proteins respond to cycloheximide • EGCG may suppress intestinal tumorigenesis *in vivo* by reducing bFGF expression and angiogenesis	targeting proteins such as bFGF
Spina, M., et al. 2008. Mechanism of inhibition of wt-dihydrofolate reductase from *E. coli* by tea epigallocatechin-gallate. *Proteins*. 72(1):240-251.	*In vitro* study using wild-type DHFR from AG-1 strain of *E. coli*. This article reports a detailed kinetic and equilibrium study on the inhibition of DHFR by green tea EGCG, taking into account inhibitory effects. <u>Studies</u>: • DHFR assays and kinetic data analysis • Inhibition of dihydrofolate reductase by tea catechin • Biocensor studies • Molecular modeling of the DHFR/EGCG complex	• EGCG strongly inhibits the activity of wt-DHFR from *E. coli* • EGCG acts as a bisubstrate inhibitor: in fact, according to one of the tested mechanisms, it is possible for inhibitor (I) to bind to free enzyme, E-DHF complex (NADPH site) and/or E-NADPH complex (DHF site) • Biphasic association and dissociation time courses of EGCG binding to immobilized DHFR are reported: this behavior is ascribed to the possible ability of EGCG to bind both to folate and NADPH sites • EGCG association rate to DHFR at	Data confirmed the selectivity of antifolate compounds with respect to the different source of enzyme (bacterial or mammalian DHFR) and the possible role of tea catechins as chemopreventative agents.

Citation	Study Design	Results	Relevance to Kao GRAS Determination
		DHF site was about 30-fold higher in comparison with the association at NADPH site	
Coyle, C.H., et al. 2008. Antioxidant effects of green tea and its polyphenols on bladder cells. *Life Sci.* 83 (1-2):12-8.	*In vitro* study utilizing normal/malignant (high/low grade) human bladder uroepithelial cells to investigate the antioxidant properties of green tea extract (GTE), Polyphenon-60 (PP-60; 60% pure catechins), and two GTE components (EGC and EGCG) following oxidative stress with H_2O_2. Studies: • Assessment of cell viability using the Sigma *In Vitro* toxicology assay kit • Measure apoptosis using the Annexin V detection kit • Measure intracellular reactive oxygen species (ROS)	• PP-60, ECG and EGCG catechins protect human bladder cells from H_2O_2-induced oxidative stress • PP-60, EGC, and EGCG significantly improved the viability of UROtsa cells compared to H_2O_2-treated cells • ECG and EGCG catechins protect human bladder cells from H_2O_2-induced apoptosis • Catechins modulate apoptosis in normal human bladder cells via alteration of reactive oxygen species (ROS) signaling • H_2O_2 stimulates ROS production in normal human bladder cells	These findings demonstrate that urothelium cell death via H_2O_2-induced oxidative stress is mediated, in part, through superoxide (O_2-), and potentially, direct H_2O_2 mechanisms, suggesting that green tea polyphenols can protect against oxidative stress/damage and bladder cell death
Nishikawa, H., Kitani S. 2008. Tea catechins have dual effect on mast cell degranulation induced by compound 48/80. *Int.*	*In vitro* study to investigate catechin's effect on intracellular ROS generation and degranulation in mast cell activation. Studies: • Measure the intracellular free Ca^{2+} concentration • Measure degranulation by monitoring release of β–hexosaminidase from cells	• The compound 48/80 activates CM-MC mast cell • C48/80 increased intracellular DCF oxidation dose and time dependently • The intracellular ROS generation by NADPH oxidase is related with C48/80-induced degranulation from CM-MC.	There is an optimal level of ROS for degranulation, and catechins have a dual function by controlling the ROS levels.

Citation	Study Design	Results	Relevance to Kao GRAS Determination
Immunopharmacol. 8(9):1207-1215.	into the supernatant • Measure intracellular ROS generation using a fluorometric assay	• Catalase above 10 units/ml removed inhibitory effects by low ROS or the degranulation by high exogenous ROS and that there is a presence of optimal amount of ROS for mast cell activation	
Lee, S.I., et al. 2008. Effect of green tea and (-)-epigallocatechin gallate on ethanol-induced toxicity in HepG2 cells. *Phytother. Res.* 22(5):669-674.	*In vitro* study in HepG2 cells to compare EGCG, L-theanine, and caffeine for their protective effects against ethanol-induced cytotoxicity, using GGT as a marker. Studies: • GGT activity was assayed calorimetrically • Cell viability was assayed using MMT • Determined intracellular GSH content	• EGCG was found to inhibit the ethanol-induced cell death more efficiently than did green tea extract itself • GTE and EGCG inhibited the GGT increase dose-dependently while L-theanine had no significant effect and caffeine enhanced GGT release even more • EGCG, 4-MP, GSH, NAC, SAM, and acivicin failed to preserve intracellular GSH upon exposure to ethanol • EGCG and 4-MP lowered the GSH significantly, even in the absence of ethanol treatments	• The cyto-protective effects of green tea could be attributed to the inhibition of GGT activity by EGCG. • Study suggests that GGT inhibitors including EGCG may provide a novel strategy for attenuating ethanol-induced liver damage.
Oh, C.J., et al. 2008. Epigallocatechin gallate, a constituent of green tea, regulates high glucose-induced	*In vitro* study with U9 37 cells to investigate the effect of EGCG on high glucose-induced apoptosis. Cells were in two separate groups; pre-treated with 1 μM EGCG for 2 hours or not. They were then exposed to 35 mM glucose for 2 days	• ROS plays a pivotal role in apoptosis and reductants can block or delay this process • Cells pre-treated with 1 μM EGCG for 2 hours were significantly more resistant than un-treated cells to high	EGCG pre-treated cells showed significant suppression of apoptotic features such as DNA

Citation	Study Design	Results	Relevance to Kao GRAS Determination
apoptosis. *Arch. Pharm. Res.* 31(1):31-40.	Studies: • Immunoblot analysis • DAPI staining • DNA fragmentation using the diphenylamine assay • PI staining to determine the portion of apoptotic cells • Determine cellular reduction status using hydrogen peroxide • Oxidative DNA damage estimated using a fluorescent binding assay • Mitochondrial damage determined by mitochondrial membrane potential transition (MPT)	glucose-induced apoptosis • When cells were exposed to glucose, apoptotic cells were increased markedly in untreated cells as compared to EGCG-treated cells • The pre-treatment of EGCG resulted in a significantly lower intracellular level of H_2O_2 as compared to control • The decrease in the efficiency of GSH recycling may be responsible for the higher concentration of intracellular peroxides in control cells treated with high glucose • EGCG appears to protect cells from oxidative DNA damage caused by high glucose • High glucose-induced cleavage of procaspase-3 into the active form of caspase-3 and caspase-3 induces degradation of PARP or lamin B. The results indicate that EGCG exhibits a protective effect on the high glucose-induced apoptosis	fragmentation, damage to mitochondrial function, and modulation of apoptotic marker proteins upon exposure to high glucose. Study indicates that EGCG may play an important role in regulating the apoptosis induced by high glucose presumably through scavenging of reactive oxygen species

Citation	Study Design	Results	Relevance to Kao GRAS Determination
Erguder, I.B., et al. 2008. Effects of aqueous green tea extract on activities of DNA turn-over enzymes in cancerous and non-cancerous human gastric and colon tissues. *Altern. Ther. Health Med.* 14(3):30-33.	*In vitro* study to investigate possible effects of green tea extract on the activities of DNA turn-over enzymes, namely adenosine deaminase (ADA) and xanthine oxidase (XO) in gastric and colon tissues from patients with stomach and colon cancer. Studies: • Six cancerous and 6 non-cancerous adjacent human gastric tissues, and 7 cancerous and 7 non-cancerous adjacent colon tissues obtained surgically were treated with aqueous green tea extract at three different concentrations (0.05%, 0.5%, and 1.25%) for 1 hour and then ADA and XO levels were measured	• In both cancerous and non-cancerous tissue, XO activities were found to elevate in correlation with increased extract concentrations in both cancer types. ADA activity was found to decrease in the cancerous part of the stomach tissue and to increase in the non-cancerous part.	Green tea may support the medical treatment of stomach and colon cancer
Mukai, D., et al. 2008. Potential anthelmintics: polyphenols from the tea plant *Camellia sinensis L.* are lethally toxic to *Caenorhabditis elegans*. *Nat. Med. (Tokyo).* 62(2):155-159.	*In vitro* study to report the isolation and identification of a new tannin gallate, with known polyphenols, from the tea plant *Camellia sinensis* L. and their toxicities to *C. elegans*, with reference to novel anthelmintic relevancies. Studies: • Identify plant material, extraction, and isolation • Enzymatic hydrolysis of compound 8 • Prepare egg-bearing adults of *C. elegans* and determine LC_{50}	• Compound 8 consists of two flavan-3-ol units and one galloyl group • A diagnostic HMBC correlation between H-3' and carbonylic C-7'' was indicative of the presence of a C-3'-O-C-7'' linkage in 8 • Green tea polyphenols are toxic to *C. elegans*	These data shows that many green tea polyphenols may be potential anthelmintics

Citation	Study Design	Results	Relevance to Kao GRAS Determination
Hisamura, F., et al. 2008. Synergistic effect of green tea polyphenols on their protection against FK-506 inducted cytotoxicity in renal cells. *Am. J. Chin. Med.* 36(3): 615-624.	*In vitro* study to evaluate the synergistic interactions of tea polyphenols on FK506-induced cell death and ROS levels in LLC-PK1 renal cells Studies: • Cytotoxicity of FK506 measured by the MTT assay • Measured ROS formation using hydrogen peroxide • Measured caspase activity using CPP32/Caspase-3 colorimetric protease assay kit	• The effect of tea polyphenols on FK 506-induced cytotoxicity in LL-PK1 cells shows a dose-dependent protective effect for EGCG and EGC • CO-treatments with EGCG and EGC, EGCG or ECG, and EGC and ECG have strong synergistic effects on the protection of FK 506-induced cell death • EGCG, EGC, and the combined treatment of EGCG and EGC show significant suppression of the increased levels of intracellular ROS. Time-dependent effects • EGCG, EGC, and combined treatment of EGCG and EGC suppressed the level of caspase-3 activity in cells treated with FK 506	Synergistic effects of the constituents of green tea extract suggest that its protective effects may reside in more than just one of its constituents
Devika, P.T., Mainzen Prince, P.S. 2008. (-)-Epigallocatechin gallate EGCG prevents isoprenaline-induced cardiac marker enzymes and	*In vivo* study using male albino Wistar rats weighing 140-160 g. Different doses of EGCG (5, 10, 20, and 30 mg/kg) were dissolved in DMSO and administered by gavage at 7, 14, 21, and 28 days before isoprenaline administration to determine dose-dependence and duration of treatment against ISO-induced myocardial infarction.	• Pretreatment with EGCG significantly reduced the increase in heart weight • Pretreatment with EGCG significantly decreased the activities of enzymes in serum CK, CK-MB, LDH, AST, and ALT • Pretreatment of EGCG significantly increased activities of enzymes (CK, LDH, AST, and ALT) in the heart	EGCG exhibits beneficial effects on enzymes and electrolytes in the heart due to antioxidant and membrane-stabilizing effects

Citation	Study Design	Results	Relevance to Kao GRAS Determination
membrane-bound ATPases. *J. Pharm. Pharmacol*. 60(1):125-133.	Studies: • Assay of marker enzymes • Assay of Na+/K+ATPase • Assay of Ca2+ ATPase • Assay of Mg2+ ATPase • Estimation of Na+, K+, Ca2+ and protein concentration • Separation and quantification of LDH isoenzymes	• EGCG pretreatment decreased the intensity of LDH 1 and LDH 2 • Pretreatment with EGCG significantly increased the activity of Ca2+ and Mg2+-ATPases • Pretreatment with EGCG significantly decreased the levels of sodium and calcium and increased the levels of potassium in the heart	
Renno, W.M., et al. 2008. Effect of green tea on kidney tubules of diabetic rats. *Br. J. Nutr*. 6:1-8.	*In vivo* study examining the long-term effect of green tea extract on streptozoticin-induced diabetic nephropathy and on the glycogen accumulation in the kidney tubules. Male Sprague-Dawley rats were randomly assigned to normal control groups (2, 6, 8, and 12 weeks) and five diabetic groups (N=10) of comparable age. A GT diabetic group received 16% concentration of GT for 12 weeks post-diabetes induction as their sole source of drinking water. Studies: • Blood glucose levels determined • Blood samples taken by cardiac puncture and used for determination of blood urea nitrogen • Serum glycosylated protein level measured using method of McFarland et	• Body weights of diabetic rats treated with GT were significantly lower than the normal control group • Diabetic rats treated with GT showed significant reduction in both serum glucose and glycosylated protein • Blood urea nitrogen level was significantly lower • GT-treated group showed significant higher Ccr • Urinary urea nitrogen excretion was significantly lower • Creatine, glucose. and protein excretion declined • The number of glycogen-filled tubules decreased almost completely in the GT-treated kidneys	Results indicate that in STZ diabetes, kidney function appears to be improved with GT consumption which also prevents glycogen accumulation in the renal tubules, probably by lowering blood levels of glucose. Therefore, GT could be beneficial therapy in the management of diabetic nephropathy

Citation	Study Design	Results	Relevance to Kao GRAS Determination
	al. • Performed all experimental protocols and group treatments • Performed specimen collection and histological staining • Quantification of glycogen-filled proximal tubules		
Call, J.A., et al. 2008. Endurance capacity in maturing mdx mice is markedly enhanced by combined voluntary wheel running and green tea extract. *J. Appl. Physiol*. [epub ahead of print].	*In vivo* study using male mdx mice testing endurance exercise and GTE (0.5% added into diet) separately and in combination to increase endurance and antioxidant activities. Three hypothesis were tested: 3 weeks of endurance exercise initiated immediately post-weaning age (21 days) will improve skeletal function and serum antioxidant capacity, and decrease muscle lipid peroxidation; GTE supplementation without running will demonstrate similar effects; GTE supplementation in combination with endurance exercise will be more beneficial than either alone. <u>Studies</u>: • Conducted voluntary wheel running experiment • Tested excised muscles for isometric contractile and mechanical properties • Determine contractile protein contents	• GTE mice ran a total distance of 128% greater than controls • Absolute myosin content, total muscle protein relative to muscle mass, and total contractile protein relative to total muscle protein were all increased in GTE mice • 22% greater antioxidant capacity • Demonstrated 64% less lipid peroxidation in the gastrocnemius, and 29% less lipid peroxidation in the heart • Had 95% greater CS activity in the quadriceps muscles • 35% great CS activity in the heart muscle • β-oxidation activity was 36% greater in the quadriceps and 35% greater in the heart muscle	Data suggest that both endurance exercise and GTE may be beneficial therapeutic strategies to improve muscle function

Citation	Study Design	Results	Relevance to Kao GRAS Determination
	and myosin heavy chain (MyHC) isoform distribution • Determine creatine kinase activity and antioxidant capacity • Determine lipid peroxidation • Determine citrate synthase activity • Determine beta-hydroxyl acyl-CoA dehydrogenase (BHAD) activity		
Leong, H., et al. 2008. Inhibition of mammary tumorigenesis in the C3(1)/SV40 mouse model by green tea. *Breast Cancer Res. Treat.* 107 (3): 359-369.	*In vivo* study using C3(1)/SV40 mice in 3 groups receiving either 0.1%, 0.3%, or 0.5% Poly E in tap water every Monday, Wednesday, and Friday beginning at 6 weeks of age as sole source of drinking water (equivalent to daily consumption of six cups of green tea by the average adult human). Animals from each group were killed at 8, 12, 15, and 20 weeks of age. <u>Studies</u>: • Measured chemoprevention with administration of green tea polyphenols • Analyzed mammary glands • Performed an enzyme-linked immunosorbant assay	• Green tea alters the natural history and histopathology of mammary tumor function • Green tea inhibits mammary ductal growth • Green tea has anti-angiogenic effects	Data strongly supports potential use of green tea as a breast cancer chemopreventative agent

Citation	Study Design	Results	Relevance to Kao GRAS Determination
Hsu, C.H., et al. 2008. Effect of green tea extract on obese women: a randomized double-blind, placebo-controlled clinical trial. *Clin. Nutr*. 27(3): 363-370.	A randomized, double-blind, placebo-controlled, clinical trial from July 2006 to June 2007 in Taipei Hospital, Taiwan. Seventy-eight of 100 obese women aged between 16 and 60 years with BMI> 27 kg/m2 and who had not received any other weight control agent within the previous 3 months of the study. Subjects were randomly divided into Groups A and B. • Group A (n=41) received GTE • Group B (n= 37) took cellulose as a placebo • Took one capsule (400 mg) three times a day for 12 weeks • BW, BMI, and WC were measured at the beginning of the study and after 12 weeks of treatment • A hormone peptides analysis was also conducted	• There was no statistical difference in percent reduction in BW, BMI, and WC between the GTE and placebo groups after 12 weeks of treatment • After treatment, the GTE group revealed significant reduction in WC, and levels of LDL-cholesterol and triglyceride, and marked increase in the levels of HDL-cholesterol, adiponectin and ghrelin • The placebo group showed significant reduction in HC and triglyceride only, and marked increase in the level of ghrelin alone	This study shows no statistical difference in percent reduction in body weight, BMI, or waist circumference between the GTE and placebo groups after 12 weeks of treatment.
Gawande, S., et al. 2008. Effect of nutrient mixture and black grapes on the pharmacokinetics of orally administered (-)-epigallocatechin-3-galate from green	*In vivo* study to determine bioavailability of EGCG. Five human volunteers, were each given a single oral dose of GTE (A), nutrient mixture (NM) containing GTE (B), and formulation B along with black grapes 250 mg C0. Blood samples were drawn at 0, 2, 4, 6, and 8 hours.	• EGCG mean plasma levels at hour 2 show significant differences between Group A and Groups B & C. C_{max} for EGCG was reached in hour 4 of each treatment. At hour 6, EGCG levels were only significant for Group C • Mean values for AUC at 8 hours show significantly higher values for C	Supplementation of nutrient mixture normally prescribed to cancer patients containing ascorbic acid, selenium, N-acetyl cysteine and other nutrients

Citation	Study Design	Results	Relevance to Kao GRAS Determination
tea extract: a human study. *Phytother. Res*. 22 (6):802-808.	Studies: • Samples collected for initial processing • Extraction and separation of EGCG from plasma • Extraction and analysis of quercetin equivalents in plasma	compared to groups A and B • The percent increase in the availability of EGCG for B and C groups was 14% and 27% respectively • For plasma levels of quercetin equivalents, the AUC of group C was significantly higher than the AUC values of groups B and A. The percent increase in the availability of quercetin for groups B and C was 9% and 12% respectively.	(formulation B) resulted in an increase of the systemic availability of EGCG by 14%, with C giving an additional 13%.
Mnich, C.D., et al. 2008. Green tea extract reduces induction of p53 and apoptosis in UVB-irradiated human skin independent of posttranscriptional controls. *Exp. Dermatol*. [epub ahead of print].	*In vivo* study to investigate the photochemopreventative effect of low-dose GTE under everyday conditions, addressing questions like GTP stability and tachyphylaxis. The study irradiated up to 100 ml/cm^2 of UVB light skin patches that were pretreated with either OM24 – containing lotion or a placebo lotion (application was applied 3 x daily for 24 days; irradiated on days 5 and 33 from 10 mJ/cm^2 to 100 mJ/cm^2). Biopsies taken from both irradiated and un-irradiated skin for both immunohistochemistry and DNA microarray analysis	• The number of UV-induced p53-positive keratinocytes was significantly reduced in OM24 compared to placebo-pretreated samples by 31.9% on say 6, and 36.3% on day 34 • The level of UV-induced apoptosis was also reduced by OM24 pretreatment on days 6 and 34 • 213 were identified genes that undergo at least a 2-fold expression change upon UV-exposure after either OM24 or placebo-pretreatment • Both analytical approaches show that OM24 pretreatment does not result in any gene expression changes that are	Study suggests GTE as a suitable everyday photochemo-preventative agent.

Citation	Study Design	Results	Relevance to Kao GRAS Determination
	Studies: • Erythema quantification • Immunohistochemistry and quantification of biopsies • Histochemical quantification • Gene expression analysis	significantly different from placebo-pretreatment	
Lee, H.J., et al. 2008. Modification of gamma-radiation response in mice by green tea polyphenols. *Phytother Res*. [epub ahead of print].	*In vivo* study using 7-week old female ICR mice for the jejunal crypt survival and apoptosis assays; 7-week male ICR mice for the endogenous spleen colony formation experiment. Animals were irradiated with 12 Gy for the jejunal crypt survival assay, 6.5 Gy for endogenous spleen colony, and 2 Gy for experiment on apoptosis Studies: • Jejunal crypt: 66 female mice (6 per group) divided into a) untreated controls b) irradiated control c) GT/ GTPs/ catechins/ DDC administration in combination with radiation. Animals were sacrificed 3.5 days after irradiation • Endogenous spleen: 81 male mice (9 per group) divided into a) irradiated control, b) GT/ GTPs/ catechins / DDC administration in combination with radiation. Mice were sacrificed 9 days after irradiation. Spleens were then	• Only the pretreatment with GT, DDC, and ECG resulted in a significant increase in the number of surviving crypts • The number of spleen colonies was higher in the mice that received the GT, GTPs, and EC • Pretreatment of GT, DDC, or polyphenols was associated with decreases in the number of cells with nuclei positively stained for apoptosis compared with control group • GT administration prior to irradiation protected the jejunal crypt, increased the formation of endogenous colonies, and reduced the frequency of radiation-induced apoptosis	Each of the catechins was a much less effective radioprotector, suggesting that total extract or a mixture of GTPS may be more effective than individual catechins

Citation	Study Design	Results	Relevance to Kao GRAS Determination
	removed • Apoptosis experiment: 44 females (4 per group) divided into a) untreated control, b) irradiated control, c) GT/ GTPs/ catechins/ DDC administration in combination with radiation. Mice were sacrificed 6 hours after irradiation		

Legend:

5-CldC: Cationic lipid-DNA (non-coding) complexes are activators of the innate immune response that increase survival of rodents with some acute viral infections and cancers

α-KDGH: α- ketoglutarate dehydrogenase is a multienzymatic complex made up of three different types of enzymes, responsible for the conversion of α -ketoglutarate in to succinyl CoA. This is an important step in the citric acid cycle as it can be used to regulate energy production

ALT: Alanine Aminotransferase is found mainly in the liver, but also in smaller amounts in the kidneys, heart, muscles, and pancreas. ALT is measured to determine if the liver is damaged or diseased

AP-1: Activator protein 1; is a transcription factor which is a heterodimeric protein composed of proteins belonging to the c-Fos, c-Jun, ATF and JDP families. AP-1 upregulates transcription of genes containing the TPA response element

AST: Aspartate Aminotransferase is an enzyme in the blood. AST is normally found in red blood cells, liver, heart, muscle tissue, pancreas, and kidneys. When body tissue or an organ such as the heart or liver is diseased or damaged, additional AST is released into the bloodstream

BSA: Bovine serum albumin (BSA) is one of the most commonly used carriers for conjugation in antibody production. It belongs to the class of serum proteins called albumins, which make up about half of the protein in plasma and are the most stable and soluble proteins in plasma

Ccr: Cinnamoyl-coa reductase

CD: Circular dichroism (CD) spectroscopy measures differences in the absorption of left-handed polarized light versus right-handed polarized light which arise due to structural asymmetry

CK: Creatine kinase catalyses the conversion of creatine to phosphocreatine, consuming adenosine triphosphate (ATP) and generating adenosine diphosphate (ADP)

CK-MB: the CK enzyme consists of two subunits, either brain or muscle type. CK-MB is one of the three isoenzymes. The expression of genes for these subunits are located on different chromosomes: *B* on 14q32 and *M* on 19q13

CREB: cAMP response element-binding: proteins are transcription factors which bind to certain DNA sequences called *cAMP response elements* (CRE) and thereby increase or decrease the transcription of certain genes

CS: Chitin synthesis

DAPI: 4',6-diamidino-2-phenylindole is a fluorescent stain that binds strongly to DNA

DCF: dichlorofluorescin oxidation is used to measure the production of reactive oxygen species (ROS) within cells

DHFR: Dihydrofolate reductase reduces dihydrofolic acid to tetrahydrofolic acid, using NADPH as an electron donor

DMSO: Dimethyl sulfoxide is a colorless liquid that is an important polar aprotic solvent that dissolves both polar and nonpolar compounds

EGF: Epidermal growth factor

EM: Electron microscope

ESI – MS/MS: Electrospray ionization tandem mass spectrometry

GGT: Gamma-glutamyl transferase

GST: Glutathione S-transferase gene

Gy: the SI unit of absorbed radiation dose- One gray is the absorption of one joule of radiation energy by one kilogram of matter

HPLC – DAD – ESI – MS: High-performance liquid chromatography-diode array detector (HPLC-DAD) coupled with electron-spray mass spectrometry (ESI-MS) is used to detect flavonoid profiles

ICDH: Iso-Citrate Dehydrogenase; enzyme which participates in the citric acid cycle by catalyzing the third step

ICR: Imprinting Control Region mouse

LDH: Lactate dehydrogenase is an enzyme that catalyses the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+

LPO: Lipid peroxidation refers to the oxidative degradation of lipids; free radicals remove electrons from the lipids in cell membranes, resulting in cell damage

MDA: Malondialdehyde is a reactive species occurs naturally and is a marker for oxidative stress

MDH: Malate dehydrogenase is an enzyme in the citric acid cycle that catalyzes the conversion of malate into oxaloacetate (using NAD+) and vice versa (this is a reversible reaction)

MMT: Methylcyclopentadienyl manganese tricarbonyl

NAC: N-acetylcysteine

OM24: a Swiss phytochemical extract; is a powerful Anti-Oxidant

RT-PCR: Reverse transcriptase Polymerase Chain Reaction

SAM: *S*-Adenosyl-L-homocysteine is an amino acid derivative used in several metabolic pathways in the organism *E. coli*.

SDH: Succinate-coenzyme Q reductase is an enzyme complex bound to the inner mitochondrial membrane. It is the only enzyme that participates in both the citric acid cycle and the mitochondrial electron transport chain

SDS: Sodium Dodecyl Sulfate

SOD: Enzyme superoxide dismutase catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide

STAT1: Member of the Signal Transducers and Activators of Transcription family of transcription factors; STAT1 is involved in upregulating genes due to a signal by either type I or type II interferons

TBARS: Thiobarbituric Acid Reactive Substances Assay

TEM: Transmission electron microscope

TRIS: Tris (hydroxymethyl) aminomethane- used as a buffer solution

PARP: Poly (ADP-ribose) polymerase is a protein involved in a number of cellular processes involving mainly DNA repair and programmed cell death

HMBC: heteronuclear multiple bond coherence experiment detects long range coupling between proton and carbon (two or three bonds away) with great sensitivity

ATTACHMENT 2

Exponent®

EXTERNAL MEMORANDUM

TO: Sharon Segal, Morgan Lewis and Bockius, LLP

FROM: Leila Barraj and Xiaoyu Bi

DATE: December 16, 2008

CONTRACT: WD00904.000

SUBJECT: Revised Catechin Intake Assessment for KAO

At the request of Morgan Lewis and Bockius, LLP, on behalf of Kao Corporation, Exponent had prepared intake estimates of catechin from bottled tea, sports drinks, and carbonated beverages and juices. The assessment assumed that all beverages were fortified at 540 mg per 500 ml. Results of the assessment were used in a GRAS petition that was reviewed by the US Food and Drug Administration (FDA). The FDA review seems to indicate that FDA conducted a confirmatory assessment and obtained 90th percentile estimates that were 50% higher than the estimates derived by Exponent for the US Population age 2 years or more. Morgan Lewis and Bockius, LLP requested that Exponent (i) provide additional information on the assumptions it used in its assessment, (ii) conduct revised assessments with alternative fortification assumptions, and (iii) investigate the potential reason for the difference between the results in Exponent's and FDA's assessments.

(i) Exponent's Initial Assessment:

As stated above, Exponent's initial assessment assumed that all beverages (bottled tea, sports drinks, and carbonated beverages and juices) were fortified at 540 mg per 500 ml. Exponent used consumption data from USDA's 1994-96 & 1998 Continuing Survey of Food Intakes by Individuals (CSFII). The CSFII survey collected consumption data on two non-consecutive days. The consumption data from that survey are reported in grams. Hence, it was necessary to transform the catechin concentrations from mg/ml basis to mg/gm or ppm basis. The ppm level for each selected food code or beverage was calculated by first determining the gram equivalent to 1 fluid ounce (from USDA's What's in the Foods You Eat Search Tool 2.0) for each beverage. Then the gram equivalent to 500 ml was calculated and applied to the concentration of 540 mg per 500 ml to each beverage. For example, the gram equivalent to 500 ml of acerola juice (food code 51200500) was calculated to be 526 g by using the conversion factor that 1 fluid ounce (12 fl oz = 355 ml) of acerola juice was equivalent to 31.1 g (USDA). The concentration of 540 mg per 500 ml therefore is equivalent to540 mg/526 g which was calculated to be 974 ppm. Similar calculations were done for the other beverages.

Catechin intakes for the following US population subgroups were estimated:

- US population (2+ years)
- Children 2-6 years
- Children 7-12 years
- Males 13-19 years
- Females 13-19 years
- Males 20+ years
- Females 20+ years

The results of that assessment are summarized in Table 1 (bottled teas), Table 2 (sports drinks), Table 3 (carbonated beverages and juice drinks), and Table 4 (bottled tea, sports drinks and carbonated beverages and juice drinks).

(ii) Revised Assessments:

Morgan Lewis and Bockius, LLP informed Exponent that the correct fortification levels for bottled teas and sport drinks were 540 mg/ 350 ml and 540 mg/ 500 ml, respectively, while the fortification levels for carbonated beverages and juices could be 540 mg/ 350 ml or 540 mg/ 500 ml. Therefore, Exponent conducted two revised analyses:

- Revised analysis 1 assumed that the fortification levels for bottled teas, sport drinks, and carbonated beverages and juices were 540 mg/ 350 ml, 540 mg/ 500 ml, and 540 mg/ 500 ml, respectively

- Revised analysis 2 assumed that the fortification levels for bottled teas, sport drinks, and carbonated beverages and juices were 540 mg/ 350 ml, 540 mg/ 500 ml, and 540 mg/ 350 ml, respectively.

Both revised analyses used the approach described above to calculate the fortification level in ppm for each food code. The results of these analyses are summarized in Tables 5 (revised analysis 1) and 6 (revised analysis 2). In addition Tables 7 and 8 summarize the intakes for bottled teas only (assuming fortification at 540 mg/350 ml) and carbonated beverages and juice drinks only (assuming fortification at 540 mg/350 ml).

(iii) Potential Reasons for Discrepancy Between Exponent's and FDA's Assessments

The FDA information provided to Morgan Lewis and Bockius, LLP seems to indicate that FDA assumed a uniform fortification level of 540 mg/350 ml for all beverages (bottled tea, sports drinks, and carbonated beverages and juices). To determine whether this was the reason for the discrepancy in intake estimates, Exponent ran an assessment using that fortification level. The assessment used the approach described above to calculate the fortification level in ppm for each food code. The results of this assessment were identical to those reported in Table 6 for revised analysis 2. Thus, the per user mean and 90th percentile estimates derived assuming all products are fortified with 540 mg/350ml (859 mg/day and 1737 mg/day) are about 43% higher than the estimates initially derived by Exponent (Mean: 602 mg/day, 90th percentile: 1217 mg/day).

Another potential reason for the discrepancy could be that FDA did not adjust the fortification level from mg/ml to ppm, thus assuming that 1 ml of beverage is equivalent to 1 gram.

Therefore Exponent conducted an assessment assuming that the fortification level in all beverages was 540 mg/350 mg (instead of 540 mg/350 ml). The results of this assessment are summarized in Table 9. The per user mean and 90th percentile estimates derived assuming all products are fortified with 540 mg /350 mg (895 mg/day and 1813 mg/day) are about 49% higher than the estimates initially derived by Exponent (Mean: 602 mg/day, 90th percentile: 1217 mg/day).

Therefore, it appears that the reason for the discrepancy between the estimates derived in Exponent's initial analysis and those derived by FDA could be due to the difference in fortification levels (540 mg/500 ml for all products vs. 540 mg/350 ml for all products) and for the fact that FDA did not adjust for the fact that 1 ml of beverage is not necessarily equivalent to 1 gm.

Table 1. Results from 2006 Report:
2-Day Average Estimated Intake of Catechin Tea Extract from Bottled Tea[1]; CSFII 1994-1996, 1998

Population Group	Per Capita	Per User	Per Capita	Per User	Unweighted Number of Users	% Users
	mg/day		mg/kg-bw/day			
US Population (2+ years)					991	7.39
Mean	21.9	297	0.3	4.3		
90th Percentile	0	545	0	7.8		
Children (2 to 6 years)					106†	1.91
Mean	2.8	148	0.2	7.9		
90th Percentile	0	246	0	12.0		
Children (7 to 12 years)					40†	3.39
Mean	6.3	187	0.2	5.4		
90th Percentile	0	256	0	7.4		
Males (13 to 19 years)					55†	8.87
Mean	28.7	323	0.4	4.7		
90th Percentile	0	550	0	8.3		
Females (13 to 19 years)					47†	8.07
Mean	20.3	251	0.4	4.4		
90th Percentile	0	492	0	7.2		
Males (20+ years)					414	9.58
Mean	32.0	334	0.4	4.1		
90th Percentile	0	560	0	7.8		
Females (20+ years)					329	7.19
Mean	19.8	275	0.3	4.2		
90th Percentile	0	495	0	7.7		

[1]Assuminng bottled tea fortified at 540 mg/ 500 ml
† Indicates estimates are not statistically valid. The unweighted number of consumers does not meet the minimum sample size required to estimate intake at the 90th percentile.

Table 2. Results from 2006 Report:
2-Day Average Estimated Intake of Catechin Tea Extract from Sports Drinks[1]; CSFII 1994-1996, 1998

Population Group	Per Capita	Per User	Per Capita	Per User	Unweighted Number of Users	% Users
	mg/day		mg/kg-bw/day			
US Population (2+ years)					422	2.20
Mean	8.2	372	0.1	6.5		
90th Percentile	0	761	0	13.6		
Children (2 to 6 years)					178†	2.95
Mean	5.6	190	0.3	10.8		
90th Percentile	0	321	0	18.8		
Children (7 to 12 years)					51†	3.64
Mean	9.2	252	0.3	7.5		
90th Percentile	0	514	0	14.5		
Males (13 to 19 years)					43†	7.50
Mean	36.4	484	0.5	6.9		
90th Percentile	0	1016	0	15.7		
Females (13 to 19 years)					14†	1.83
Mean	5.8	319	0.1	5.9		
90th Percentile	0	873	0	15.9		
Males (20+ years)					106†	2.55
Mean	11.5	451	0.1	5.6		
90th Percentile	0	1014	0	11.8		
Females (20+ years)					30†	0.69
Mean	1.8	268	0.03	4.4		
90th Percentile	0	510	0	7.9		

[1]Assuminng sports drinks are fortified at 540 mg/ 500 ml
† Indicates estimates are not statistically valid. The unweighted number of consumers does not meet the minimum sample size required to estimate intake at the 90th percentile.

Table 3. Results from 2006 Report:
2-Day Average Estimated Intake of Catechin Tea Extract from Carbonated Beverages and Juice drinks[1]; CSFII 1994-1996, 1998

Population Group	Per Capita	Per User	Per Capita	Per User	Unweighted Number of Users	% Users
	mg/day		mg/kg-bw/day			
US Population (2+ years)					15292	84.06
Mean	491	584	8.6	10.2		
90th Percentile	1085	1172	19.7	21.2		
Children (2 to 6 years)					5454	90.27
Mean	339	375	19.2	21.2		
90th Percentile	668	697	38.2	39.7		
Children (7 to 12 years)					1358	88.64
Mean	433	489	12.4	14.0		
90th Percentile	859	898	24.7	25.3		
Males (13 to 19 years)					575	94.80
Mean	903	953	13.6	14.3		
90th Percentile	1734	1775	26.1	26.2		
Females (13 to 19 years)					584	94.02
Mean	609	648	10.8	11.5		
90th Percentile	1241	1269	21.4	22.2		
Males (20+ years)					3766	83.3
Mean	561	674	6.8	8.2		
90th Percentile	1233	1331	15.4	16.3		
Females (20+ years)					3555	79.53
Mean	401	504	6.0	7.6		
90th Percentile	937	1012	14.1	15.5		

[1]Assuminng all beverages fortified at 540 mg/ 500 ml

Table 4. Results from 2006 Report:
2-Day Average Estimated Intake of Catechin Tea Extract from Combined Tea, Sport Drinks, Carbonated Beverages and Juices Combined[1]; CSFII 1994-1996, 1998

Population Group	Per Capita	Per User	Per Capita	Per User	Unweighted Number of Users	% Users
	mg/day		mg/kg-bw/day			
US Population (2+ years)					15,476	85.27%
Mean	513	602	8.90	10.4		
90th Percentile	1,145	1,217	20.2	21.5		
Children (2 to 6 years)					5,470	90.47%
Mean	342	378	19.3	21.3		
90th Percentile	669	701	38.4	39.9		
Children (7 to 12 years)					1,360	88.76%
Mean	440	495	12.6	14.2		
90th Percentile	875	918	24.8	25.6		
Males (13 to 19 years)					579	95.32%
Mean	932	977	14.0	14.7		
90th Percentile	1,780	1,792	26.3	27.0		
Females (13 to 19 years)					587	94.61%
Mean	630	666	11.2	11.8		
90th Percentile	1,272	1,278	22.2	22.3		
Males (20+ years)					3,843	84.67%
Mean	593	701	7.20	8.50		
90th Percentile	1,283	1,372	16.0	17.1		
Females (20+ years)					3,637	81.25%
Mean	420	517	6.30	7.80		
90th Percentile	958	1,036	14.6	16.0		

[1]All beverages fortified at 540 mg/ 500 ml

Table 5. 2-Day Average Estimated Intake of Catechin Tea Extract from Combined Tea, Sport Drinks, Carbonated Beverages and Juices Combined[1]; CSFII 1994-1996, 1998

	Per Capita	Per User	Per Capita	Per User	Unweighted Number of Users	% Users
Population Group	**mg/day**		**mg/kg-bw/day**			
US Population (2+ years)					15,476	85.27%
Mean	523	613	9.03	10.6		
90th Percentile	1,155	1,242	20.5	21.9		
Children (2 to 6 years)					5,470	90.47%
Mean	343	379	19.4	21.4		
90th Percentile	671	699	38.5	40.0		
Children (7 to 12 years)					1,360	88.76%
Mean	442	498	12.6	14.2		
90th Percentile	886	923	24.9	25.7		
Males (13 to 19 years)					579	95.32%
Mean	944	990	14.2	14.9		
90th Percentile	1,802	1,833	26.9	27.1		
Females (13 to 19 years)					587	94.61%
Mean	638	675	11.3	12.0		
90th Percentile	1,274	1,285	22.3	23.1		
Males (20+ years)					3,843	84.67%
Mean	607	717	7.38	8.72		
90th Percentile	1,333	1,405	16.2	17.3		
Females (20+ years)					3,637	81.25%
Mean	429	528	6.46	7.95		
90th Percentile	967	1,080	14.8	16.3		

[1]Fortification Level
Teas: 540 mg/ 350 ml
Sport drinks: 540 mg/ 500ml
Carbonated beverages & Juices: 540 mg/ 500 ml

Table 6. 2-Day Average Estimated Intake of Catechin Tea Extract from Combined Tea, Sport Drinks, Carbonated Beverages and Juices Combined[1]; CSFII 1994-1996, 1998

Population Group	Per Capita	Per User	Per Capita	Per User	Unweighted Number of Users	% Users
	mg/day		mg/kg-bw/day			
US Population (2+ years)					15,476	85.27%
Mean	733	859	12.70	14.9		
90th Percentile	1,635	1,737	28.8	30.7		
Children (2 to 6 years)					5,470	90.47%
Mean	488	539	27.6	30.5		
90th Percentile	955	1,002	54.9	56.9		
Children (7 to 12 years)					1,360	88.76%
Mean	628	707	17.9	20.2		
90th Percentile	1,249	1,311	35.4	36.6		
Males (13 to 19 years)					579	95.32%
Mean	1,330	1,396	20.0	21.0		
90th Percentile	2,542	2,559	37.6	38.5		
Females (13 to 19 years)					587	94.61%
Mean	899	950	16.0	16.9		
90th Percentile	1,816	1,825	31.7	31.9		
Males (20+ years)					3,843	84.67%
Mean	847	1,001	10.30	12.16		
90th Percentile	1,833	1,959	22.9	24.4		
Females (20+ years)					3,637	81.25%
Mean	600	739	9.04	11.13		
90th Percentile	1,368	1,479	20.9	22.9		

[1]Fortification Levels
Teas: 540 mg/ 350 ml
Sport drinks: 540 mg/ 500ml
Carbonated beverages & Juices: 540 mg/ 350 ml

Table 7. 2-Day Average Estimated Intake of Catechin Tea Extract from Bottled Tea[1]; CSFII 1994-1996, 1998

Population Group	Per Capita	Per User	Per Capita	Per User	Unweighted Number of Users	% Users
	mg/day		mg/kg-bw/day			
US Population (2+ years)					991	7.39%
Mean	31.3	424	0.454	6.15		
90th Percentile	0.00	779	0.00	11.1		
Children (2 to 6 years)					106	1.91%
Mean	4.02	211	0.216	11.3		
90th Percentile	0.00	352	0.00	17.1		
Children (7 to 12 years)					40	3.39%
Mean	9.04	267	0.260	7.65		
90th Percentile	0.00	366	0.00	10.6		
Males (13 to 19 years)					55	8.87%
Mean	41.0	462	0.599	6.75		
90th Percentile	0.00	785	0.00	11.8		
Females (13 to 19 years)					47	8.07%
Mean	28.9	359	0.511	6.33		
90th Percentile	0.00	702	0.00	10.3		
Males (20+ years)					414	9.58%
Mean	45.7	477	0.559	5.84		
90th Percentile	0.00	800	0.00	11.11		
Females (20+ years)					329	7.19%
Mean	28.2	393	0.428	5.95		
90th Percentile	0.00	707	0.00	10.8		

[1]Bottled tea fortified at 540 mg/ 350 ml

Table 8. 2-Day Average Estimated Intake of Catechin Tea Extract from Carbonated Beverages and Juices Drinks[1]; CSFII 1994-1996,1998

Population Group	Per Capita	Per User	Per Capita	Per User	Unweighted Number of Users	% Users
	mg/day		mg/kg-bw/day			
US Population (2+ years)					15,292	84.06%
Mean	701	835	12.2	14.6		
90th Percentile	1550	1673	28.2	30.3		
Children (2 to 6 years)					5,454	90.27%
Mean	484	536	27.4	30.3		
90th Percentile	954	996	54.5	56.7		
Children (7 to 12 years)					1,358	88.64%
Mean	619	698	17.7	20.0		
90th Percentile	1227	1283	35.3	36.2		
Males (13 to 19 years)					575	94.80%
Mean	1290	1360	19.4	20.5		
90th Percentile	2477	2534	37.2	37.5		
Females (13 to 19 years)					584	94.02%
Mean	870	926	15.4	16.4		
90th Percentile	1772	1815	30.6	31.7		
Males (20+ years)					3,766	83.30%
Mean	801	962	9.7	11.7		
90th Percentile	1761	1900	21.9	23.3		
Females (20+ years)					3,555	79.53%
Mean	572	719	8.6	10.8		
90th Percentile	1338	1447	20.1	22.2		

[1]Carbonated beverages and juices fortified at 540 mg/ 350ml

Table 9. Duplicating FDA's Results:
2-Day Average Estimated Intake of Catechin Tea Extract from Combined Tea, Sport Drinks, Carbonated Beverages and Juices Combined[4]; CSFII 1994-1996,1998

	Per Capita	Per User	Per Capita	Per User	Unweighted Number of Users	% Users
Population Group	**mg/day**		**mg/kg-bw/day**			
US Population (2+ years)					15,476	85.27%
Mean	763	895	13.25	15.5		
90th Percentile	1,705	1,813	30.2	32.1		
Children (2 to 6 years)					5,470	90.47%
Mean	511	565	28.9	31.9		
90th Percentile	999	1,046	57.3	59.7		
Children (7 to 12 years)					1,360	88.76%
Mean	657	740	18.8	21.1		
90th Percentile	1,315	1,361	37.1	38.1		
Males (13 to 19 years)					579	95.32%
Mean	1,391	1,460	20.9	22.0		
90th Percentile	2,653	2,680	39.2	39.9		
Females (13 to 19 years)					587	94.61%
Mean	940	993	16.7	17.6		
90th Percentile	1,881	1,903	33.1	33.6		
Males (20+ years)					3,843	84.67%
Mean	882	1,042	10.73	12.67		
90th Percentile	1,909	2,041	24.0	25.5		
Females (20+ years)					3,637	81.25%
Mean	623	767	9.39	11.56		
90th Percentile	1,417	1,539	21.8	23.9		

[4]All beverages fortified at 540 mg/350 mg (that is assuming that 1 ml = 1 g).

Harry, Molly *

From: Mahinka, Stephen Paul [smahinka@morganlewis.com]
Sent: Monday, January 12, 2009 8:32 PM
To: Harry, Molly *
Cc: Sanzo, Kathleen M.; Segal, Ph.D., Sharon A.; Gaynor, Paulette M
Subject: RE: Clarifications for GRN 000259 (Catechins from Green Tea Extract)

Molly:

Thanks very much for your reply. Your comments respond to the only questions we have at this point and, consequently, we do not believe that scheduling a teleconference would be useful.

We certainly understand the resource constraints and other obligations which you and the reviewing officials have. For the reasons I've mentioned in my December 24 email, however, we believe it is not unreasonable to expect and urge that the Agency complete its review before the running of the full review period.

Best regards.

Steve Mahinka

Stephen Paul Mahinka
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From: Harry, Molly * [mailto:Molly.Harry@fda.hhs.gov]
Sent: Friday, January 09, 2009 10:53 AM
To: Mahinka, Stephen Paul
Cc: Sanzo, Kathleen M.; Segal, Ph.D., Sharon A.; Gaynor, Paulette M
Subject: RE: Clarifications for GRN 000259 (Catechins from Green Tea Extract)

Dear Mr. Mahinka,

Thank you for your reply to our e-mail of January 7, 2009 suggesting a date for the teleconference you requested. In answer to your questions in your e-mail below, we offer the following comments.

Your responses to the list of clarifications that we requested on December 5, 2008 were helpful. The review of GRN 000259 is actively ongoing. If we have additional questions or need further clarifications we will forward them to you as soon as they are raised.

The filing date for GRN 000259 was September 8, 2008. We are working as expeditiously as possible, given our other priorities, to complete the review of this notice within 180 days from the date it was filed.

I hope my explanations have answered your questions. If you still desire to have the teleconference with us, please suggest a date and time and we will do our best to schedule it.

Sincerely,

Molly Harry, M.S.
Division of Biotechnology and
GRAS Notice Review

From: Mahinka, Stephen Paul [mailto:smahinka@morganlewis.com]
Sent: Thursday, January 08, 2009 11:17 AM
To: Harry, Molly *
Cc: Sanzo, Kathleen M.; Segal, Ph.D., Sharon A.; Gaynor, Paulette M
Subject: Re: Clarifications for GRN 000259 (Catechins from Green Tea Extract)

Molly:

Thanks very much for your reply. We hope you had a nice holiday as well.

While we understand it is very difficult to get a large review team together, we are puzzled as to why this is necessary. Our only interests - "agenda" if you will - are to confirm that the GRAS Notice will be reviewed expeditiously and there will be no further extended review time, in view of the history and circumstances that I mentioned in my prior emails. We also simply wanted to confirm that our responses were helpful and complete.

We doubt an entire team is needed for what we assume is a short and simple call, and so perhaps we need not wait until the end of the month to do so.

In any event, and most importantly, we also assume that the GRAS Notice is being actively reviewed now by the Agency, and that any confirming phone call we might have presents no delays to ongoing review, so that we can receive a response by the Agency as quickly as possible.

Please let me know if our understanding is correct, both as to the
Agency's review being undertaken now and, in light of our very narrow questions, whether a smaller Agency group would be appropriate and available sooner, so that we can arrange a brief call on that you suggest or earlier.

Thanks very much.

Steve

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From: Harry, Molly *
To: Mahinka, Stephen Paul
Cc: Sanzo, Kathleen M.; Segal, Ph.D., Sharon A.; Gaynor, Paulette M
Sent: Wed Jan 07 13:41:18 2009
Subject: RE: Clarifications for GRN 000259 (Catechins from Green Tea Extract)
Dear Mr. Mahinka,

Happy new year to you, I hope you had a good holiday period.

Thank you for sending the response to the clarifications that we requested. You indicated in your e-mails of

December 23-30, 2008, that you will like a telephone conference with our office. The preferred dates you indicated were January 14, 15, or 16. Unfortunately we are unable to schedule the teleconference for any of these days as all of the members of the review team will not be available on those days. We have tentatively blocked our calendars for Monday January 26, 2009 at 11:00 am-12:00 pm. Please confirm as soon as possible if this date and time are convenient for you.

Additionally, we will like you to provide us an agenda of what you would like accomplished during the teleconference call. This will enable us determine who needs to be at the meeting from our office.

Sincerely,

Molly Harry, M.S.
Division of Biotechnology and
GRAS Notice Review

From: Mahinka, Stephen Paul [mailto:smahinka@morganlewis.com]
Sent: Wednesday, December 31, 2008 2:23 PM
To: Martin, Robert L; Gaynor, Paulette M
Cc: Sanzo, Kathleen M.; Harry, Molly *; Segal, Ph.D., Sharon A.
Subject: RE: Clarifications for GRN 000259 (Catechins from Green Tea Extract)

Bob:

Thanks very much.

Our best wishes for the new year.

Steve Mahinka

Stephen Paul Mahinka
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From: Martin, Robert L [mailto:robert.martin@fda.hhs.gov]
Sent: Wednesday, December 31, 2008 5:39 AM
To: Mahinka, Stephen Paul; Gaynor, Paulette M
Cc: Sanzo, Kathleen M.; Harry, Molly *; Segal, Ph.D., Sharon A.
Subject: RE: Clarifications for GRN 000259 (Catechins from Green Tea Extract)

Mr. Mahinka, we will try to schedule our meeting for one of these dates. We will contact you as soon as we know which date is compatible with our schedule(s).

Thanks.
Robert L. Martin
301-436-1219

From: Mahinka, Stephen Paul [mailto:smahinka@morganlewis.com]
Sent: Tuesday, December 30, 2008 10:54 AM
To: Martin, Robert L; Gaynor, Paulette M
Cc: Sanzo, Kathleen M.; Harry, Molly *; Segal, Ph.D., Sharon A.
Subject: RE: Clarifications for GRN 000259 (Catechins from Green Tea Extract)

We can be available any time after 10:30 a.m. on Jan. 14, and any time on Jan. 15 or Jan. 16.

Best regards.

Steve Mahinka

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From: Martin, Robert L [mailto:robert.martin@fda.hhs.gov]
Sent: Tuesday, December 30, 2008 7:33 AM
To: Mahinka, Stephen Paul; Gaynor, Paulette M
Cc: Sanzo, Kathleen M.; Harry, Molly *; Segal, Ph.D., Sharon A.
Subject: RE: Clarifications for GRN 000259 (Catechins from Green Tea Extract)

Mr. Mahinka, I am just now looking over our leave schedule and note that we are not be able to accommodate you on any of these dates as some of the people who will be participating in this meeting are not expected to return to work until January 12. Please provide us with three alternate dates some time after January 12.

Thanks.
Robert L. Martin
301-436-1219

From: Mahinka, Stephen Paul [mailto:smahinka@morganlewis.com]
Sent: Monday, December 29, 2008 4:24 PM
To: Martin, Robert L; Gaynor, Paulette M
Cc: Sanzo, Kathleen M.; Harry, Molly *; Segal, Ph.D., Sharon A.
Subject: RE: Clarifications for GRN 000259 (Catechins from Green Tea Extract)

I've checked with my colleagues, and we suggest 9:30 a.m. on January 5, 6, or 9.

As we'll be in different locations, we can set up a call-in number, once you've let us know what date is most convenient.

Thanks for arranging the discussion with relevant Agency reviewing staff.

Best regards.

Steve Mahinka

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From: Martin, Robert L [mailto:robert.martin@fda.hhs.gov]
Sent: Monday, December 29, 2008 7:12 AM
To: Mahinka, Stephen Paul; Gaynor, Paulette M
Cc: Sanzo, Kathleen M.; Harry, Molly *; Segal, Ph.D., Sharon A.
Subject: RE: Clarifications for GRN 000259 (Catechins from Green Tea Extract)

Mr. Mahinka, please provide the three dates. We will then schedule the meeting.

Thanks.
Robert L. Martin
301-436-1219

From: Mahinka, Stephen Paul [mailto:smahinka@morganlewis.com]
Sent: Wednesday, December 24, 2008 11:56 AM
To: Martin, Robert L; Gaynor, Paulette M
Cc: Sanzo, Kathleen M.; Harry, Molly *; Segal, Ph.D., Sharon A.
Subject: Re: Clarifications for GRN 000259 (Catechins from Green Tea Extract)

I quite understand the scheduling concerns for the remainder of the month, and very much appreciate your prompt reply.

Our principal concern, which we're happy to discuss next month, is that our client has had before the Agency essentially the same Notice for the same substance for the same intended uses for nearly two years. While there have been necessary and appropriate clarifications that we have responded to during the Agency's review, and the lengthy delay occasioned by the USP
Committee's proposed and now withdrawn statement, our client is, we believe quite understandably and properly, concerned with further delays that would continue to extend the Agency's review period.

In view of this history and particular circumstances, our view is that the Agency should now be in a position to quickly review the minor clarifications and promptly provide our client with a positive response to the Notice.

If you consider it helpful to further discuss directly the timing for a reply to this Notice, I will provide you promptly with some suggested times and dates to discuss early next month, after conferring with my colleagues on our availability, to provide the maximum flexibility for you.

Best regards.

Steve Mahinka

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smahinka@morganlewis.com | www.morganlewis.com

From: Martin, Robert L
To: Mahinka, Stephen Paul; Gaynor, Paulette M
Cc: Sanzo, Kathleen M.; Harry, Molly * ; Segal, Ph.D., Sharon A.
Sent: Wed Dec 24 08:53:52 2008
Subject: RE: Clarifications for GRN 000259 (Catechins from Green Tea Extract)
Mr. Mahinka, we are willing to discuss with you our differences of opinions as to what constitutes an acceptable filing notice, etc. However, given that some of our people will be out of the office until early January 2009, I ask for your understanding in arranging a suitable time for this discussion. If you would provide us with three times and dates that you and others will be available in January 2009, we will check our calendars and try to accommodate your preferred times.

Thanks.
Robert L. Martin, Ph.D.
301-436-1219

From: Mahinka, Stephen Paul [mailto:smahinka@morganlewis.com]
Sent: Tuesday, December 23, 2008 5:19 PM
To: Gaynor, Paulette M
Cc: Sanzo, Kathleen M.; Martin, Robert L; Harry, Molly *; Segal, Ph.D., Sharon A.
Subject: RE: Clarifications for GRN 000259 (Catechins from Green Tea Extract)

Dr. Gaynor:

Thanks very much for your email acknowledgement.

I am puzzled by your apparent reference to December 19 as a filing date. Our GRAS Notice was filed on August 14, 2008 and accepted shortly thereafter. The questions provided to us on December 5 were only minor clarifications, to which we have responded promptly. To completely restart a lengthy review period following any clarifying inquiry by the Agency essentially transforms a regular process into one that is capricious and indeterminate. While it is understandable that major revisions or additions to any filing might result in such a restarting, in the circumstances here, with only minor clarifying questions, and particularly where we have made essentially the same filing for the same ingredient for the same uses multiple times for nearly two years, such a restart and lengthy extension are inappropriate and unwarranted.

We would be happy to discuss the above concern directly. Please let me know what might be a convenient time to do so.

Steve Mahinka

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From: Gaynor, Paulette M [mailto:paulette.gaynor@fda.hhs.gov]

Sent: Tuesday, December 23, 2008 4:58 PM
To: Segal, Ph.D., Sharon A.
Cc: Mahinka, Stephen Paul; Sanzo, Kathleen M.; Martin, Robert L; Harry, Molly *
Subject: RE: Clarifications for GRN 000259 (Catechins from Green Tea Extract)

Dr. Segal,

Thank you for the electronic copies of this information. To let you know we received the paper copies of this information on December 19, 2008.
Regarding our review, this notice is in our queue and we strive to respond within 180 days of the filing date.

Sincerely,

Paulette Gaynor, Ph.D.

From: Segal, Ph.D., Sharon A. [mailto:ssegal@morganlewis.com]
Sent: Friday, December 19, 2008 4:06 PM
To: Gaynor, Paulette M
Cc: Mahinka, Stephen Paul; Sanzo, Kathleen M.
Subject: Clarifications for GRN 000259 (Catechins from Green Tea Extract)

Dear Ms. Gaynor,

I understand that you are now the contact for Kao's GRAS Notice (GRN000259). On behalf of Kao, today we submitted a response to the requests for clarification conveyed in Ms. Molly Harry's e-mail to Kathleen Sanzo dated December 5, 2008 concerning the GRAS Notice for Kao's Catechins from Green Tea Extract ("CGT"). We understand that Ms. Harry is out of the office until Jan. 5, and we wanted you to have an electronic copy of the submission now. As indicated to you by Steve Mahinka, we are hopeful that the review of this GRAS Notice can be expedited.

Best regards,

Sharon A. Segal, Ph.D.
Director of Regulatory Science
Morgan, Lewis & Bockius LLP
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Harry, Molly *

From: Mahinka, Stephen Paul [smahinka@morganlewis.com]
Sent: Friday, February 20, 2009 11:40 PM
To: Gaynor, Paulette M; Harry, Molly *
Subject: Kao Corp. GRAS Notice GRN 000259 -- Telephone Message

Paulette and Molly:

Thanks very much for your telephone message earlier today. I'm sorry I was unavailable in meetings.

We appreciate your prompt response and status report, and confirmation that the accepted filing date is Sept. 8, 2008 for running of the review period.

Our Director of Regulatory Science, Dr. Sharon Segal, placed the calls for us, but I appreciate your appropriate caution in responding to me. It's certainly fine with us to return Dr. Segal's calls, or those of Kathy Sanzo, in my absence. It would be more convenient for us to have any emails or letters be addressed to me, since I can circulate them more readily to our client.

We also greatly appreciate your confirmation that the GRAS Notice is under active review, notwithstanding the many competing issues presented for your attention.

Best regards.

Steve Mahinka

Stephen Paul Mahinka
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