GR

ORIGINAL SUBMISSION

Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, NW
Washington, DC 20004
Tel: 202.739.3000
Fax: 202.739.3001
www.morganlewis.com

Morgan Lewis
COUNSELORS AT LAW

RECEIVED
AUG 15 2008
BY:

Kathleen M. Sanzo
202 739.5209
ksanzo@morganlewis.com

August 14, 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: Dr. Negash Belay

Re: Generally Recognized as Safe ("GRAS") Notice for Green Tea Catechin – Kao Corporation

Dear Dr. Belay:

Pursuant to 62 Fed. Reg. 18938 (April 17, 1997), and on behalf of our client Kao Corporation ("Kao"), of Tokyo, Japan, enclosed please find three (3) copies of a GRAS Notice for Catechins from Green Tea ("CGT"). Kao has determined that CGT is GRAS for use as a natural extract in beverages, including bottled teas, sports drinks, carbonated soft drinks, and juice, based on scientific procedures among experts qualified by scientific training and expertise. This determination is supported by a long history of safe use of green tea and its extracts and the opinion of an outside panel of experts qualified by scientific training and experience to evaluate the safety of CGT as a food ingredient.

Please let me know if you have any questions regarding the enclosed GRAS Notice. We greatly appreciate the Agency's prompt review of this Notice.

Sincerely,

Kathleen M. Sanzo
Counsel for Kao Corporation

Enclosures

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

Stephen Paul Mahinka, Esq.
Morgan, Lewis & Bockius LLP

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

GENERALLY RECOGNIZED AS SAFE STATUS OF CATECHINS FROM GREEN TEA

Prepared by

Kao Corporation

August 14, 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. GCT 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 601 mg/day or 10.4 mg/kg-bw/day (mean) and 1217 mg/day, or 21.5 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 CFR §182.20. As described in Section III.A, CGT is manufactured by traditional extraction methods using common additives and processing aids.

The safety of GCT is supported by a number of published studies on Kao's GCT and on a 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, corroborates 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 the 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 at 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 500-20000 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 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 72

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 96

E. Other Studies Investigating the Mechanisms of Action for Green Tea Catechins 97

F. Reports that May Appear Inconsistent 98

1. Marketing Experience 98

2. USP Dietary Supplements Information Expert Committee Proposed Caution Statement 98

3. The Anecdotal Case Reports that Served as the Sole Basis for the Proposed Caution Statement Are Inadequate and Fraught with Limitations. 99

4. The Causality Assessment Algorithm ("Naranjo") Used by the USP Committee Is Not Generally Accepted. 100

5. The USP Committee's Proposed Toxicological Mechanism for the Observed Hepatotoxicity of Green Tea Extracts is Faulty and Unsupported. 100

6. Predominant Adverse Cases Were Reported Following Consumption of Only Two Specific Products 102

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. 103

VII. SAFETY SUMMARY AND CONCLUSIONS 104

VIII. LIST OF REFERENCES 108

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

14. Aug. 2008

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
Facimile: +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,

---

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]

---

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, 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.

---

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

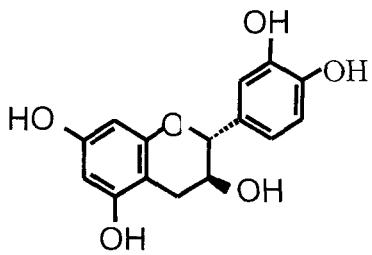
A. Identity

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.

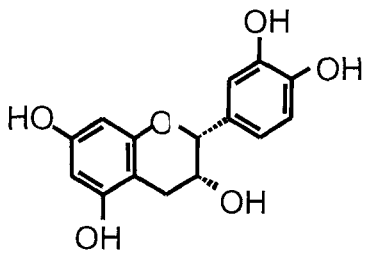
B. Chemical Abstract Service (CAS) Registry Number

CGT has not been assigned a CAS number.

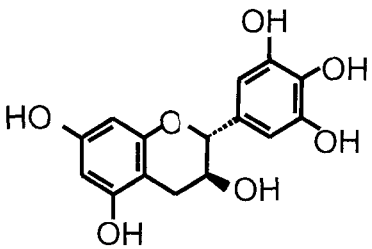
C. Structural Formula



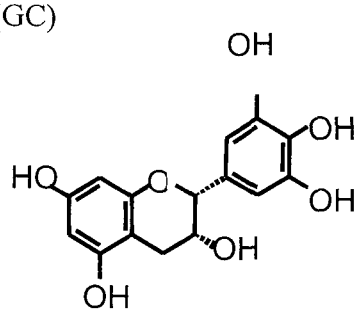
Catechin (C)



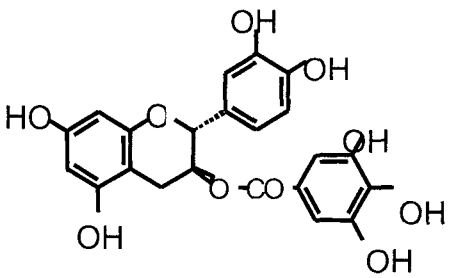
Epicatechin (EC)



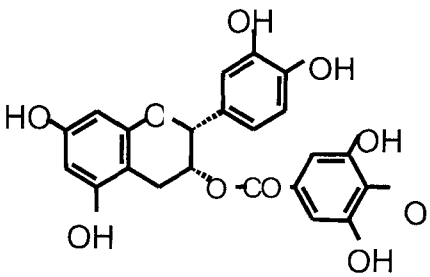
Gallocatechin (GC)



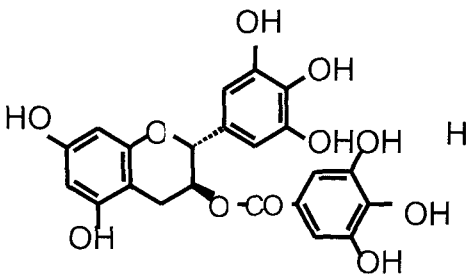
Epigallocatechin (EGC)



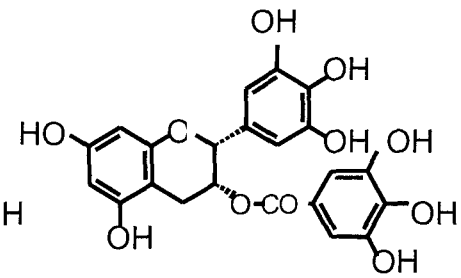
Catechin gallate (CG)



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)

BEST ORIGINAL COPY

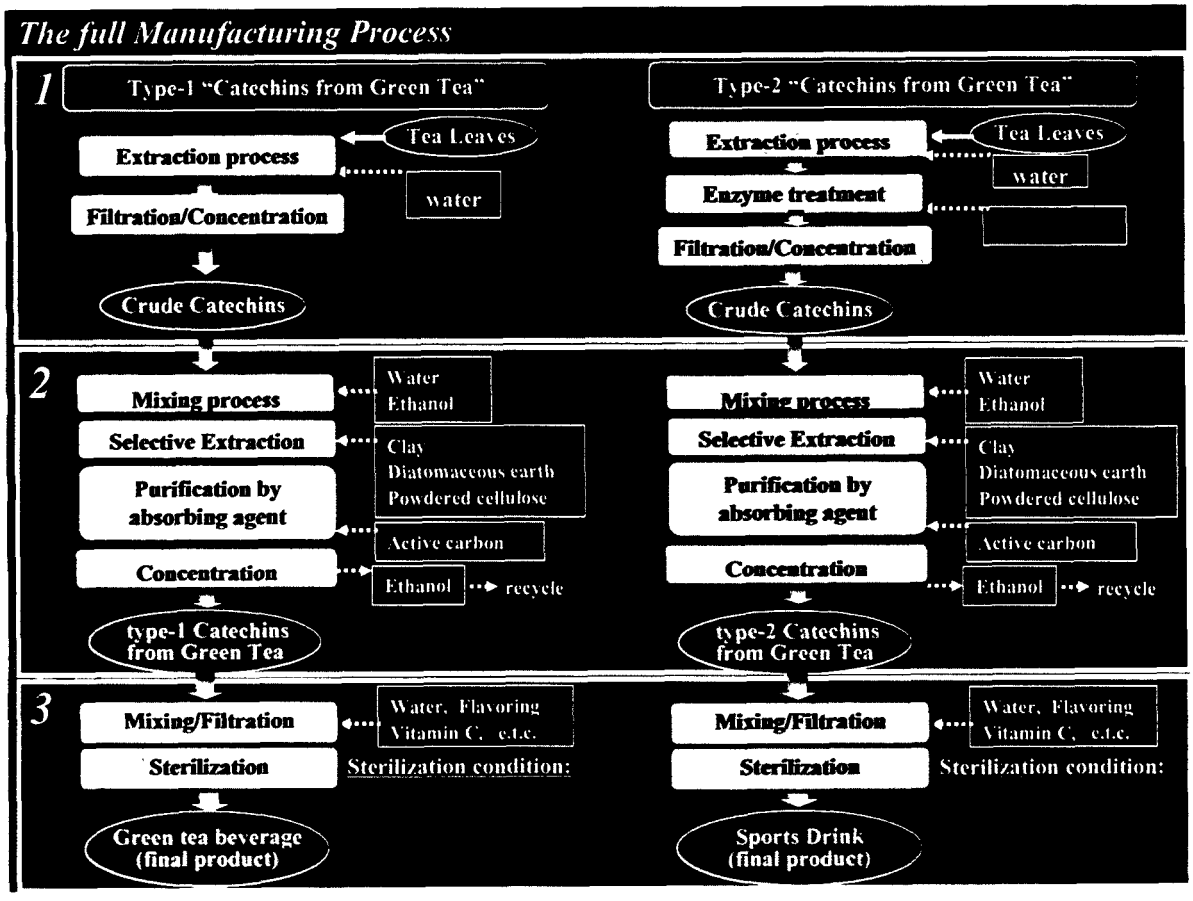


Figure 1

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]

---

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

(Continued . . .)

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.

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.

Constituent	Regulatory Status
	CAS No.: 64-17-5
	GRAS for use as a direct food substance (21 C.F.R. § 184.1293
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 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]

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%	67.4%
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%

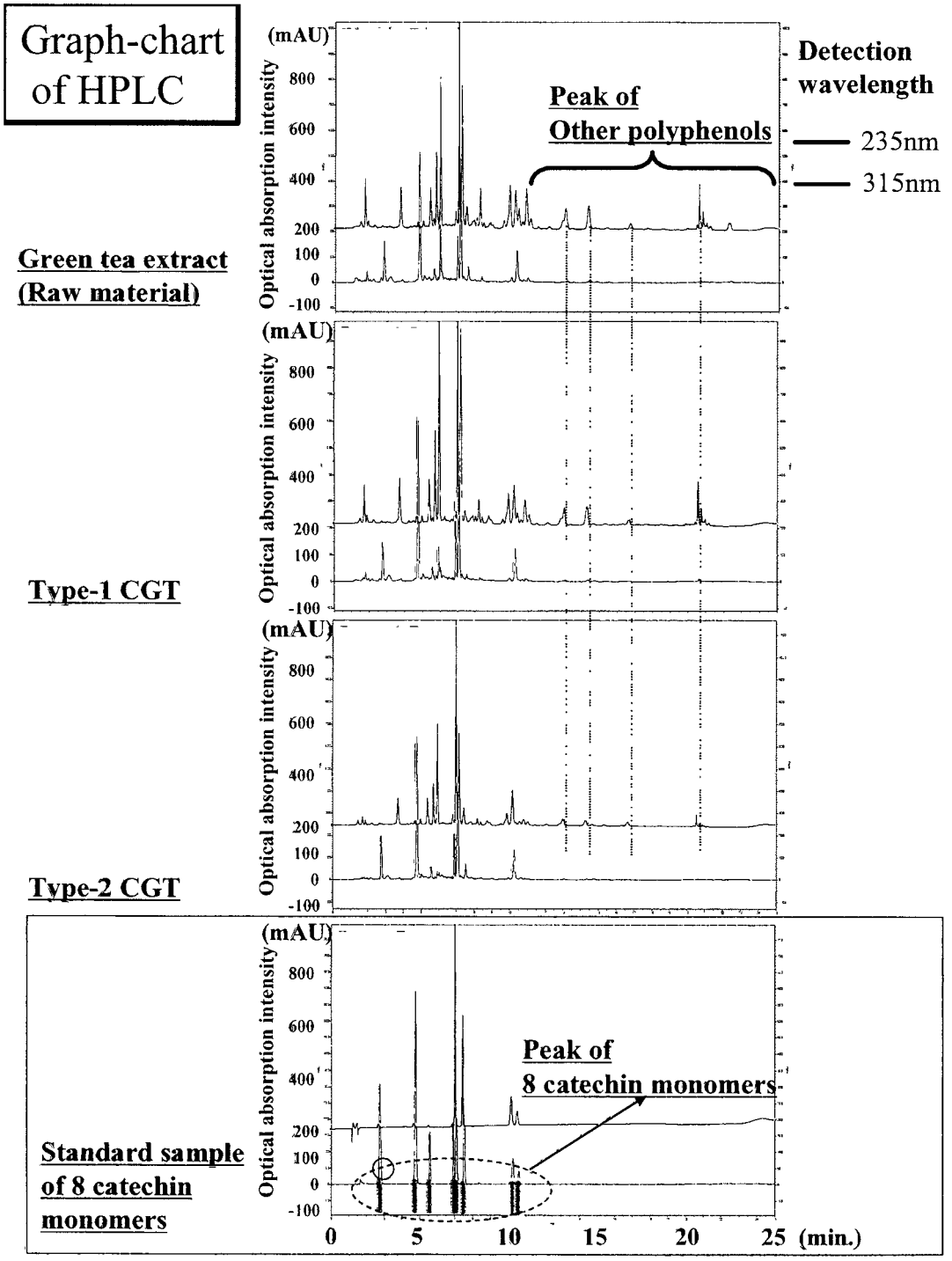
HPLC Graph of CGT:

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

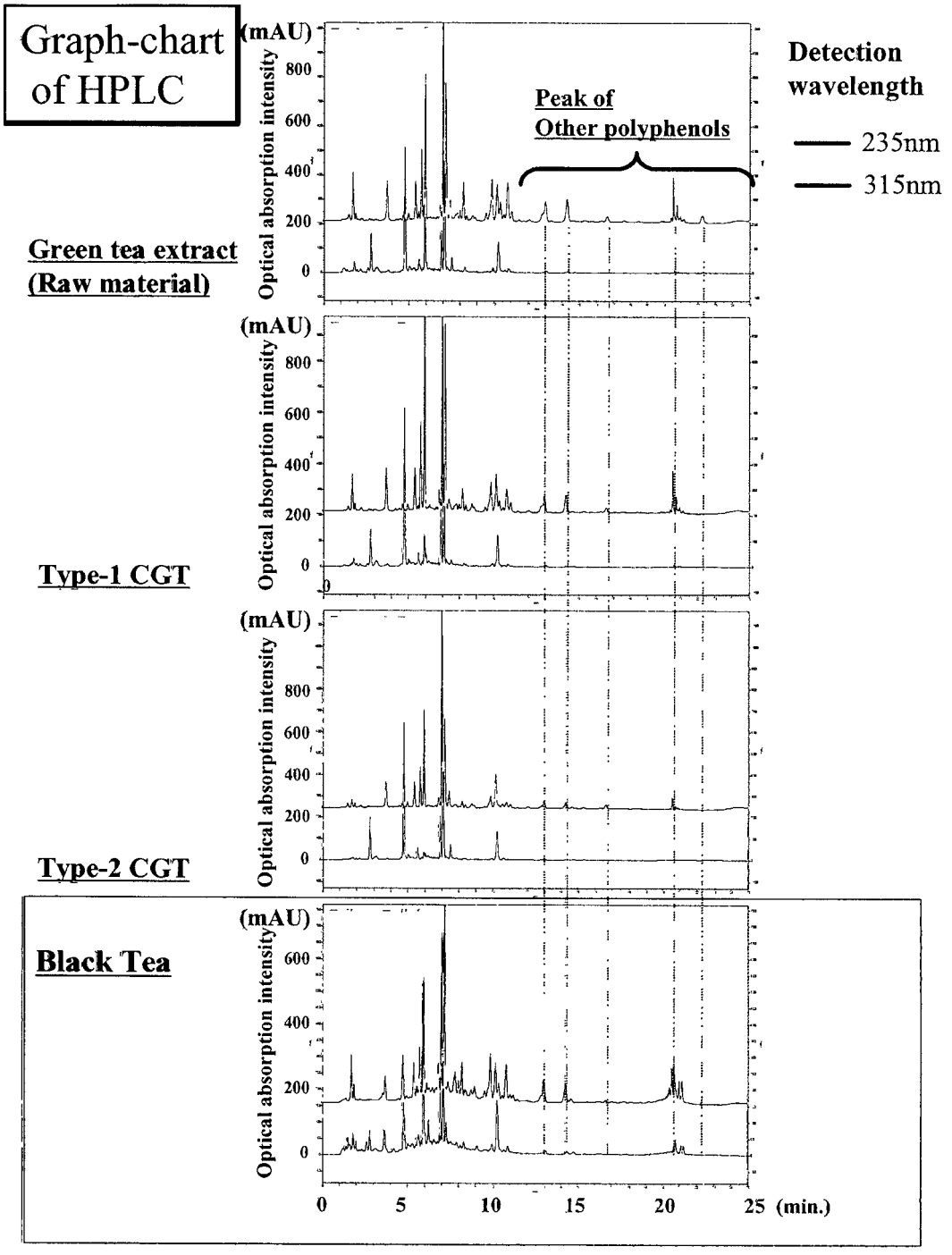
22 This Na_2C calorimetric method was derived based on the methods set forth under Japanese Law. Food Sanitation Law (2002) (Japan), pp. 849-50.

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
(mAU)
Optical absorption intensity
800
600
400
200
0
Peak of Other polyphenols
Detection wavelength
235nm
315nm
Green tea extract (Raw material)
Type-1 CGT
Type-2 CGT
Black Tea
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)	599mg (100%)	595mg (99%)	588mg (98%)	581mg (97%)	581mg (97%)
Total catechin monomers (Steel Can /340 mL)	581mg (100%)	575mg (99%)	569mg (98%)	571mg (98%)	571mg (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)	625mg (100%)	606mg (97%)	594mg (95%)	581mg (93%)	569mg (91%)
Total catechin monomers (Steel Can /480 mL)	600mg (100%)	600mg (100%)	582mg (97%)	570mg (95%)	570mg (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 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		
	Mean (90th Percentile) mg/day	Mean (90th Percentile) mg/kg-bw/day	Unweighted Number of Users (% Users)
#1. Bottled Tea			
US Population (2+ years)	**297 (545)**	**4.3 (7.8)**	**991 (7.39)**
Children (2 to 6 years)	148 (246)	7.9 (12.0)	106† (1.919)
Children (7 to 12 years)	187 (256)	5.4 (7.4)	40† (3.39)
Males (13 to 19 years)	323 (550)	4.7 (8.3)	55† (8.87)
Females (13 to 19 years)	251(492)	4.4 (7.2)	47† (8.07)
Males (20+ years)	334 (560)	4.1 (7.8)	414 (9.58)
Females (20+ years)	275 (495)	4.2 (7.7	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)	**584 (1172)**	**10.2 (21.2)**	**15292 (84.06)**
Children (2 to 6 years)	375 (697)	21.2 (39.7)	5454 (90.27)
Children (7 to 12 years)	489 (898)	14.0 (25.3)	1358 (88.64)
Males (13 to 19 years)	953 (1775)	14.3 (26.2)	575 (94.80)
Females (13 to 19 years)	648 (1269)	11.5 (22.2)	584 (94.02)
Males (20+ years)	674 (1331)	8.2 (16.3)	3766 (83.3)
Females (20+ years)	504 (1012)	7.6 (15.5)	3555 (79.53)
#4. Combined Bottled Tea, Sports Drinks, Carbonated Beverages and Juices			
US Population (2+ years)	**602 (1217)**	**10.4 (21.5)**	**16635 (84.29)**
Children (2 to 6 years)	378 (701)	21.3 (39.9)	4955 (90.92)
Children (7 to 12 years)	495 (918)	14.2(25.6)	1490 (87.74)
Males (13 to 19 years)	977 (1792)	14.7 (27.0)	
Females (13 to 19 years)	666 (1278)	11.8 (22.3)	
Males (20+ years)	701 (1372)	8.5 (17.1)	
Females (20+ years)	517 (1036)	7.8 (16.0)	

† 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.

000035

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

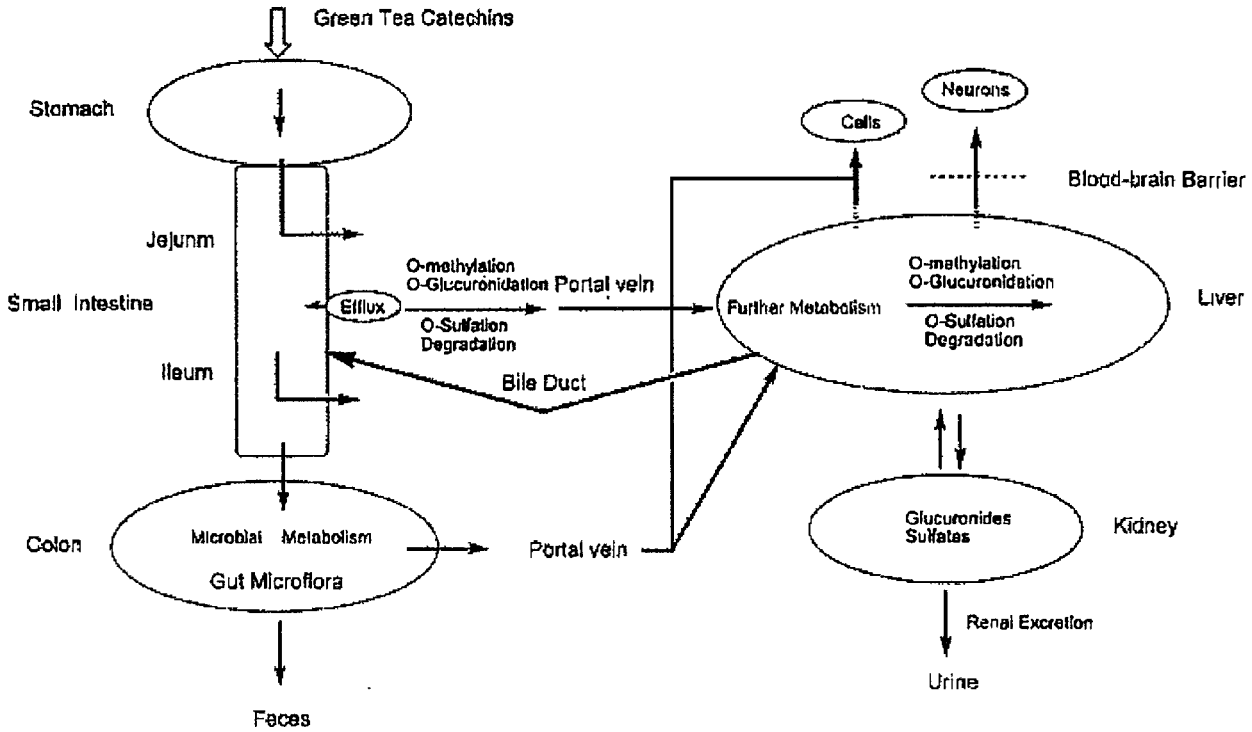
mg group. However, the R value in each group was not statistically different from one (R=1), meaning no accumulation has occurred with the increased dosage. Through concentration values were zero at all time points in the 200 mg group and nearly all in the 400 mg group, with detectable EGCG concentrations 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 $t_{1/2}$ 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 $t_{1/2}$ of 29 minutes and 28 minutes, respectively. Consistent with the observations in the plasma, the $t_{1/2}$ 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.

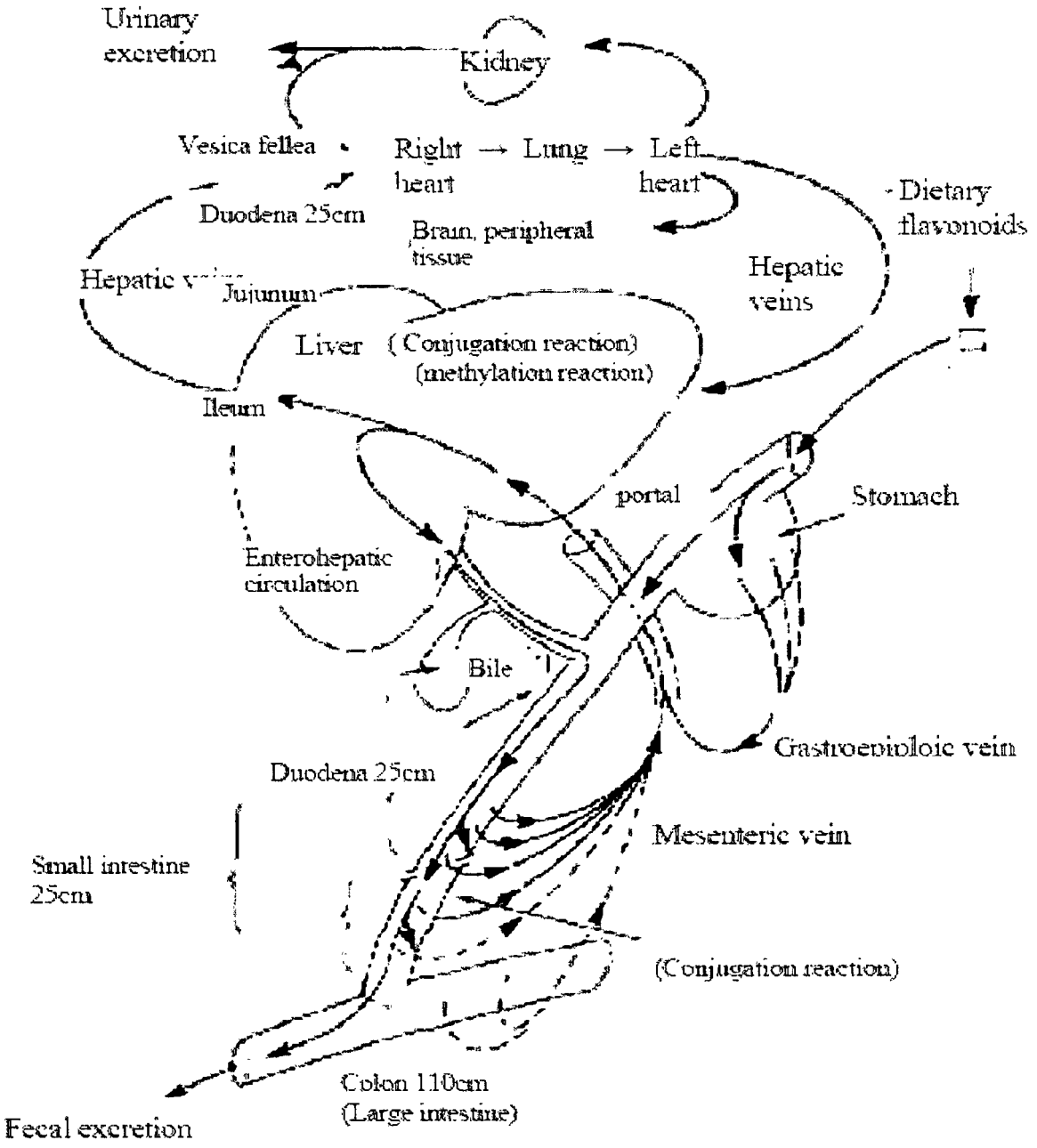
The results of CGT studies in dogs are generally in agreement with previous studies in rodents, and indicate that, after oral administration, EGCG (as parent compound and metabolites) is widely distributed to tissues (Swezey et al. 2003). Four male beagle dogs were administered doses of [^{3}H]EGCG by 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-sulphate 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-sulphate, 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-sulphate, 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 biliar 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).

Figure 5. Absorption and Metabolic Pathway of Dietary Flavanoids



Source: Miyazawa et al. (2000).

BEST ORIGINAL COPY

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 CTG 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 sterlization temperatures. The Type-2 CGT used in the toxicity studies is non-heat-treated.

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

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.

000056

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 paramter. • 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.	

000057

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.

000058

Citation	Design/Duration	Test Substance	Test Animal	Endpoints	Results	Comments
Kirkpatrick, J., et al. WIL Reseach 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.

000059

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 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.

00060

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 to 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.	

000061

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.	

000062

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

---

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 decrease 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

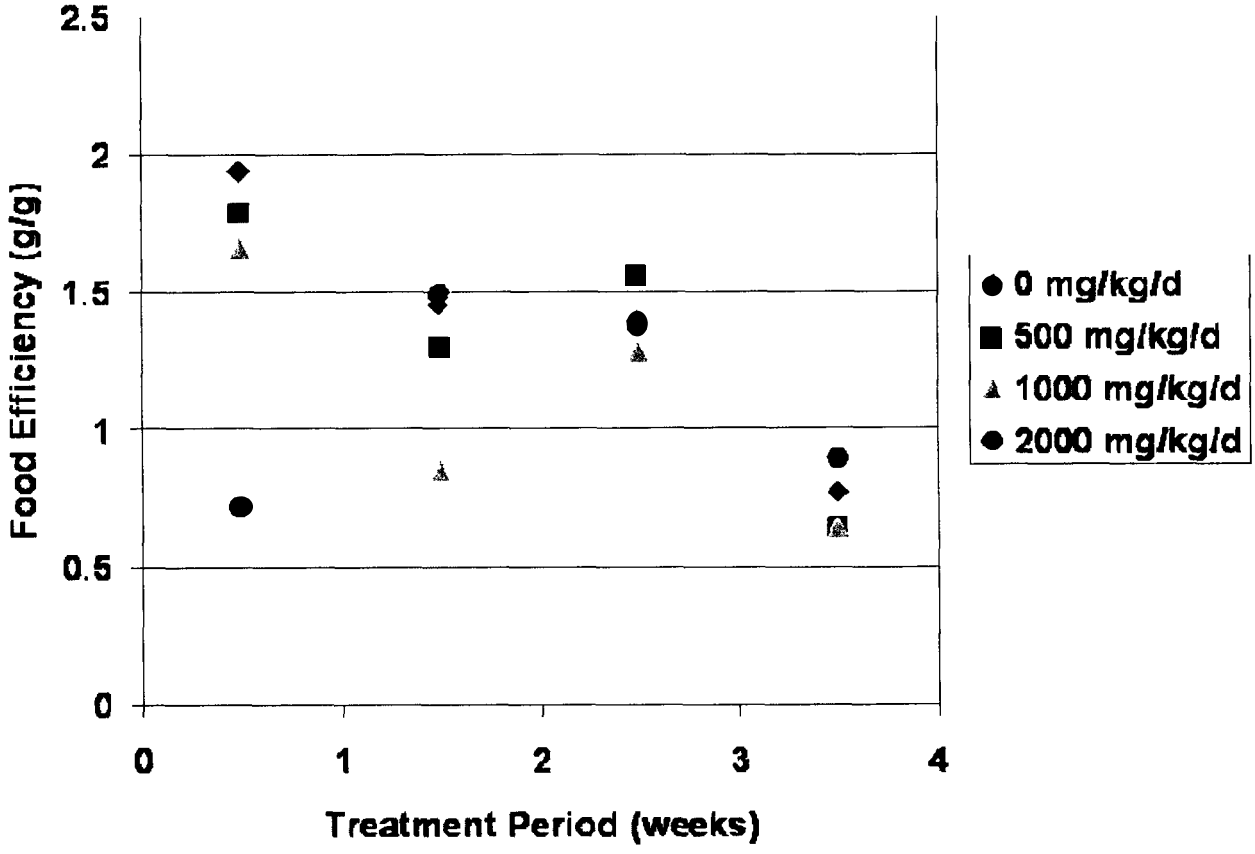
---

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 smeara; brain (cerebrum level 1, cerebrum level 2, cerebellum with medulla/pons); epididymidesb; 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 5

---

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).

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.

---

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

000071

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.

000072

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.

000073

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 signfiicantly 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.

---

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%.

000077

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 hypothryoidism 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 fetusus 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

---

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

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 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 GTC 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 concomittant exposure to potentially heptotoxic 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, 2000mg/kg/d	1st study: oral administration for 6 wks 2nd study: oral administration for 12 wks	No sign of evidence of hepatoxicity (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 histologica. 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 administratino 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.

BEST ORIGINAL COPY

000083

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 prinflammatory cytokine formation.
Fiorini, R.N., et al. 2005. Short-term adminstration of (-)-epigallocatechin gallate reduced hepatic steatosis and protects agains 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 a increase in resting hepatic energy stores (ATP), and increase in glutathione level.

BEST ORIGINAL COPY

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 0.1wt% 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, fibrosis) by 45-73%.
Kuzu, N., et al. 2007. Epigallcatechin 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.

BEST ORIGINAL COPY

000085

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 depostion.
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 hepatoxicity 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.

BEST ORIGINAL COPY

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 (1mg/kg)	EGCG	25mg/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 (500mg/kg)	Healthya Green Tea	540 mg/340 mL	Drinking Healthya green tea for 2 weeks before galactoamine intoxination	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-intoxinated 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.

BEST ORIGINAL COPY

Citation	Animal	Pretreatment for Liver Injury	Test Article	Catechin Dose (mg)	Catechin Treatment	Summary of Results
Wang, Y., et al. 2006. (-)-Epigallcatechin-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 (15mg/kg)	EGCG	5mg/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%, GCG 2%)	0, 0.5, 1.0, or 2.0% in diet	12 days	Cholesterol absorption reduced by 24%, suggesting that the hepatic LDL recptor 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

BEST ORIGINAL COPY

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).

BEST ORIGINAL COPY

000089

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% enthanolic dry green tea extract, one 80% ethanolic dry green tea extract with lipohilic 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 constitutents in the reported liver disorders."
Gelati, G., et al. 2006. Cellular and *in vivo* hepatoxicity 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. Epigallcatechin-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.

BEST ORIGINAL COPY

000090

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 geen 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 catchins 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.

BEST ORIGINAL COPY

000093

Study	Characteristics of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
						hematology paramter. • 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: Clinical chemistry: 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 Clinical Chemistry: 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

BEST ORIGINAL COPY

000094

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.

000095

BEST ORIGINAL COPY

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.

BEST ORIGINAL COPY

000096

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, erythrocytesk 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).
Kozuma, K., et al. 2005. Effects of intake of a beverage containing 540 mg catechins on the body	Kao's sports drink	Multicenter, randomized, double-blind, placebo-controlled,	226 healthy males and females Males: 36	At baseline and every 4 weeks: • Anthropometry (height, body weight,	At baseline and every 4 weeks: Blood chemistry: TG, T-CHO, LDL-	• Significant decreases in body weight, BMI, body fat ration, hip circumference, total fat area, visceral fat area, and subcutaneous fat area were

BEST ORIGINAL COPY

000097

Study	Characteristics of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
composition of obese women and men. *Progress in Medicine*. 25:1945-57.		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	treated, 73 control Females: 24 treated, 93 control Mean age: 26.8 yrs Mean BMI: 26.8 kg/m^2	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	CHO, HDL-CHO, RLP-CHO, NEFA, FBS, HbA_{1C}, total keton body; acetoaceetic 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	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."
Chow, H.H., et al. 2003. Pharmacokinetics and safety of green tea	Refer to composition of Polyphenon E in Table 9 above	Five groups (8 subjects/group): 800 mg EGCG once daily;	40 healthy subjects (8 subjects/group) Between 2 and 4	• Adverse event reports • Pharmacokinetics • Blood chemistry	At weeks 0 and 4: Hematology WBC and RBC	• All reported events (including excess gas, upset stomach, nausea, heartburn, stomach ache, abdominal pain,

BEST ORIGINAL COPY

Study	Characteristics of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
polyphenols after multiple-dose administration of epigaallocatchin gallate and polyphenon E in healthy individuals. *Clin. Cancer Res.* 9:3312-19.		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	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 pounds (Polyphenon E twice daily)		Clinical Chemistry 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	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.

BEST ORIGINAL COPY

000099

Study	Characteristics of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
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 catchins, 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 mg/day) High-dose group: 3 bottles of catechin drink per day (total intake of catechins:	129 healthy adults	• Dieatary 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 electroylytes (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 groups.

BEST ORIGINAL COPY

000100

Study	Characteristics of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
		665.9 mg/day) 2-week run-in, 12-week treatment, 8-week withdrawal				
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 eventgs	None	No significant side effects or adverse effects were documented.
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.

BEST ORIGINAL COPY

000101

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.	0mg: -2.2±0.6 278mg: 0.4±0.9 570mg: -2.6±0.6 840mg: 1.7±1.4 Not significant	0mg: -3.1±1.5 278mg: 0.7±0.9 570mg: -2.9±1.1 840mg: -0.8±2.8 Not significant	0mg: -4.2±1.7 278mg: 0.7±1.1 570mg: 0.0±3.7 840mg: -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.

BEST ORIGINAL COPY

000102

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 catchins 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

BEST ORIGINAL COPY

000103

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 affect 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 gluronidase. 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 interations.

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 our 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 superceded 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 powedered 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 heptatotoxic 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 bioavailibity, 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 500-2000 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 monmers 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 serveral systems. While positive responses were observed in two *in vitro* tests (chromosomal aberration assay and mouse lymphome 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 hepatoxicity 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 hepatoxic 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 catchins 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 hepatoxins.

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.

VIII. LIST OF REFERENCES

1. 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.

2. Arteel, G.E., et al. 2002. Green tea extract protects against early alcohol-induced liver injury in rats. *Biol. Chem.* 383:663-70.

3. 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.

4. 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.

5. 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]

6. 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.

7. 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.

8. Bu-abbas, A., et al., 1994. Selective induction of rat hepatic CYP1 and CYP4 proteins and of perixisomal proliferation by green tea. *Carcinogenesis.* 15:2575-9.

9. Bun, S.S., et al. 2006. Effect of green tea extracts on liver functions in Wistar rats. *Food Chem. Toxicol.* 44:1108-13

10. 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.

11. Cai, Y., et al., 2002. Contribution of presystemic hepatic extraction to the low oral bioavailability of green tea catechins in rats. *Drug Metab. Dispos.* 30:1246-1249.

12. 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]

13. Chang, P. 2003. Genotoxicity and toxicity of the potential cancer preventive agent polyphenon E. *Environmental and Molecular Mutagenesis.* 41: 43-54.

14. Chen, J-H., et al. 2004. Green tea polyphenols prevent toxin-induced hepatoxicity in mice by down-regulating inducible nitric oxide-derived prooxidants. *Am. J. Clin. Nutr.* 80:742-51.

15. Chen, L., et al., 1997. Absorption, distribution, and elimination of tea polyphenols in rats. *Drug Metab. Dispos.* 25(9):1045-1050.

16. Chengeles, C.P., et al. 2008. 28-Day oral (gavage) toxicity studies o fgreen tea catechins prepared for beverages in rats. *Food Chem. Toxicol.* 46(3):978-89.

17. Chow, H. 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.

18. Chow, H.-H., et al. 2007. Modulation of human glutathione *S*-transferases by polyphenon E intervention. *Cancer Epidemiol. Biomarkers Prev.* 16(8):1662-6.

19. Chow, H.H., et al., 2006. Effects of repeated green tea catechin administration on human cytochrome P450 activity. *Cancer Epidemiol. Biomarkers Prev.* 15(12):2473-6.

20. Chow, H.-H.S., et al., 2001. Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and Polyphenon E. *Cancer Epidemiol. Biomarkers Prev.* 10(1):53-58.

21. Chow, H.-H.S., 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(9):3312-3319.

22. Chow, H.-H.S., et al., 2005. Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of Polyphenon E in healthy individuals. *Clin Cancer Res.* 11(12):4627-4633.

23. Chu, K.O., et al., 2006. Pharmacokinetic studies of green tea catechins in maternal plasma and fetuses in rats. *J Pharm Sci.* 95(6):1372-81.

24. Chu, K.O., et al., 2007. Uptake and distribution of catechins in fetal organs following in utero exposure in rats. *Hum. Reprod.* 22(1):280-7.

25. Coyle, C.H., et al. 2008. Antioxidant effects of green tea and its polyphenols on bladder cells. *Life Sci.* 83 (1-2):12-8.

26. Devika, P.T., Mainzen Prince, P.S. 2008. (-)-Epigallocatechin gallate EGCG prevents isoprenaline-induced cardiac marker enzymes and membrane-bound ATPases. *J. Pharm. Pharmacol.* 60(1):125-133.

27. Devika, P.T., Stanley Mainzen Prince, P. 2008. (-) Epigallocatechin gallate (EGCG) prevents 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.

28. Donovan, J.L., et al., 2004. Green tea (Camellia sinensis) extract does not alter cytochrome P450 3A4 or 2D6 activity in healthy volunteers. *Drug Metab. Dispos.* 32(9):906-8.

29. Ehrnhoefer, D.E., et al. 2008. EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers. *Nat. Struc. Mol. Biol.* 15(6): 558-566.

30. 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.

31. 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.

32. Faqui, A.S., et al. 2001. Developmental toxicity of Polyphenon E in rats. *Toxicologist* 60(1):220 [abstract].

33. Federico, A., et al. 2007. A case of hepatotoxicity caused by green tea. Letter to the Editor. *Free Rad. Biol. and Med.* 43: 474.

34. Feng, W. Y., 2006. Metabolism of green tea catechins: an overview. *Curr. Drug Metab.* 7(7):755-809.

35. Gawande, S., et al. 2008. Effect of nutrient mixture and black grapes on the pharmacokinetics of orally administered (-)-epigallocatechin-3-galate from green tea extract: a human study. *Phytother. Res.* 22 (6);802-808.

36. Gelati, G., et al. 2006. Cellular and *in vivo* hepatoxicity caused by green tea phenolic acids and catechins. *Food Chem. Toxicol.* 40:570-80.

37. 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.

38. Hill, A.M., et al. 2007. Can EGCG reduce abdominal fat in obese subjects? *J. Amer. Coll. Nutr.* 26(4):396S-402S.

39. Hirose, M., and Ito, N. 1999. Forestomach and glandular stomach carcinogenesis. In: Kitchin, K.T. Carcinogenicity: Testing, Predicting, and Interpreting Chemical Effects. Boca Raton, FL: CRC Press, pp. 467-98.

40. 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.

41. Horn, J.R., et al. 2007. Philip D. Hansten and Lingtak-Neander Chan proposal for a new tool to evaluate drug interaction cases. *Ann. Pharmacother.* 41:674-80.

42. 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.

43. Hurley, J. WIL Research Lab Study WIL-101096. Acute Oral Toxicity Study of KH-1 (HFD) in Albino Rats

44. Imai, K. 1997. Cancer prevention effects of drinking green tea among a Japanese population. *Preventive Medicine.* 26: 769-775.

45. Isbrucker, R.A., et al. 2006a. Safety studies on epigallocatechin gallate (EGCG) preparations. Part 1: genotoxicity. *Food Chem. Toxicol.* 44(5):626-35.

46. Isbrucker, R.A., et al. 2006b. Safety studies on epigallocatechin gallate (EGCG) preparations. Part 2: dermal, acute and short-term toxicity studies. *Food Chem. Toxicol.* 44(5):636-50.

47. Isbrucker, R.A., et al. 2006c. Safety studies on epigallocatechin gallate (EGCG) reparations. Part 3: teratogenicity and reproductive toxicity studies in rats. *Food Chem. Toxicol.* 44(5):651-61.

48. Iso, H., et al., 2006. The Relationship between Green Tea and Total Caffeine Intake and Risk for Self-Reported Type 2 Diabetes among Japanese Adults, *Annals of Internal Medicine* 144(8):554-563.

49. Jia, Y., Alayash, A.I.. 2008. Effects of (-)-epigallocatechin gallate on the redox reactions of human hemoglobin. *Free Radic. Biol. Med.* [epub ahead of print]

50. Jimenez-Saenz, M., and Martinez-Sanchez, C. 2007. Green tea extracts and acute liver failure: The need for caution in their use and diagnostic assessment. *Liver Transplantation.* 13:1067.

51. Jin, X., et al. 2008. Green tea consumption and liver disease: a systematic review. Liver International. May 14. [Epub ahead of print].

52. Kajimoto, O., et al. 2005. Tea catechins with a galloyl moiety reduce body weight and fat. *J. Health Sci.* 51(2):161-171.

53. Kane-Gill, S.L., et al. 2005. Are the Naranjo criteria reliable and valid for determination of adverse drug reactions in the intensive care unit? *Ann. Pharmacother.* 39:1823-27.

54. Kapiszewska, M., et al. 2006. High tea consumption diminishes salivary 17β-estradiol concentration in Polish women. *Br. J. Nutr.* 95:989-95.

55. 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.

56. Katsuo, K. 1987. Cultural history of tea. *J. Food Sci.* 117; 14-20.

57. Kaviarasan, S., et al. 2007. Epigallcatechin-3-gallate(-) protects chang liver cells against ethanol-induced cytotoxicity and apoptosis. *Basic Clin. Pharmacol. Toxicol.* 100(3):151-6.

58. Kawai, Y., et al. 2008. Galloylated catechins as potent inhibitors of hypochlorous acid-induced DNA damage. *Chem. Res. Toxicol.* 21(7):1407-1414.

59. Kirkpatrick, J., et al. WIL Reseach Labs study WIL-101097. 14-Day Repeated Dose Oral Toxicity Test with KH-1 (HFD) in Rats.

60. Kirkpatrick, J.B. 2007. A 6-Month Repeated Dose Oral (Gavage) Toxicity Study of KH-4(HFD) and KH-4(decaffeinated-HFD) in Rats (With Functional Observational Battery and Motor Activity Determinations). WIL Research Laboratories, LLC. Final Report.

61. Knapp, J.F. 2008. A study of the effects of KH-4 (HFD) on embryo/fetal development in rats. WIL Research Laboratories, LLC. Final Report.

62. 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.

63. Kurahashi, N., et al., 2008. Green Tea Consumption and Prostate Cancer Risk in Japanese Men: A Prospective Study. *Amer. J. Epidemiol.* 167(1):71-7.

64. Kuriyama, S., et al., 2006. Green Tea Consumption and Mortality Due to Cardiovascular Disease, Cancer, and All Causes in Japan. *J. Amer. Med. Assoc.* 296(10):1255-1265.

65. Kuroiwa, Y., et al. 2007. Combined treatment with green tea catechins and sodium nitrite selectively promotes rat forestomach carcinogenesis after initiation with *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine. *Cancer Sci.* 98(7):949-57.

66. Kuzu, N., et al. 2007. Epigallcatechin gallate attenuates experimental non-alcoholic steatohepatitis induced by high fat diet. *J. Gastroenterol. Hepatol.* 22(11):2009-14.

67. 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.

68. Lambert, J.D., et al. 2007a. Metabolism of dietary polyphenols and possible interactions with drugs. *Current Drug Metab.* 3:499-507.

69. Lambert, J.D., et al. 2007b. Biotransformation of green tea polyphenols and the biological activities of those metabolites. *Mol. Pharm.* 4(6):819-25.

70. Lambert, J.D., et al., 2003. Epigallocatechin-3-gallate is absorbed but extensively glucuronidated following oral administration to mice. *J. Nutr.* 133(12):4172-7.

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

72. Lee, H.J., et al. 2008. Modification of gamma-radiation response in mice by green tea polyphenols. *Phytother Res.* [epub ahead of print]

73. Lee, M., et al., 1995. Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiol. Biomarkers Prev.* 4:393-399.

74. Lee, M.-J., et al., 2002. Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. *Cancer Epidemiol. Biomarkers Prev.* 11(10 Pt 1):1025-1032.

75. 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.

76. 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.

77. Li, C., et al., 2001. Analysis of urinary metabolites of tea catechins by liquid chromatography/electrospray ionization mass spectrometry. *Chem. Res. Toxicol.* 14:702-707.

78. Lin, L.-C., et al., 2007. Pharmacokinetics of (-)-epigallocatechin-3-gallate in conscious and freely moving rats and its brain regional distribution. *J Agric. Food Chem.* 55(4):1517-24.

79. Lu, H., et al., 2003a. Glucuronides of tea catechins: enzymology of biosynthesis and biological activities. *Drug Metab. Dispos.* 31:452-461.

80. Lu, H., et al. 2003b. Enzymology of methylation of tea catechins and inhibition of catechol-*O*-methyltransferase by (-)-epigallocatechin gallate. *Drug Metab. Dispos.* 31:572-579.

81. Maki, K.C., et al. Effect of daily consumption of a tea catechin containing sports drink on exercise-induced changes in body composition and fat distribution in overweight and obese adults. (Provident Clinical Research and Consulting, Inc.) [Manuscript in preparation.]

82. Maliakal, P.P., et. al., 2001. Tea consumption modulates hepatic drug metabolizing enzymes in Wistar rats. *J Pharm. Pharmacol.* 53:569-77.

83. Mata-Bilbao, M.D., et al., 2008. Absorption and pharmacokinetics of green tea catechins in beagles. *Br. J Nutr.* Jan 21;1-7. [Epub ahead of print]

84. Matsumoto, N., et al. 1999. Single dose oral toxicity and oral mucous irritation study of green tea extract. *Jpn. Pharmacol. Ther.* 27(9):1479-81. (Japanese)

85. Matsumoto, N., et al. 1999. Repeated dose toxicity study of green tea extract in rat. *Jpn. Pharmacol. Ther.* 27(11):1701-7.

86. Matsuyama, T., et al. 2008. Catechin safety improved higher levels of fatness, blood pressure and cholesterol in children. *Obesity* 16(6):1338-1348.

87. Ministry of Health, Labor and Welfare, Permit for Labeling a product as a Food for Specified Health Use (2003), green tea.

88. Ministry of Health, Labor and Welfare, Permit for Labeling a product as a Food for Specified Health Use (2003), oolong tea.

89. 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.

90. Miyazawa, T. 2000. Absorption and metabolism of natural antioxidants (Review). *Kagaku to Seibutu.* 38(2): 104-P.114. (Japanese)

91. Miyazawa, T. and K. Nakagawa, 1998. Structure-related emission spectrometric analysis of the chemiluminescence of catechins, theaflavins and anthocyanins. *Biosci. Biotech. Biochem.* 62(4):829-832.

92. Miyazawa, T., et al., 2000. Absorption and metabolism of natural antioxidants. *Kagaku to Seibutu.* 38(2):104-114. [Article in Japanese]

93. 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]

94. Molinari, M., et al. 2006. Acute liver failure induced by green tea extracts: case report and review of the literature. *Liver Transpl.* 12:1892-5.

95. 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.

96. Mukhtar H. and Ahmad N., 2000. Tea polyphenols: prevention of cancer and optimizing health. *Am. J. Clin. Nutr.* 71(suppl):1698S-1702S.

97. Muramatsu, K. 1991. Science of Tea. Asakura Publishing Co., Ltd. (Tokyo) p. 153 (Japanese).

98. Nagao, T. 2001. Tea catechins suppress accumulation of body fat in humans. *J. of Oleo Sci.* 50(9): 717-728.

99. Nagao, T., et al. 2007. A green tea extract high in catchins reduces body fat and cardiovascular risks in humans. *Obesity.* 15:1473-83.

100. 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.

101. 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 histologica. immunohistochemical and electrophysiological study. *Histochem. Cell Biol.* 129(4):489-501.

102. Nakagawa, K. and Miyazawa,T. 1997. Absorption and distribution of tea catechin, (-)-epigallocatechin-3-gallate in the rat. *J. Nutr. Sci. Vitaminol.*, 43:679-684.

103. Nakagawa, K. 1997. Dose-dependent incorporation of tea catechins, (-)-epigallocatechin-3-gallate and (-)-epigallocatechin, into human plasma, *Biosci. Biotech. Biochem.* 61:1981-1985.

104. Netsch, M.I., et al., 2006. Induction of CYP1A1 by green tea extract in human intestinal cell lines. *Planta. Med.* 72(6):514-20.

105. Nishikawa, H., Kitani S. 2008. Tea catechins have dual effect on mast cell degranulation induced by compound 48/80. *Int. Immunopharmacol.* 8(9):1207-1215.

106. Ogura, R., et al. 2008. Genotoxicity studies on green tea catechin. *Food Chem. Toxicol.* 46(6):2190-2200.

107. Oh, C.J., et al. 2008. Epigallocatechin gallate, a constituent of green tea, regulates high glucose-induced apoptosis. *Arch. Pharm. Res.* 31(1):31-40.

108. Okushio, K., et al., 1996. Absorption of tea catechins into rat portal vein. *Biol. Pharm. Bull.* 19(2):326-329.

109. Otsuka, K., et al. 2002. Effects of tea catechins on body fat metabolism in women. *Jpn.. J. Nutritional Assessment.* 19(3):365-76.

110. Oz, H.S., and Chen, T.S. 2008. Green-tea polyphenols downreguloate cyclooxygenase and Bcl-w activitgy in acetaminophen-induced hepatotoxicity. *Dig. Dis. Sci.* [Epub ahead of print].

111. Oz, H.S., et al. 2004. Diverse antioxidants protect against acetaminophen hepatotoxicity. *J. Biochem. Mol. Toxicol.* 18(6):361-8.

112. Pietta, P. 1998. Relationship between rate and extent of catechin absorption and plasma antioxidant status. *Biochem. Mol. Biol. Int.* 46: 896-903.

113. 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(6):1830-8.

114. Renno, W.M., et al. 2008. Effect of green tea on kidney tubules of diabetic rats. *Br. J. Nutr.* 6:1-8.

115. Saitou, N. 1998. Study on tea drinks: contents of catechins, caffeine, and total amino acid. *Report of Consumer Science Research Institute of Hyogo Prefecture.* 13: 29-42. (Japanese).

116. Sakamoto, Y., et al. 2001. Goitrogenic effects of green tea extract catechins by dietary administration in rats. *Arch. Toxicol.* 75:591-6.

117. Sakata, R., et al. Green tea with high-density catchin improves liver funciton and fat infiltration in non-alcoholic liver disease patients: a double-blind placebo-controlled study. (Kurume University, Fukuoka, Japan. Final report in progress.)

118. Sarma, D.N., et al. 2008. US Pharmacopeia Safety Review of Green Tea Extracts. Drug Safety. 31(6):469-484.

119. Sato, Y., et al. 1989. Possible contribution of green tea drinking habits to the prevention of stroke. *Tohoku J. Exp. Med.* 57(4):337-43.

120. 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.

121. Siu, A.W., et al. 2008. Glutamate-induced retinal lipid and protein damage: the protective effects of catechin. *Neurosci. Lett.* 432(3): 193-197.

122. Sohn, O.S., et al., 1994. Effects of green tea and black tea on hepatic xenobiotic metabolizing systems in the male F344 rat. *Xenobiotica.* 24:119-27.

123. Spina, M., et al. 2008. Mechanism of inhibition of wt-dihydrofolate reductase from *E. coli* by tea epigallocatechin-gallate. *Proteins.* 72(1):240-251

124. Suga, K. 1998. Phase I clinical trial with green tea tablets in a Japanese healthy population. *Cancer Prevention International.* 3:79-88.

125. Suganuma, M., et al., 1998. Wide distribution of [3H](-)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. *Carcinogenesis.* 19(10):1771-6.

126. Sugiyama, K., et al. 1998. Green tea suppresses D-galactosamine-induced liver injury in rats. *Biosci. Biotechnol. Biochem.* 62(3):609-11.

127. Sukhthankar, M., et al. 2008. A green tea component suppresses posttranslational expression of basic fibroblast growth factor in colorectal cancer. *Gastroenterology.* 134(7): 1972-1980.

128. Swezey, R.R., et al., 2003. Absorption, tissue distribution and elimination of 4-[(3)h]-epigallocatechin gallate in beagle dogs. *Int. J. Toxicol.* 22(3):187-193.

129. 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.

130. Takashi T., et al. 2002. Reduction in body fat in humans by long-term ingestion of catechins. *Progress in Medicine.* 26(11):1459-1464.

131. Tsuchida, T., et al. 2002. Reduction in body fat in humans by long-term ingestion of catechins. *Progress in Medicine.* 26(11):1459-1464

132. Ullmann U., et al., 2003. A single ascending dose study of epigallocatechin gallate in healthy volunteers. *J. Int. Med. Res.* 31(2):88-101.

133. Ullmann U., et al., 2004. Plasma-kinetic characteristics of purified and isolated green tea catechin epigallocatechin gallate (EGCG) after 10 days repeated dosing in healthy volunteers. *Int. J. Vitam. Nutr. Res.* 74(4):269-278.

134. Unno, T., et al., 1996. Analysis of (-)-epigallocatechin gallate in human serum obtained after ingesting green tea. *Biosci. Biotech. Biochem.* 60(12) P.2066-2068.

135. Wang, C.C., et al. 2007. Tea epigallocatechin-3-gallat increases 8-isoprostane leel and induced caudal regression in developing rat embryos. *Free Rad. Biol. and Med.* 43:519-27.

136. Wang, Y., et al. 2006. (-)-Epigallcatechin-3-gallate protects mice from concanavalin A-induced hepatitis through suppressing immune-mediated liver injury. *Clin. Exper. Immunol.* 145:485-92.

137. Weinshilboum, R.M., et al., 1999. Methylation pharmacogenetics: catechol *O*-methyltransferase, thiopurine methyltransferase, and histamine *N*-methyltransferase. *Annu. Rev. Pharmacol. Toxicol.* 39:19-52.

138. Williams, S.N., et al., 2003. A combination of tea (*Camellia Sinesis*) catechins is required for optimal inhibition of induced CYP1A1 expression by green tea extract. *J. Agric. Food Chem.* 51(22):6627-34.

139. World Health Organization (WHO. 2006. *The World Health Report 2006 - Working Together for Health.* Available at: http://www.who.int/whr/2006/en/.

140. World Health Organization. 1987. International Programme on Chemical Safety. Environmental Health Criteria 70. Principles for the Safety Assessment of Food Additives and Contaminants in Food. Available at: http://www.inchem.org/documents/ehc/ehc/ehc70.htm.

141. Yamaguchi, M. et al. Kao Study 03-159-2. 14-Day Repeated Oral Toxicity Test with KH-1 (FD).

142. Yamaguchi, M., et al. Kao Study 03-158-2. A Single Dose Oral Toxicity Study of KH-1 (FD) in Rats.

143. Yamaguchi, M., et al. Kao Study 03-158-01. Acute Oral Toxicity with UHG(FD) in Rats.

144. Yamaguchi, M., et al. Kao Study 03-159-1. 14-Day Repeated Oral Toxicity Test with UHG (Type 2).

145. Yamamoto, T., et al. 1997. Chemistry and Applications of Green Tea. CRC Press (New York) p. 1.

146. Yang, C.S. 1999. Tea and health. *Nutrition*. 15(11): 946-948.

147. Yang, C.S. 1998. Blood and urine levels of tea catechins after ingestion of different amount of green tea by human volunteers. *Cancer Epi. Biomarkers Prev.* 17:351-354.

148. Yang, S.P. and G.M. Raner, 1998. Cytochrome P450 expression and activivties in human tongue cells and their modulation. *Toxicol. Appl. Pharmacol.* 202(2):140-50.

149. 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.

150. 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.

151. Zhen, M-C., et al. 2007. Green tea polyphenol EGCG inhibits oxidative damage and preventive effects on carbon tetrachloride-induced hepatic fibrosis. *J. Nutr. Biochem.* 18:795-805.

152. Zhu, B.T., et al., 2000a. *O*-Methylation of tea polyphenols catalyzed by human placental cytosolic catechol-*O*-methyltransferase. *Drug Metab.Dispos.* 28:1024-1030.

153. Zhu, M., et al., 2000b. Oral absorption and bioavailability of tea catechins. *Planta Med.* 66(5):444-7.

A

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

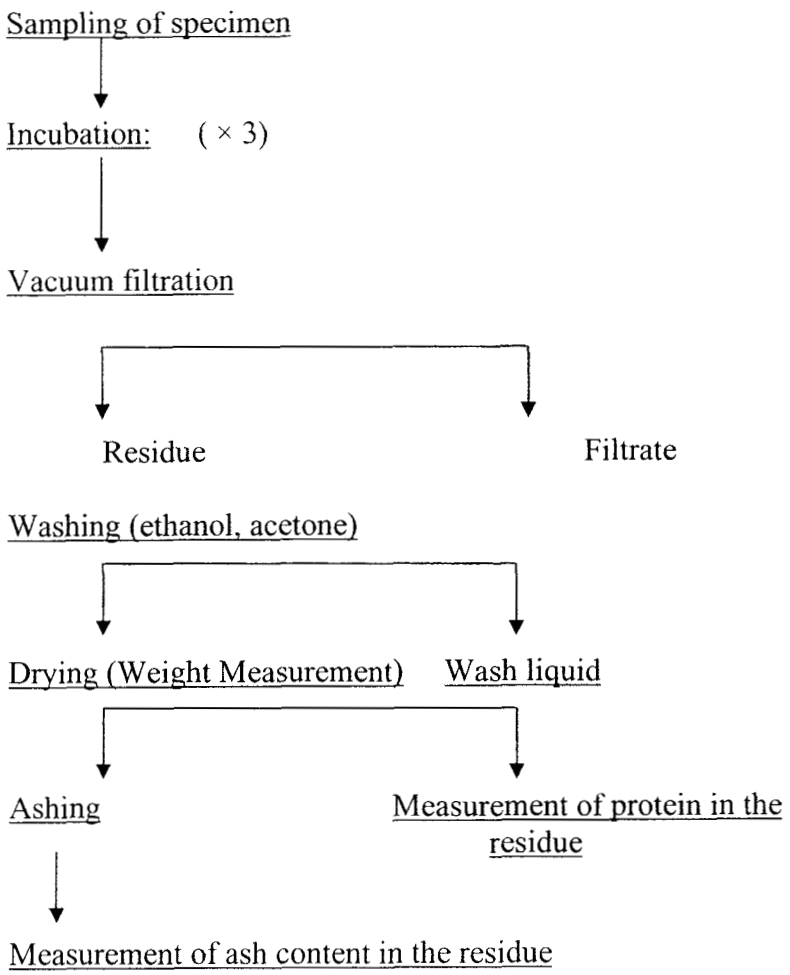
Equipment used
Kjeltec Auto Sampler System Analyzer

*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

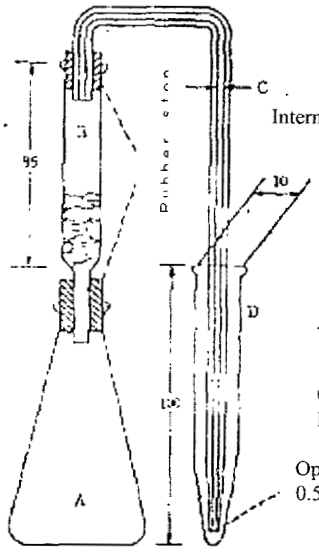
      Take the sample, humidify on a water-bath until it is syrupy after evaporative concentrations. Transfer it into a decomposition flask by using water, add and dissolve sulfuric acid and nitric acid, and then continue heating until the solution becomes almost colorless or pale yellow, adding nitric acid 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).



A Generation flask (inner volume. 100 to 125 ml)
B: Absorption tube (Stuff the glass wool dampened with lead acetate solution.)
C: Gas-inducing tube
D: Absorption receiver

Opening
0.5

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.

B

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 SWEETNER	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

C

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?

D

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 JUIICE	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 & VEGETAGLE 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,SWEETEND(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	SRAWBERRY-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-FORTIFD	Carb bevs/juices
92582050	FRUIT-FLAVORED DRINK, VITAMIN & MINERAL FORTIFIED	Carb bevs/juices
92582100	CITRUS JUICE DRINK, CALCUIM FORTIFIED	Carb bevs/juices
92582110	ORANGE BREAKFAST DRINK, CALCIUM FORTIFIED	Carb bevs/juices

E

Appendix E

GRAS Expert Panel Critical Evaluation of Tea Catechin

THE GENERALLY RECOGNIZED AS SAFE STATUS OF CATECHINS FROM GREEN TEA

OPINION OF AN EXPERT PANEL

August 4, 2008

Introduction

Kao Corporation convened a panel of independent, internationally recognized biomedical scientists (the Expert Panel), qualified by their scientific training and experience to evaluate the safety of food and food ingredients, to independently and collectively critically evaluate available published and unpublished information on catechins from green tea ("CGT") to determine the safety and suitability and generally recognized as safe ("GRAS") status based on scientific procedures of the intended uses of catechins from green tea.

The members of the Expert Panel included Professors Joseph F. Borzelleca of the Virginia Commonwealth University School of Medicine, James W. Anderson of the University of Kentucky School of Medicine, and Michael W. Pariza of the University of Wisconsin, Madison. The curricula vitae of the panelists are provided in Appendix 1.

Morgan, Lewis & Bockius LLP ("Morgan Lewis") conducted a thorough search of the scientific literature on green tea catechin and related compounds through July 2008. The results of these searches were available to the Expert Panel. Kao Corporation and Morgan Lewis also provided to the panelists a comprehensive package of appropriate information including the methods of manufacture, specifications, batch analyses, proposed uses, exposure estimates, and safety (the GRAS dossier). The Expert Panel independently and collectively critically evaluated these materials and other materials deemed appropriate or necessary and consulted by telephone. The Expert Panel unanimously concluded that the intended uses of catechins from green tea, meeting appropriate food grade material specifications, and manufactured and used as described herein and in accordance with current Good Manufacturing Practice, are safe and GRAS based on scientific procedures, corroborated by history of safe use.

A summary of the basis for this GRAS determination follows.

Identity and Characterization

CGT is a mixture of eight catechin monomers that is extracted from crude green tea extract and includes polyphenols, caffeine, organic acids, protein and amino acids, saccharide, fiber, fat, and ash.

Manufacturing, Specifications, Batch Analyses

Green tea catechin is manufactured from aqueous green tea extract (the primary ingredient), which is mixed with a water/ethanol solution to dissolve the catechin monomers present in the green tea extract. Following dissolution of the catechin monomers, the ethanol and water solution containing the extracted catechin monomers is filtered by centrifugation to remove suspended solids, and the catechin monomers are then purified by adsorption to activated carbon.

The extract containing the highly concentrated catechin monomers is dried and the residual ethanol is allowed to evaporate. The extract is then sterilized (92° C for 2 minutes), inspected, and packaged as a brown aqueous solution. The product formed, CGT, is a water-soluble complex mixture of tea catechins. For the purpose of altering the taste of some beverages, a tannase-treated version of the CGT is sometimes employed in the manufacturing process (and the product is referred to as "Tannase-treated CGT"). Specifications for CGT and Tannase-treated CGT are presented in the GRAS dossier.

We have reviewed the specifications for Kao's CGT, and conclude that they are appropriate to ensure that the product meets suitable toxicological and microbiological standards for a product of this type.

Batch analyses demonstrate consistency of production. CGT is stable for 18 months as a liquid and six months in beverages.

All ingredients used in the manufacture of CGT are used in food and beverage manufacturing and are approved by the U.S. FDA.

Regulatory Status

Green tea extract has been granted GRAS status by the U.S. Food and Drug Administration ("FDA") (21 C.F.R. § 182.20). FDA has affirmed clay as GRAS for use as an indirect food substance (21 C.F.R. § 186.1256), and ethanol as GRAS for use as a direct food substance (21 C.F.R. § 184.1293). Active carbon and diatomaceous earth are widely used in the beverage manufacturing industry worldwide to refine beverages. Powdered cellulose has served as a food ingredient in the U.S. for nearly 85 years. Tannase is used as a processing aid in the production of some tea-containing beverages.

Exposure, Intended Uses, and Estimated Daily Intake (EDI)

CGT is intended for use as a natural extract in beverages, including bottled teas, sports drinks, carbonated soft drinks, and juice. The estimated intake of Catechins from Green Tea for the U.S. population was determined from appropriate databases including USDA's 1994-96 and 1998 CSFII surveys and reported in the dossier. These findings are summarized in the context of four scenarios:

Intake Scenario	Mg CGT/kg-bw/day, Mean (90th percentile)
Scenario 1: Consumers (US Population, 2+ years) of all bottled tea in the U.S. will switch to CGT beverage	4.3 (7.8)
Scenario 2: Consumers (US Population, 2+ years) of all bottled of sports drinks in the U.S. will switch to CGT beverage	6.5 (13.5)
Scenario 3: Consumers (US Population, 2+ years) of all bottled of carbonated beverages and juices in the U.S. will switch to CGT beverage	10.2 (21.2)
Scenario 4: Consumers (US Population, 2+ years) of all bottled of combined bottled tea, sports drinks, carbonated beverages, and juices in the U.S. will switch to proposed beverage	10.4 (21.5)

As of November 2007, Kao has sold 910 million bottles of beverages containing CGT in Japan

Self-Limiting Levels of Use

Although no limits on the use of CGT have been established, it is intended to be included in beverages at a level of 540 mg per bottle (typical beverage use). Its bitter, astringent taste likely would limit its use at levels exceeding 540 mg per bottle. Use levels will be in accordance with Good Manufacturing Practices.

Biological Data Relating to Safety

The safety of CGT has been established by published and unpublished studies, including acute oral (rat), subacute (rat, 14 days and 28 days), subchronic (rat, 6 months), developmental toxicity (rat), and genotoxicity (bacterial reverse mutation assay (Ames test), chromosomal aberration assay in cultured Chinese hamster ovary cells, mouse lymphoma L5178/tk assay, and mouse bone marrow micronucleus test). Due to a heat-sterilization step 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"). Published studies on catechin mixtures similar in composition to CGT demonstrate a lack of toxicity of the constituents of CGT in oral subchronic studies (rat, 90 days, 6 months), lack of irritation potential, and lack of reproductive/developmental toxicity (rat). In addition, published studies provide information on the absorption, distribution, metabolism, excretion, and toxicity of individual catechins or similar catechin mixtures.

Safety data are limited to *in vitro* and animal studies. However studies assessing the efficacy of CGT in humans for fat reduction also report useful safety data including hematology, clinical chemistry, and adverse event reporting. These data support the safety of the intended uses of CGT.

Absorption, Distribution, Metabolism and Excretion

Catechins from green tea are poorly absorbed from the gastrointestinal tract (< 10% of the oral dose), distributed in the gastrointestinal mucosa, and excreted in feces via bile or as unabsorbed catechins. Five to 8% of ingested CGT is absorbed within a relatively short time and partially conjugated with glucuronide and sulfate in microepithelial cells.

Acute Oral Toxicity Studies of Green Tea Catechin

The acute oral toxicity of HT-CGT was assessed in male and female Sprague-Dawley rats at a dose of 2000 mg/kg-bw (the limit dose based on OECD Guideline 401). No deaths or signs of clinical abnormalities were reported.

In an additional study of HT-CGT in male and female albino rats (strain not specified) at doses of either 2000 mg/kg-bw or 5000 mg/kg-bw (the limit dose based on Red Book Guidelines), one male and one female administered 5000 mg/kg-bw died 1 and 2 days after administration. In the 2000 mg/kg-bw group, 2 out of 6 females died 2 or 3 days after administration, and no males died. Based on the above results, the acute oral LD_{50} of HT-CGT was estimated to be greater than 2000 mg/kg-bw but less than 5000 mg/kg-bw

An estimated LD_{50} of 4647.29 mg/kg-bw in mice (strain not specified) was reported for Polyphenon 30, a mixture of catechins similar to CGT.

14-Day Oral Toxicity Studies of Green Tea Catechin (HT-CGT and UT-CGT)

CGT was administered by gavage to male and female Sprague-Dawley rats for 14 days at doses of 500, 1000, or 2000 mg/kg-bw/day. The No-Observed-Adverse-Event-Level ("NOAEL") for HT-CGT was determined to be 500 mg/kg-bw/day for males based on decreased body weight and body weight gain in the absence of decreased food consumption, and 2000 mg/kg-bw/day for females, the highest dose tested. A NOAEL of 2000 mg/kg-bw/day was observed for UT-CGT

28-Day Oral Toxicity Studies of Catechins from Green Tea (CGT)

CGT (UT-CGT, HT-CGT, or decaffeinated HT-CGT) was administered by gavage to male and female Sprague-Dawley rats for 28 days at doses of 0, 500, 1000, or 2000 mg/kg-bw/day (study consistent with OECD Guideline 407).

The NOAEL for HT-CGT was 1000 mg/kg-bw/day based on the lack of *local toxicity* (e.g., gastric erosions). The NOAEL for *systemic toxicity* was 2000 mg/kg-bw/day, the highest dose tested. Study findings for the 2000 mg/kg/day decaffeinated version of HT-CGT 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.

The NOAEL for UT-CGT was 2000 mg/kg bw/day (the highest dose tested).

90-Day Oral Toxicity Study of Catechins from Green Tea (CGT)

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, groups of 10 F344 rats per sex each were administered "Sunphenon 100S," a green tea catechin extract manufactured by Taiyo Kagaku Co., Ltd. that is substantially equivalent to Kao's CGT with regard to total catechins and individual catechin monomer content, in the diet at concentrations of 0 (control), 0.3%, 1.25%, and 5.0% for 90 days. No test article-related clinical observations or deaths were found during the study. 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.

While a number of hematological or clinical chemistry parameters were significantly altered as compared to the control animals, the only changes considered to possibly be related to treatment include increased alanine transaminase (ALT), and alkaline phosphatase (ALP) in the 5.0% group males and increased aspartate transaminase (AST), ALT, and ALP in the 5.0% 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, but there were no clinically significant histopathological lesions of the liver observed in these animals. The remaining changes were not dose-related and/or were not accompanied by changes in other relevant parameters or histopathological evidence of toxicity.

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).

Six-Month Oral Toxicity Study of Catechins from Green Tea (CGT)

CGT (HT-CGT) was administered by gavage to groups of 10 male and 10 female Sprague-Dawley rats for 6 months at doses of 0, 120, 400, and 1200 mg/kg-bw/day. Another group of 10 animals/sex received 1200 mg/kg-bw/day decaffeinated CGT (D-HT-CGT) (study consistent with OECD Guidelines).

The NOAEL for HT-CGT was 400 mg/kg-bw/day based on reduced weight gain in females at 1200 mg/kg-bw/day. The reduced weight gain was not accompanied by changes in food consumption.

Genotoxicity of Catechins from Green Tea

Three *in vitro* tests were performed with either UT-CGT or HT-CGT. Positive responses were observed in both the chromosomal aberration assay (in Chinese hamster lung cells, CHU/IU) and the mouse lymphoma L5178Y/tk assay but none were reported in the Ames test. The bone micronucleus test in ICR CD mice—an *in vivo* test—revealed no evidence of mutagenicity.

The results of these studies were qualitatively equivalent for UT-CGT and HT-CGT. Although positive findings were reported in the *in vitro* assays, the *in vivo* study failed to demonstrate genotoxicity. A published study on Polyphenon E reported negative results for a bacterial (*Salmonella typhimurium-E. coli*) mutagenicity assay, but positive results for the Big Blue CII transgenic mutation assay and mouse micronucleus test.

Developmental Toxicity of Catechins from Green Tea

The effects of HT-CGT on maternal toxicity and embryo/fetal development were studied in Sprague-Dawley rats after maternal exposure during gestation days 6 through 17. Groups of 25 bred females received 0, 200, 600, 02 2000 mg/kg-bw/day by gavage. All maternal animals survived to the scheduled necropsy on gestation day 20, and there were no test-article-related macroscopic findings. No test article-related fetal malformations or developmental variations were observed in this study. A NOAEL of 200 mg/kg-bw/day for maternal toxicity was estimated from this study based on the magnitude of body weight reductions observed, even though there was a corresponding decrease in food consumption. A NOAEL of 2000 mg/kg-bw/day, the highest dose tested, was estimated for embryo/fetal developmental toxicity (study consistent with the International Conference on Harmonisation Tripartite Guideline on Detection of Toxicity to Reproduction for Medicinal Products, Section 4.1.3).

Irritation and Sensitization

No evidence of irritation of the oral mucosa of hamster cheek pouches either immediately after treatment or 24 hours thereafter was reported for Polyphenon E.

Sensitization studies on CGT or related substances were not identified in the literature search.

Studies in Humans

A series of short- and longer-term clinical investigations on Kao's CGT and substances similar to CGT examining tolerability, safety, and nutritional effects in humans including clinical chemistry, hematology, urinalysis, and self-reported adverse event data demonstrated tolerability and no adverse health effects at daily intakes of up to 900 mg/day for 8, 12, or 24 weeks (studies of similar substances were carried out for up to 6 months and 1 year).

The Expert Panel also critically evaluated available information on several anecdotal case reports on the adverse effects of the green tea extract-containing dietary supplements, Exolise, Tealine, TRA, Green Lite, Hydroxycut, Fitofruit, and Chinese Green Tea. There were no case reports on Kao CGT. The Expert Panel concluded that the case reports were not pivotal in its assessment of the safety of Kao CGT because of the complexity of the patients' histories (*e.g.,* concomitant medications, alcohol use, pre-existing disease or risk factors) and because there were multiple

ingredients, in addition to green tea extract, contained in several of these dietary supplements. There were no published reports on adverse effects of Kao's CGT.

Summary and Discussion

Catechins from Green Tea is a mixture of eight catechins extracted from green tea leaves, which also includes polyphenols, caffeine, organic acids, protein and amino acids, saccharide, and ash. CGT is intended for use as a natural extract in beverages, including bottled teas, sports drinks, carbonated soft drinks, and juice. For the purpose of modifying taste in some of these beverages, CGT is sometimes slightly altered by treatment with tannase. The constituents of CGT are poorly absorbed, distributed in the gastrointestinal mucosa, and excreted in feces via bile. There is no evidence that CGT accumulates in the body and elicits toxicity.

A critical evaluation of available information on the safety of CGT in animals and in humans demonstrates that CGT presents no safety issues.

The upper estimated daily intake (i.e., scenario 4 described above) from all proposed uses is 10.4 mg/kg bw/day at the mean and 21.5 mg/kg bw/day at the 90th percentile.

Conclusion

Based on its independent and collective critical evaluation of the data and information currently available on CGT, the Expert Panel concludes that the intended uses of Kao CGT, manufactured in accordance with current Good Manufacturing Practice and meeting appropriate specifications for food grade material, are safe.

The Expert Panel further concludes that the intended uses of Kao CGT, manufactured in accordance with current Good Manufacturing Practice and meeting appropriate specifications for food grade material, are Generally Recognized as Safe (GRAS) based on scientific procedures, corroborated by history of safe use.

It is the opinion of the Expert Panel that other experts qualified by scientific training and

05 August 2008

Joseph F. Borzelleca, Ph.D., Chair
Professor Emeritus, Pharmacology & Toxicology
Virginia Commonwealth University
School of Medicine

James W. Anderson, M.D.
Professor Emeritus, Medicine and Clinical Nutrition;
Coordinator, HMR Weight Management Program
Endocrine Division
University of Kentucky School of Medicine

Michael W. Pariza, Ph.D.
Wisconsin Distinguished Professor of Food Microbiology and Toxicology
Director, Food Research Institute
University of Wisconsin, Madison

Conclusion

Based on its independent and collective critical evaluation of the data and information currently available on CGT, the Expert Panel concludes that the intended uses of Kao CGT, manufactured in accordance with current Good Manufacturing Practice and meeting appropriate specifications for food grade material, are safe.

The Expert Panel further concludes that the intended uses of Kao CGT, manufactured in accordance with current Good Manufacturing Practice and meeting appropriate specifications for food grade material, are Generally Recognized as Safe (GRAS) based on scientific procedures, corroborated by history of safe use.

It is the opinion of the Expert Panel that other experts qualified by scientific training and experience, and evaluating the same data and information, would concur with these conclusions.

_____

Joseph F. Borzelleca, Ph.D., Chair
Professor Emeritus, Pharmacology & Toxicology
Virginia Commonwealth University
School of Medicine

_____ 8 Aug 2008

James W. Anderson, M.D.
Professor Emeritus, Medicine and Clinical Nutrition;
Coordinator, HMR Weight Management Program
Endocrine Division
University of Kentucky School of Medicine

_____

Michael W. Pariza, Ph.D.
Wisconsin Distinguished Professor of Food Microbiology and Toxicology
Director, Food Research Institute
University of Wisconsin, Madison

Conclusion

Based on its independent and collective critical evaluation of the data and information currently available on CGT, the Expert Panel concludes that the intended uses of Kao CGT, manufactured in accordance with current Good Manufacturing Practice and meeting appropriate specifications for food grade material, are safe.

The Expert Panel further concludes that the intended uses of Kao CGT, manufactured in accordance with current Good Manufacturing Practice and meeting appropriate specifications for food grade material, are Generally Recognized as Safe (GRAS) based on scientific procedures, corroborated by history of safe use.

It is the opinion of the Expert Panel that other experts qualified by scientific training and experience, and evaluating the same data and information, would concur with these conclusions.

_____

Joseph F. Borzelleca, Ph.D., Chair
Professor Emeritus, Pharmacology & Toxicology
Virginia Commonwealth University
School of Medicine

_____

James W. Anderson, M.D.
Professor Emeritus, Medicine and Clinical Nutrition;
Coordinator, HMR Weight Management Program
Endocrine Division
University of Kentucky School of Medicine

_____

Michael W. Pariza, Ph.D.
Wisconsin Distinguished Professor of Food Microbiology and Toxicology
University of Wisconsin, Madison

F

Appendix F

Curricula Vitae of Expert Panel Members

Curricula Vitae for Joseph F. Borzelleca, Ph.D.

Curriculum Vitae

Joseph F. Borzelleca, Ph.D.

Personal History

Name: Joseph Francis Borzelleca S.S. No: upon request

University Address:
Dept. of Pharmacology andToxicology
Medical College of Virginia
Virginia Commonwealth University
Box 980613
Richmond, VA 23298-0613

Educational Background

B.S.	St. Joseph's University, Philadelphia, PA Biology, Chemistry	1952
M.S.	School of Graduate Studies Thomas Jefferson University Jefferson Medical Co llege, Philadelphia, PA Pharmacology, Physiology	1954
Ph.D.	School of Graduate Studies Thomas Jefferson University Jefferson Medical College, Philadelphia, PA Pharmacology, Biochemistry	1956

Academic Appointments

Department of Pharmacology Medical College of Pennsylvania Philadelphia, PA	Instructor-Associate	1956-1959
Department of Pharmacology and Toxicology Medical College of Virginia Richmond, VA 23298-0613	Assistant Professor	1959-1962
	Associate Professor	1962-1967
	Professor	1967-
	Head, Division of Toxicology	1972-1986
	Professor Emeritus	01 July 1996-

Professional Certification

Fellow, Academy of Toxicological Sciences

Professional Affiliations

Societies

Academy of Toxicological Sciences* **
American Association for the Advancement of Science
American Chemical Society
American College of Toxicology*
American Society of Pharmacology and Experimental Therapeutics**
(Environmental Pharmacology Committee; Liaison Committee, SOT; Toxicology Committee)
Institute of Food Technologists (Professional Member)
International Society of Regulatory Toxicology and Pharmacology*
(Member of Council)
Sigma XI
Society of Experimental Biology and Medicine*
(Councilor; Program Chairman of Southeastern Section)
Society for Risk Analysis
Society of Toxicology* **
(Member and/or Chairman: Awards, Education, Legislative Affairs, Membership, Nominating Committees; Secretary of the Society, Councilor, and President; President,
Food Safety Specialty Section)
Virginia Academy of Science*
(Chairman, Medical Sciences Division)

* Held elected office
** Held appointed office or position

Board of Directors

ILSI (until 2002)

Board of Scientific and Policy Advisors

American Council on Science and Health (until 2000)

Journals

Editor, Food Chemical Toxicology, 1992-

Editorial Board
Environmental Carcinogenesis Reviews, 1981-2000
Journal of Environmental Pathology,Toxicology and Oncology 1977- 2000
Journal of Environmental Science and Health, 1979-2004
Journal of the American College of Toxicology, 1982-
Journal of Toxicology: Cutaneous and Ocular Toxicology, 1982- 1992
Journal of Applied Toxicology, 1989-
Pharmacology, 1978-
Pharmacology and Drug Development, 1980-
Toxicology and Applied Pharmacology, 1975-1978

Consultantships (Past, Present)

Governmental

Food and Drug Administration
National Institute of Mental Health
National Cancer Institute
Environmental Protection Agency
Department of Labor - OSHA (Chairman, Carcinogens Standards Committee)
U.S. Army - Research and Development Command

Non-Governmental

National Academy of Sciences - NRC
Committee on Toxicology (Member, Chairman)/Board on Toxicology and Environmental Health Hazards
Safe Drinking Water Committee
Evaluation of Household Substances Committee (1138 Committee)
Food Protection Committee
Food Additives Survey Committee
Committee on Risk-Based Criteria for Non-RCRA Hazardous Wastes
Committee on Risk Assessment of Flame-Retardant Chemicals
Food Chemicals Codex Committee

Federation of American Societies of Experimental Biology
Select Committee on GRAS Substances
Flavors and Extracts
Biotechnology Product Safety
Caprenin GRAS Committee

World Health Organization
Joint Meeting on Pesticide Residues (JMPR) (Member, Chairman)

NATO/CCMS Drinking Water Committee

Industrial

Chemical Companies; Trade Associations

University Activities

Related to Instruction

Prepared a laboratory manual in pharmacology (animal and human studies) (1960)
Introduced the use of closed circuit TV and TV tapes in pharmacology (1960)
Introduced clinical pharmacological experiments into the medical and dental programs (1960)
Planning and participation in continuing education program (Schools of Dentistry, Medicine and Pharmacy)
Planning and administration: each of the three major efforts in pharmacology (dental, medical, pharmacy) since 1960.
Graduate Program - assisted in developing graduate training program in toxicology

Current Teaching Activities

Present lectures on Toxicological Issues, Food Intake and Control

Not Directly Related to Instruction

Elected senator from the graduate school, then vice-president of the University Senate
Served on various committees (e.g. Curriculum, Search, Animal Care,) in each of the four major schools (Dentistry, Graduate, Medical, Pharmacy)

Research

Research was continuously funded from 1956. Sources of support included governmental (U.S.P.H.S.; N.I.H; E.P.A.; N.I.D.A.) and non-governmental (industrial). (A list of publications is attached).

Awards

DOD - US Army - Chemical Research Development and Engineering Center
Distinguished Service Award, 1986

National Italian - American Foundation Award
Excellence in Medicine and Community Service, 1987

Thomas Jefferson University
Distinguished Alumnus Award, 1987

Virginia Commonwealth University - School of Basic Health Sciences
Outstanding Faculty Award, 1987

Virginia Commonwealth University, Dept. of Pharmacology and Toxicology
Professor of the Year- 1992

American College of Toxicology
Distinguished Service Award - 1997

Virginia's Life Achievement in Science Award- April 2001

Bernard L. Oser Food Ingredient Safety Award by the Institute of Food Technologists- June 2001

International Society for Regulatory Toxicology and Pharmacology's International Achievement Award for 2001- December 2001

Society of Toxicology - Education Award- March 2002

PUBLICATIONS

Borzelleca, J.F. and Manthei, R.W.: Factors influencing pentobarbital sleeping time in mice. Arch. Int. Pharmacodyn. Ther. 111: 296, 1957.

Borzelleca, J.F.: Studies of the contribution of bladder absorption to the physiological changes induced by pentobarbital. J. Pharm. Exp. Ther. 129: 305, 1960.

Borzelleca, J.F.: The absorption of nicotine from the urinary bladder of the dog. Arch. Int. Pharmacodyn. Ther. 133: 444, 1961.

Borzelleca, J.F., Bowman, E.R. and McKennis, H., Jr.: The cardiovascular and respiratory effects of (-)-cotinine. J. Pharmacol. Exp. Ther. 137: 313, 1962.

Borzelleca, J.F.: Drug absorption from the urinary tract of the rat. Nicotine. Arch. Int. Pharmacodyn. Ther. 143: 595, 1963.

Borzelleca, J.F.: Influence of saline and glucose infusions on the course of barbiturate intoxication. Arch. Int. Pharmacodyn. Ther. 146: 163, 1963.

Larson, P.S., Borzelleca, J.F., Bowman, E.R., Crawford, E.M., Smith, R.B., Jr. and Henningar, G.R.: Toxicologic studies on a preparation of p-tertiary octylphenoxy-polyethoxy ethanols (Trition X-405). Toxicol. Appl. Pharmacol. 5: 782, 1963.

Borzelleca, J.F., Larson, P.S., Henningar, G.R., Hug, E.G., Crawford, E.M. and Smith, R.B., Jr.: Studies on the chronic oral toxicity of monomeric ethyl acrylate and methyl methacrylate. Toxicol. Appl. Pharmacol. 6: 29, 1964.

Borzelleca, J.F. and Cherrick, H.: The excretion of drugs in saliva. Antibiotics. J. Oral Ther. Pharmacol. 2: 180, 1965.

Borzelleca, J.F. and Lester, D.: Acute toxicity of some perhalogenated acetones. Toxicol. Appl. Pharmacol. 7: 592, 1965.

Borzelleca, J.F.: Drug movement from the isolated urinary bladder of the rabbit. Arch. Int. Pharmacodyn. Ther. 154: 40, 1965.

Borzelleca, J.F.: Rabbit urinary bladder potentials. Invest. Urology 3: 77, 1965.

Borzelleca, J.F.: Studies on the mechanisms of drug movement from the isolated urinary bladder. J. Pharmacol. Exp. Ther. 148: 111, 1965.

Lowenthal, W. and Borzelleca, J.F.: Drug absorption from the rectum. I. J. Pharm. Sci. 54: 1790, 1965.

Ambrose, A.M., Borzelleca, J.F., Larson, P.S., Smith, R.B., Jr. and Hennigar, G.R.: Toxicologic studies on monochloroacetaldehyde: 2,4-dinitrophenylhydrazone, a foliar fungicide. Toxicol. Appl. Pharmacol. 8: 472, 1966.

Borzelleca, J.F. and Doyle, C.H.: Excretion of drugs in saliva. Salicylate, barbiturate, sulfanilamide. J. Oral. Ther. Pharmacol. 3: 104, 1966.

Borzelleca, J.F. and Lowenthal, W.: Drug absorption from the rectum. II. J. Pharm. Sci. 55: 151, 1966.

Wooles, W.R. and Borzelleca, J.F.: Prolongation of barbiturate sleeping time in mice by stimulation of the reticuloendothelial system. J. Reticuloendothel. Soc. 3: 41, 1966.

PUBLICATIONS

Wooles, W.R., Borzelleca, J.F. and Branham, G.W.: The effects of acute and prolonged salicylate administration on liver and plasma triglyceride levels and dietary-induced hypercholesterolemia. Toxicol. Appl. Pharmacol. 10: 1, 1967.

Borzelleca, J.F., Harris, T. and Bernstein, S.: The effect of DMSO on drug movement through the wall of the urinary bladder of the rabbit. J. Invest. Urol. 6: 43, 1968.

Borzelleca, J.F.: The excretion of glucose in saliva. Dog. J. Oral Ther. Pharmacol. 4: 338, 1968.

Kim, K.S., Borzelleca, J.F., McKennis, H. and Bowman, E.R.: Pharmacological effects of some nicotine metabolites and related compounds. J. Pharmacol. Exp. Ther. 161: 59, 1968.

Marcus, S. and Borzelleca, J.F.: Observations of reserpine-induced bradycardia. Arch. Int. Pharamacodyn. Ther. 174: 12, 1968.

Schwartz, S.L. and Borzelleca, J.F.: Adrenergic blood pressure response in the shark. Science 163: 395, 1969.

Ambrose, A.M., Borzelleca, J.F., Larson, P.S. and Hennigar, G.R.: The toxicology of a foliar fungicide, GC-4072. Toxicol. Appl. Pharmacol. 17: 323, 1970.

Borzelleca, J.F. and Putney, J.W., Jr.: A model for the movement of salicylate across the parotid epithelium. J. Pharmacol. Exp. Ther. 174: 527, 1970.

Borzelleca, J.F. and Putney, J.W., Jr.: Studies on the biotransformation of salicylic acid by the salivary gland. Arch. Int. Pharmacodyn. Ther. 188: 127, 1970.

Lowenthal, W., Borzelleca, J.F. and Corder, C.D., Jr.: Drug absorption from the rectum. III. Aspirin and some aspirin derivatives. J. Pharm. Sci. 59: 1353, 1970.

Putney, J.W., Jr. and Borzelleca, J.F.: A method for the determination of small quantities of salicylate metabolites in the presence of a great excess of salicylic acid. Arch. Int. Pharmacodyn. Ther. 188: 119, 1970.

Wynn, J.E., van't Riet, B. and Borzelleca, J.F.: Excretion and toxicity of EGTA and EDTA after oral administration to rats. Toxicol. Appl. Pharmacol. 16: 807, 1970.

Borzelleca, J.F., Larson, P.S., Crawford, E.M., Hennigar, G.R., Jr., Kuchar, E.J. and Klein, H.H.: Toxicologic and metabolism studies on pentachloronitrobenzene. Toxicol. Appl. Pharmacol. 18: 522, 1971.

Putney, J.W., Jr. and Borzelleca, J.F.: On the mechanisms of ^{14}C-nicotine distribution in rat submaxillary gland in vitro. J. Pharmacol. Exp. Ther. 178: 180, 1971.

Ambrose, A.M., Larson, P.S., Borzelleca, J.F., Smith, R.B., Jr. and Hennigar, G.R.: Toxicologic studies on 2,4-dichlorophenyl-p-nitrophenyl ether. Toxicol. Appl. Pharmacol. 19: 263, 1971.

Putney, J.W., Jr. and Borzelleca, J.F.: On the mechanisms of ^{14}C- salicylic acid distribution in rat submaxillary gland in vitro. J. Pharmacol. Exp. Ther. 117: 263, 1971.

Egle, J.L., Jr., Putney, J.W., Jr. and Borzelleca, J.F.: Cardiac rate and rhythm in mice affected by haloalkane propellants. J.A.M.A. 222: 786, 1972.

Ambrose, A.M., Larson, P.S., Borzelleca, J.F. and Hennigar, G.R.: Toxicologic studies on 3',4'-dichloropropionanilide. Toxicol. Appl. Pharmacol. 23: 650, 1972.

PUBLICATIONS

Putney, J.W., Jr. and Borzelleca, J.F.: On the mechanisms of ^{14}C-salicylic acid excretion by the rat submaxillary gland. J. Pharmacol. Exp. Ther. 182: 515, 1972.

Putney, J.W., Jr. and Borzelleca, J.F.: Active accumulation of ^{14}C-salicylic acid by rat kidney cortex in vitro. J. Pharmacol. Exp. Ther. 186: 600, 1973.

Borzelleca, J.F.: Safety evaluation and toxicological tests and procedures. J.A.O.A.C. 58: 692, 1975.

Jordan, R.L., Sperling, F., Klein H.H., Borzelleca, J.F. A study of the potential teratogenic effects of pentachloronitrobenzene in rats.
Toxicol. Appl. Pharmacol. 33 :222-30,1975

Allen, M.A., Wrenn, J.M., Putney, J.W., Jr. and Borzelleca, J.F.: A study of the mechanism of transport of diphenylhydantoin in the rat submaxillary gland in vitro. J. Pharmacol. Exp. Ther. 197: 408, 1976.

Adams, M.D., Wedig, J.H., Jordan, R.L., Smith, L.W., Henderson, R. and Borzelleca, J.F.: Urinary excretion and metabolism of salts of 2-pyridinethiol-1-oxide following intravenous administration to female yorkshire pigs. Toxicol. Appl. Pharmacol. 36: 523, 1976.

Ambrose, A.M., Larson, P.S., Borzelleca, J.F. and Hennigar, G.R.: Long-term toxicologic assessment of nickel in rats and dogs. J. Food Sci. Technol. 13: 181, 1976.

Egle, J.L., Jr., Long, J.E., Simon, G.S. and Borzelleca, J.F.: An evaluation of the cardiac sensitizing potential of a fabric protector in aerosol form, containing 1,1,1-trichloroethane. Toxicol. Appl. Pharmacol. 38: 369, 1976.

Egle, J.L., Jr., Fernandez, S.B., Guzelian, P.S. and Borzelleca, J.F.: Distribution and excretion of chlordecone (Kepone) in the rat. Drug Metab. Dispos. 6:91, 1976

Weinberg, A.D., Dimen, E.M., Borzelleca, J.F. and Harris, L.S.: Weight and activity in male mice after daily inhalation of cannabis smoke in an automated smoke exposure chamber. J. Pharm. & Pharmac. 29: 477, 1977.

Siu, R.G., Borzelleca, J.F., Carr, C.J., Day, H.G., Fomon, S.J, Irving, G.W. Jr, La Du, B.N. Jr., McCoy, J.R., Miller, S.A., Plaa, G.L., Shimkin, M.B., Wood, J.L. Evaluation of health aspects of GRAS food ingredients: lessons learned and questions unanswered. Fed Proc. 36 :2519-62,1977

Weinberg, A.D., Dimen, E.M., Simon, G.S., Harris, L.S. and Borzelleca, J.F.: Measurements of weight and activity in male mice following inhalation of cannabis smoke in a controlled smoke exposure chamber. Toxicol. Appl. Pharmacol. 42: 301, 1977.

Allen, M.A., Wrenn, J.M., Putney, J.W., Jr. and Borzelleca, J.F.: A study of the mechanisms of transport of benzylpenicillin in the rat submaxillary gland. Arch. Int. Pharmacodyn. 233: 180, 1978.

Bowman, F.J., Borzelleca, J.F. and Munson, A.E.: The toxicity of some halomethanes in mice. Toxicol. Appl. Pharmacol. 44: 213, 1978.

Egle, J.L., Jr., Fernandez, S.B., Guzelian, P.S. and Borzelleca, J.F.: Distribution and excretion of chlordecone (Kepone) in the rat. Drug Metab. Dispos. 6: 91, 1978.

PUBLICATIONS

McConnell, W.R. and Borzelleca, J.F.: A study of the mechanism of transport of Δ9-tetrahydrocannabinol in the rat submaxillary gland in vivo. Arch. Int. Pharmacodyn. Ther. 235: 180, 1978.

McConnell, W.R., Dewey, W.L., Harris, L.S. and Borzelleca, J.F.: A study of the effect of delta-9-tetrahydrocannabinol (delta-9-THC) on mammalian salivary flow. J. Pharmacol. Exp. Ther. 206: 567, 1978.

Schumann, A.M. and Borzelleca, J.F.: An assessment of the methemoglobin and Heinz body inducing capacity of pentachloronitrobenzene in the cat. Toxicol. Appl. Pharmacol. 44: 523, 1978.

Simon, G.S., Tardiff, R.G. and Borzelleca, J.F.: Potential mutagenic and adverse male reproductive effects of 1,2,3,4-tetrabromobutane. A dominant lethal study in the rat. Toxicol. Appl. Pharmacol. 44: 661, 1978.

Carmines, E.L., Carchman, R.A. and Borzelleca, J.F.: Kepone: Cellular sites of action. Toxicol. Appl. Pharmacol. 49: 543, 1979.

Egle, J.L., Jr., Guzelian, P.S. and Borzelleca, J.F.: Time course of the acute toxic effects of sublethal doses of chlordecone (Kepone). Toxicol. Appl. Pharmacol. 48: 533, 1979.

Larson, P.S., Egle, J.L., Jr., Hennigar, G.R. and Borzelleca, J.F.: Acute and subchronic toxicity of mirex in the rat, dog, and rabbit. Toxicol. Appl. Pharmacol. 49: 271, 1979.

Larson, P.S., Egle, J.L., Jr., Hennigar, G.R., Lane, R.W. and Borzelleca, J.F.: Acute, subchronic and chronic toxicity of chlordecone. Toxicol. Appl. Pharmacol. 48: 29, 1979.

Simon, G.S., Kuchar, E.J., Klein, H.H. and Borzelleca, J.F.: Distribution and clearance of pentachloronitrobenzene in chickens. Toxicol. Appl. Pharmacol. 50: 401,1979.

Simon, G.S., Tardiff, R.G. and Borzelleca, J.F.: Failure of hexachlorobenzene to induce dominant lethal mutations in the rat. Toxicol. Appl. Pharmacol. 47: 415, 1979.

Borzelleca, J.F. and Skalsky, H.L.: The excretion of pesticides in saliva and its value in assessing exposure. J. Environ. Sci. Health, B15(6), 843, 1980.

Borzelleca, J.F., Egle, J.L., Jr., Hennigar, G.R., Klein, H.H., Kuchar, E.J., Lane, R.W. and Larson, P.S.: A toxicologic evaluation of 5-ethoxy-3- trichloromethyl-1,2,4-triadiazole (ETMT). Toxicol. Appl. Pharmacol. 56: 164, 1980.

Carmines, E.L., Carchman, R.A. and Borzelleca, J.F.: A method for the evaluation of dose-effect data utilizing a programmable calculator. J. Environ. Pathol. Toxicol. 4:23, 1980.

Kessler, F.K., Laskin, D.L., Borzelleca, J.F. and Carchman, R.A.: Assessment of somatogenotoxicity of povidone-iodine using two in vitro assays. J. Environ. Pathol. Toxicol. 3: 327, 1980.

Skalsky, H.L., Wrenn, J.M. and Borzelleca, J.F.: In vitro and in vivo evaluation of the movement of Kepone in the rat submaxillary gland. J. Environ. Pathol. Toxicol. 3: 529, 1980.

Smith, L.W. and Borzelleca, J.F.: Excretion of cadmium and mercury in rat saliva. Toxicol. Appl. Pharamacol. 54: 134, 1980.

Smith, L.W. and Borzelleca, J.F.: In vitro stimulation of oxygen consumption in rat submaxillary gland by pilocarpine. J. Dent. Res. (59)9: 1531, 1980.

PUBLICATIONS

Smith, L.W. and Borzelleca, J.F.: Movement of cadmiun in rat submaxillary slices. Toxicol. Appl. Pharmacol. 55: 403, 1980.

Smith, L.W. and Borzelleca, J.F.: Movement of mercury in rat submaxillary slices. Toxicology 18: 169, 1980.

Borzelleca, J.F.: Report of the NATO/CCMS drinking water pilot study on health aspects of drinking water contaminants. Sci. Tot. Environ. 18: 205, 1981.

Carmines, E.L., Carchman, R.A. and Borzelleca, J.F.: Investigations into the mechanism of paraquat toxicity utilizing a cell culture system. Toxicol. Appl. Pharmacol. 58: 353, 1981.

Simon, G.S., Borzelleca, J.F. and Dewey, W.L.: Narcotics and diabetes II. Streptozotocin-induced diabetes selectively alters the potency of certain narcotic analgesics. Mechanism of diabetes: morphine interaction. J. Pharmacol. Exp. Ther. 218: 324, 1981.

Balster, R.L. and Borzelleca, J.F.The behavioral toxicity of trihalomethane contaminants of drinking water in mice. Environ. Health Perspect. 46: 127, 1982.

Kauffmann, B.M., White, K.L., Jr., Sanders, V.M., Douglas, K.A., Sain, L.E., Borzelleca, J.F.and Munson A.E.: Humoral and cell-mediated immune status in mice exposed to chloral hydrate. Environ. Health Perspect. 44: 147, 1982.

Lane, R.W., Riddle, B.L. and Borzelleca, J.F.: Effects of 1,2-dichloroethane and 1,1,1-trichloroethane in drinking water on reproduction and development in mice. Toxicol. Appl. Pharmacol. 63: 409, 1982.

Munson, A.E., Sain, L.E., Sanders, V.M., Kauffmann, B.M., White, K.L., Jr., Page, D.G., Barnes, D.W., and Borzelleca, J.F.: Toxicology of organic drinking water contaminants: trichloromethane, bromodichloromethane, dibromochloromethane and tribromomethane. Environ. Health Perspect. 46: 117, 1982.

Sanders, V.M., Kauffmann, B.M., White, K.L., Douglas, K.A., Barnes, D.W., Sain, L.E., Bradshaw, T.J., Borzelleca, J.F. and Munson, A.E.: Toxicology of chloral hydrate in the mouse. Environ. Health Perspect. 44: 137, 1982.

Sanders, V.M., Tucker, A.N., White, K.L., Jr., Kauffmann, B.M., Hallett, P., Carchman, R.A., Borzelleca, J.F. and Munson, A.E.: Humoral and cell-mediated immune status in mice exposed to trichloroethylene in the drinking water. Toxicol. Appl. Pharmacol. 62: 358, 1982.

Borzelleca, J.F.: A review of volatile organic contaminant data. Proc. AWWA Water Quality Tech. Conf. 225, 1983.

Charles, J.L., Kram, D., Borzelleca, J.F. and Carchman, R.A.: The kinetics of in vivo sister chromatid exchange induction in mouse bone marrow cells by alkylating agents. I. Cyclophosphamide. Environ. Mutagen. 5: 825, 1983.

Borzelleca, J.F., Condie, L.W. and Hayes, J.R.: Toxicological evaluation of selected chlorinated phenols. Proceedings of the 5th International Water Disinfection Conference, Williamsburg, VA, 1984 .

Borzelleca, J.F.: Food safety: regulations, research, and results. Va. Med. 111: 390, 1984.

Seyler, D.E., East, J.M., Condie, L.W. and Borzelleca, J.F.: The use of in vitro methods for assessing reproductive toxicity of dichlorophenols. Toxicol. Letters 20: 309, 1984.

PUBLICATIONS

Shopp, G.M., White, K.L., Jr., Holsapple, M.P., Barnes, D.W., Duke, S.S., Anderson, A.C., Condie, L.W., Jr., Hayes, J.R. and Borzelleca, J.F.: Naphthalene toxicity in CD-1 mice: general toxicology and immunotoxicology. Fund. Appl. Toxicol. 4: 406, 1984.

Borzelleca, J.F. and Hogan, G.K.: Chronic toxicity/carcinogencity study of FD&C Blue No. 2 in mice. Food Chem. Toxicol. 23: 719, 1985.

Borzelleca, J.F., Hayes, J.R., Condie, L.W. and Egle, J.L., Jr.: Acute toxicity of monochlorophenols, dichlorophenols and pentachlorophenol in the mouse. Toxicol. Letters 29: 39, 1985.

Borzelleca, J.F., Hayes, J.R., Condie, L.W. and Egle, J.L.: Acute and subchronic toxicity of 2,4-dichlorophenol in CD-1 mice. Fund. Appl. Toxicol. 5: 478, 1985.

Borzelleca, J.F., Hogan, G.K. and Koestner A.: Chronic toxicity/carcinogencity study of FD&C Blue No. 2 in rats. Food Chem. Toxicol. 23: 551, 1985.

Hayes, J.R. and Borzelleca, J.F.: Nutrient interaction with drugs and other xenobiotics, J. Am. Diet. Assoc. 85: 3 335, 1985.

Lane, R.W., Simon, Glen, S.S., Dougherty, R.W., Egle, J.L. and Borzelleca, J.F.: Reproductive toxicity and lack of dominant lethal effects of 2,4-dinitrotolune in the male rat. Drug Chem. Toxicol. 4: 265, 1985.

Borzelleca, J.F., Goldenthal, E.I. and Wazeter, F.X.: A multigeneration study of FD&C Blue No. 2 in rats. Food Chem. Toxicol. 24: 159, 1986.

Charles, J.L., Jacobson-Kram, D., Condie, L.W., Jr., Borzelleca, J.F. and Carchman, R. A.: The kinetics of in vitro sister chromatid exchange induction in mouse bone marrow cells by ethylnitrosourea and methylnitosourea. Toxicol. Appl. Pharmacol. 84: 56, 1986.

Hayes, J.R., Condie, L.W., Jr. and Borzelleca J.F.: The subchronic toxicity of tetrachorethylene (perchloroethylene) administered in the drinking water of rats. Fundam. Appl. Toxicol. 7: 119, 1986.

Hayes, J.R., Condie, L.W., Jr. and Borzelleca, J.F.: Acute, 14-day repeated dosing and 90-day subchronic toxicity studies of carbon tetrachloride in CD-1 mice. Fundam. Appl. Toxicol. 7: 454, 1986.

Hayes, J.R., Condie, L.W., Jr. and Borzelleca, J.F.: Acute, 14-day repeated dosing, and 90-day subchronic toxicity studies of potassium picloram. Fund. Appl. Toxicol. 7: 464, 1986.

Hayes, J.R., Condie, L.W., Jr. and Borzelleca, J.F.: Toxicology of haloacetonitriles. Environ. Health Perspect. 69: 183, 1986.

Lane, R.W., Sturm, R.J., Borzelleca, J.F. and Carchman, R.A.: Effect of in vitro differentiation on phorbol diester receptor number in human promyelocytic leukemia (HL-60) cells. Cancer Res. 46: 3782, 1986.

Simon, G.S., Egle, J.L., Jr., Dougherty, R.W. and Borzelleca, J.F.: Dominant lethal assay of chlordecone and its distribution in the male reproductive tissues of the rat. Toxicol. Letters 30: 237, 1986.

Tarka, S.M., Jr., Applebaum, R.S. and Borzelleca, J.F.: Evaluation of the perinatal, postnatal and teratogenic effects of coca powder and theobromine in Sprague-Dawley/CD rats. Food Chem. Toxicol. 24: 375, 1986.

PUBLICATIONS

Tarka, S.M., Jr., Applebaum, R.S. and Borzelleca, J.F.: Evaluation of the teratogenic potential of cocoa powder and theobromine in New Zealand white rabbits. Food Chem. Toxicol. 24: 363, 1986.

Borzelleca, J.F., Capen, C.C. and Hallagan, J.B.: Lifetime toxicity/carcinogenicity study of FD&C Red no. 3 (erythrosine) in rats. Food Chem. Toxicol. 25: 723, 1987.

Borzelleca, J.F., Capen, C.C., and Hallagan, J.B.: Lifetime toxicity/carcinogenicity study of FD&C Red No. 3 (erythrosine) in mice. Food Chem. Toxicol. 25: 735, 1987.

Hayes, J.R., Condie, L.W., Jr., Egle, J.L., Jr. and Borzelleca, J.F.: The acute and subchronic toxicity in rats of trans-1,2 dichloroethylene in drinking water. J. Am. Coll. Toxicol. 6: 471, 1987.

Borzelleca, J.F. and Hallagan, J.B.: Chronic toxicity/carcinogenicity studies of FD&C Yellow No. 5 (tartrazine) in rats. Food Chem. Toxicol. 26: 179, 1988.

Borzelleca, J.F., Condie, L.W., Jr. and Egle, J.L.: Short-term toxicity (one-and ten-day gavage) of barium chloride in male and female rats. J. Am. Coll. Toxicol. 7: 675-685, 1988.

Condie, L.W., Jr., Hill, J.R. and Borzelleca, J.F.: Oral toxicology studies with xylene isomers and mixed xylenes. Drug and Chem. Toxicol. 11: 329, 1988.

Borzelleca, J.F. and Hallagan, J.B.: A chronic toxicity/carcinogenicity study of FD&C yellow no. 5 (tartrazine) in mice. Food Chem. Toxicol. 26:189, 1988.

Borzelleca, J.F., Clark, E.C. and Condie, L.W., Jr.: Short-term toxicity (1 and 10 days) of cadmium chloride in male and female rats: gavage and drinking water. J. Am. Coll. Toxicol. 8: 377, 1989.

Borzelleca, J.F., Condie, L.W., Jr., Clarke, E.C. and Egle, J.L.: Short-term toxicity (one and ten day gavage) of potassium dichromate in male and female rats. J. Am. Coll. Toxicol. 8: 1197, 1989.

Borzelleca, J.F., Olson, J.W.A. and Reno, F.A.: Lifetime toxicity/carcinogenicity study of FD&C red No. 40 (allura red) in Sprague-Dawley rats. Food Chem. Toxicol. 27: 701, 1989.

Borzelleca, J.F.: Status of colors and flavors used in the confectionery industry. Proc. 106th Annual Convention of the National Confectioners Association of the United States. 33, 1989.

O'Hara, T.M., Borzelleca, J.F., Clark, E.C., Sheppard, M.A. and Condie, L.W., Jr.: A $CCl_4/CHCl_3$ interaction study in isolated hepatocytes: selection of a vehicle. Fund. Appl. Toxicol. 13: 605, 1989.

Lamb, R.G., Borzelleca, J.F., Condie, L.W. and Gennings, C.: Toxic interactions between carbon tetrachloride and chloroform in cultured rat hepatocytes. Toxicol. Appl. Pharmacol. 101: 106, 1989.

Borzelleca, J.F. and Hallagan, J.B.: Multigeneration study of FD&C red no. 3 (erythrosine) in sprague-dawley rats. Fd. Chem. Tox. 28: 813, 1990

Lauer, W.C., Johns, F.J., Wolfe, G.W., Myers, B.A., Condie, L.W., Borzelleca, J.F.: Comprehensive health effects testing program for Denver's potable water reuse demonstration project. J. Toxicol. Environ. Health. 30: 305-21, 1990

PUBLICATIONS

Borzelleca, J.F., Depukat, K. and Hallagan, J.B.: Lifetime toxicity/carcinogenicity studies of FD&C blue no. 1 (brilliant blue FCF) in rats and mice. Fd. Chem. Toxic. 28: 221,1990.

Borzelleca, J.F., O'Hara, T.M., Gennings, C., Granger, R.H., Sheppard, M.A. and Condie, L.W., Jr.: Interactions of water contaminants. I. Plasma enzyme activity and response surface methodology following gavage administration of CCl_4 and $CHCl_3$ or TCE singly and in combination in the rat. Fund. Appl. Toxicol. 14: 477, 1990.

Borzelleca, J.F., Olson, J.W.A. and Reno, F.A.: Lifetime toxicity/carcinogenicity study of FD&C red no. 40 (allura red) in mice. Fd. Chem. Tox. 29:313, 1991.

O'Hara, T.M., Sheppard, M.A., Clarke, E.C., Borzelleca J.F., Gennings, C. and Condie, L.W., Jr.: A CCl_4/$CHCl_3$ interaction study in isolated hepatocytes: non-induced, and phenobarbital pretreated cells. J. Appl. Toxicol. 11: 147, 1991

Borzelleca, J.F.: Assessment of Safety/Risk of Chemicals- Inception and Evolution of the ADI and Dose-Response Modeling Procedures- Commentary. Toxicol. Letters 59:1, 1991

Borzelleca, J.F.: The safety evaluation of macronutrient substitutes. Crit. Rev. Food Sci. Nutr. 32: 127, 1992

Borzelleca, J.F.: Macronutrient substitutes: safety evaluation. Regul. Toxicol. Pharmacol. 16: 253, 1992

Waddell, W.J., Borzelleca, J.F., Doull, J., Grasso, P., LeBourhis, B., Levy, P.S. and Tamburro, C.H.: Alcohol and Cancer. Br. J. Cancer. 66: 1200, 1992

Borzelleca, J.F: Evaluation of the safety of tara gum as a food ingredient: a review of the literature. J. Am. Coll. Toxicol. 12 (1): 81,1993

Borzelleca, J.F. and Egle, J. L. Jr.: An evaluation of the reproductive and developmental effects of tara gum in rats. J. Am. Coll. Toxicol. 12 (1): 91, 1993

Borzelleca, J.F.: Interactions of environmental chemicals and toxins. In: Proceedings of the Second Princess Chulabhorn Science Congress:" Environment, Science and Technology: the Challenges of the 21st Century." 1993

Borzelleca, J.F., Egle, J.L., Jr., Harris, L.S., Johnson, D.N., Terrill, J.B. and Belleville, J.A.N.: Toxicological evaluation of mu-agonists Part I. Assessment of toxicity following 30 days of repeated oral dosing of male and female rats with levo-alpha-acetylmethadol HCl (LAAM). J. Appl. Toxicol. 14 (6): 435, 1994

Conn, R.E., Kolstad, J.J., Borzelleca, J.F., Dixler, D.S., Filer, L.J., Jr, LaDu, B.N., Jr, and Pariza, M.W.: Safety Assessment of Polylactide (PLA) for Use as a Food-contact Polymer. Fd. Chem. Tox. 33:273-283, 1995

Hallagan, J.B., Allen, D.C., and Borzelleca, J.F.: The safety and regulatory status of food, drug and cosmetics color additives exempt from certification. Food Chem. Toxicol. 33:515, 1995

Borzelleca, J.F.: Post-marketing surveillance of macronutrient substitutes. Food Technol. 49: 107-113, 1995

Borzelleca, J.F., Egle, J.L.,Jr., Harris, L.S. and Belleville, J.A.N.: Toxicological evaluation of u-agonists. Part II: Assessment of toxicity following 30 days of repeated oral dosing of male and female rats with levo-alpha-noracetylmethadol HCl (NorLAAM). J. Appl. Toxicol. 15(5): 339-355, 1995

PUBLICATIONS

Moore, K.A., Lichtman, A.H., Poklis, A., and Borzelleca, J.F.: alpha-Benzyl-N-methylphenethylamine (BNMPA), an impurity of illicit methamphetamine synthesis: pharmacological evaluation and interaction with methamphetamine. Drug Alcohol Depend. 39: 83-89, 1995

Borzelleca, J.F., Filer, L.J., Jr., Kinoshita, F.K., Gerrish, T.C., Kuo, P.K., and LaDu, B.N.: Evaluation of the safety of sodium pectate as a food ingredient. Food Chem. Toxicol. 34: 21-25, 1996

Borzelleca, J.F.: A proposed model for safety assessment of macronutrient substitutes. Regul. Toxicol. Pharmacol. 23:S15-S18, 1996

Steinberg, M., Borzelleca, J.F., et al: A new approach to the safety assessment of pharmaceutical excipients. Regul. Toxicol. Pharmacol. 24: 149-154, 1996

Borzelleca, J.F., Cheney, M., Davidson, M.H., Degnan, F.H., Derfler, P.S., Forbes, A.L., Glinsmann, W., Heckman, J.H., Miller, S.A., Rulis, A.M., Masten, L.W.: Panel discussion: what is the future of macronutrient substitutes in North America? Regul Toxicol Pharmacol. 23(1 Pt 2):S51-9, 1996

Berndt, W.O., Borzelleca, J.F., Flamm, W.G., and Munro, I.C.: Erythritol: A review of biological and toxicological studies. Regul. Toxicol. Pharmacol. 24:S191-198, 1996

Hallagan, J.B., LaDu, B.N., Pariza, M.W., Putnam, J.M., and Borzelleca, J.F.: Assessment of cassia gum. Food Chem. Tox. 35: 625-632, 1997

Graham, D.M., Pariza, M.W., Glaze, W.H., Newell, G.W., Erdman, J.W., and Borzelleca, J.F.: Use of ozone in food processing. Food Technol. June 1997

Pariza, M.W., Borzelleca, J.F. et al: Examination of Dietary Recommendations for Salt-Cured, Smoked, and Nitrite-Preserved Foods. CAST Issue Paper Number 8, November 1997

Munro, I.C., Bernt, W.O., J.F. Borzelleca, Flamm, G., Lynch, B.S., Kennepohl, E., Bar, E.A. and Modderman, J.: Erythritol: An Interpretive Summary of biochemical, Metabolic, Toxicological and Clinical Data. Food Chem. Toxicol. 36: 1139-1174, 1998

Jeppsen, R.B., Borzelleca, J.F.: Safety evaluation of ferrous bisglycinate chelate. Food Chem Toxicol. 37: 723-31, 1999.

Borzelleca, JF: Paracelsus: Herald of Modern Toxicology. Toxicological Sciences 53: 2-4. 1999

Lien, E.L., Boyle, F.G., Wrenn, J.M., Perry, R.W., Thompson, C.A., Borzelleca J.F.: Comparison of AIN-76A and AIN-93G diets: a 13-week study in rats. Food Chem Toxicol. 39: 385-92, 2001

Zipprich, J.L., Harris, S.A., Fox, J.C. and Borzelleca, J.F.: An analysis of factors that influence personal exposure to nitrogen oxides in residents of Richmond, Virginia. J. Expo. Anal. Environ. Epidemiol. . 2002

Schoneker, D.R., DeMerlis, C.C. and Borzelleca, J.F.: Evaluation of the toxicity of polyvinylacetate phthalate in experimental animals. Food Chem. Toxicol. 41: 405-413, 2003

Rodwell, D.E., Kelly, C.M., DeMerlis, C.C., Schoneker, D.R. and Borzelleca, J.F.:Effects of polyvinyl alcohol administered in the diet to rats on fertility, early embryonic development, growth and development. Food Chem. Toxicol. 41: 729-737, 2003

PUBLICATIONS

Kelly, C.M., DeMerlis, C.C., Schoneker, D.R. and Borzelleca, J.F.: Subchronic toxicity study in rats and genotoxicity tests with polyvinyl alcohol. Food Chem. Toxicol. 41: 719-726, 2003

Freeman, C., Weiner, M.L., Kotkoskie, L.A. Borzelleca, J.F. and Butt, M. Subchronic and developmental studies in rats with Ac-Di-Sol Croscarmellose Sodium. Int. J. Toxicol. 22: 149-158, 2003

Ruckman, S.A., Rocabayera, X., Borzelleca, J.F. and Sandusky, C.B. Toxicological and metabolic investigations of the safety of N-alpha-Lauroyl-L-arginine ethyl ester monohydrochloride (LAE). Food Chem. Toxicol. 42: 245-259, 2004

Yasunaga, K., Glinsmann, W. H., Seo, Y., Katsuragi, Y., Kobayashi, S., Flickinger, B., Kennepohl, E., Yasukawa, T., and Borzelleca, J.F. Safety aspects regarding the consumption of high-dose dietary diacylglycerol oil in men and women in a double-blind controlled trial in comparison with consumption of a triacylglycerol control oil. Food Chem. Toxicol. 42: 1419-1429, 2004

Anderson, J.W., Nicolosi, R.J. and Borzelleca, J.F. Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy. Food Chem. Toxicol. 43: 187-201, 2005

DeMerlis, C.C., Schoneker, D.R., and Borzelleca, J.F. A subchronic toxicity study in rats and genotoxicity test with Surelease® aqueous ethylcellulose disperson. Food Chem. Toxicol., 43: 1355-1364, 2005.

Borzelleca, J.F., Peters, D., Hall, W. A 13-week dietary toxicity and toxicokinetic study with l-theanine in rats. Food Chem Toxicol. 44: 1158-66, 2006.

Griffiths, J.C., Borzelleca, J.F., St Cyr, J. Sub-chronic (13-week) oral toxicity study with D-ribose in Wistar rats. Food Chem Toxicol. 45: 144-52, 2007

Griffiths, J.C., Borzelleca, J.F., St Cyr, J. Lack of oral embryotoxicity/teratogenicity with D-ribose in Wistar rats. Food Chem Toxicol. 45: 388-95, 2007

Doull, J., Borzelleca, J.F., Becker, R., Daston, G., DeSesso, J., Fan, A., Fenner-Crisp, P., Holsapple, M., Holson, J., Llewellyn, C.G., MacGregor, J., Seed, J., Walls, I., Woo, Y.T., Olin, S. Framework for use of toxicity screening tools in context-based decision-making. Food Chem Toxicol. 45: 759-96, 2007

Hall, W.C., Elder, B., Walker, C.L., Cai, S.L., Peters, D.G., Goodman, D.G., Ulland, B.M., Borzelleca, J.F. Spontaneous renal tubular hyperplastic and neoplastic lesions in three Sprague-Dawley rats from a 90-day toxicity study. Toxicol Pathol. 35: 233-41, 2007

ABSTRACTS

Borzelleca, J.F. and Manthei, R.W.: Influence of dehydration on pentobarbital sleeping time in mice. Fed. Proc. 15: 403, 1956.

Borzelleca, J.F.: The effect of blood pH on barbiturate sleeping time in mice. Fed. Proc. 16: 284, 1957.

Borzelleca, J.F.: Drug absorption from the urinary bladder. Fed. Proc. 18: 370, 1959.

Borzelleca, J.F.: Nicotine absorption from the urinary bladder of the dog. Fed. Proc. 19: 391, 1960.

Borzelleca, J.F., Bowman, E.R. and McKennis, H., Jr.: Depressor effects arising from (-)-cotinine. Pharmacologist 2: 72, 1960.

Borzelleca, J.F.: Influence of saline infusions on the course of barbiturate intoxication. Pharmacologist 3: 63, 1961.

Borzelleca, J.F.: Drug absorption from the urinary tract of the rat. Nicotine. Fed. Proc. 21: 451, 1962.

Borzelleca, J.F.: Drug movement from the isolated urinary bladder of the rabbit. Fed. Proc. 22: 661, 1963.

Borzelleca, J.F.: Studies on the mechanisms of drug movement from the isolated urinary bladder. Pharmacologist 6: 178, 1964.

Kim, K.S., Borzelleca, J.F., McKennis, H., Jr. and Bowman, E.R.: Effects of cotinine and other nicotine metabolites in vitro on duodenum and ileum segments. Fed. Proc. 23: 330, 1964.

Borzelleca, J.F. and Doyle, H.: Salivary excretion of drugs. Fed. Proc. 24: 546, 1965.

Cherrick, H. and Borzelleca, J.F.: Salivary excretion of drugs. Antibiotics. Toxicol. Appl. Pharmacol. 7: 481, 1965.

Wooles, W.R. and Borzelleca, J.F.: Prolongation of barbiturate sleeping time in mice by stimulation of the RES. J.R.E.S, 1: 574, 1965.

Borzelleca, J.F.: Salivary excretion of glucose, salicylate, penicillin. Fed. Proc. 24: 564, 1966.

Lowenthal, W. and Borzelleca, J.F.: Rectal absorption of salicylates. Toxicol. Appl. Pharmacol. 8: 347, 1966.

Bernstein, S. and Borzelleca, J.F.: The effect of dimethylsulfoxide on drug transfer from the urinary bladder. Va. J. Sci. 18: 195, 1967.

Kim, K.S. and Borzelleca, J.F.: Pharmacological effects of some nicotine metabolites and related compounds. Fed. Proc. 26: 683, 1967.

Mullen, K. and Borzelleca, J.F.: Predictive model for blood glucose concentration in the dog. Va. J. Sci. 18: 200, 1967.

Schwartz, S.L. and Borzelleca, J.F.: Adrenergic blood pressure responses in the shark. Proc. Shark Res. Panel of Am. Inst. Biol. Sci., 26 April 1968.

Schwartz, S.L. and Borzelleca, J.F.: Adrenergic responses in the shark. Toxicol. Appl. Pharmacol. 12: 307, 1968.

ABSTRACTS

Wynn, J.E., van't Riet, B. and Borzelleca, J.F.: Excretion and toxicity of EGTA and EDTA after oral administration to rats. Fed. Proc. 271: 465, 1968.

van't Riet, B., O'Rear, C.E., Wynn, J.E. and Borzelleca, J.F.: Effect of EGTA and EDTA on bladder stone formation in rats. Toxicol. Appl. Pharmacol. 14: 638, 1969.

Borzelleca, J.F. and van't Riet, B.: Hydrolysis and excretion of esters of EDTA and EGTA after oral administration to rats. Va. J. Sci. 29: 143, 1970.

Borzelleca, J.F., Larson, P.S., Hennigar, G.R. and Kuchar, E.J.: A toxicological evaluation of pentachloronitrobenaene (PCNB). Pharmacologist 12: 208, 1970.

Borzelleca, J.F.: The role of pharmacology in the training of toxicologists. Pharmacologist 12: 217, 1970.

Putney, J.W., Jr. and Borzelleca, J.F.: A model for drug movement across the salivary epithelium. Va. J. Sci. 21: 147, 1970.

Putney, J.W., Jr. and Borzelleca, J.F.: Factors modifying excretion of salicylate by the dog, comparison of urinary and salivary routes. Toxicol. Appl. Pharmacol. 16: 23, 1970.

Putney, J.W., Jr. and Borzelleca, J.F.: Studies on salicylate biotransformation by the salivary gland. Pharmacologist 12: 272, 1970.

Borzelleca, J.F., Larson, P.S., Hennigar, G.R. and Kuchar, E.J.: A toxicologic evaluation of 5-ethoxy-3-trichloromethyl- 1,2,4-thiadiazole (terrazole). Toxicol. Appl. Pharmacol. 19: 79, 1971.

Putney, J.W., Jr. and Borzelleca, J.F.: Mechanisms of ^{14}C-salicylate uptake by submaxillary gland slices. Fed. Proc. 30: 448, 1971.

Putney, J.W., Jr. and Borzelleca, J.F.: Active uptake of ^{14}C-salicylic acid by rat kidney cortex slices. Fed. Proc. 31: 518, 1972.

Putney, J.W., Jr. and Borzelleca, J.F.: Participation of extracellular hydrogen ion in the efflux of nicotine-^{14}C from submaxillary gland cells. Pharmacologist 13: 518, 1972.

Allen, M.A. and Borzelleca, J.F.: On the method of benzyl penicillin-^{14}C potassium distribution in rat submaxillary gland. Fed. Proc. 32: 733, 1973.

Allen, M.A. and Borzelleca, J.F.: On the method of diphenyl hydantoin distribution in rat submaxillary gland. Pharmacologist 15: 229, 1973.

Jordan, R.L. and Borzelleca, J.F.: Teratogenic studies with pentachloronitrobenzene in rats. Toxicol. Appl. Pharmacol. 25: 454, 1973.

Allen, M.A. and Borzelleca, J.F.: Diphenylhydantoin distribution in rat submaxillary gland: influence of age. Fed. Proc. 33: 525, 1974.

Burnett, C.M., Agersborg, H.P.H., Jr., Borzelleca, J.F., Egle, Jr., E., Ebert, A.G., Pierce, E.C., Kirschman, J.C. and Scala, R.A.: Teratogenic studies with certified colors in rats and rabbits. Toxicol. Appl. Pharmacol. 29: 121, 1974.

ABSTRACTS

Pierce, E.G., Agersborg, H.P.K., Jr., Borzelleca, J.F., Burnett, C.M., Egle, E., Ebert, A.G., Kirschman, J.C. and Scala, R.A.: Multigeneration reproduction studies with certified colors in rats. Toxicol. Appl. Pharmacol. 29: 121, 1974.

Adams, M., Wedig, J.H., Jordan, R., Smith, L., Henderson, R. and Borzelleca, J.F.: Excretion and metabolism of three 2-6-^{14}C Omadines following intravenous injection in female Yorkshire pigs. Toxicol. Appl. Pharmacol. 33: 180, 1975.

Egle, J.L., Jr., Borzelleca, J.F. and Long, J.E.: An evaluation of the cardiac sensitizing potential of Scotchgard brand fabric protector. Toxicol. Appl. Pharmacol. 33: 154, 1975.

Jordan, R.L. and Borzelleca, J.F.: Teratogenic studies with zinc Omadine in swine. Anat. Rec. 18: 388, 1975.

McConnell, W.R., Borzelleca, J.F. and Chambers, J.W.: The effects of delta-9-tetrahydrocannabinol (THC) on electrically stimulated saliva from cat submaxillary gland. Fed. Proc. 34: 782, 1975.

Smith, L.W., Borzelleca, J.F. and Bowman, E.R.: Application of isolated cell suspensions to the study of membrane phenomena in mammalian salivary cells. Fed. Proc. 34: 752, 1975.

Wrenn, J.M. and Borzelleca, J.F.: Effect of phenobarbital and pentobarbital on the transport of diphenylhydantoin in salivary tissues and saliva. Fed. Proc. 34: 573, 1975.

Egle, J.L., Jr., Gochberg, B.J. and Borzelleca, J.F.: The distribution of ^{14}C-Kepone in the rat. Pharmacologist 18: 195, 1976.

McConnell, W.R. and Borzelleca, J.F.: On the method of 3H-delta-9 tetrahydrocannabinol (^{3}H-delta-9-THC) distribution in the submaxillary gland of the rat. Pharmacologist 18: 149, 1976.

McConnell, W.R., Borzelleca, J.F. and Dewey, W.L.: The mechanism by which delta-9-tetrahydrocannabinol (THC) produces a decrease in salivary flow following electrical stimulation. Fed. Proc. 35: 644, 1976.

McCoy, W.D., Kuchar, E.J., Klein, H.H. and Borzelleca, J.F.: Biotransformation and distribution of pentachloronitrobenzene in chickens. Toxicol. Appl. Pharmacol. 37: 175, 1976.

Schumann, A.M. and Borzelleca, J.F.: The potential methemoglobin and Heinz body inducing capacity of pentachloronitrobenzene (PCNB) in the cat. Toxicol. Appl. Pharmacol. 37: 171, 1976.

Schumann, A.M., Bloom, A.S., Dewey, W.L., Harris, L.S. and Borzelleca, J.F.: Development of central catecholamine systems in the postnatal rat brain. Pharmacologist 18: 243, 1976.

Smith, L.W. and Borzelleca, J.F.: Uptake of cadmium in rat submaxillary slices. The Pharmacologist 18: 196, 1976.

Bagshaw, B., Schumann, A., Borzelleca, J. and Dewey, W.: The effects of chloroform and bromoform on the noradrenergic and dopaminergic systems of the mouse brain. Pharmacologist 9: 200, 1977.

Barrett, B.A., Sanders, V.M., Borzelleca, J.F., Munson, E.: Growth rates and tumor takes in mice with transplanted tumors exposed to halomethanes. Va. J. Sci. 28: 100, 1977.

Brady, K.T., Sanders, V.M, Borzelleca, J.F. and Munson, A.E.: The acute toxicity of the halomethanes: drinking water contaminants. Va. J. Sci. 28: 100, 1977.

ABSTRACTS

Martin, B.R., Dewey, W.L., Beckner, J.S. and Borzelleca, J.F.: Synthesis and metabolism of brain serotonin in mice following acute exposure to several haloalkanes. Toxicol. Appl. Pharmacol. 19: 200, 1977.

Munson, A., Sanders, V., Borzelleca, J. and Barnes, D.: Toxicologic studies on adult and neonatal mice exposed to the trichloromethanes: drinking water contaminants. Pharmacologist 19: 200, 1977.

Munson, A.E., Sanders, V.M., Barrett, B.A. and Borzelleca, J.F.: Functional activity of the reticuloendothelial system in mice exposed to haloalkanes for ninety days. J. Reticuloendothel. Soc. 22: 17a, 1977.

Sanders, V.M., Barrett, B.A., Borzelleca, J.F. and Munson, A.E.: Reticuloendothelial system activity and cell mediated immune responsiveness in mice exposed to polychlorinated biphenyls. J. Reticuloendothel. Soc. 22: 16a, 1977.

Schumann, A.M., Dewey, W.L. and Borzelleca, J.F.: The effects of triethyllead on central catecholamine function in the adult rat. Toxicol. Appl. Pharmacol. 41: 208, 1977.

Schumann, A.M., Dewey, W.L., Borzelleca, J.F. and Alphin, R.S.: The effects of lead acetate on central catecholamine function in the postnatal mouse. Fed. Proc. 36: 405, 1977.

Smith, L.W. and Borzelleca, J.F.: The excretion of cadmium and mercury in saliva. Toxicol. Appl. Pharmacol. 41: 153, 1977.

Smith, L.W., Ismay, J.A. and Borzelleca, J.F.: Movement of mercury in rat submaxillary slices. Fed. Proc. 36: 355, 1977.

Carmines, E.L., Burkhalter, J.A., Carchman, R.A. and Borzelleca, J.F.: Inhibitory effects of chloroform on P388D macrophage cell. Fed. Proc. 37: 320, 1978.

Dougherty, R.W., Simon, G.S., Campbell, K.I. and Borzelleca, J.F.: Failure of 2,4-dinitrotoluene to induce dominant lethal mutations in the rat. Pharmacologist 20: 155, 1978.

Larson, P.S., Hennigar, G.R., Lane, R.W. and Borzelleca, J.F.: Acute, subchronic and chronic toxicological studies with kepone. Toxicol. Appl. Pharmacol. 95: 331, 1978.

Munson, A.E., Sanders, V.M., Borzelleca, J.F., Tardiff, R.G. and Barrett, B.A.: Reticuloendothelial system function in mice exposed to four haloalkane drinking water contaminants. Toxicol. Appl. Pharmacol. 45: 329, 1978.

Schuller, G.B., Kauffmann, B.M., Borzelleca, J.F., Sanders, V.M. and Munson, A.E.: Effect of four haloalkanes on humoral and cell mediated immunity in mice.
Toxicol. Appl. Pharmacol. 45: 329, 1978.

Simon, G.S., Carchman, R.A. and Borzelleca, J.F.: Diabetes: responses to selected pharmacologic agents. Pharmacologist 20: 151, 1978.

Simon, G.S., Kipps, B.R., Tardiff, R.G. and Borzelleca, J.F.: Failure of Kepone and hexachlorobenzene to induce dominant lethal mutations in the rat. Toxicol. Appl. Pharmacol. 45: 330: 1978.

Smith, S.H., Sanders, V.M., Barrett, B.A., Borzelleca, J.F. and Munson, A.E.: Immunotoxicological evaluation on mice exposed to polychlorinated biphenyls. Toxicol. Appl. Pharmacol. 45: 330, 1978.

ABSTRACTS

Zimmerman, M.L., Lane, R.W., Skalsky, H.L. and Borzelleca, J.F.: Excretion of carbaryl into saliva and its effect on cholinesterase. l0th Inter-American Conf. on Toxicol. and Occupational Med., p. 47, 1978.

Zimmerman, M.L., May, R.G. and Borzelleca, J.F.: Excretion of carbaryl into the saliva of the rat. Toxicol. Appl. Pharmacol. 45: 35, 1978.

Balster, R.L., Burkhalter, J. and Borzelleca, J.F.: Behavioral toxicity evaluation of four halomethane contaminants of drinking water in adult mice. Fed. Proc. 38: 846, 1979.

Carmines, E.L., Carchman, R.A. and Borzelleca, J.F.: in vitro effects of Kepone. Va. J. Sci. 30: 89, 1979.

Borzelleca, J.F., Skalsky, H.L. and Riddle, B.L.: Effects of dibromochloromethane in drinking water on reproduction and development in mice. Fed. Proc. 39: 999, 1980.

Carmines, E.L., Carchman, R.A. and Borzelleca, J.F.: Analysis of the interactions between paraquat and DNA. Fed. Proc. 39: 545, 1980.

Balster, R.L., Kallman, M.J. and Borzelleca, J.F.: Behavioral toxicity evaluation of trihalomethane contaminants of drinking water. Health Effects of Drinking Water Symposiun, 1981.

Carchman, R.A., Cardlin, E.L., Skalsky, H.L. and Borzelleca, J.F.: The effects .of selected water disinfectant products on testicular DNA metabolism. Health Effects of Drinking Water Symposium, 1981.

Kallman, M.J., Balster, R.L., Kaempf, G.L. and Borzelleca, J.F.: Behavioral toxicity evaluation of chloral in adult mice. Fed. Proc. 40: 698, 1981.

Tarka, S.M., Jr., Keeney, P.G., and Borzelleca, J.F.: The effect of pretreatment with dietary cocoa on growth and reproductive performance in young and adult rats. Fed. Proc. 40: 668, 1981

Lane, R.W., Carchman, R.A. and Borzelleca, J.F.: Characterization of DNA metabolism in mouse primary spermatocytes. Toxicologist 1: 39(#143), 1981.

Riddle, B.L., Carchman, R.A. and Borzelleca, J.F.: Effects of 1,2-dichloroethane and 1,1,1-trichloroethane in drinking water on reproduction and development in mice. Toxicologist 1: 26 (#95), 1981.

Tarka, S.M., Jr., Keeney, P.G. and Borzelleca, J.F.: A comparison of the effects of methylxanthine-containing food stuffs on reproductive capacity in rats. Toxicologist 1: 147 (#533), 1981.

Borzelleca, J.F., Hallagan, J., Reese, C., Goldenthal, E. and Hogan, G.: Chronic oral toxicity/carcinogenicity studies of food, drug and cosmetic colors in CD-1 mice. J. Am. Coll. Tox. 2: 240 (#108), 1982.

Charles, J.L., Carchman, R.A., Kram, D. and Borzelleca, J.F.: Time course of in vivo induction of sister chromatid exchange by ethylnitrosourea and methylnitrosourea. Toxicologist 2: 175 (#613), 1982.

Hayes, J.R., Condie, L.W., Jr., and Borzelleca, J.F.: Kinetics of naphthalene (NTL) covalent binding to hepatic DNA, RNA and protein in CD-1 mice. J. Am. Coll. Tox. 3: 144, 1982.

ABSTRACTS

Lane, R.W., Coles, R.B., Carchman, R.A. and Borzelleca, J.F.: Phorbol diester receptors on HL-60 human promyelocytic leukemia cells. Toxicologist 2: 105 (#373), 1982.

Seyler, D., East, J. and Borzelleca, J.F.: Cadmium depression of mouse in vitro fertilization. Toxicologist 2: 238 (#764), 1982.

Borzelleca, J.F., Hallagan, J., Reese, C., Goldenthal, E. and Hogan, G.: Chronic oral toxicity/carcinogenicity studies of food, drug and cosmetic colors in CD rats. Toxicologist 3: 129 (#514),1983 .

Condie, L.W., Hayes, J.R. and Borzelleca, J.F.: Acute and subchronic oral toxicity of 2,4-dichlorophenol (2,4-DCP) in male and female CD-1 mice. Pharmacologist 25: 228, 1983.

Hayes, J.R. and Borzelleca, J.F. Implications of nutrient-drug interactions. Proc. Ann. Meeting Inst. Food Technol., 1983.

Hayes, J.R. and Borzelleca, J.F.: Diet-nutrient interactions. Proc. Ann. Meeting Am. Diet. Assoc., 1983.

Hayes, J.R., Condie, L.W., Jr., and Borzelleca, J.F.: Pharmacokinetics of oral naphthalene (NTL) in CD-1 mice. Toxicologist 3: 161 (#644), 1983.

Kallman, M.J., Borzelleca, J.F. and Condie, L., Jr.: Behavioral toxicity of naphthalene in adult mice. J. Am. Coll. Toxicol. 2: 247 (#136), 1983.

Kessler, F.K., Charles, J.L., Borzelleca, J.F. and Carchman, R.A.: Effects of chlorinated phenols on mouse bone marrow sister chromatid exchange. J. Am. Coll. Toxicol. 2: 249 (#142), 1983.

Lane, R.W., Carchman, R.A. and Borzelleca, J.F.: Phorbol diester (PDE) binding and oxygen metabolism of differentiated HL-60 cells. Toxicologist 3: 144 (#575), 1983.

Shopp, G.M., White, K.L., Jr., Holsapple, M.P., Barnes, D.W., Condie, L.W., Jr. and Borzelleca, J.F.: General toxicology and immunotoxicology of mice exposed to naphthalene (NAP). Toxicologist, 3: 57 (#226), 1983.

Smith, B., Lane, R.W., Carchman, R.A. and Borzelleca, J.F.: A comparison of the reversibility of phorbol diester induced changes in macrophage morphology. Toxicologist 3: 144 (#574), 1983.

Borzelleca, J.F., Hayes, J,R. and Condie, L.: Toxicological evaluation of selected chlorinated phenols and haloacetonitriles. Proc. of 5th International Conf. on Water Chlorination: Environmental Impact and Health Effects 1: 100, 1984.

Hayes, J.R., Condie, L. and Borzelleca, J.F.: Subchronic toxicity of carbon tetrachloride administered by oral gavage to CD-1 mice. Toxicologist 4: 183 (#730), 1984.

Hayes, J.R., Condie, L.W. and Borzelleca, J.F.: Acute and 14-day continuous dosing toxicity of dichloroacetonitrile (DCA) and dibromoacetonitrile (DBA). Pharmacologist 26: 233, 1984.

Condie, L.W. Hayes, J.R. and Borzelleca, J.F.: Acute, 14-day and subchronic toxicity of potassium picloram (PIC) administered to rats via the drinking water. Toxicologist 5: 222, 1985

ABSTRACTS

Capen, C.C., Nishikawa, S., Ingbar, S.H., Braverman, L.E., and Borzelleca, J.F.: Mechanisms of thyroid oncogenesis by chronic erythrosine (red. no. 3) feeding: ultrastructural and morphometric evaluation of thyroid glands and changes in circulating levels of thyroid hormones and thyrotropin (TSH). Abstract No. 48, 75th Annual Meeting of the International Academy of Pathology, New Orleans, 10-14 March, and published in Laboratory Investigations 54: 54 a, 1986.

Lamb, R.G., Bush, S.R., Condie, L.W., and Borzelleca, J.F.: Influence of chlorinated hydrocarbon mixtures on cultured hepatocyte function. Pharmacologist 28: 180, 1986.

Lamb, R.G., Coleman, J.B., Condie, L.W., and Borzelleca, J.F.: Influence of chlorinated hydrocarbons on cultured hepatocyte function. Toxicologist 6: 116 (#470), 1986.

Granger, R.H., Coleman, J.B., Condie, L.W., Lamb, R.G. and Borzelleca, J.F.: Effect of vehicle on the relative uptake of haloalkanes administered by gavage. Toxicologist 7: 265 (#1060), 1987.

Lamb, R.G., Coleman, J.B., Granger, H., Condie, L.W. and Borzelleca, J.F.: The influence of chlorinated hydrocarbons on hepatocyte function in vivo and in vitro. Toxicologist 7: 267 (#1068), 1987.

Coleman, J.B., Condie, L.W., Borzelleca, J.F. and Lamb, R.G.: The influence of structural analogues of carbon tetrachloride (CCl_4) on hepatocyte functions in vitro. Toxicologist 8: 96 (#381), 1988.

Granger, R.H., O'Hara, T.M., Condie, L.W., and Borzelleca, J.F.: A study of the joint action of carbon tetrachloride (CCl_4) and trichloroethylene (C_2HCl_3) following simultaneous gavage administration in the rat. Toxicologist 8: 95 (#378), 1988.

O'Hara, T.M., Granger, R.H., Condie, L.W. and Borzelleca, J.F.: A study of the joint hepatotoxic action of carbon tetrachloride (CCl_4) and chloroform ($CHCl_3$) following simultaneous gavage administration in the rat. Toxicologist 8: 96 (#380), 1988.

Borzelleca, J.F., O'Hara, T.M., Gennings, C. and Condie, L.W., A CCl_4-$CHCl_3$ interaction study in isolated hepatocytes-the role of P-450 metabolism. Toxicologist 9: 58 (#229), 1989.

Lamb, R.G., Gennings, C., Borzelleca, J.F., and Condie, L.W.: Toxic Interactions between carbon tetrachloride (CCl_4) and chloroform ($CHCl_3$). Toxicologist 9: 59 (#233), 1989.

O'Hara, T.M., Borzelleca, J.F. and Condie, L.W.: A CCl_4/$CHCl_3$ interaction study in isolated hepatocytes-selection of a vehicle. Toxicologist 9: 59 (#235), 1989.

Borzelleca, J.F., Gennings, C., Bercz, P. and Lamb, R.G.: Toxic interactions between carbon tetrachloride (CCl_4) and perchloroethylene (PCE) in cultured rat hepatocytes. Toxicologist 10: 54 (#213), 1990.

Lamb, R.G., Gennings, C., Borzelleca, J.F. and Bercz, P.: Toxic interactions between carbon tetrachloride (CCl_4) and trichloroethylene (TCE) in cultured rat hepatocytes. Toxicologist 10: 53 (#212), 1990.

Wolfe, G., Myers, B., Lemen, J., Lauer, W., Johns, F., Condie, L. and Borzelleca, J.: Preliminary report of the findings of the health effects for Denver's potable reuse demonstration project. Toxicologist 10: 176 (#704), 1990.

ABSTRACTS

Egle, J.L., Jr., Borzelleca, J.F. and Harris, L.S.: Acute and subchronic toxicity of Levo-alpha-acetyl-methadol (LAAM) and Levo-alpha-acetyl-normethadol (NORLAAM) in male and female rats. Toxicologist 11: 149 (#521), 1991

Weiner, M.L., Steinberg, M., Borzelleca, J.F., Enters, E.K., Hager, D.F., Kinoshita, F.K., Loper, A., Mitchell, D.B. and Tamulinas, C.B.: Proposed safety evaluation guidelines for new excipients. Toxicologist 13: 213 (#796), 1994

Borzelleca, J.F.: The safety evaluation of macronutrient substitutes. IFT Annual Meeting Abstracts #15-2, 1994

Borzelleca, J.F.: Fat replacers. ACS meeting, 1995

Rice, R.G., Graham, D.M., Glaze, W.H., Pariza, M.W., Newell, G.W., Erdman, J.W., and Borzelleca, J.F.: Ozone preservation of Foods and Foodstuffs. 13th Ozone World Congress, October 1997, Kyoto, Japan

Lien, E., Boyle, F., Perry, Thompson, C., Borzelleca, J.F., and Wrenn, J.: Comparison of AIN-76A and AIN-93G Diets in Rats; a 13 Week Study. Fed. Proc., 1998

Munro, E.C., Berndt, W.O., Borzelleca, J.F., Flamm, G., Lynch, B.S., Kennepohl, E., Bar, A. and Modderman, J.: Erythritol: An interpretive summary of biochemical, metabolic, toxicological and clinical data. Toxicologist 38, 1999

A subchronic toxicity study In rats and genotoxicity tests with Surelease® aqueous ehylcellulose dispersion, ACT & SOT Annual Meetings, March 2003

BOOKS AND BOOK CHAPTERS

Munson, A.E., Barrett, B.A. and Borzelleca, J. F.: In vitro experimental approaches to detection of sensitive agents. In: Cutaneous Toxicity, (V. Drill, ed.), Academic Press, Inc., San Francisco, p. 175, 1977.

Skalsky, H.L., Lane, R.W. and Borzelleca, J.F.: "Excretion of carbaryl into saliva of the rat and its effect on cholinesterase". In: Toxicology and Occupational Medicine (W.B. Deichman, ed.), p. 349, 1979.

Borzelleca, J.F. and Carmines, E.L.: "New drug evaluation: safety assessment". In: Program for Applied Research on Fertility Regulation, 1980.

Hayes, J.F. and Borzelleca, J.F.: "Biodisposition of environmental chemicals by animals". In: Animal Products in Human Nutrition (D. Beitz and R. Hansen, eds.), Chap. 11, p. 225. Academic Press, New York, 1982.

Borzelleca, J.F.: "Neurobehavior toxicological testing". Pharmacodependence and neurobehavioral toxicology. Quo Vadis ?, Symposium "Quo Vadis ?", Sanofi Group, Montpellier, France, p. 115, 1983.

Schwartz, S.L. and Borzelleca, J.F.: "Toxicology of polyvinylpyrrolidone". Proceedings of the International Symposium on Povidone (G.A. Digenis, Ed.), College of Pharmacy, University of Kentucky, Lexington, KY, p. 234,
1983.

Borzelleca, J.F., Hallagan, J. and Reese, C.:"Food, Drug and Cosmetic Colors: Toxicological Considerations." ACS Symposium Series, No. 234, Xenobiotics in Foods and Feeds. (Finley, J.W. and Schwass, D.E., eds.), Chap. 20, p.311. ACS, Washington, D.C., 1983

Borzelleca, J.F.: "Extrapolation of animal data to man". In: Toxicology Laboratory Design and Management for the 80's and Beyond (Tegeris, A.S., Ed); Vol. 1 of Concepts in Toxicology, Homburger, F., Series Ed.), 1984.

Borzelleca, J.F.: "Current concepts in reproductive toxicology". In: Clinics in Laboratory Medicine, Symposium on Environmental and Occupational Health Hazards, Vol. 4 (R.V. Blanke, ed.), W.B. Saunders Co., Philadelphia, 1984.

Borzelleca, J.F., Condie, L.W., and Hayes, J.R.: "Toxicological evaluation of selected chlorinated phenols". In Water Chlorination, Chemistry, Environmental Impact and Health Effects. (R.L. Jolley, R.J. Bull, W.P. Davis, S. Katz, M.H. Roberts, Jr., V.A. Jacobs). Volume 5, Chap. 26, p.331. Lewis Publishers, Inc., Ann Arbor, Michigan, 1985.

Robinson, B.V., Sullivan, F.M., Borzelleca, J.F. and Schwartz, S.L.: PVP: A Critical Review of the Kinetics and Toxicology of Polyvinylpyrrolidone (Povidone). Lewis Publishers, Inc., Ann Arbor, Michigan. 1990

Borzelleca, J.F. and Hallagan, J.B.: "Safety and Regulatory Status of Food, Drug, and Cosmetic Colors." ACS Symposium Series, No. 484, Food Safety Assessment. (Finley, J.W., Robinson, S.F., and Armstrong, D.J., eds.), Chap. 31, p.377. ACS, Washington, DC. 1992

Borzelleca, J.F.:"Foods of the Future: What Will We Be Eating in the Next Century?" In Practical Handbook of Nutrition in Clinical Practice (Kirby, D.F. and Dudrick, S.J., eds.), Chap. 16, p.279. CRC Press, Inc., Boca Raton, Fl. 1994

Borzelleca, J.F.: "History of Toxicology." In Principles and Methods of Toxicology (Hayes, A.W., editor), edition 3, Chap. 1, p 1-18, Raven Press, New York, NY. 1994

BOOKS AND BOOK CHAPTERS

Matt, D.W. and Borzelleca, J.F.: "Toxic Effects on the Female Reproductive System During Pregnancy, Parturition, and Lactation." In Reproductive Toxicology (Witorsch, R.J., editor), edition 2, chapter 10, p. 175. Raven Press, New York, NY. 1995

Borzelleca, J.F.: "Food-Borne Health Risks: Food Additives, Pesticides and Microbes." In Nutrition Policy in Public Health (Bronner, F., editor). Chap. 3, p.33, Springer Publishing Co. New York, NY. 1997

Rice, R.G., Graham, D.M., Glaze, W.H., Pariza, M.W., Newell, G.W., Erdman, J.W., and Borzelleca, J.F.: Ozone Preservation of Foods and Foodstuffs. 13th Ozone World Congress, Kyoto, Japan, October 1997

Borzelleca, J.F. and Weiner, M.L. : "Development of Safety Evaluation Guidelines." In Excipient Toxicity and Safety (Weiner, M. L. and Kotkoskie, L. A., editors). Chapter 5, p.101. Marcel Dekker, Inc., New York, N.Y. 1999

Borzelleca, J.F., Glinsmann, W.H. and Kennepohl, E. Safety Aspects of Diacylglycerol Oil. In Diacylglycerol Oil. (Katsuragi, Y., Yasukawa, T., Matsuo, N., Flickinger, B.D., Tokimitsu, I. And Matlock, M.G., editors), Chapter 17, p. 165, AOCS Press, Champaign, Ill, 2004

Schleef, R.R., Vanscheeuwijck, P.M., Schlage, W.K., Borzelleca, J.F., Coggins, C.R.E., and Haussmann, HJ. Animals models for three major cigarette-smoke-induced diseases. Inhalation Toxicology, Editor, Harry Salem. 2005

Contributing authorship on the following publications of the Life Sciences Research Office, Federation of American Societies of Experimental Biology (FASEB):

Evaluation of the health aspects of iron and iron salts as food ingredients. 1973.

Evaluation of the health aspects of butylated hydroxytoluene as a food ingredient. 1973.

Evaluation of the health aspects of certain zinc salts as food ingredients. 1973.

Evaluation of the health aspect of pulps as they may migrate to food from packaging materials. 1973.

Evaluation of the health aspects of propylene glycol and propylene glycol monostearate as food ingredients. 1973.

Evaluation of the health aspects of alginates as food ingredients. 1973.

Evaluation of the health aspects of agar-agar as a food ingredient. 1973.

Evaluation of the health aspects of certain red and brown algae as food ingredients. 1973.

Evaluation of the health aspects of cellulose and certain cellulose derivatives of food ingredients. 1973.

Iodine in foods: chemical methodology and sources of iodine in the human diet. 1974.

Evaluation of the health aspects of aconitic acid as a food ingredient. 1974.

Evaluation of the health aspects of stannous chloride as a food ingredient. 1974.

Evaluation of the health aspects of licorice, glycyrrhiza and ammoniated glycrrhizin as food ingredients. 1974.

Evaluation of the health aspects of caprylic acid as a food ingredient. 1974.

Evaluation of the health aspects of sorbose as a food ingredient. 1974.

Evaluation of the health aspects of sulfuric acid and sulfates as food ingredients. 1974.

Evaluation of the health aspects of potassium iodide, potassium iodate, and calcium iodate as food ingredients. 1975.

Evaluation of the health aspects of dextran as food ingredients. 1975.

Evaluation of the health aspects of calcium oxide and calcium hydroxide as food ingredients. 1975.

Evaluation of the health aspects of succinic acid as a food ingredient. 1975.

Evaluation of the health aspects of certain calcium salts as food ingredients. 1975.

Evaluation of the health aspects of glycerin and glycerides as food ingredients 1975

Evaluation of the health aspects of dextrin and corn dextrin as food ingredients. 1975.

Evaluation of the health aspects of sodium thiosulfate as a food ingredient. 1975.

Contributing authorship on the following publications of the Life Sciences Research Office, Federation of American Societies of Experimental Biology (FASEB):

Evaluation of the health aspects of gelatin as a food ingredient. 1975.

Evaluation of the health aspects of bile salts and ox bile extract as food ingredients. 1975.

Evaluation of the health aspects of choline chloride and choline bitartrate as food ingredients.1975.

Evaluation of the health aspects of aluminum compounds as food ingredients. 1975.

Evaluation of the health aspects of tallow, hydrogenated tallow, stearic acid, and calcium stearate as food ingredients. 1975.

Evaluation of the health aspects of phosphates as food ingredients. 1975.

Evaluation of the health aspects of the tocopherols and a-tocopheryl acetate as food ingredients. 1975.

Evaluation of the health aspects of sorbic acid and its salts as food ingredients. 1975.

Evaluation of the health aspects of hydrogenated fish oil as a food ingredient. 1975.

Evaluation of the health aspects of beeswax (yellow or white) as a food ingredient. 1975.

Evaluation of the health aspects of inositol as a food ingredient. 1975.

Evaluation of the health aspects of malic acid as a food ingredient. 1975.

Evaluation of the health aspects of Japan Wax as a substance migrating to food from cotton or cotton fabrics used in dry food packaging. 1976.

Evaluation of the health aspects of carnauba wax as a food ingredient. 1976.

Evaluation of the health aspects of sulfamic acid as it may migrate to foods from packaging materials. 1976.

Evaluation of the health aspects of hydrosulfites as they may migrate to foods from packaging materials. 1976.

Evaluation of the health aspects of gum guaiac as a food ingredient. 1976.

Evaluation of the health aspects of tall oil as it may migrate to foods from packaging materials. 1976

Evaluation of the health aspects of corn sugar (dextrose), corn syrup and invert sugar as food ingredients. 1976.

Evaluation of the health aspects of sucrose as a food ingredient. 1976.

Evaluation of the health aspects of sulfiting agents as food ingredients. 1976.

Evaluation of the health aspects of glycerophosphates as food ingredients. 1976.

Evaluation of the health aspects of magnesium salts as food ingredients. 1976.

Contributing authorship on the following publications of the Life Sciences Research Office, Federation of American Societies of Experimental Biology (FASEB):

Evaluation of the health aspects of sodium hydroxide and potassium hydroxide as food ingredients. 1976.

Evaluation of the health aspects of adipic acid as a food ingredient. 1976.

Evaluation of the health aspects of hydrogenated soybean oil as a food ingredient.

Evaluation of the health aspects of formic acid, sodium formate, and ethyl formate as food ingredients. 1976.

Evaluation of the health aspects of lard and lard oil as they may migrate to foods from packaging materials. 1976.

Evaluation of the health aspects of pyridoxine and pyridoxine hydrochloride as food ingredients. 1977.

Evaluation of the health aspects of papain as a food ingredient. 1977.

Evaluation of the health aspects of hypophosphites as food ingredients. 1977.

Evaluation of the health aspects of coconut oil, peanut oil, and oleic acid as they migrate to food from packaging materials, and linoleic acid as a food ingredient. 1977.

Evaluation of the health aspects of pectin and pectinates as food ingredients. 1977.

Evaluation of the health aspects of tannic acid as a food ingredient. 1977.

Evaluation of the health aspects of rennet as a food ingredient. 1977.

Evaluation of the health aspects of acetic acid and sodium acetate as food ingredients. 1977.

Evaluation of the health aspects of sodium oleate and sodium palmitate as substances migrating to food from paper and paperboard used in food packaging. 1977.

Evaluation of the health aspects of corn silk as a food ingredient. 1977.

Evaluation of the health aspects of bentonite and clay (kaolin) as food ingredients.1977

Evaluation of the health aspects of citric acid, sodium citrate, potassium citrate, calcium citrate, ammonium citrate, triethyl citrate, isopropyl citrate, and stearyl citrate as food ingredients. 1977.

Evaluation of the health aspects of lactic acid and calcium lactate as food ingredients. 1978.

Evaluation of the health aspects of calcium pantothenate, sodium pantothenate, and D-pantothenyl acohol as food ingredients. 1978.

Evaluation of the health aspects of Vitamin B12 as a food ingredient. 1978.

Evaluation of the health aspects of Vitamin D2 and Vitamin D3 as food ingredients. 1978.

Evaluation of the health aspects of caffeine as a food ingredient. 1978.

Evaluation of the health aspects of certain glutamates as food ingredients. 1978.

Contributing authorship on the following publications of the Life Sciences Research Office, Federation of American Societies of Experimental Biology (FASEB):

Evaluation of the health aspects of protein hydrolyzates as food ingredients. 1978.

Evaluation of the health aspects of butylated hydroxyanisole as a food ingredient. 1978.

Evaluation of the health aspects of sodium, potassium, magnesium and zinc gluconates as food ingredients. 1978.

Evaluation of the health aspects of urea as a food ingredient. 1978.

Evaluation of the health aspects of thiamin hydrochloride and thiamin mononitrate as food ingredients. 1978.

Evaluation of the health aspects of biotin as a food ingredient. 1978.

Evaluation of the health aspects of ascorbic acid, sodium ascorbate, calcium ascorbate, erythorbic acid, sodium erythorbate, and ascorbyl palmitate as food ingredients. 1979.

Evaluation of the health aspects of propionic acid, calcium propionate, sodium propionate, dilauryl thiodipropionate, and thiodipropionic acid as food ingredients. 1979.

Evaluation of the health aspects of casein, sodium caseinate, and calcium caseinate as food ingredients. 1979.

Evaluation of the health aspects of nickel as a food ingredient. 1979

Evaluation of the health aspects of soy protein isolates as food ingredients. 1979.

Evaluation of the health aspects of carotene (B-carotene) as a food ingredient. 1979.

Evaluation of the health aspects of nitrogen, helium, propane, n-butane, isobutane, and nitrous oxide as gases used in foods. 1979.

Evaluation of the health aspects of hydrogen peroxide as a food ingredient. 1979.

Evaluation of the health aspects of riboflavin and riboflavin-5-1-phosphate as food ingredients. 1979.

Evaluation of the health aspects of starch and modified starches as food ingredients. 1979.

Evaluation of the health aspects of carbon dioxide as a food ingredient. 1979.

Evaluation of the health aspects of sodium chloride and potassium chloride as food ingredients. 1979.

Evaluation of the health aspects of certain silicates as food ingredients. 1979.

Evaluation of the health aspects of manganous salts as food ingredients. 1979.

Evaluation of the health aspects of copper gluconate, copper sulfate, and cuprous iodide as food ingredients. 1979.

Evaluation of the health aspects of hydrochloric acid as a food ingredient. 1979.

Contributing authorship on the following publications of the Life Sciences Research Office, Federation of American Societies of Experimental Biology (FASEB):

Evaluation of the health aspects of lecithin as a food ingredient. 1979.

Evaluation of the health aspects of potassium acid tartrate, sodium potassium tartrate, sodium tartrate and tartaric acid as food ingredients. 1979.

Evaluation of the health aspects of starter distillate and diacetyl as food ingredients. 1980.

Vitamin A, Vitamin A Acetate, and Vitamin A Palmitate as food ingredients. 1980.

Evaluation of the health aspects of iron and iron salts as food ingredients. 1980.

Evaluation of the health aspects of protein hydrolyzates as food ingredients. 1980.

Evaluation of the health aspects of collagen as a food ingredient. 1981.

Evaluation of the health aspects of methyl polysilicones as food ingredients. 1981.

Evaluation of the health aspects of soya fatty acid amines as food ingredients. 1981.

Evaluation of the health aspects of activated carbon (charcoal) as a food processing aid. 1981.

Evaluation of the health aspects of smoke flavoring solutions and smoked yeast flavoring as food ingredients. 1981.

Evaluation of the health aspects of cornmint oil as a food ingredient. 1981.

Evaluation of the health aspects of a mixture. Evaluation of the health aspects of diferrous, dipotassium ferrous, and potassium ferrocyanides as finding agents in wine production. 1981.

Evaluation of the health aspects of wheat gluten, corn gluten, and zein as food ingredients. 1981.

Evaluation of the health aspects of peptones as food ingredients. 1981.

Evaluation of the health aspects of shellac and shellac wax as food ingredients. 1981.

Evaluation of the health aspects of sodium metasilicate and sodium zinc metasilicate as food ingredients. 1981.

Evaluation of the health aspects of oat gum, okra gum, quince seed gum, and psyllium seed husk gum as food ingredients. 1982.

Contributing Authorship on the Following Publications of the National Academy of Sciences:

Principles and Procedures for Evaluating the Toxicity of Household Substances.
Committee for the Revision of NAS Publication 1138, Committee on Toxicology, Assembly of Life Sciences, National Research Council, National Academy of Sciences
National Academy Press, Washington, D.C. 1977

Drinking Water and Health.
Safe Drinking Water Committee, Board on Toxicology and Environmental Health Hazards, Assembly of Life Sciences, National Research Council, National Academy of Sciences
Volume 1, 1977; Volume 2, 1980, Volume 3, 1980
National Academy Press, Washington, D.C.

Estimating Consumer Exposure to Food Additives and Monitoring Trends in Use.
Food Additives Survey Committee, Food and Nutrition Board, Institute of Medicine, National Academy of Sciences. National Academy Press, Washington, D.C. 1992

Examination of Dietary Recommendations for Salt-Cured, Smoked, and Nitrite-Preserved Foods.
Pariza, M.W., Borzelleca, J.F., Cassens, R.G., Filer, L.J., and Kritchevsky, D.,
CAST Issue Paper Number 8, November 1997

Curricula Vitae for Michael W. Pariza

MICHAEL W. PARIZA - CURRICULUM VITAE

Academic Rank: Director, Food Research Institute; Wisconsin Distinguished Professor of Food Microbiology and Toxicology, Department of Food Science

Education:

Ph.D.	Kansas State University	1973	Microbiology
M.Sc.	Kansas State University	1969	Microbiology
B.Sc.	University of Wisconsin-Madison	1967	Bacteriology

Professional Experience.

- Kansas State University: Graduate Teaching Assistant, Graduate Research Assistant, 1967-1969.
- U.S. Army (Armed Forces Institute of Pathology, Washington, D.C.): Biological Sciences Assistant, 1969-1971.
- Kansas State University: Graduate Teaching Assistant, Graduate Research Assistant, 1971-1973.
- The University of Wisconsin-Madison (McArdle Laboratory for Cancer Research): NIH Postdoctoral Traineeship with Professor Van R. Potter, 1973-1976.
- The University of Wisconsin-Madison (The Food Research Institute, Department of Food Microbiology and Toxicology): Assistant Professor, 1976-1981; Associate Professor, 1981-1984; Professor, 1984-present; Associate Department Chair, 1981-1982; Department Chair, 1982-2006; Director, Food Research Institute, 1986-present; Department of Food Science, 2007 to present.
- Wisconsin Distinguished Professor, 1993-present.
- Affiliate appointments at the University of Wisconsin: Wisconsin Comprehensive Cancer Center; Environmental Toxicology Center; Department of Nutritional Sciences.

Membership in Scientific Organizations:

The American Association for Cancer Research
The American Society for Microbiology
The American Association for the Advancement of Science
The Society of Sigma Xi
The Institute of Food Technologists
FASEB (The American Institute of Nutrition; The American Society for Clinical Nutrition)
The American Chemical Society
The American Oil Chemists' Society

Special Professional Recognition (1996-present):

- Recognized by Thomson-ISI (publisher of Web of Science) as one of the most "Highly Cited Researchers" of the past two decades
- Associate Editor, **Regulatory Pharmacology and Toxicology**
- Member, International Editorial Board, **Food Chemicals Toxicology**
- Member, 1993 and 1998 Program Planning Committees, American Association for Cancer Research.
- Member, Institute of Medicine's Food Forum, 1993-1998.
- Member, Board of Trustees, International Life Sciences Institute – North America, 1986-present.
- Member, National Academy of Sciences Committee on Comparative Toxicity of Naturally Occurring Carcinogens, 1993-1996.
- Testified at a hearing on "The Need for FDA Reform" held by the Subcommittee on Health and Environment, Committee on Commerce, U.S. House of Representatives, Washington, D.C., February 27, 1996.
- Invited participant (presented 2 lectures) at a symposium on conjugated linoleic acid sponsored by the American Oil Chemists Society, April 29, 1996, in Indianapolis.
- Invited participant and session moderator at a Workshop on Individual Fatty Acids and Cancer sponsored by the International Life Sciences Institute-North America, June 4-5, 1996, Washington, D.C.
- Recipient of 1996 Marqueta C. Huyck Endowed Lectureship, Wayne State University, Detroit, MI. Lecture title, "CLA: The fat that reduces obesity."

- Invited participant at an international symposium, "Fundamentals of Cancer Prevention," sponsored by the Princess Takamatsu Cancer Research Fund, Tokyo, Japan, November, 1996.
- Speaker at February 1997 dinner meeting of the Chicago Section of the Institute of Food Technologists, Chicago, IL.
- Lecturer at 1997 symposium series, "Modern Views in Nutrition," Iowa State University, Ames, IA.
- Chair of Symposium on "Enhancing the Regulatory Approval Process for Food Ingredient Technologies," sponsored by the National Academy of Sciences and the Institute of Medicine (Food and Nutrition Board, Food Forum), May 6-7, 1997.
- Elected Institute of Food Technologists Fellow, 1997.
- Invited lecture at 6th International Congress on Clinical Nutrition, at Banff, Alberta, Canada, July, 1997.
- Invited lecture at 16th International Congress of Nutrition, Montreal, Canada, July, 1997.
- Chair of Organizing Committee for the first CLA Forum, held at the University of Wisconsin-Madison, August, 1997 (highlights published in INFORM 9:69-73, 1998).
- Invited lecture at symposium sponsored by Best Foods, New Jersey, September, 1997.
- Invited lecture at Michigan State University, October, 1997.
- Invited lecture at the Japanese National Cancer Center, January, 1998.
- Invited lecture at the University of Illinois, February, 1998.
- Invited lecture, "Functional Foods: Technology, Functionality and Health Benefits," at Experimental Biology 98 symposium held in San Francisco, CA sponsored by ILSI (International Life Sciences Institute), April, 1998.
- Invited lecture on conjugated linoleic acid research for the Board of the Finnish Food Foundation, Helsinki, Finland, May, 1998.
- Two invited lectures at the Finnish Food Congress in Helsinki, Finland: "Food Safety: Risks and Management" (keynote address), and *E. coli* O157:H7 research at FRI, May, 1998.
- Invited lecture on conjugated linoleic acid at the National Cancer Institute, Bethesda, MD, May, 1998.
- Chair of session, "Modification of Cancer Risk by Inulin and Oligofructose," at conference entitled "Nutritional and Health Benefits of Inulin and Oligofructose" held at NIH (National Institute of Health) in Bethesda, MD, May, 1998.
- Invited lecture on conjugated linoleic acid at The Toxicology Forum, Aspen, CO, July, 1998.
- Invited lecture on conjugated linoleic acid at a symposium at Iowa State University, Ames, IA, September, 1998.
- Invited lecture on conjugated linoleic acid at a symposium sponsored by the Society of Toxicology, Reston, VA, October, 1998
- Co-organizer of the first "International Workshop on Conjugated Linoleic Acid Analysis," held in Washington, DC, January, 1999.
- Invited lecture on conjugated linoleic acid at the 1999 annual meeting of the American Oil Chemists Society.
- Invited lecture on conjugated linoleic acid at the 1999 annual meeting of the Institute of Food Technologists (IFT).
- Invited lecture on conjugated linoleic acid at the 40th International Conference on the Biochemistry of Lipids, Dijon, France, September, 1999.
- Invited lecture on conjugated linoleic acid at the 1999 Steenbock Symposium.
- Invited lecture at American Chemical Society conference on conjugated linoleic acid, August 2000.
- Featured speaker at American Chemical Society symposia on dietary supplements and functional foods for science writers, August 2000 and January 2001.
- Featured speaker at University of Wisconsin-Madison alumni fundraiser, held near Orlando, Florida in March 2001.
- Presented 2 invited lectures at the Food and Drug Administration in Washington, DC, April 2001.
- Invited participant at international conference on conjugated linoleic acid held in Alesund, Norway, June 2001.
- Summary speaker at food bioterrorism conference, organized by the International Life Sciences Institute-North America, held in Washington, DC, December 2001.
- Invited participant and speaker at a workshop on conjugated linoleic acid, organized by the National Institutes of Health, held in Bethesda, MD, May 2002.
- Invited lectures on CLA in Amsterdam and Copenhagen, August 2002
- Invited lecture on acrylamide in food, conference sponsored by U. Maryland, held in Chicago, October 2002

- Invited lecture on acrylamide in food, Frito-Lay Corporation, Dallas, March 2003
- Keynote speaker at international conference on CLA, Winnipeg, Canada, March 2003
- Organizer and speaker at conference on acrylamide in food, International Association for Food Protection, August 2003
- First recipient of Bruce P. Wasserman Lectureship, Rutgers University, February 2004.
- Invited lecture on acrylamide in food, American Chemical Society meeting, Anaheim, CA April 2004
- Invited lectures on CLA, Spain, May 2004
- Invited lecture at Institute of Food Technologists Annual Meeting, July 2004
- Keynote speaker at international conference on CLA, Winnipeg, Canada, October 2004
- Recognized in 2005 by Thompson-ISI as one of the most "Highly Cited Researchers" of the past two decades
- Invited lecture at Institute of Food Technologists Annual Meeting, July 2005
- Invited participant in 1st meeting of the CLA Network, September 2005
- Invited lecture on FDA regulatory process, Toxicology Forum, Brussels, November 2005
- Invited lecture at Institute of Food Technologists Annual Meeting, June 2006
- Keynote speaker at international conference on CLA, Cork, Ireland, June 2006
- Invited speaker at Food Processing Conference, Florida, October 2006
- Recipient of 2007 Bernard L. Oser Food Ingredient Safety Award from the Institute of Food Technologists.

Research Focus:

Discovered the biological activity of conjugated linoleic acid (CLA), thus founding the modern field of CLA research (see http://www.wisc.edu/fri/clarefs.htm); food toxicology with emphasis on acrylamide formation in cooked food

Patents (Note:

1. Pariza, M.W., and Ha, Y.L. Methods of preventing oxidation, quenching singlet oxygen, and inhibiting mold growth and novel compositions thereof. U.S. 5,017,614
2. Pariza, M.W., and Ha, Y.L. Methods of chelating metal and novel compositions therefor. U.S. 5,070,104
3. Pariza, M.W., and Ha, Y.L. Octadecadienoic phospholipid esters, antioxidant and mold inhibiting compositions. U.S. 5,208,356
4. Cook, M.E., and Pariza, M.W. Methods for preventing weight loss, reduction in weight gain, and anorexia due to immune stimulation. U.S. 5,430,066
5. Cook, M.E. and Pariza, M.W. Method for increasing the efficiency of feed conversion in animals. U.S. 5,428,072
6. Cook, M.E., Pariza, M.W., Lee, K.N., and Wentworth, B.C. Method for controlling bird populations. U.S. 5,504,114.
7. Cook, M.E., Pariza, M.W., and Park Y. Method for reducing body fat in animals. U.S. patent application. U.S. 5,554,646.
8. Cook, M.E., Pariza, M.W., Yang, X., and DeVoney, D. Methods of treating animals to maintain or increase CD-4 and CD-8 cell populations. U.S. 5,674,901.
9. Cook, M.E., and Pariza, M.W. Dietetic foods containing conjugated linoleic acids. U.S. 5,760,082.
10. Cook, M.E., Cook, E.B., Stahl, J.L., Graziano, F.M., and Pariza, M.W. Methods of attenuating the allergic response in animals. U.S. 5,585,400.
11. Cook, M.E. and Pariza, M.W. Methods of treating animals to maintain or enhance bone mineral content and compositions for use therein. U.S. 5,804,210.
12. Satter, L.D., Dhiman, T.R., and Pariza, M.W. Method of increasing the CLA content of cow's milk. U.S. 5,770,247.
13. Cook, M.E., Pariza, M.W., and Jerome, D.L. Use of CLA to reduce the incidence of valgus and vargus leg deformities in poultry. U.S. 5,760,083.
14. Pariza, M.W. and Yang, X. Method of producing conjugated fatty acids. U.S. 5,856,149.
15. Pariza, M.W. and Lee, K.N. Method to reduce secretion of apolipoprotein. U.S. 5,837,733.
16. Cook, M.E., Pariza, M.W., Kim, S., and DeVoney, D. Methods of treating animals to enhance natural killer lymphocyte function. U.S. 5,914,346.
17. Cook, M.E., Park, Y., and Pariza, M.W. Method for controlling body fat and/or body weight in animals and pharmaceutical compositions for use therein. U.S. 5,855,917.

18. Cook, M.E., Jerome, D.L., Pariza, M.W., and Buege, D.R. Method for increasing fat firmness and improving meat quality in animals. U.S. 5,851,572.
19. Cook, M.E. and Pariza, M.W. Method for maintaining an existing level of body fat and/or body weight. U.S. 5,814,663.
20. Cook, M.E. Pariza, M.W., Yang, X., and DeVoney, D. Methods of treating animals to maintain or increase CD-4 and CD-8 cell populations. U.S. 5,827,885.
21. Cook, M.E., Jerome, D., and Pariza, M.W. Method for selectively altering body fat level, feed efficiently, or weight gain. U.S. 6,020,378.
22. Pariza, M.W. and Yang, X. Methods of treating animals to maintain or increase CD-4 and CD-8 cell populations. U.S. 6,020,376.
23. Pariza, M.W. and Yang, X. Method of producing conjugated fatty acids. U.S. 5,856,149.
24. Pariza, M.W. and Yang, X-Y. Method of producing conjugated fatty acids U.S 6,060,304.
25. Cook, M.E., Jerome, D.L., Pariza, M.W., Buege, D.R., and Mozdziak, P. Method of increasing fat firmness and improving meat quality in animals. U.S. 6,060,087
26. Cook, M.E., Whigham, L.D., and Pariza, M.W. Selective inhibition of cyclooxygenase-2. U.S. 6,077,868.
27. Cook, M.E., Aydin, R., and Pariza, M.W. Eggs enriched with conjugated linoleic acid. U.S. 6,113,973.
28. Cook, M., Yang, M., and Pariza, M.W. Method of increasing longevity and preventing body weight wasting in autoimmune disease by using conjugated linoleic acid. U.S. 6,395,782.

-- (Note: Foreign patent equivalents on each of these have issued or are pending.) --

Publications:

1. Pariza, M. W. and Iandolo, J. J. 1969. Coagulase production by injured *Staphylococcus aureus* MF-31 during recovery. *Appl. Microbiol.* 17:836-838.
2. Rosenthal, L. J., Martin, S. E., Pariza, M. W. and Iandolo, J. J. 1972. Ribosome synthesis in thermally shocked cells of *Staphylococcus aureus*. *J. Bacteriol.* 109:243-249.
3. Pariza, M. W. and Iandolo, J. J. 1974. Base ratio and DNA homology studies on six *Staphylococcus aureus*. *Appl. Microbiol.* 27:317-323.
4. Pariza, M. W. and Iandolo, J. J. 1974. Determination of genome size of selected bacteriophages of *Staphylococcus aureus*. *Appl. Microbiol.* 28:510-512.
5. Pariza, M. W., Becker, J. E., Yager, J. D., Jr., Bonney, R. J. and Potter, V. R. 1974. Enzyme induction in primary cultures of rat liver parenchymal cells, pp. 267-284. In W. Nakahara, T. Ono, T. Sugimura and H. Sugano (eds.) *Differentiation and control of malignancy of tumor cells*. University of Tokyo Press, Tokyo, Japan.
6. Kletzien, R. F., Pariza, M. W., Becker, J. E. and Potter, V. R. 1975. A method using 3-0-methyl-D-glucose and phloretin for the determination of intracellular water space of cells in monolayer cultures. *Anal. Biochem.* 68:537-544.
7. Kletzien, R. F., Pariza, M. W., Becker, J. E. and Potter, V. R. 1975. A "permissive" effect of dexamethasone on the glucagon induction of amino acid transport in cultured hepatocytes. *Nature* 256:46-47.
8. Pariza, M. W., Yager, J. D., Jr., Goldfarb, S., Gurr, J. A., Yanagi, S., Grossman, S. H. Becker, J. E., Barber, R. A. and Potter, V. R. 1975. Biochemical, autoradiographic and electron microscopic studies on adult rat liver parenchymal cells in primary culture, pp. 137-167. In L. E. Gerschenson and E. B. Thompson (eds.) *Gene expression and carcinogenesis in cultured liver*. Academic Press, New York.
9. Yager, J. D., Jr., Pariza, M. W., Becker, J. E. and Potter, V. R. 1975. DNA synthesis in primary cultures of parenchymal cells isolated from regenerating rat liver, pp. 148-151. In R. Lesch and W. Reutter (eds.) *Liver regeneration after experimental injury*. Stratton Intercontinental Medical Book Corporation, New York.
10. Pariza, M. W., Yanagi, S., Gurr, J. A., Morris, H. P. and Potter, V. R. 1976. Ornithine decarboxylase activity and DNA synthesis in Morris hepatomas 5123-C and 7800. *Life Sci.* 18:39-48.
11. Kletzien, R. F., Pariza, M. W., Becker, J. E., Butcher, F. R. and Potter, V. R. 1976. Induction of amino acid transport in primary cultures of adult rat liver parenchymal cells by insulin. *J. Biol. Chem.* 251:3014-3020.
12. Pariza, M. W., Yanagi, S., Gurr, J. A., Morris, H. P. and Potter, V. R. 1976. Fasting does not abolish the diurnal oscillation of ornithine decarboxylase in Morris hepatoma 5123-C. *Life Sci.*19:1553-1558.
13. Pariza, M. W., Butcher, F. R., Kletzien, R. F., Becker, J. E. and Potter, V. R. 1976. Induction and decay of glucagon-induced amino acid transport in primary cultures of adult rat liver cells: Paradoxical effects of cycloheximide and puromycin. *Proc. Natl. Acad. Sci. U.S.A.* 73:4511-4515.

14. Pariza, M. W., Kletzien, R. F., Butcher, F. R. and Potter, V. R. 1976. Inductions by hormones added singly, simultaneously or sequentially: What cultured hepatocytes can tell us about metabolic regulation in the whole animal. *Adv. Enzyme Regulation* 14:103-115.
15. Kletzien, R. F., Pariza, M. W., Becker, J. E. and Potter, V. R. 1976. Hormonal regulation of amino acid transport and gluconeogenesis in primary cultures of adult rat liver parenchymal cells. *J. Cell. Physiol.* 89:641-646.
16. Pariza, M. W., Butcher, F. R., Becker, J. E. and Potter, V. R. 1977. Cyclic AMP-independent induction of amino acid transport by epinephrine in primary cultures of adult rat liver cells. *Proc. Natl. Acad. Sci U.S.A.* 74:234-237.
17. Pariza, M. W., Kletzien, R. F. and Potter, V. R. 1977. A model for the "permissive" effect of glucocorticoids on the glucagon induction of amino acid transport in cultured hepatocytes, pp. 379-388. In R. T. Acton and J. D. Lynn (eds.) Proc. International Cell Culture Congress. *Academic Press*, New York.
18. Goldfarb, S., Barber, T. A., Pariza, M. W. and Pugh, T. W. 1978. Lipid synthesis and ultrastructure of adult rat hepatocytes during their first twenty-four hours in culture. *Exptl. Cell Res.* 117:39-46.
19. Giger, O. and Pariza, M. W. 1978. Depression of amino acid transport in cultured rat hepatocytes by purified enterotoxin from *Clostridium perfringens*. *Biochem. Biophys. Res. Commun.* 82:378-383.
20. Saccone, G. T. P. and Pariza, M. W. 1978. Effects of dietary butylated hydroxytoluene and phenobarbital on the activities of ornithine decarboxylase and thymidine kinase in rat liver and lung. *Cancer Lett.* 5:145-152.
21. Pariza, M. W., Ashoor, S. H., Chu, F. S. and Lund, D. B. 1979. Effects of temperature and time on mutagen formation in pan-fried hamburger. *Cancer Lett.* 7:63-69.
22. Pariza, M. W., Ashoor, S. H. and Chu, F. S. 1979. Mutagens in heat-processed meat, bakery and cereal products. *Food Cosmet. Toxicol.* 17:429-430.
23. Pariza, M. W. 1979. Food safety: from the eye of a hurricane. Professional Nutritionist 11:11-14 (commissioned article).
24. Giger, O. and Pariza, M. W. 1980. Mechanism of action of *Clostridium perfringens* enterotoxin: effects on membrane permeability and amino acid transport in primary culture of adult rat hepatocytes. *Biochim. Biophys. Acta* 595:264-276.
25. Ashoor, S. H., Dietrich, R. A., Chu, F. S. and Pariza, M. W. 1980. Proline enhances mutagen formation in ground beef during frying. *Life Sci.* 26:1801-1805.
26. Gayda, D. P. and Pariza, M. W. 1980. Rat hepatocytes in primary monolayer culture: a tool for investigating the regulation of carcinogen metabolism, pp. 1165-1168. In M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette and P. J. O'Brien (eds.) Microsomes, drug oxidations and chemical carcinogenesis, Vol. II. *Academic Press*, New York.
27. Saccone, G. T. P. and Pariza, M. W. 1981. Enhancement of hepatic microsome-mediated bacterial mutagenesis by the rat liver soluble protein fraction. *Mutat. Res.* 88:135-145.
28. Gayda, D. P. and Pariza, M. W. 1981. Activation of aflatoxin B_1 by primary cultures of adult rat hepatocytes: effects of hepatocyte density. *Chem. Biol. Interactions* 35:255-265.
29. Saccone, G. T. P., DasGupta, B. R. and Pariza, M. W. 1981. Enhancement of N-hydroxy-2-aminofluorene bacterial mutagenicity by the soluble protein fraction from rat liver and partial purification of the enhancement activity. *Cancer Res.* 41:4600-4605.
30. Pariza, M. W. 1982. Mutagens in heated foods. *Food Technol.* 36:53-56.
31. DasGupta, B. R. and Pariza, M. W. 1982. Purification of two *Clostridium perfringens* enterotoxin-like proteins and their effects on membrane permeability in primary cultures of adult rat hepatocytes. *Infect. Immun.* 38:592-597.
32. Pariza, M. W. (Consulting Reviewer). 1982. *Oncology Overview* on mutagens and carcinogens in cooked, smoked and charred foods. National Technical Information Service, Springfield, VA. Document #PB82-922914.
33. Hargraves, W. A., Dietrich, R. A. and Pariza, M. W. 1982. A new chromatographic method for separating mutagens from commercial beef extract and fried ground beef. In H. F. Stitch (ed.) *Carcinogens and Mutagens in the Environment*, Vol. I, Food Products. CRC Press, Inc., pp. 223-229.
34. Hargraves, W. A. and Pariza, M. W. 1983. Purification and mass spectral characterization of bacterial mutagens from commercial beef extract. *Cancer Res.* 43:1467-1472.
35. Gayda, D. P. and Pariza, M. W. 1983. Activation of 2-amino-3-methylimidazo [4,5-f]quinoline and 2-aminofluorene for bacterial mutagenesis by primary monolayer cultures of adult rat hepatocytes. *Mutat. Res.* 118:7-14.

36. Gayda, D. P. and Pariza, M. W. 1983. Effects of carcinogens on hormonal regulation of gene expression in primary cultures of adult rat hepatocytes. *Carcinogenesis* 4:1127-1131.
37. Schwartz, S. J., von Elbe, J. H., Pariza, M. W., Goldsworthy, T. J. and Pitot, H. C. 1983. Inability of red beet betalain pigments to initiate or promote hepatocarcinogenesis. *Fd. Chem. Toxic.* 21:531-535.
38. Pariza, M. W. 1983. Carcinogenicity/toxicity testing and the safety of foods. *Food Technol.* 37(1):84-86.
39. Pariza, M. W., Loretz, L. J., Storkson, J. M. and Holland, N. C. 1983. Mutagens and modulators of mutagenesis in fried ground beef. *Cancer Res.* 43:2444s-2446s.
40. Pariza, M. W. and Foster, E. M 1983. Determining the safety of enzymes used in food processing. *J Food Prot.* 46:453-468.
41. Pariza, M. W. 1984. A perspective on diet, nutrition, and cancer. *J. Amer Med. Assoc.* 251:1455-1458 (commissioned article).
42. Loretz, L. J. and Pariza, M. W 1984. Effect of glutathione levels, sulfate levels and metabolic inhibitors on covalent binding of 2-amino-3-methylimidazo[4,5-f] quinoline and 2-acetylaminofluorene to cell macromolecules in primary monolayer cultures of adult rat hepatocytes. *Carcinogenesis* 5:895-899.
43. Hargraves, W. A. and Pariza, M. W. 1984. Mutagens in cooked foods. *J. Environ. Sci. Health* C2(1):1-49.
44 Pariza, M. W. and Hargraves, W. A. 1985. A beef-derived mutagenesis modulator inhibits initiation of mouse epidermal tumors by 7,12-dimethylbenz[a]anthracene. *Carcinogenesis* 6:591-593.
45. Pariza, M. W. 1985. Diet and cancer. American Council on Science and Health, New York, NY.
46. Pariza, M. W., Hargraves, W. A., Benjamin, H., Cristou, M., Jefcoate, C. R., Storkson, J., Albright, K., Kraus, D., Sharp, P., Boissonneault, G. A. and Elson, C. E. 1986. Modulation of carcinogenesis by dietary factors: I. Inhibition of carcinogenesis by a beef-derived mutagenesis modulator; and II. Role of net energy in enhancement of carcinogenesis by dietary fat. *Environ. Health Perspectives* 67:25-29.
47. Pariza, M. W., Hargraves, W. A. and Boissonneault, G. A. 1986. Modulation of carcinogenesis by a beef-derived mutagenesis modulator, and by dietary fat, in *Genetic Toxicology of the Diet*, I. Knudsen (ed.), Alan R. Liss, Inc., pp. 265-271.
48. Boissonneault, G. A., Elson, C. E. and Pariza, M. W. 1986. Net energy effects of dietary fat on chemically-induced mammary carcinogenesis in F344 rats. *JNCI* 76:335-338.
49. Pariza, M. W. 1986. Analyzing current recommendations on diet, nutrition and cancer. *Fd. Nutrit. News* 58:1-3 (commissioned article).
50. Pariza, M. W. 1986. Calories and energy expenditure in carcinogenesis. *Contemporary Nutrit.* Vol. XI, No. 4 (commissioned article).
51. Pariza, M. W. 1986. Calorie restriction, ad libitum feeding, and cancer. FASEB Symposium on Nutrition and Cancer. *Proc. Soc. Exptl. Biol. Med.* 183:293-298.
52. Pariza, M. W. 1986. Diet and cancer: science vs policy. *Pediatric Basics* 44:10-15.
53. Pariza, M. W. 1986. Symposium on calories and energy expenditure in carcinogenesis-- executive summary. *Nutrit. Today*, July/August, 21-23.
54. Poirier, L. A., P. M. Newberne, and M. W. Pariza, (eds.). 1987. *Role of Essential Nutrients in Carcinogenesis*, Plenum Press, New York, 562 pp.
55. Boissonneault, G. A., Elson, C. E. and Pariza, M. W. 1987. Dietary fat and neoplasia: The role of net energy in enhancement of carcinogenesis; Effects of fat and calories on the immune system, In: *Role of Essential Nutrients in Carcinogenesis,* L. Poirier, P. Newberne and M. Pariza (eds.), Plenum Press, New York, pp. 85-98.
56. Pariza, M. W. (Task Force Chair). 1987. Diet and health. Council for Agricultural Science and Technology, Ames, Iowa.
57. Pariza, M. W. and Boutwell, R. K. 1987. Calories and energy expenditure in carcinogenesis: Historical Perspective. *Amer. J. Clin. Nutrit.* (supplement) 45:151-156.
58. Pariza, M. W. 1987. Fat, calories, and mammary carcinogenesis. *Amer. J. Clin. Nutrit.* (supplement) 45:261-263.
59. Pariza, M. W. 1987. Dietary fat, calorie restriction, *ad libitum* feeding, and cancer risk. *Nutrit. Rev.* 45:1-7 (commissioned lead article).
60. Ha, Y. L., Grimm, N. K. and Pariza, M. W. 1987. Anticarcinogens from fried ground beef: Heat-altered derivatives of linoleic acid. *Carcinogenesis* 8:1881-1887.
61. Pariza, M. W. 1988. Effects of calorie intake and expenditure on carcinogenesis, In: *Horticulture and Human Health. Contributions of Fruits and Vegetables.* Proc. 1st Int. Symp. Horticulture and Human Health. B. Quebedeaux and F. A. Bliss (eds.), Prentice-Hall, Englewood Cliffs, N.J., pp. 144-149.

62. Pariza, M. W. 1988. Dietary fat and cancer risk: Evidence and research needs. *Ann. Rev. Nutr.* 8:167-183.
63. Benjamin, H., Storkson, J., Tallas, P. G., and Pariza, M. W. 1988. Reduction of benzo(a)pyrene-induced forestomach neoplasms in mice given nitrite and dietary soy sauce. *Fd. Chem. Toxicol.* 26:671-678.
64. Ha, Y. L., Grimm, N. K., and Pariza, M. W. 1989. Newly recognized anticarcinogenic fatty acids: Identification and quantification in natural and processed cheeses. *J. Ag. Fd. Chem.* 37:75-81.
65 Johnson, E. A. and M. W. Pariza. 1989. Microbiological principles for the safety of foods, In: *International Food Regulation Handbook. Policy, Science, Law.* R. D. Middlekauff and P. Shubik (eds.), Marcel Dekker, Inc., New York, pp. 135-174.
66. Pariza, M. W. 1989. A perspective on diet and cancer, In: *Food Toxicology. A Perspective on Relative Risks.* S. L. Taylor and R. A. Scanlan (eds.), Marcel Dekker, Inc., New York, pp. 1-10.
67 Ha, Y. L., Storkson, J. and Pariza, M. W. 1990. Inhibition of benzo()pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. *Cancer Res.* 50:1097-1101.
68 Pariza, M. W. and Ha, Y. L. 1990. Newly recognized anticarcinogenic fatty acids, In: *Antimutagenesis and Anticarcinogenesis Mechanisms II.* Y. Kuroda, D. M. Shankel and M. D. Waters (eds.), Plenum Press, New York, pp. 167-170.
69. Pariza, M. W., H. -U. Aeschbacher, J. S. Felton, and S. Sato (eds). 1990. *Mutagens and Carcinogens in the Diet*, Wiley-Liss, Inc., New York, 332 pp.
70. Pariza, M. W. and Ha, Y. L. 1990. Conjugated dienoic derivatives of linoleic acid: Mechanism of anticarcinogenic effect, In: *Mutagens and Carcinogens in the Diet.* M. W. Pariza, H.-U. Aeschbacher, J. S. Felton, and S. Sato (eds.), Wiley-Liss, Inc., New York, pp. 217-221.
71. Pariza, M. W., and Ha, Y. L. 1990. Conjugated dienoic derivatives of linoleic acid: a new class of anticarcinogens. *Medical Oncology Tumor Pharmacology.* 7:169-172.
72. Pariza, M. W. 1990. Diet and cancer, In: *Foodborne Diseases.* D. O Cliver (ed.), Academic Press, Inc., New York, New York, pp. 308-317.
73. Pariza, M. W. 1990 Perspectives on food safety and biotechnology, In: *Biotechnology and Food Safety*, Proceedings of the Second International Symposium. D. D. Bills and S. D. Kung (eds.), Butterworth-Heinemann, Boston, pp. 47-52.
74. Ha, Y. L., and Pariza, M. W. 1990. Anticarcinogenic conjugated dienoic derivatives of linoleic acid found in grilled ground beef: Isolation, identification, and mechanism of action, In: *The First Korean Conference on Science and Technology.* The Korean Federation and Science Association, Seoul, Korea, pp. 442-445.
75. Pariza, M. W. 1990. Evaluating the relative safety of biotechnologically produced foods. In: *Agricultural Biotechnology, Food Safety and Nutritional Quality for the Consumer*, NABC Report 2. Union Press of Binghampton, New York, pp. 167-173.
76. Benjamin, H., Storkson, J., Nagahara, A. and Pariza, M. W. 1991. Inhibition of benzo[a]pyrene-induced mouse forestomach neoplasia by dietary soy sauce. *Cancer Res.* 51:2940-2942.
77. Pariza, M. W. 1991. CLA, a new cancer inhibitor in dairy products. *Bull. Int. Dairy Fed.* 257:29-30.
78. Pariza, M. W. and Ha, Y. 1991. Fatty acids that inhibit cancer: Conjugated dienoic derivatives of linoleic acid. In: *Nutrients and Cancer Prevention*, K. N. Prasad and F. L. Meyskens, Jr. (eds.), Humana Press, Clifton, NJ, pp. 113-117.
79. Pariza, M. W., Ha, Y. L., Benjamin, H., Sword, J. T., Grüter, A., Chin, S. F., Storkson, J., Faith, N., and Albright, K. 1991. Formation and action of anticarcinogenic fatty acids. In: *Nutritional and Toxicological Consequences of Food Processing*, M. Friedman (ed.), Plenum Publishing Corp., New York, New York, pp. 269-272.
80. Pariza, M. W. 1991. Human health concerns associated with pesticidal transgenic plants. In: *Pesticidal Transgenic Plants: Product Development, Risk Assessment, and Data Needs.* U. S. EPA Conference Proceedings. Document #EPA/21T-1024, pp. 107-111.
81. Ip, C., Chin, S. F., Scimeca, J. A., and Pariza, M. W. 1991. Mammary cancer prevention by conjugated dienoic derivatives of linoleic acid. *Cancer Res.* 51:6118-6124.
82. Ha, Y. L. and Pariza, M. W. 1991. Naturally-occurring novel anticarcinogens: Conjugated dienoic derivatives of linoleic acid (CLA). *J. Korean Soc. Food Nutr.* 20:401-407.
83. Pariza, M. W. 1992. A new approach to evaluating carcinogenic risk. *Proc. Natl. Acad. Sci. U.S.A.* 89:860-861.
84. Pariza, M. W. 1992. Risk assessment. *Crit. Rev. Food Sci. Nutr.* 31:205-209.
85. Pariza, M. W. 1992. Chapter 4: Risk-benefit perceptions. In: *Food Safety Assessment*, J. W. Finley, S. F. Robinson, and D. J. Armstrong (eds.), American Chemical Society, Washington, DC, pp. 36-40.

86. Nagahara, A., Benjamin, H., Storkson, J., Krewson, J., Sheng, K., Liu, W., and Pariza, M. W. 1992. Inhibition of benzo[a]pyrene-induced mouse forestomach neoplasia by a principal flavor component of Japanese-style fermented soy sauce. *Cancer Res.* 52:1754-1756.
87. Pariza, M. W. 1992. Foods of new biotechnology vs traditional products: microbiological aspects. *Food Technol.* 46:100-102.
88. Pariza, M. W. 1992. Designer foods: effects on development of cancer. *J. Natl. Cancer Inst. Monographs* 12:105-107
89. Pariza, M. W. 1992. Chemoprevention by conjugated dienoic isomers of linoleic acid. In: *Cancer Chemoprevention*, L. Wattenberg, M. Lipkin, C. W. Boone and G. J. Kelloff (eds.), CRC Press, Inc., Boca Raton, FL, pp. 279-283.
90. Chin, S. F., Liu, W. Storkson, J. M., Ha, Y. L. and Pariza, M. W. 1992. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *J. Food Comp. Anal.* 5:185-197
91. Foster, E. M. and Pariza, M. W. 1993. Food safety: federal inspection programs. Commissioned by the Council on Scientific Affairs, American Medical Association. *Arch. Fam. Med.* 2:210-214.
92. Cook, M. E., Miller, C. C., Park, Y. and Pariza, M. 1993. Immune modulation by altered nutritient metabolism: nutritional control of immune-induced growth depression. *Poultry Sci.* 72:1301-1305.
93. Pariza, M. W. 1993. Diet and cancer: where do matters stand? Commissioned by the Council on Scientific Affairs, American Medical Association, Chicago, IL. *Arch. Intern. Med.* 153:50-56.
94. Chin, S. F., Storkson, J. M., and Pariza, M. W. 1993. Conjugated dienoic derivatives of linoleic acid. Chapter 21. In: *Food Flavor and Safety: Molecular Analysis and Design*, A. M. Spanier, H. Okai, and M. Tamura (eds.), American Chemical Society, Washington, DC, pp. 262-271.
95. Pariza, M. W. 1993. Chapter 88: Diet, cancer, and food safety. In: *Modern Nutrition in Health and Disease*, 8th Edition. M. E. Shils, J. A. Olson, and M. Shike (eds.), Lea and Febiger, Philadelphia, pp. 1545-1558.
96. Pariza, M. W. 1993. CLA and HEMF: newly recognized anticarcinogenic antioxidants. In: *Active Oxygens, Lipid Peroxides, and Antioxidants*, K. Yagi (ed.), Japan Sci. Soc. Press, Tokyo/CRC Press, Boca Raton, FL, pp. 359-365.
97. Pariza, M. W. 1994. Chapter 28. Fermentation-derived anticarcinogenic flavor compound. In: *Food Phytochemicals for Cancer Prevention I*, American Chemical Society Symposium Series 546, M. T. Huang, T. Osawa, C. T. Ho, and R. T. Rosen (eds.), American Chemical Society, Washington, DC, pp. 349-352.
98. Bonorden, W. R. and Pariza, M. W. 1994. Antioxidant nutrients and protection from free radicals. In: *Nutritional Toxicology, Target Organ Toxicology Series*, F. N. Kotsonis, M. Mackey, and J. J. Hjelle (eds.), Raven Press, Ltd., New York, pp. 19-48.
99. Miller, C. C., Park, Y., Pariza, M. W., and Cook, M. E. 1994. Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection. *Biochem. Biophys. Res. Commun.* 198:1107-1112.
100. Lee, K. N., Kritchevsky, D., and Pariza, M. W. 1994. Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* 108:19-25.
101. Chin, S. F., Storkson, J. M., Liu, W., Albright, K. J., and Pariza, M. W. 1994. Conjugated linoleic acid (9, 11- and 10, 12-octadecadienoic acid) is produced in conventional but not germ-free rats fed linoleic acid. *J. Nutr.* 124:694-701.
102. Chin, S. F., Storkson, J. M., Albright, K. J., Cook, M. E., and Pariza, M. W. 1994. Conjugated linoleic acid is a growth factor for rats as shown by enhanced weight gain and improved feed efficiency. *J. Nutr.* 124:2344-2349.
103. Yurawecz, M. P., Hood, J. K., Roach, J. A. G., Mossoba, M. M., Daniels, D. H., Ku, Y., Pariza, M. W. and Chin, S. F. 1994. Conversion of allylic hydroxy oleate to conjugated linoleic acid and methoxy oleate by acid-catalyzed methylation procedures. *J. Am. Oil Chem. Soc.* 71:1149-1155.
104. Conn, R. E., Kolstad, J. J., Borzelleca, J. F., Dixler, D. S., Filer Jr., L. J., LaDu, Jr., B. N., and Pariza, M. W. 1995. Safety assessment of polylactide (PLA) for use as a food-contact polymer. *Fd. Chem. Toxicol.* 33:273-283.
105. Cohen, S. M., Clydesdale, F. M., Winter, C., Graham, J. D., Weil Jr., W. B., Kroger, M., Pariza, M. W., Crawford, L. M., Avery, D., Scheuplein, R. J. and Weisberger, E. 1995. Delaney Reform. (Letter.) *Science* 268:1829-1830.
106. Liew, C., Schut, H. A. J., Chin, S. F., Pariza, M. W. and Dashwood, R. H. 1995. Protection of conjugated linoleic acids against 2-amino-3-methylimidazo[4,5-*f*]quinoline-induced colon carcinogenesis in the F344 rat: a study of inhibitory mechanisms. *Carcinogenesis* 16:3037-3043.

107. Pariza, M. W. 1996. Chapter 57: Toxic substances in foods. In: *Present Knowledge in Nutrition, Seventh Edition.* E. E. Ziegler and L. J. Filer, Jr. (eds.), ILSI Press, Washington, DC, pp. 563-573.
108. Pariza, M. W. 1996. Chapter 3: Dietary lipids and cancer: Lessons from the past, directions for the future. In: *Food Lipids and Health.* IFT Basic Symposium Series. R. E. McDonald and D. B. Min (eds.), Marcel Dekker, Inc., New York, pp. 35-41.
109. Pariza, M. W. 1996. Diet in infancy and cancer development. In: *Infant Nutrition in the Prevention of Chronic Pathology.* Third International Symposium held in Alicante, Spain on September 20, 1995. M. Moya, G. Sawatzki, A. Motulsky and J. Morán (eds.), Ediciones Ergon, S.A., Madrid, Spain, pp. 15-20.
110. Pariza, M. W 1996. Conjugated linoleic acid: A unique anticarcinogenic fatty acid in dairy products. In: *Yogurt: Myth Versus Reality.* Proceedings of a conference presented by the National Yogurt Association held in Washington, DC on October 26-27, 1993. D. Curtis (ed.), National Yogurt Association, McLean, Virginia, pp. 111-116.
111. Estabrook, R. W., Birt, D., Carlson, G. P , Cohen, S. M., Conn, E. E., Farnsworth, N. R., Gaylor, D. W., Hall, R. L., Higginson, J , Hodgson, E., Kolonel, L. N., Krewski, D., McQueen, C. A., Pariza, M. W., Sipes, I. G., Wagner, B., Watkins, P. B., Weinstein, I. B., and Zeise, L. 1996. *Carcinogens and Anticarcinogens in the Human Diet.* National Academy Press, Washington, DC.
112. Pariza, M. W. 1996. Toxic substances. In: *Food Chemistry, Third Edition.* O. R. Fennema (ed.), Marcel Dekker, Inc., New York, pp. 825-840.
113. Lee, K. N., Pariza, M. W., and Ntambi, J. M. 1996. Differential expression of hepatic stearoyl-CoA desaturase gene 1 in male and female mice. *Biochim. Biophys. Acta* 1304:85-88.
114. Pariza, M. W. 1997. Conjugated linoleic acid, a newly recognised nutrient. *Chem. & Ind.* 12:464-466 (June 16 issue).
115. Hallagan, J. B., La Du, B. N., Pariza, M. W., Putnam, J. M., and Borzelleca, J. F. 1997. Assessment of Cassia Gum. *Fd. Chem. Toxicol.* (Review Section) 35:625-632.
116. Pariza, M. W. 1997. Conjugated linoleic acid: An anticarcinogenic nutrient. In: *Food Factors for Cancer Prevention.* H. Ohigashi, T. Osawa, J. Terao, S. Watanabe, and T. Yoshikawa (eds.), Springer-Verlag Tokyo, Inc., Tokyo, Japan, pp. 365-366.
117. Park, Y., Albright, K. J., Liu, W., Storkson, J. M., Cook, M. E., and Pariza, M. W. 1997. Effect of conjugated linoleic acid on body composition in mice. *Lipids* 32:853-858.
118. Kataoka, S., Liu, W., Albright, K., Storkson, J., and Pariza, M. 1997. Inhibition of benzo[a]pyrene-induced mouse forestomach neoplasia and reduction of H_2O_2 concentration in human polymorphonuclear leucocytes by flavour components of Japanese-style fermented soy sauce. *Fd. Chem. Toxicol.* 35:449-457.
119. Pariza, M. W. 1997. Animal studies: summary, gaps, and future research. *Am. J. Clin. Nutr.* 66(suppl):1539S-40S.
120. Garcia, H. S., Storkson, J. M., Pariza, M. W., and Hill Jr., C. G. 1998. Enrichment of butteroil with conjugated linoleic acid via enzymatic interesterification (acidolysis) reactions. *Biotech. Letters* 20:393-395.
121. Cook, M. E. and Pariza, M. W. 1998. The role of conjugated linoleic acid (CLA) in health. *Int. Dairy J.* 8:459-462.
122. Park, Y. and Pariza, M. W. 1998. Evidence that commercial calf and horse sera can contain substantial amounts of *trans*-10,*cis*-12 conjugated linoleic acid. *Lipids* 33:817-819.
123. Lee, K. N., Pariza, M. W., and Ntambi, J. M. 1998. Conjugated linoleic acid decreases hepatic stearoyl-CoA desaturase mRNA expression. *Biochem. Biophys. Res. Commun.* 248:817-821.
124. Pariza, M. W., Ponakala, S. V., Gerlat, P.A., and Andress, S. 1998. Predicting the functionality of direct food additives. *Food Technol.* 52:56-60.
125. Dhiman, T. R., Helmink, E. D., McMahon, D.J ., Fife, R. L., and Pariza, M. W. 1999. Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds. *J. Dairy Sci.* 82:412-419.
126. Park, Y., Storkson, J. M., Albright, K. J., Liu, W., and Pariza, M. W. 1999. Evidence that the *trans*-10,*cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids* 34:235–241.
127. Park, Y., Albright, K. J., Storkson, J. M., Liu, W., Cook, M. E., and Pariza, M. W. 1999. Changes in body composition in mice during feeding and withdrawal of conjugated linoleic acid. *Lipids* 34:243–248.
128. Yurawecz, M. P., M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson (eds.). 1999. *Advances in Conjugated Linoleic Acid Research, Vol. 1*, AOCS Press, Champaign, IL.
129. Pariza, M. W. 1999. Chapter 2: The biological activities of conjugated linoleic acid. In: *Advances in Conjugated Linoleic Acid Research, Vol. 1.* M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson (eds.), AOCS Press, Champaign, IL, pp. 12-20.

130. Cook, M. E., DeVoney, D., Drake, B., Pariza, M. W., Whigham, L., and Yang, M. 1999. Chapter 17: Dietary control of immune-induced cachexia: conjugated linoleic acid and immunity. In: *Advances in Conjugated Linoleic Acid Research, Vol. 1*. M. P. Yurawecz, M. M. Mossoba, J. K.G. Kramer, M. W. Pariza, and G. J. Nelson (eds.), AOCS Press, Champaign, IL, pp. 226-237.
131. Dhiman, T. R., Anand, G. R., Satter, L. D., and Pariza, M. W. 1999. Conjugated linoleic acid content of milk from cows fed different diets. *J. Dairy Sci.* 82:2146-2156.
132. Pariza, M. W., Park, Y., and Cook, M. E. 1999. Conjugated linoleic acid and the control of cancer and obesity. *Toxicol. Sci.* 52 (Supplement):107-110.
133. Pariza, M. W., Park, Y., and Cook, M. E. 2000. Mechanisms of action of conjugated linoleic acid: evidence and speculation. *Proc. Soc. Exp. Biol. Med.* 223:8-13.
134. Pariza, M. W. and Park, Y. 2000. Conjugated linoleic acid, lipid metabolism and adipocytes. In: *Adipocyte Biology and Hormone Signaling* J. M. Ntambi (ed.), IOS Press, Tokyo, Japan, pp 159-164.
135. Park, Y., Storkson, J. M., Ntambi, J. M., Cook, M. E., Sih, C. J. and Pariza, M. W. 2000. Inhibition of hepatic stearoyl-CoA desaturase activity by *trans*-10,*cis*-12 conjugated linoleic acid and its derivatives. *Biochim. Biophys. Acta* 1486:285-292.
136. Choi, Y., Kim, Y-C, Han, Y-B, Park, Y., Pariza, M. W. and Ntambi, J. M. 2000. The *trans*-10,*cis*-12 isomer of conjugated linoleic acid downregulates stearoyl-CoA desaturase gene expression in 3T3-L1 adipocytes. *J. Nutr.* 130:1920-1924.
137. Yang, M., Pariza, M. W. and Cook, M. E. 2000. Dietary conjugated linoleic acid protects against end stage disease of systemic lupus erythematosus in the NZB/W F_1 mouse. *Immunopharmacol. Immunotoxicol.* 22:433-449.
138. Dhiman, T. R., Satter, L. D., Pariza, M. W., Galli, M. P., Albright, K. and Tolosa, M. X. 2000. Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. *J Dairy Sci.* 83:1016-1027.
139. Ohshita, K., Nakajima, Y., Yamakoshi, J., Kataoka, S., Kikuchi, M., and Pariza, M. W. 2000. Safety evaluation of yeast glutaminase. *Fd. Chem. Toxicol.* 38:610-670.
140. Pariza, M. W. 2000. Food safety and biotechnology. In: *The Cambridge World History of Food, Vol. 2*. K. F. Kiple and K. C. Ornelas (eds.), Cambridge University Press, New York, NY, pp. 1662-1667.
141. Whigham, L. D., Cook, E. B., Stahl, J. L., Saban, R., Bjorling, D. E., Pariza, M. W., and Cook, M. E. 2001. CLA reduces antigen-induced histamine and PGE2 release from sensitized guinea pig tracheae. *Am. J. Physiol. Reg.* 280:R908-R912.
142. Park, Y., Albright, K. J., Cai, Z. Y., and Pariza, M. W. 2001. Comparison of methylation procedures for conjugated linoleic acid and artifact formation by commercial (trimethylsilyl)diazomethane. *J. Agric. Food Chem.* 49:1158-1164.
143. Aydin, R., Pariza, M. W., and Cook, M. E. 2001. Olive oil prevents the adverse effects of dietary conjugated linoleic acid on chick hatchability and egg quality. *J. Nutr.* 131:800-806.
144. Stahl, J. L., Cook, E. B., Pariza, M. W.*, Cook, M. E., and Graziano, F. M. 2001. Effect of L-tryptophan supplementation on eosinophils and eotaxin in guinea pigs. *Exptl. Biol. Med.* 226:177-184. *(incorrectly listed in journal title as Michael A. Pariza)
145. Pariza, M. W. and Johnson, E. A. 2001. Evaluating the safety of microbial enzyme preparations used in food processing: Update for a new century. *Regulat. Toxicol. and Pharmacol.* 33:173-186.
146. Choi, Y. J., Park, Y., Pariza, M. W., and Ntambi, J. M. 2001. Regulation of stearoyl-CoA desaturase activity by the *trans*-10,*cis*-12 isomer of conjugated linoleic acid in HepG2 cells. *Biochem. Biophys. Res. Commun.* 284:689-693.
147. Pariza, M. W., Park, Y., and Cook, M. E. 2001. The biologically active isomers of conjugated linoleic acid. *Prog. in Lipid Res.* 40:283-298.
148. Doyle, M. E. and Pariza, M. W. 2001. Foodborne microbial pathogens and the Food Research Institute. *Adv. Appl. Microbiol.* 49:143-161.
149. Kang, K. and Pariza, M. W. 2001. *trans*-10,*cis*-12-conjugated linoleic acid reduces leptin secretion from 3T3-L1 adipocytes. *Biochem. Biophys. Res. Commun.* 287:377-382.
150. Park, Y. and Pariza, M. W. 2001. The effects of dietary conjugated nonadecadienoic acid on body composition in mice. *Biochim. Biophys. Acta* 1533:171-174.
151. Park, Y. and Pariza, M. W. 2001. Lipoxygenase inhibitors inhibit heparin-releasable lipoprotein lipase activity in 3T3-L1 adipocytes and enhance body fat reduction in mice by conjugated linoleic acid. *Biochim. Biophys. Acta* 1534:27-33.

152. Doyle, M. E. and Pariza, M. W. 2001. Chapter 1: Antioxidant nutrients and protection from free radicals. In: *Nutritional Toxicology, Second Edition*. F. N. Kotsonis and M. A. Mackey (eds.), Taylor & Francis, London and New York, pp. 1-30.
153. Peters, J. M., Park, Y., Gonzalez, F. J. and Pariza, M. W. 2001. Influence of conjugated linoleic acid on body composition and target gene expression in peroxisome proliferator-activated receptor α-null mice. *Biochim Biophys. Acta* 1533:233-242.
154. Evans, M., Park, Y., Pariza, M., Curtis, L., Kuebler, B., and McIntosh, M. 2001. *trans*-10,*cis*-12 conjugated linoleic acid reduces trigylceride content while differentially affecting peroxisome proliferator activated receptor γ2 and aP2 expression in 3T3-L1 preadipocytes. *Lipids* 36:1223-1232.
155. Whigham, L. D., Higbee, A., Bjorling, D. E., Park, Y., Pariza, M. W., and Cook, M. E. 2002. Decreased antigen-induced eicosanoid release in conjugated linoleic acid-fed guinea pigs. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 282(4):R1104-R1112.
156. Pariza, M. W. 2002. Commentary: Conjugated linoleic acid may be useful in treating diabetes by controlling body fat and weight gain. *Diabetes Technol. Therap.* 4:335-338.
157. Choi, Y., Park, Y., Storkson, J. M., Pariza, M. W., and Ntambi, J. M. 2002. Inhibition of stearoyl-CoA desaturase activity by the *cis*-9, *trans*-11 isomer and the *trans*-10, *cis*-12 isomer of conjugated linoleic acid in MDA-MB-231 and MCF-7 human breast cancer cells. *Biochem. Biophys. Res. Commun.* 294:785-790.
158. Kim, M. R., Park, Y., Albright, K. J., and Pariza, M. W. 2002. Differential responses of hamsters and rats fed high-fat or low-fat diets supplemented with conjugated linoleic acid. *Nutr. Res.* 22(6):715-722.
159. Ntambi, J. M., Choi, Y., Park, Y., Peters, J. M., and Pariza, M. W. 2002. Effects of conjugated linoleic acid (CLA) on immune responses, body composition and stearoyl-CoA desaturase. *Can. J. Appl. Physiol.* 27(6):617-627.
160. Xu, X., Storkson, J., Kim, S., Sugimoto, K., Park, Y., and Pariza, M. W. 2003. Short-term intake of conjugated linoleic acid inhibits lipoprotein lipase and glucose metabolism but does not enhance lipolysis in mouse adipose tissue. *J. Nutr.* 133(3):663-667.
161. Kang, K. H., Liu, W., Albright, K. J., Park, Y., and Pariza, M. W. 2003. trans-10,cis-12 CLA inhibits differentiation of 3T3-L1 adipocytes and decreases PPAR gamma expression. *Biochem. Biophys. Res. Commun.* 303(3):795-799.
162. Brown, J. M., Boysen, M. S., Jensen, S S., Morrison, R. F., Storkson, J., Currie. R. L., Pariza, M., Mandrup, S., and McIntosh, M. K. 2003. Isomer-specific regulation of metabolism and PPARγ signaling by CLA in human preadipocytes. *J. Lipid Res.* 44:1287-1300.
163. Pariza, M. W., Park, Y., Xu, X., Ntambi, J., and Kang, K. 2003. Chapter 14: Speculation on the mechanisms of action of conjugated linoleic acid, pp. 251-258. In: *Advances in Conjugated Linoleic Acid Research, Vol. 2.* J. L. Sebedio, W. W. Christie, and R. Adlof (eds.), AOCS Press, Champaign, IL.
164. Kang K, Miyazaki M, Ntambi JM, Pariza MW. 2004. Evidence that the anti-obesity effect of conjugated linoleic acid is independent of effects on stearoyl-CoA desaturase1 expression and enzyme activity. *Biochem. Biophys. Res. Commun.* 315(3):532–537.
165. Pariza, M. W. 2004. Perspective on the safety and effectiveness of conjugated linoleic acid. *Am. J. Clin. Nutr.* 79(Suppl):1132S–1136S.
166. Park Y., J. M. Storkson, W. Liu, K. J. Albright, M. E. Cook, and M. W. Pariza. 2004. Structure–activity relationship of conjugated linoleic acid and its cognates in inhibiting heparin-releasable lipoprotein lipase and glycerol release from fully differentiated 3T3-L1 adipocytes. *J. Nutr. Biochem.* 15(9):561–568.
167. Dhiman, T. R., S. Zaman, K. C. Olson, H. R. Bingham, A. L. Ure, and M. W. Pariza. 2005. Influence of feeding soybean oil on conjugated linoleic acid content in beef. *J. Agric. Food Chem.* 53(3):684–689.
168. Graves, E., A. Hitt, M. W. Pariza, M. E. Cook, and D. O. McCarthy. 2005. Conjugated linoleic acid preserves gastrocnemius muscle mass in mice bearing the colon-26 adenocarcinoma. *Res. Nursing Health* 28(1):48–55.
169. Park, Y., J. M. Storkson, K. J. Albright, W. Liu and M. W. Pariza. 2005. Biological activities of conjugated fatty acids: conjugated eicosadienoic (conj. 20:2delta$^{c11,t13/t12,c14}$), eicosatrienoic (conj. 20:3delta^{c8,t12,c14}), and heneicosadienoic (conj. 21:2delta$^{c12,t14/c13,t15}$) acids and other metabolites of conjugated linoleic acid. *Biochim. Biophys. Acta* 1687:120–129.
170. Storkson, J. M., Y. Park, M. E. Cook, and M. W. Pariza. 2005. Effects of trans-10,cis-12 conjugated linoleic acid and cognates on apolipoprotein B secretion in HepG2 cells. *Nutr. Res.* 25(4):387–399.
171. Park, Y., K. J. Albright, and M. W. Pariza. 2005. Effects of conjugated linoleic acid on long term feeding in Fischer 344 rats. *Food Chem. Toxicol.* 43(8):1273–1279.

172. Li, G.M., D. Butz, B.Y. Dong, Y. Park, M.W. Pariza, and M.E. Cook. 2006. Selective conjugated fatty acids inhibit guinea pig platelet aggregation. *European J. Pharmacol.* 545 (2-3): 93-99

Appendix F

Curricula Vitae of Expert Panel Members

Curricula Vitae for James W. Anderson

Curriculum Vitae

JAMES W. ANDERSON, M.D.

Metabolic Research Group

EDUCATION:	1958	B.A. West Virginia University
	1961	M.D. Northwestern University
	1965	M.S. Mayo Graduate School
TRAINING:	1961-62	Internship, Presbyterian Hospital, Denver
	1962-65	Fellow in Medicine and Endocrinology Mayo Clinic, Rochester, Minnesota
MILITARY:	1965-68	U.S. Army, Denver, Colorado (Captain) Chief, Metabolic-Renal Section, Research Endocrinologist, Nutrition Research Laboratory, Fitzsimons Hospital
FACULTY APPOINTMENTS:		
	1973-	Chief, Endocrine-Metabolic Section VA Medical Center, Lexington, Kentucky
	1978-	Professor of Medicine University of Kentucky College of Medicine Professor of Clinical Nutrition University of Kentucky, Lexington, Kentucky
	1985-	Medical Director, HMR Fasting Program, University of Kentucky, Lexington, Kentucky
	1983-84	Visiting Scientist, Massachusetts Institute of Technology Cambridge, Massachusetts
	1980-83	Chief, Medical Service VA Medical Center, Lexington, KY
	1973-78	Associate Professor of Medicine University of Kentucky College of Medicine
	1968-73	Assistant Professor of Medicine University of California Medical School San Francisco, California

CERTIFICATION AND LICENSE:

1975	American Board Internal Medicine
1973	Kentucky

PROFESSIONAL ORGANIZATIONS:

Alpha Omega Alpha
American College of Nutrition - Fellow
American College of Physicians - Fellow
American Diabetes Association
American Federation for Clinical Research
American Society for Clinical Nutrition
Endocrine Society
Obesity Research Network
Society for Experimental Biology and Medicine

EDITORIAL BOARD:

1974-77	American Journal of Clinical Nutrition
1987-	Diabetes: Self-Management
1989-	Prevention
1990-96	Diabetes in the News
1991-97	Medicine, Exercise, Nutrition and Health
1997	Vegetarian Nutrition
1998	PRIME Health & Fitness

REVIEWER:

American Journal of Clinical Nutrition
American Journal of Medicine
Annals of Internal Medicine
Archives of Internal Medicine
Diabetes
Diabetes Care
Diabetologia
Journal of the American Medical Association
Journal of Laboratory and Clinical Medicine
Journal of Nutrition
Journal of American College of Nutrition
Journal of American Dietetic Association
Journal of Clinical Endocrinology and Metabolism
Metabolism
New England Journal of Medicine

EDITOR: 1979-96 HCF Nutrition Newsletter (Circulation 10,000)

PROFESSIONAL SERVICE:

2000-	Chairman of Board, President, Obesity Research Network
2000-	American College of Nutrition: Secretary
1996-	Obesity Research Network; Co-Founder & Coordinator
1997-00	American College of Nutrition: Board of Directors
1994	Special Study Section: NIDDK for "NIDDM" Primary Prevention Trial

1988-2000	Diabetes Advisory Committee Kentucky Diabetes Control Program
1986	LSRO-FDA Expert Panel on Dietary Fiber
1985-86	NIH Diabetes and Hypertension Treatment Panel
1982-86	Scientific Advisory Committee, Wheat Industry Council
1975-76	Kentucky Diabetes Association President
1974-present	Director, Metabolic Research Group

GRANTS AS PRINCIPAL INVESTIGATOR:

2000-	VA Cooperative Study #465, Glycemic Control and Complications in Diabetes Mellitus Type 2 VADT (VA Diabetes Trial) 85 to be enrolled
2000-	NIH - R21 Program Project, "Soy protein in early diabetic nephropathy" -
2000-	Bupropion SR Obesity Study. PI for 6 Site Study ; Sponsor: Glaxo-Wellcome
1991-1998	VA Cooperative Study #363, The VA HDL Intervention Trial (HIT): Secondary prevention of Coronary Heart Disease in Men with Low HDL-Cholesterol and Desirable LDL-Cholesterol ·
1991-1993	VA Cooperative Study #344A, Implantable Insulin Pump Study -
1990-1993	NIH Program Project, "Diabetes Diet, Glycemia and Microcirculation in Humans" for Third Year
1986-1989	NIH - HL 37902 Program Project. "Dietary Fiber and Cardiovascular Risk Factors"
1970-1988	Veterans Administration Medical Center Merit Review Grants for 18 consecutive years
1977-1980	National Institute of Arthritis, Metabolism and Digestive Diseases Grant, "High Carbohydrate, High Fiber Diets for Diabetes"
1974-	University of Kentucky Diabetes Research Fund Grants from Foundations, Corporations and Individuals, (1995-2001)

COMMITTEES:

University	2000-	Task Force on Coordination of Clinical Research & Patient Care
	1996-	Executive Committee-Nutrition Graduate Program
	1994-95	Medical Center Clinical Sciences Area, Advisory Committee
	1991-93	University of Kentucky/Veterans Affairs Research Advisory Committee
	1990-93	University of Kentucky Press Committee
	1989-	Nutrition Focus Committee Department of Nutrition and Food Science
	1987-	Coordinated Program Advisory Committee, Department of Nutrition and Food Science
	1979-82	Faculty, Promotions and Tenure Committee

VAMC	1993-98	SDTU Ad-Hoc Committee, Chairman
	1986	Pharmacy Committee
	1981-83	Medical Intensive Care Committee, Chairman
	1981-83	Ambulatory Care Committee, Chairman
	1980-83	Clinical Executive Board
	1974-93	Founder and Director of the SDTU
University Hospital	1993-00	Nutrition Committee
Department of Medicine		
	1992-95	Problem Based Learning Committee
	1988-90	Board of Directors, Association for Medical Research
	1986-96	Faculty Promotions Committee
	1980-88	Chairman's Advisory Committee

TEACHING:

Undergraduate	1973-	Ward teaching attending 20 weeks per year (average) Four formal lectures per year
Graduate	1973-	Ward teaching attending 20 weeks per year (average) Four to six conferences per year
	1980-83	VA Morning Report for Residents Supervise 6 days/week
Postgraduate	1974-	Annual Endocrine Postgraduate Conference (UK)
	1975-	Each year I chair one or two National or International Workshops on Nutrition, Diabetes, Dyslipidemia or Obesity
	1981-90	Annual High Fiber Diet Workshop - Director (UKMC)

PUBLIC SERVICE:

1996-	Downtown Christian Unity Taskforce
1994-96	Chairman, Board of Trustees, Georgetown College, Georgetown, Kentucky
1990-96	Executive Committee, Board of Trustees, Georgetown College, Georgetown, Kentucky
1989-96	Board of Trustees, Georgetown College, Georgetown, Kentucky
1974-	Deacon, Sunday School Teacher, Calvary Baptist Church

PUBLICATIONS-REFEREED JOURNALS

1. Anderson JW, Kilbourn KG, Robinson J, and Wright PH. Diabetic acidosis in rats treated with anti-insulin serum. Clin Sci 24:417-430, 1963.

2. Anderson JW, Sawyer KC Jr, Sheridan DP. Cystic fibrosis of the pancreas with diabetes mellitus. Rocky Mountain Med J 60:32-35, 1963.

3. Anderson JW, McConahey WM, Alarcon-Segovia D, Emslander RF, Wakim KG. Diagnostic value of thyroid antibodies. J Clin Endocrinol Metab 27:937-944, 1967.

4. Anderson JW. Hyperglycemic diabetic stupor: A spectrum of disorders. Mil Med 133:538-542, 1968.

5. Anderson JW, Herman RH. Classification of reactive hypoglycemia. Am J Clin Nutr 22:646-650, 1969.

6. Gorman CA, Anderson JW, Flock EV, Owen CA Jr, Wakim KG. Effect of experimentally induced thyroiditis on biosynthesis of thyroxine in rats. Acta Endocrinol 62:11-20, 1969.

7. Pastore RA, Anderson JW, Herman RH. Anterior and posterior hypopituitarism associated with sickle cell trait. Ann Intern Med 71:593-598, 1969.

8. Anderson JW, Wakim KG, McConahey WM. The influence of experimental thyroiditis on thyroid function. Mayo Clinic Proc 44:711-724, 1969.

9. Anderson JW, Herman RH, Newcomer KL. Improvement of glucose tolerance of fasting obese patients given oral potassium. Am J Clin Nutr 22:1589:1596, 1969.

10. Anderson JW, Zakim D. The influence of alloxan-diabetes and fasting on glycolytic and gluconeogenic enzyme activities of rat intestinal mucosa and liver. Biochem Biophys Acta 201:236-241, 1970.

11. Anderson JW. Pyruvate carboxylase and phosphoenolpyruvate carboxykinase in rat intestinal mucosa. Biochem Biophys Acta 208:165-167, 1970.

12. Everett ED, Newcomer KL, Anderson JW, Bergin J, Overholdt EL. Goodpasture's syndrome. Response to mercaptopurine and prednisone. JAMA 213:1849-1852, 1970.

13. Anderson JW, Herman RH. Treatment of reactive hypoglycemia and sulfonylureas. Am J Med Sci 261:16-23, 1971.

14. Ho W, Anderson JW. Phosphofructokinase in rat jejunal mucosa: Subcellular distribution, isolation, and characterization. Biochem Biophys Acta 227:354-363, 1971.

15. Anderson JW, Herman RH, Tyrrell JB, Cohen RB. Hexokinase: A compartmented enzyme. Am J Clin Nutr 24:642-650, 1971.

16. Tyrrell JB, Anderson JW. Glycolytic and pentose phosphate pathway enzymes in jejunal mucosa, adaptive responses to alloxan diabetes and fasting in the rat. Endocrinology 89:1178-1185, 1971.

17 Anderson JW, Herman RH. Effect of fasting, caloric restriction and refeeding on glucose tolerance of normal men. Am J Clin Nutr 25:41-52, 1972.

18. Anderson JW, Stowring L. Glycolytic and gluconeogenic enzyme activities in renal cortex of diabetic rats. Am J Physiol 224:930-936, 1973.

19. Anderson JW, Rosendall AF. Gluconeogenesis in jejunal mucosa of guinea pig. Biochem Biophys Acta 304:384-388, 1973.

20. Anderson JW, Herman RH, Zakim D. Effect of high glucose and high sucrose diets on glucose tolerance of normal men. Am J Clin Nutr 26:600-607, 1973.

21. Anderson JW, Tyrrell JB. Hexokinase activity of rat intestinal mucosa: Demonstration of four isozymes and changes in subcellular distribution with fasting and refeeding. Gastroenterology 65:69-76, 1973.

22. Murphy ED, Anderson JW. Tissue glycolytic and glucoenogenic enzyme activites in mildly and moderatley diabetic rats, influence of tolbutamide administration. Endocrinology 94:27-34, 1974.

23. Anderson JW. Glucose metabolism in jejunal mucosa of fed, fasted, and streptozotocin-diabetic rats. Am J Physiol 226-229, 1974.

24. Anderson JW, Jones AL. Biochemical and ultrastructural study of glycogen in jejunal mucosa of diabetic rats. Proc Soc Exp Biol Med 145:268-272, 1974.

25. Anderson JW. Endocrine manifestations of lung tumors. J Ky Med Assoc 72:208-211, 1974.

26. Anderson JW. Alterations in metabolic fate of glucose in the liver of diabetic animals. Am J Clin Nutr 27:746-755, 1974.

27. Anderson JW. Metabolic abnormalities contributing to diabetic complications. I. Glucose metabolism in insulin-insensitive pathways. Am J Clin Nutr 28:273-280, 1975.

28. Anderson JW, King P. Subcellular distribution of hexokinase activity in rat jejunal mucosa: Response to diabetes and dietary changes. Biochem Med 12:1-11, 1975.

29. Anderson JW, Herman RH. Effects of carbohydrate restriction on glucose tolerance of normal men and reactive hypoglycemic patients. Am J Clin Nutr 28:748-755, 1975.

30. Anderson JW. Hyperglycemic nonketotic coma. J Ky Med Assoc 73:211-213, 1975.

31. Anderson JW, Karounos D, Yoneyama T. Dichloracetate-induced changes in liver of normal and diabetic rats. Proc Soc Exp Biol Med 149:814-821, 1975.

32. Anderson JW. Metabolic abnormalities contributing to diabetic complications. II. Peripheral Nerves. Am J Clin Nutr 29:402-408, 1976.

33. Kiehm TG, Anderson JW, Ward K. Beneficial effects of a high-carbohydrate high-fiber diet on hyperglycemic diabetic men. Am J Clin Nutr 29:895-899, 1976.

34. Anderson JW. High polysaccharide diet studies in patients with diabetes and vascular disease. Cereal Foods World 22:12-15, 1977.

35. Anderson JW. Effect of carbohydrate restriction and high-carbohydrate diets on men with chemical diabetes. Am J Clin Nutr 30:402-408, 1977.

36. Lin WJ, Anderson JW. Effects of high sucrose or starch-bran diets on glucose and lipid metabolism of normal and diabetic rats. J Nutr 107:584-595, 1977.

37 Anderson JW, Ward K. Long-term effects of high-carbohydrate, high-fiber diets on glucose and lipid metabolism: a preliminary report on patients with diabetes. Diabetes Care 1:77-82, 1978.

38. Anderson JW, Lin WJ, Ward K. Composition of foods commonly used in diets for persons with diabetes. Diabetes Care 1:293-302, 1978.

39. Anderson JW, Chen WJ. Plant fiber: Carbohydrate in lipid metabolism. Am J Clin Nutr 32:346-363, 1979.

40. Chen WJ, Anderson JW. Effects of guar gum and wheat bran on lipid metabolism of rats. J Nutr 109:1028-1034, 1979.

41. Anderson JW, Midgley WR, Wedman B. Fiber and diabetes. Diabetes Care 2:369-379, 1979.

42. Chen WJ, Anderson JW. Effects of plant fiber in decreasing plasma total cholesterol and increasing high density lipoprotein cholesterol. Proc Soc Exp Biol Med 162:310-313, 1979.

43. Anderson JW, Ward K. High-carbohydrate, high-fiber diets for insulin-treated men with diabetes mellitus. Am J Clin Nutr 32:2312-2321, 1979.

44. Anderson JW, Ferguson SK, Karounos D, O'Malley L, Sieling B, Chen WJ. Mineral and vitamin status on high-fiber diets: Long-term studies of diabetic patients. Diabetes Care 3:38-40, 1980.

45. Dominic JA, Miller RE, Anderson JW, McAllister R. Pharmacology of verapamil. Pharmacology 20:196-202, 1980.

46. Anderson JW, Chen WJ, Sieling B. Hypolipidemic effects of high-carbohydrate, high-fiber diets. Metabolism 29:551-558, 1980.

47. Anderson JW, Sieling B. High fiber diets for obese diabetic patients. Obesity/Bariatric Med 9:109-113, 1980.

48. Anderson JW. High-fiber diets for diabetic and hypertriglyceridemic patients. Can Med Assoc J 123:975-979, 1980.

49. Anderson JW. Sieling B. High-fiber diets for diabetics: Unconventional but effective. Geriatrics 36:64-72, 1981.

50. Kirby RW, Anderson JW, Sieling B, Rees ED, Chen WL, Miller RE, Kay RM. Oat-bran intake selectively lowers serum low-density lipoprotein cholesterol concentrations of hypercholesterolemic men. Am J Clin Nutr 34:824-829, 1981.

51. Chen WL, Anderson JW. Soluble and insoluble plant fiber in selected cereals and vegetables. Am J Clin Nutr 34:1077-1082, 1981.

52. Leichter SB, Anderson JW. Exogenous but not endogenous cyclic GMP inhibits hepatic pyruvate kinase activity. Biochem Biophys Acta 659:199-203, 1981.

53. Chen WL, Anderson JW, Gould MR. Effects of oat bran, oat-gum, and pectin on lipid metabolism of cholesterol-fed rats. Nutr Reports Internatl 24:1093-1098, 1981.

54. Anderson JW, Story L, Sieling B, Chen WJ. Plant fiber content of selected breakfast cereals. Diabetes Care 4:490-492, 1981.

55. Anderson JW. Plant fiber and blood pressure. Ann Intern Med 98:842-846, 1983.

56. Wrobel SB, Griffen WO, Anderson JW, Whitaker EB, Wiegert HT, Searle M. Surgical treatment of morbid obesity: Economic, psychological, ethical, preventive, medical aspects of health care. Yale J Biol Med 56:231-241, 1983.

57. Anderson JW. Nutrition choices for diabetic patients. J Am Coll Nutr 2:307-308, 1983.

58. Anderson JW, Story L, Sieling B, Chen WJ. Hypocholesterolemic effects of high-fiber diets rich in water-soluble plant fibers: Long-term studies with oat-bran and bean-supplemented diets for hypercholesterolemic men. J Can Diet Assoc 45:140-149, 1984.

59. Chen WJ, Anderson JW, Jennings D. Propionate may mediate the hypocholesterolemic effects of certain soluble plant fiber in cholesterol-fed rats. Proc Soc Exper Biol Med 175:215-218, 1984.

60. Anderson JW, Bridges SR. Short-chain fatty acid fermentation products of plant fiber affect glucose metabolism of isolated rat hepatocytes. Proc Soc Exper Biol Med 177:372-376, 1984.

61. Anderson JW, Story L, Sieling B, Chen WJ, Petro MS, Story J. Hypocholesterolemic effects of oat-bran or bean intake for hypercholesterolemic men. Am J Clin Nutr 40:1146-1155, 1984.

62. Colwell JA, Bingham SF, Abraira C, Anderson JW, Kwaan HC. VA Cooperative study on antiplatelet agents in diabetic patients after amputation for gangrene: I. Design, methods and baseline characteristics. Controlled Clin Trials 5:165-183, 1984.

63. Story L, Anderson JW, Chen WJ, Karounos D, Sieling B. Adherence to high-carbohydrate, high-fiber diets: Long-term studies of non-obese diabetic men. J Am Diet Assoc 85:1105-1110, 1985

64. Anderson JW. Health implications of wheat fiber. Am J Clin Nutr 41:1103-1112, 1985.

65. Anderson JW. Physiologic and metabolic effects of dietary fiber. Fed Proc 44:2902-2906, 1985.

66. Colwell JA, Bingham SF, Abraira C, Anderson JW, Comstock JP, Kwaan HC, Nutall F VA Cooperative study on antiplatelet agents in diabetic patients after amputation for gangrene: II. Effects of aspirin and dipyridamole on atherosclerotic vascular disease rates. Diabetes Care 9:140-148, 1986

67. Anderson JW. Fiber and health: an overview. Am J Gastroenterol 81:892-897, 1986.

68. Anderson JW. Fiber and health: an overview. Nutrition Today Nov/Dec:22-26, 1986.

69. Anderson JW, Bryant CA. Dietary fiber: diabetes and obesity. Am J Gastroenterol 81:898-906, 1986.

70. Anderson JW, Tietyen-Clark J. Dietary fiber: Hyperlipidemia, hypertension and coronary heart disease. Am J Gastroenterol 81:907-919, 1986.

71. Anderson JW, Gustafson N, Bryant CA, Tietyen-Clark J. Dietary fiber and diabetes: A comprehensive review and practical application. J Am Diet Assoc 87:1189-1197, 1987.

72. Anderson JW. Dietary fiber, lipids, and atherosclerosis. Am J Cardiol 60:17G-22G, 1987.

73. Anderson JW, Gustafson NJ. High-carbohydrate, high-fiber diet. Is it practical and effective in treating hyperlipidemia? Post-grad Med 82:40-55, 1987.

74. Anderson JW, Gustafson NJ. Hypocholesterolemic effects of oat and bean products. Am J Clin Nutr 48:749-753, 1988.

75. Anderson JW, Bridges SR. Dietary fiber content of selected foods. Am J Clin Nutr 47:440-447, 1988.

76. Anderson JW, Zettwoch N, Feldman T, Tietyen-Clark J, Oeltgen P, Bishop CW. Cholesterol-lowering effects of psyllium hydrophilic mucilloid for hypercholesterolemic men. Arch Intern Med 148:292-296, 1988.

77. Jennings CD, Boleyn K, Bridges SR, Wood PJ, Anderson JW. A comparison of the lipid-lowering and the intestinal morphologic effects of cholestyramine, chitosan and oat gum in rats. Proc Soc Exp Biol Med. Arch Intern Med 189:13-20, 1988.

78. Anderson JW, Geil PB. New perspectives in nutrition management of diabetes mellitus. Am J Med 85:159-165, 1988.

79. Anderson JW, Gustafson NJ. Hypocholesterolemic effects of oat and bean products. Michigan Dry Bean Digest 13:2-5, 1989.

80. Anderson JW, Bridges SR, Tietyen J, Gustafson NJ. Dietary fiber content of a simulated American diet and selected research diets. Am J Clin Nutr 49:352-357, 1989

81. Anderson JW, Story L, Zettwoch N, Gustafson NJ, Jefferson BS. Metabolic effects of fructose supplementation in diabetic individuals. Diabetes Care 12:337-344, 1989.

82. Anderson JW. Recent advances in carbohydrate nutrition and metabolism in diabetes mellitus. J Am Coll Nutr 8(Suppl):61-67, 1989.

83. Anderson JW, Gustafson NJ. Adherence to high-carbohydrate, high-fiber diets. Diabetes Educ 15:429-434, 1989.

84. Colwell JA, Bingham SF, Abraira C, Anderson JW, Comstock JP, Kwaan HD, Nuttall FV. VA Cooperative study on antiplatelet agents in diabetic patients after amputation for gangrene: V. unobserved, sudden, and unexpected deaths. J Diabet Complications 3:191-197, 1989.

85. Wood PJ, Anderson JW, Braaten JT, Cave NA, Scott FW, Vachon C. Physiological effects of β-D-glucan rich fractions from oats. Cereal Foods World 34:878-882, 1989.

86. Anderson JW, Gustafson NJ, Spencer DB, Tietyen J. Serum lipid response of hypercholesterolemic men to single and divided doses of canned beans. Am J Clin Nutr 51:1013-1019, 1990.

87. Anderson JW, Wood CL. Oat brain and serum cholesterol. (Letter). N Eng J Med 322:1747-1748, 1990.

88. Anderson JW, Smith BM, Geil PB. High-fiber diets for diabetes. Safe and effective treatment. Post-grad Med 88:157-168, 1990.

89. Fukagawa NK, Anderson JW, Hagemen G, Young VR, Minaker KL. High-carbohydrate, high-fiber diets increase peripheral insulin sensitivity in healthy young and old adults. Am J Clin Nutr 52:524, 528, 1990.

90. Anderson JW, Spencer DB, Hamilton CC, Smith SF, Tietyen J, Bryant CA, Oeltgen P. Oat-bran cereal lowers serum total and LDL cholesterol in hypercholesterolemic men. Am J Clin Nutr 52:495-499, 1990.

91. Wright RS, Anderson JW, Bridges SR. Propionate inhibits hepatic lipid synthesis. Proc Soc Exp Biol Med 190:26-29, 1990.

92. Anderson JW, Hamilton CC, Horn JL, Spencer DB, Dillon DW, Zeigler JA. Metabolic effects of insoluble oat fiber on lean men with Type II diabetes. Cereal Chemistry 68:291-294, 1991.

93. Anderson JW, Floore TL, Geil PB, O'Neal DS, Balm TK. Hypercholesterolemic effects of different bulk-forming hydrophilic fibers as adjuncts to dietary therapy in mild to moderate hypercholesterolemia. Arch Intern Med 151:1597-1602, 1991.

94. Anderson JW, Gilinsky NH, Deakins DA, Smith SF, O'Neal DS, Dillon DW, Oeltgen PR. Lipid responses of hypercholesterolemic men to oat bran and wheat bran intake. Am J Clin Nutr 54:936-943, 1991.

95. Anderson JW, Zeigler JA, Deakins DA. Floore TL, Dillon DW, Wood CL, Oeltgen PR. Whitley RJ. Metabolic effects of high-carbohydrate, high-fiber diets for insulin-dependent diabetic individuals. Am J Clin Nutr 54:936-943, 1991.

96. Anderson JW, Riddell-Lawrence S, Floore TL, Dillon DW, Oeltgen PR. Bakery products lower serum cholesterol concentration in hypercholesterolemic men. Am J Clin Nutr 54:836-840, 1991.

97. Anderson JW, Hamilton CC, Crown-Weber E, Riddlemoser M, Gustafson NJ. Safety and effectiveness of a multidisciplinary very-low-calorie diet program for selected obese individuals. J Am Diet Assoc 91:1582-1584, 1991.

98. Anderson JW, Hamilton CC, Brinkman-Kaplan V. Benefits and risks of an intensive very-low-calorie diet program for severe obesity. Am J Gastroenterol 87:6-15, 1992.

99. Anderson JW, Brinkman VL, Hamilton CC. Weight loss and 2-y follow-up for 80 morbidly obese patients treated with intensive very-low-calorie diet and an education program. Am J Clin Nutr 56:1S-3S, 1992.

100. Anderson JW, Riddell-Mason S, Gustafson NJ, Smith SF. Cholesterol-lowering effects of psyllium-enriched cereal as an adjunct to a prudent diet in the treatment of mild to moderate hypercholesterolemia. Am J Clin Nutr 56:93-98, 1992.

101. Bridges SR, Anderson JW, Deakins DA, Wood CL. Oat bran increases serum acetate of hypercholesterolemic men. Am J Clin Nutr 56:455-459, 1992.

102. Hamilton CC, Geil PB, Anderson JW. Management of obesity in diabetes mellitus. Diabetes Educ 18:407-410, 1992.

103. Geil PB, Anderson JW. Health benefits of dietary fiber. Med Exer Nutr Health 1:257-271, 1992.

104. Anderson JW, Garriety TF, Wood CL, Whitis SE, Smith BM, Oeltgen PR. Prospective, randomized, controlled comparison of the effects of low-fat and low-fat plus high-fiber diets on serum lipid levels. Am J Clin Nutr 56:887-894, 1992.

105. Anderson JW. Diet, lipids and cardiovascular disease in women. J Am Coll Nutr 12:433-437, 1993.

106. Anderson JW, Jones AE, Riddell-Mason S. Ten different fibers have significantly different effects on serum and liver lipids in cholesterol-fed rats. J Nutr 124:78-83, 1994.

107. Anderson JW, Brinkman-Kaplan VL, Lee H, Wood CL. Food-containing hypocaloric diets are as effective as liquid-supplement diets for obese individuals with NIDDM. Diabetes Care 17:602-604, 1994.

108. Anderson JW, Brinkman-Kaplan VL, Lee H, Wood CL. Relationship of weight loss to cardiovascular risk factors in morbidly obese individuals. J Am Coll Nutr 14:256-261, 1994.

109. Geil PB, Anderson JW. Nutrition and health benefits of dry beans: A review. J Am Coll Nutr 14:549-558, 1994.

110. Anderson JW, Smith BM, Gustafson NJ. Health benefits and practical aspects of high-fiber diets. Am J Clin Nutr 59(Suppl):1242S-1247S, 1994.

111. Geil PB, Anderson JW, Gustafson NJ. Women and men with hypercholesterolemia respond similarly to an AHA step I diet. J Am Diet Assoc 95:436-441, 1995.

112. Anderson JW, O'Neal DS, Riddell-Mason S, Floore TL, Dillon DW, Oeltgen PR. Postprandial serum glucose, insulin and lipoprotein responses to high- and low-fiber diets. Metabolism 44:848-854, 1995.

113. Rubins HB, Robins SJ, Collins D, Wittes J, Iranmanesh A, Wilt TJ, Mann D, Mayo-Smith M, Faas FH, Elam MB, Rutan GH, Anderson JW, Kashyap MI, Schectman G. Distribution of lipids in 8,500 men with coronary heart disease: Implications for treatment. Am J Cardiol 75:1196-1201, 1995.

114. Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. N Eng J Med 333:276-282, 1995.

115. Collins RW, Anderson JW. Medication cost savings associated with weight loss for obese non-insulin-dependent diabetic men and women. Prev Med 24:369-374, 1995.

116. Bridges RB, Anderson JW, Saxe SR, Gregory K, Bridges SR. Periodontal status of diabetic and non-diabetic men: Effects of smoking, glycemic control and socioeconomic factors. J Peridontol 67:1185-1192, 1996.

117. Saudek CD, Duckworth WC, Giobbie-Hurder AG, Henderson WG, Henry RR, Kelley DE, Edelman SV, Zieve FJ, Adler RA, Anderson JW, Anderson RJ, Hamilton BP, Donner TW, Kirkman MS, Morgan NA. Comparison of the implantable insulin pump with multiple dose insulin in the treatment of non-insulin-dependent diabetes mellitus. JAMA 276:1322-1327, 1996.

118. Wilt TJ, Riley WA, Rubins HB, Robins SJ, Collins D, Elam M, Rutan G, Anderson JW. Carotid atherosclerosis in men with low levels of HDL cholesterol. Stroke 28:1-7, 1997.

119. Olson BR, Anderson SM, Becker MP, Anderson JW, Hunninghake DB, Jenkins DJA, LaRosa JC, Rippe JM, Roberts DCK, Stoy DB, Summerbell CD, Truswell AS, Wolever TMS, Morris DH, Fulgoni III VL. Psyllium-enriched cereals lower total blood cholesterol and LDL-cholesterol, but not HDL-cholesterol, in hypercholesterolemic adults: Result of a meta-analysis. J Nutr 127:1973-1980, 1997.

120. Bagdade JD, Kelley DE, Henry RR, Eckel RH, Ritter MC. Effects of multiple daily insulin injections and intraperitoneal insulin therapy on cholesteryl ester transfer and lipoprotein lipase activities in NIDDM. Diabetes 46:414-420, 1997.

121. Anderson JW, Emmett J, Turner J, Smith BM. Soy protein effects on renal function and proteinuria for individuals with non-insulin-dependent diabetes mellitus. Am J Clin Nutr 68:1347S-1353S, 1998.

122. Anderson JW, Pi-Sunyer FX, Danforth E, Dujovne CA, Greenway F, Hill JO, Lucas CP, O'Neal PM, Smith DK. Clinical trial design for obesity agents: A workshop report. Obes Res 6:311-315, 1998.

123. Anderson JW. Guidelines for approval of anti-obesity drugs affecting atherosclerosis and/or lipids. Am J Cardiol 81:29F-30F, 1998.

124. Duckworth WC, Saudek CD, Giobbie-Hurder AG, Henderson WG, Henry RR, Kelley DE, Edelman SV, Zieve FJ, Adler RA, Anderson JW, Anderson RJ, Hamilton BP, Donner TW, Kirkman MS, Morgan NA. Implantable pumps and cardiovascular risk factors. Diabetes Care 21:1596-1602, 1998.

125. Anderson JW, Diwadkar VA, Bridges SR. Selective effects of different antioxidants on oxidation of lipoproteins from rats. Proc Soc Exp Biol Med 218:376-381, 1998.

126. Thompson JS, Duckworth WC, Saudek CD, Giobbie-Hurder AG. Surgical experience with implantable insulin pumps. Am J of Surg 176:622-626, 1998.

127. Anderson JW, Gilliland SE, Tijssens R, Elbers FJ. Effect of fermented milk (yogurt) containing lactobacillus acidophilus L1 on serum cholesterol in hypercholesterolemic humans: Report on a placebo-controlled study. J Am Coll Nutr 18:43-50, 1999.

128. Chen LH, Osio J, Anderson JW. Blood antioxidant defense system and dietary survey of elderly diabetic men. Arch Gerontol Geriatr 28:65-83, 1999.

129. Hill JO, Hauptman J, Allison M, Anderson JW, Fujioka K, O'Neal P, Perry A, Smith DK, Zavoral JH, Aronne LJ. Orlistat, a lipase inhibitor, for weight maintenance after conventional dieting: a 1-year study. Am J Clin Nutr 69:1108-1116, 1999.

130. Anderson JW, Hanna TJ. Impact of nondigestible carbohydrates on serum lipoproteins and risk for cardiovascular disease. J Nutr 129:1457S-1466S, 1999.

131. Rubins HB, Robins SJ, Collins D, Anderson JW, Elam MB, Faas FH, FyeCI, Linares E, Schaefer E, Schectman G, Wilt T, Wittes J. The VA HDL intervention trial: Gemfibrozil for the secondary prevention of coronary heart disease in men with low HDL-cholesterol. N Eng J Med 341:410-417, 1999.

132. Anderson JW, Smith BM, Washnock CS. Cardiovascular and renal benefits of dry bean and soy bean intake. Am J Clin Nutr 7:1-5, 1999.

133. Anderson JW, Johnstone BM, Remley DT. Breast feeding and cognitive development: A meta-analysis. Am J Clin Nutr 70:525-535, 1999.

134. Anderson JW, Allgood LD, Turner J, Daggy BP. Effects of psyllium on glycemic and serum lipid responses in men with type II diabetes and hypercholesterolemia. Am J Clin Nutr 70:466-473, 1999.

135. Anderson JW, Hanna TJ. Whole grains and protection against coronary heart disease: what are the active components and mechanisms? Am J Clin Nutr 70:307-308, 1999.

136. Gowri MS, Van der Westhuyzen DR, Bridges SR, Anderson JW. Decreased protection by HDL from poorly controlled Type 2 diabetic subjects against LDL oxidation may be due to its abnormal composition. Atheroscl Thromb 19:2226-2233, 1999.

137. Diwadkar VA, Anderson JW, Bridges SR, Gowri MS, Oelgten PR. Postprandial low-density lipoproteins in type 2 diabetes are oxidized more extensively than fasting diabetes and control samples. Proc Soc Exp Biol Med 222:178-184, 1999.

138. Anderson JW, Gowri MS, Turner J, Nichols L, Diwadkar VA, Chow CK, Oeltgen PR. Antioxidant supplementation effects on low-density lipoprotein oxidation for individuals with non-insulin-dependent diabetes mellitus. J Am Coll Nutr 18:451-461, 1999.

139. Anderson JW, Vichitbandra S, Qian W, Kryscio RJ. Long-term weight maintenance after an intensivie weight-loss program. J Am Coll Nutr 18:620-627, 1999.

140. Anderson JW, Allgood LD, Lawrence A, Altringer LA, Jerdack GR, Hengehold DA, Morel JG. Cholesterol-lowering effects of psyllium intake adjunctive to diet therapy in men and women with hypercholesterolemia: meta-analysis of 8 controlled trials. Am J Clin Nutr 71:472-479, 2000.

141. Anderson JW, Hanna TJ, Peng X, Kryscio RJ. Whole grain foods and heart disease risk. J Am Coll Nutr 19(Suppl 3):291S-299S, 2000.

142. Anderson JW, Konz EC. Benefits and risks of anti-obesity agents. (Letter to Editor). Am J Clin Nutr 71:844-853, 2000.

143. Anderson JW, Davidson MH, Blonde L, Brown WV, Howard WJ, Ginsberg H, Allgood LD, Weingand KW. Long-term cholesterol-lowering effects of psyllium as an adjunct to diet therapy in the treatment of hypercholesterolemia. Am J Clin Nutr 71:1433-1438, 2000.

144. Anderson JW, Konz EC, Jenkins DJA. Health advantages and disadvantages of weight-reducing diets: A computer analysis and critical review. J Am Coll Nutr 19:578-590, 2000.

145. Heshka S, Greenway F, Anderson JW, Atkinson RL, Hill JO, Phinney SD, Miller-Kovach K, Pi-Sunyer X. Self-help weight loss versus a structured commercial program after 26 weeks: a randomized controlled study. Am J Med 109:282-287, 2000.

146. Anderson JW. Dietary fiber prevents carbohydrate-induced hypertriglyceridemia. Curr Atheroscl Reports 2:536-541, 2000.

147. Daly A, Konz EK, Anderson JW, Soler NS. Successful long-term management of substantial weight loss: One program's experience. (Letter) J Am Diet Assoc 100:1456, 2000.

147. Anderson JW, Konz EC, Frederich RC, Wood CL. Long-term weight-loss maintenance: a meta-analysis of US studies. Am J Clin Nutr 74:579-584, 2001.

148. Anderson JW, Konz EC. Obesity and disease management: Effects of weight loss on co-morbid conditions. Obes Res 9 (suppl 4):326S-334S, 2001.

149. Anderson JW, Greenway FL, Fujioka K, Gadde KM, McKenny J, O'Neil PM. Bupropion SR significantly enhances weight loss: a 24-week double-blind, placebo-controlled trial with placebo group randomized to bupropion SR during 24-week extension. Submitted for publication.

150. Anderson JW, Hoie LH. Very-low and low-energy dietary preparations: comparisons with other dietary and pharmacological approaches to weight management. Submitted for publication.

151. Reynolds LR, Konz EC, Frederich RC, Anderson JW. Rosiglitazone amplifies the benefits of lifestyle intervention measures in long standing type 2 diabetes mellitus. Diab Ob Met. In press.

152. Rubins HB, Robins SJ, Collins D, Nelson DB, Elam MB, Schaefer EJ, Faas FH, Anderson JW. Diabetes, plasma insulin and cardiovascular disease: Subgroup analysis for the VA-HDL intervention trial (VA-HIT). Submitted for publication.

153. Anderson JW, Ward AK. Pulses and lipidemia: Short and long term effects – a potential in the prevention of cardiovascular disease. Brit J Nutr. In press.

154. Miles JW, Haupman J, Anderson JW, et al. Adjunctive use of orlistat with metformin in obese type 2 diabetic subjects. Submitted for publication.

155. Anderson JW, Kendall C, Jenkins DJA. Importance of weight management in type 2 diabetes. Submitted for publication.

BOOKS, CHAPTERS, CONFERENCE REPORTS, OTHER PUBLICATIONS

1. Anderson JW. Influence of experimental thyroiditis on thyroid function. MS Thesis, Mayo Graduate School, University of Minnesota, Minneapolis, MN, 1-61, 1965.

2. Anderson JW. Influence of high carbohydrate diets on glucose tolerance of normal and diabetic men. Proceedings of the International Sugar Research Symposium, Washington, DC, 44-52, 1974

3. Anderson JW, Ward K. HCF diets: Guidelines for high carbohydrate, fiber diets. Diabetes Research Foundation, Lexington, KY, 1-40, 1978.

4. Anderson JW. Reactive hypoglycemia. In: Conn HF, ed. Current therapy. Philadelphia: WB Saunders Co., 421-423, 1979.

5. Anderson JW. High carbohydrate, high fiber diets for patients with diabetes. In: Camerini-Davolos RA, Hanover B, eds. Treatment of early diabetes. New York: Plenum Press, 263-273, 1979.

6. Anderson JW, Sieling B. HCF diets: A professional guide to high carbohydrate, high fiber diets. Diabetes Foundation, Lexington, KY, 1-54, 1979.

7. Anderson JW, Ward K, Seiling B. HCF diets: A user's guide to high carbohydrate, high fiber diets. Diabetes Foundation, Lexington, KY, 1-44, 1979.

8. Midgley W, Anderson JW. Fiber in your future? Diabetes Forecast 32:32FF, 1979.

9. Gould MR, Anderson JW, O'Mahony S. Biofunctional properties of oats. In: Inglett GE, Munch L, eds. Cereals for food and beverages. New York: Academic Press, 447-460, 1980.

10. Anderson JW. Newer approaches to diabetes diets, high-fiber diets. Med Times 108:41-44, 1980.

11. Anderson JW. Dietary fiber and diabetes. In: Spiller GA, Spiller KR, eds. Medical aspects of dietary fiber. New York: 193-221, 1980.

12. Anderson JW, Chen W-JLI, Sieling B. Plant fiber in foods. HCF Diabetes Research Foundation, Lexington, KY, 1-48, 1980.

13. Anderson JW, Sieling B, Chen W-JLI. User's guide to HCF diets. HCF Diabetes Research Foundation, Lexington, KY, 1-48, 1980.

14. Anderson JW. The role of dietary carbohydrate and fiber in the control of diabetes. Adv Intern Med 26:67-95, 1981.

15. Anderson JW. Diabetes Mellitus. In: Trowell HC, Burkitt DP, eds. Western disease: Their emergence and prevention. London: Edward Arnold, 373-391, 1981.

16. Anderson JW. Fiber, carbohydrate and diabetes. Nutrition and the MD July, 1981.

17. Anderson JW, Sieling B, Chen W-JL. Professional guide to HCF diets. HCF Diabetes Research Foundation, Lexington, KY, 1-79, 1981.

18. Anderson JW. Plant fiber treatment for metabolic diseases. In: Cohen MR, Foa PP, eds. Special topics in endocrinology and metabolism. New York: Alan R. Liss Inc., 2:1-42, 1981.

19 Anderson JW. Use of high fiber diets in the treatment of diabetes. In: Jansen GR, Brown G, eds. Proceedings from 1980 Lillian Smith Conference. Fort Collins, Colorado State University, 103-116, 1981.

20. Anderson JW. High fiber diets in diabetes. Continuing Education 14:22-27, 1981.

21. Anderson JW. Fiber, diabetes and hyperlipidemia. In: Garry PJ, ed. Human nutrition: Clinical and biochemical aspects. Washington: American Association for Clinical Chemistry, 132-147, 1981.

22. Anderson JW. Diabetes: A practical new guide to healthy living. London, United Kingdom, Martin Dunitz, Lt., 1981, 4th printing, 1982; New York, Arco, 1981, 2nd reprint, 1983; Toronto, Canada, Prentice Hall, 1981; Sydney, Australia, Methuen, 1982, 1st reprint, 1983; Auckland, New Zealand, Methuen, 1982; New York, Warner Books (paperback), 1983; Vienna, Austria, Verlag ORAC (Austrian translation), 1983; Jyvaskyla, Findland, Gummerus (Finnish translation), 1983; Athens, Greece, Psichogios, (Greek translation), 1983; Mexico City, Mexico, Fondo Educativo, (Spanish translation), 1983; Craighall, South Africa, Delta Books, 1983; Stockholm, Sweden, Askild and Varnehll, (Swedish translation), 1983;

23. Anderson JW. Plant fiber: Dietary effects on glucose and lipid metabolism. Proc Fourth International Conference on Human Functioning, Wichita, KS, 1981.

24. Anderson JW. Dietary fiber and diabetes. In: Vahouny GV, Kirtchevsky D, eds. Dietary fiber in health and disease. New York: Plenum Press, 151-167, 1982.

25. Anderson JW. High-carbohydrate diet effects on glucose and triglyceride metabolism of normal and diabetic men. In: Reiser S, ed. Metabolic effects of utilizable dietary carbohydrates. New York: Marcel Dekker, 285-313, 1982.

26. Anderson JW, Chen W-JL. Legumes and their soluble fiber: Effect on cholesterol-rich lipoproteins. In: Furda I, ed. Unconventional sources of dietary fiber. New York: American Chemical Society, 49-59, 1983.

27. Anderson JW, Chen W-JL. Legumes and their soluble fiber: Effect on cholesterol-rich lipoproteins. Michigan Dry Bean Digest, 11:4-6;13, 1986.

28. Anderson JW. A diabetic in the family? Health. July:49-52, 1983.

29. Anderson JW. High fiber diets. Saturday Evening Post. Nov:54-56, 1983.

30. Anderson JW. Dietary fiber and diabetes. In: Wallace G, Bell L, eds. Fibre in human and animal nutrition. Bulletin 20 Wellington, New Zealand: Royal Society of New Zealand, 183-187, 1983.

31. Anderson JW. Glycemic response to different carbohydreates: Consequences for management of diabetes. Clin Nutr 3:59-64, 1984.

32. Anderson JW. Dr. Anderson's HCF diet. London: Martin Dunitz, Ltd., 1-124, 1984.

33. Anderson JW. Fiber: Food for health. Diabetes '85. Summer:1-2, 1985.

34. Anderson JW. Feeling better with oats. Saturday Evening Post Sept: 54-55, 82, 1985.

35. Anderson JW, Sieling. Nutrition and diabetes. In: Weinenger J, Briggs G, eds. Nutrition update. New York: John Wiley & Sons, Inc., 2:49-69, 1985.

36. Anderson JW. Hyperlipidemia and diabetes: Nutrition considerations. In: Peterson C, Jovanovic L, eds. Nutrition and diabetes. New York: Alan R. Liss Inc., 133-159, 1985.

37. Anderson JW. High-fiber diets for obese diabetic men on insulin therapy: short-term and long-term effects. In: Vahouny GV, ed. Dietary fiber and obesity. New York: Alan R. Liss Inc., 49-68, 1985.

38. Anderson JW. Fiber-rich foods can help control diabetes. Diabetes in the news Dec, 1985.

39. Anderson JW. Dietary fiber and diabetes: An update. In: Jansen GR, ed. Proceedings 1985, Lillian F. Smith Conference, Ft. Collins, CO, Colorado State University, 1985.

40. Colwell JA, Bingham SF, Abraira C, Anderson JW, Kwaan HC. VA Cooperative study on antiplatelet agents in diabetic patients after amputation for gangrene: III. Definitions and review of design and baseline characteristics. In: Pfeiffer EF, Lipsett MB, eds. Macrovascular disease in diabetes mellitus. International Workshop, Garmisch-Grainau, 69-73, 1985.

41. Anderson JW, Chen W-JL. Cholesterol-lowering properties of oat products. In: Webster F, ed. Oats: Chemisty and technology. American Association of Cereal Chemists Inc., 309-333, 1986.

42. Tietyen J, Anderson JW. Adding fiber to your diet. Diabetes self-management, April:33-35, 1986.

43. Anderson JW. Dietary fiber in nutrition management of diabetes. In: Vahouny GV, Kritchevsky D, eds. Dietary fiber (basic and clinical aspects). New York: Plenum, 343-360, 1986.

44. Chen W-JI, Anderson JW. Hypocholesterolemic effects of soluble fibers. In: Vahouny GV, Kritcheysky D. Dietary Fiber (basic and clinical aspects). New York: Plenum, 275-286, 1986.

45. Anderson JW. Nutrition management of metabolic conditions. (Professional guide). HCF Diabetes Foundation, Lexington, KY, 1-139, 1986.

46. Anderson JW. Plant fiber in food. HCF Diabetes Foundation, Lexington, KY, 1-33, 1986.

47. Anderson JW, Gustafson NJ. Intensive nutrition management of hypercholesterolemia. Practical Cardiology 12:59-69, 1986.

48. Anderson JW, Gustafson NJ. A guide to intensive nutrition management of obesity in diabetes mellitus. Internal Medicine for the Specialist 7:100-117, 1986.

49. Anderson JW, Gustafson NJ. Type II diabetes: Current nutrition management concepts. Geriatrics 41:28-38, 1986.

50. Anderson JW, Tietyen-Clark J. Soluble dietary fiber: Metabolic an physiologic considerations. Contemporary Nutrition 11:1-2, 1986.

51. Anderson JW. Treatment of diabetes with high fiber diets. In: Spiller GA, ed. Handbook of dietary fiber in human nutrition. Boca Raton: CRC Press, Inc., 349-359, 1986.

52. Anderson JW. Dr. Anderson's life-saving diet. Los Angeles: The Body Press, 1-128, 1986.

53. Anderson JW. Carbohydrate metabolism. In: Advances in Clinical Nutrition. Bethesda, MD, General Clinical Research Center Branch, Division of Research Resources, National Institutes of Health, 64-72, 1986.

54. Anderson JW. Dietary fiber and current health perspectives. In: Paul Bass, ed. The role of fiber in the American diet in health and in various pathologies. Proceedings of Symposium, Phoenix, AZ, 23-26, 1986.

55. Anderson JW. Use more fiber in your diet. Diabetes in the news 11:3, 1987.

56. Anderson JW, Gustafson NJ. Diabetes Mellitus Type II; Nutrition can be your primary treatment. Consultant 27:40-44, 1987.

57. Anderson JW, Gustafson NJ. Dietary fiber in disease prevention and treatment. Comprehensive Therapy 13:43-53, 1987.

58. Anderson JW, Gustafson NJ. Sugar in high-fiber cereals: How much is too much? Diabetes Self-Management March/April:6-9, 1987.

59. Anderson JW. The HCF Guide Book. The HCF Diabetes Foundation, Lexington,KY, 1-63, 1987.

60. Anderson JW, Tietyen-Clark J. The promise of fiber. Diabetes Forecast April:47-50, 1987.

61. Anderson JW. HCF Exchanges (A sensible plan for healthy eating). HCF Diabetes Foundation, Lexington, KY, 1-19, 1987.

62. Runyan JW, Anderson JW, Bild D, Christlieb AR, Espenshade J, Gans JA, Horan M, Hostetter TH, Julius S, LaBarbera RJ, Levine D, Rifkin H, Silverman RE, Roccella E, Moriarty P. Hypertension in diabetes. National High Blood Pressure Education Program US Dept HHS, Bethesda, MD, 1-28. 1987.

63. Anderson JW, Tietyen-Clark J. Nutrition management of hyperlipidemia in diabetes. Food and Nutrition News 59:1-8, 1987

64. Anderson JW. Nutrition management of diabetes mellitus. In: Shils ME, Young VR, eds. Modern Nutrition in Health and Disease. Philadelphia: Lea and Febiger, 1201-1229, 1988.

65. Anderson JW. Benefits of dietary fiber in diabetic individuals. In: Leff S, ed. Advances in Diet and Nutrition: 2. Proceedings from Second International Conference on Diet and Nutrition, Jerusalem, Israel, 7-11, 1988.

66. Anderson JW, Gustafson NJ. Alternative lipid-lowering diets. Practical Cardiology 14:85-97, 1988.

67. Anderson JW. HCF Lifestyle Record Book for Food and Physical Activity. The HCF Nutrition Research Foundation, 1-64, 1988.

68. Anderson JW. HCF Fiber Facts. The HCF Nutrition Research Foundation, 1-12, 1988.

69. Anderson JW. A Diet that Can Save Your Life. The Saturday Evening Post Sept:90-91, 1988.

70. Anderson JW. Foreword. In: Earnest B, Schlesinger S, eds. The Low-Cholesterol Oat Plan. New York: Hearst Books, 7-11, 1988.

71. Anderson JW, Gustafson NJ. Dietary fiber and heart disease: Current management concepts and recommendations. Top Clin Nutr 3:21-29, 1988.

72. Anderson JW. Be Heart Smart. HCF Nutrition Research Foundation, 1-20, 1989.

73. Ridell-Lawrence SR, Anderson JW. Adding fiber to the American diet. Pharmacy Times Sept:31-34, 1989.

74. Anderson JW. Dietary fiber and diabetes mellitus. Proceedings from the 14th International Congress of Nutrition, Vol I, Seoul, Korea, 206-209, 1989.

75. Anderson JW. Dietary fiber and nutrition management of diabetes mellitus. Kellogg International Symposium on Dietary Fiber, Center for Academic Publications, Tokyo, Japan, 59-70, 1990.

76. Anderson JW. Sorting Out Fiber. The Saturday Evening Post March: 1990.

77. Kritchevsky D, Bonfield C, Anderson JW. Dietary Fiber: Chemistry, Physiology, and Health Effects. New York: Plenum Press, 1990.

78. Anderson JW, Deakins DA, Bridges SR. Soluble fiber: Hypocholesterolemic effects and proposed mechanisms. In: Dietary Fiber: Chemistry, Physiology, and Health Effects. New York: Plenum Press, 339-363, 1990.

79 Anderson JW, Geil PB Diabetes Diet: Past, Present and Future. Diabetes in the News June:11-14, 1990.

80. Anderson JW, Deakins DA, Floore TL, Smith BM, Whitis SE. Dietary fiber and coronary heart disease. Critical Reviews in Food Science and Nutrition 29:95-147, 1990.

81. Anderson JW, Floore TL. Lipoproteins and diet in the pathogenesis of atherosclerosis. In: Diana JA, ed. Tobacco, Smoking and Atherosclerosis. Pathogenesis and Cellular Mechanisms. New York: Plenum Press, 245-258, 1990.

82. Anderson JW. Plant Fiber in Foods. HCF Nutrition Research Foundation, Lexington, KY, 1990.

83. Anderson JW, Siesel AE. Hypocholesterolemic effects of oat products. In: Furda I, Brine CJ, eds. New Developments in Dietary Fiber, Physiological, Physiochemical, and Analytical Aspects. New York: Plenum Press, 17-36, 1990.

84. Anderson JW. Health benefits of soluble fibre. Kellogg Nutrition Symposium, Toronto, Ontario, 21-53, 1990.

85. Anderson JW. Dietary fiber and human health. Hot Science 25:1480-1495, 1990.

86. Anderson JW. Dietary fiber and coronary heart disease. In: Russell RM, Stone NJ, eds. The Role of Psyllium in a Healthy Diet. Little Falls, NJ: Health Learning Systems, Inc. Colloquium Proceedings, 11-15, 1990.

87. Zeigler JA, Smith BM, Anderson JW. Glycemic control and serum lipids: Practical nutrition implications for diabetes management. Practical Diabetology, May/June:10-14, 1991.

88 Anderson JW, Riddell-Lawrence S. Refined bakery products lower blood cholesterol in hypercholesterolemic men. American Institute of Baking Technical Bulletin Dec, 1991.

89. Anderson JW. Dietary fiber: Recommendations for the 1990s. Nestle Worldview; Nestle Information Service 3:1-8, 1991.

90. Anderson JW, Akanji AO. Dietary fiber - an overview. Diabetes Care 14:1126-1131, 1991.

91. Anderson JW. Food labeling: What do we do now? Medicine Exercise Nutrition and Health 1:63, 1992.

92. Hamilton CC, Anderson JW. Fiber and weight management. J Florida MA 79:379-381, 1992.

93. Anderson JW. Dietary fiber and diabetes: What else do we need to know? Editoral. Diabetes Research and Clinical Practice 17:71-73, 1992.

94. Anderson JW, Akanji AO. Treatment of diabetes with high fiber diets. In: Spiller GA, ed. CRC Handbook of Dietary Fiber in Human Nutrition. Boca Ration, FL: CRC Press, Inc., 443-470, 1993.

95. Anderson JW, Bridges SR. Hypocholesterolemic effects of oat bran in humans. In: Wood P, ed. Oat Bran. St. Paul: American Association of Cereal Chemists, Inc., 139-157, 1993.

96. Anderson JW. Why do diabetic individuals eat so much protein and fat? Editoral. Med Exerc Nutr Health 2:65-68, 1993

97. Anderson JW. Eat more carbohydrate and fiber - for the health of it. International Newsletter on Carbohydrates, Fiber and Health 1:49-72. 1993.

98. Anderson JW, Geil PB. Nutrition management of diabetes mellitus. In: Shils ME, ed. Modern Nutrition in Health and Disease, 8th edition. Philadelphia: Lea & Febiger, 1259-1286, 1994.

99. Anderson JW. Summary and first commentary on article by Bonanome A, et al. Am J Clin Nutr 54:586-590, 1991. Diabetes Spectrum 6:304. 1993.

100. Anderson JW, deAngulo MO. Diabetes mellitus in adults. In: Rakel RE, ed. Conn's Current Therapy. Philadelphia: WB Saunders Co., 519-527, 1994.

101. Anderson JW, Akanji AO. Reversibility of obesity, diabetes, hyperlipidemia and coronary heart disease. In: Temple NJ, Burkitt DP, eds. Western diseases: Their dietary prevention and reversibility. Totowa, NJ: The Humana Press, Inc., 317-348, 1994.

102. Anderson JW. Dr. Anderson's high fiber prevention plan. Lexington, KY, University of Kentucky Press, 250, 1994.

103. Anderson JW. New developments in dietary fiber: Cardiovascular disease and diabetes. Proceedings from IV International Symposium on Dietary Fiber, Mexico City, 51-69, 1994.

104. Anderson JW. Cholesterol-lowering effects of soluble fiber in humans. In: Kritchevsky D, Bonfield C, eds. Dietary Fiber in Health and Disease. St. Paul: Eagan Press, 126-136, 1995.

105. Anderson JW. Short chain fatty acids and lipid metabolism: Human studies: In: Cummings JH, Rombeau JL, Sakata T, eds. Physiological and Clinical Aspects of Short Chain Fatty Acid. New York: Cambridge University Press, 509-523, 1995.

106. Anderson JW. Dietary fiber and cardiovascular disease in the elderly. Cardiology in the elderly 3:16-20, 1995.

107. Anderson JW. Book Review: Werback M, Nutritional Influences on Illness: A Sourcebook of Clinical Research, 2nd edition. J Nutr Ed, 1995.

108. Anderson JW, Denke MD, Foreyt JP, Smith BM. Cutting dietary fats: Advice that really works. Patient Care Jan:16-31, 1995.

109. Anderson JW, Smith BM. Fantastic fiber. Women's health digest 1: 24-27, 1995.

110. Anderson JW, Johnstone BM, Cook-Newell ME. Response to correspondence by Krauss, Chait and Stone. N Eng J Med 333:1715-1716, 1995.

111. Smith BM, Anderson JW. The role of fiber in the diabetic diet. Postgraduate Healthcare, Greenwich, CT: MRA Publications, Inc., 7-11, 1995.

112. Anderson JW. Dietary fibre, complex carbohydrate and coronary artery disease. Can J Cardiol 11(SupplG) 55-61, 1995.

113. Anderson JW. Phytoestrogen effects in humans relative to risk for cardiovascular disease, breast cancer, osteroporosis, and menopausal symptoms. In: Pavlik EJ, ed. Estrogens, Progestins, and their Antagonists. Boston: Birkhauser, 51-71, 1996.

114. Anderson JW, Breecher MM. Dr. Anderson's antioxidant, antiaging health program. New York: Carroll & Graf, 1-270, 1996.

115. Geil PB, Anderson JW. Dry beans: Nutrition and health implications. Michigan Dry Bean Digest 21:2-6, 1997.

116. Anderson JW. Introduction, The Soy Gourmet, Robertson, Robin, viiii-xv, 1998.

117. Green CS, Hanna TJ, Anderson JW. Benefits of soy protein in diabetes: Role in preventing and treating kidney disease. Its Role in Health and Wellness. Ohio Soybean Council, P.O.1998.

118. Anderson JW, Smith BM, Thompson PK. Soy protein and risk for coronary heart disease. Role in preventing and treating kidney disease. Its Role in Health and Wellness. Ohio Soybean Council, P.O. Box 479, Columbus, Ohio 43216-0479, 1998.

119. Anderson JW. Nutritional management of diabetes mellitus. In: Shils ME, Olson JA, Shike M, Ross AC, Young VA, eds. Modern Nutrition in Health and Disease. Baltimore: Williams and Wilkins, 1365-1395, 1999.

120. Anderson JW, Konz EC. Orlistat: A new generation of drugs for the treatment of obesity. Today's Therapeutic Trends, 17:243-255, 1999.

121. Anderson JW. Fiber therapy in IBS and other GI disorders. Participate, The International Foundation for Functional Gastrointestinal Disorders, 8:2-4, 1999.

122. Anderson JW. The effects of soy protein on renal health. The Soy Connection, 7:3-6, 1999.

123. Anderson JW, Smith BM, Moore KA, Hanna TJ. Soy foods and health promotion. In: Watson, T. Vegetables, Fruits, and Herbs in Health Promotion. Boca Raton, FL: CRC Press, 9:117-134, 2000.

124. Anderson JW, Hanna TJ. Soy protein and diabetic nephropathy. Seoul, Korea. Soybean and Human Health, S-8:113-128, Nov 2000.

125. Starr C, Allison DB, Anderson JW, Aronnie LJ, Campfield LA, Vash PD. Taking advantage of antiobesity medications. Patient Care 11:34-62, 2000.

126. Saunders CS, Anderson JW, Quella SK, Yetley EA. Sorting out health claims about soy Patient Care 12:14-28, 2000.

127. Anderson JW, Randles KM. Treatment of diabetes with high-fiber diets. In: Spiller GA, ed. CRC handbook of dietary fiber in human nutrition. 3rd ed. Boca Raton, FL: CRC Press, 373-400, 2001.

128. Anderson JW. The health benefits of soluble fiber: Emphasis on reducing the risk of heart disease. Lexington, KY: University of Kentucky CME, 1-32, 2001.

129. Stephenson, TJ, Anderson JW. Soy and diabetes, kidney disease, and hypertension. Book chapter. In press.

130. Anderson JW. Soy protein decreases risk for coronary heart disease. European General Practitioners. Monograph. In press.

131. Stephenson TJ, Anderson JW. Phytoestrogens: Renal effects – diabetic nephropathy. Anderson JJB, ed. Phytoestrogen monograph. Champaign, IL. American Oil Chemical Association. In press.

132. Anderson JW. Health benefits of whole grains: overview. Kritchevsky D, ed. Vahouny Fiber Monograph. In press.

133. Anderson JW. Fiber workshop: Dyslipidemia. Kritchevsky D, ed. Vahouny Fiber Monograph. In press.

134. Anderson JW. Whole grain intake and risk of coronary heart disease. In Slaven J. Whole grains in health and disease. Monograph. In press.

G

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)

Health, Labor and Welfare

Ministry Food Product Evaluation No. 306001

Permit for labeling a product

as a Food for Specified Health Use

Name of applicant: Kao Corporation

With respect to "Healthya Green Tea" for which application was made on April 18, 2001, I give the applicant permission to indicate "Food for Specified Health Use" on the label of the product concerned as described below, according to Article 12, Paragraph 1 of the Nutrition Improvement Law (Law No. 248 that was enacted in 1952).

March 6, 2003

Chikara Sakaguchi

Minister of Health, Labor and Welfare

[Stamped with the Minister's seal]

Details

Permit number: No. 466

Content of labeling: As this green tea contains a large amount of tea catechin, it is suitable for people who are concerned about body fat.

Others:

As for the "amount of daily intake", the following labeling shall be made.

"Please drink this tea with one bottle per day as a rough standard."

As for the "precautions to take in its intake", the following labeling shall be made.

" • The intake of a large amount of this tea neither cures disease nor promotes health.

• One bottle of this tea contains 80 mg of caffeine, so please observe the recommended daily intake."

If any new fact concerning the health care effect or safety of this food product is found, it shall be promptly reported to the Office of Health Policy on Newly Developed Food in the Policy Planning Division of Dept. of Food Safety, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labor and Welfare.

厚生労働省発食第0306001号

特定保健用食品表示許可書

申請者　花王株式会社

平成１３年４月１８日付で申請のあった「ヘルシア緑茶」について、栄養改善法（昭和２７年法律第２４８号）第１２条第１項の規定により、下記のとおり特定保健用食品の表示をすることを許可する。

平成１５年３月６日

厚生労働大臣　坂口　力

記

許可番号　第４６６号

表示内容　この緑茶は茶カテキンを豊富に含んでいるので、体脂肪が気になる方に適しています。

その他

一日当たりの摂取目安量として、次のような表示を行うこと。

『１本を目安にお飲みください。』

摂取をする上での注意事項として、次のような表示を行うこと。

『・多量摂取により疾病が治癒したり、より健康が増進するものではありません。
・本品は１本当たりカフェインを８０ｍｇ含有します。１日当たりの摂取目安量をお守りください。』

なお、当該食品の保健の効果又は安全性につき、新たな知見を入手した際には、遅滞なく厚生労働省医薬局食品保健部企画課新開発食品保健対策室まで報告すること。

Health, Labor and Welfare

Ministry's Food Product Evaluation No. 306002

Permit for labeling a product

as a Food for Specified Health Use

Name of applicant: Kao Corporation

With respect to "Healthya Oolong Tea" for which application was made on April 18, 2001, I give the applicant permission to indicate "Food for Specified Health Use" on the label of the product concerned as described below, according to Article 12, Paragraph 1 of the Nutrition Improvement Law (Law No. 248 that was enacted in 1952).

Chikara Sakaguchi

Minister of Health, Labor and Welfare

[Stamped with the Minister's seal]

Details

Permit number: No. 467

Content of labeling: As this oolong tea contains a large amount of tea catechin, it is suitable for people who are concerned

about body fat.

Others:

As for the "amount of daily intake", the following labeling must be made.

"Please drink this tea with one bottle per day as a rough standard."

As for the "Precautions to take in its intake", the following labeling must be made.

" • The intake of a large amount of this tea neither cures disease nor promotes health.

• One bottle of this product contains 80 mg of caffeine, so please observe the recommended daily intake."

If any new fact concerning the health care effect or safety of this food product is found, it must be promptly informed to the Office of Health Policy on Newly Developed Food in the Policy Planning Division of Dept. of Food Safety, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labor and Welfare.

厚生労働省発食第0306002号

特定保健用食品表示許可書

申請者　花王株式会社

平成１３年４月１８日付で申請のあった「ヘルシア烏龍茶」について、栄養改善法（昭和２７年法律第２４８号）第１２条第１項の規定により、下記のとおり特定保健用食品の表示をすることを許可する。

平成１５年３月６日

厚生労働大臣　坂口　力

記

許可番号　第４６７号

表示内容　この烏龍茶は茶カテキンを豊富に含んでいるので、体脂肪が気になる方に適しています。

その他

一日当たりの摂取目安量として、次のような表示を行うこと。

『１本を目安にお飲みください。』

摂取をする上での注意事項として、次のような表示を行うこと。

『・多量摂取により疾病が治癒したり、より健康が増進するものではありません。
・本品は１本当たりカフェインを８０ｍｇ含有します。１日当たりの摂取目安量をお守りください。』

なお、当該食品の保健の効果又は安全性につき、新たな知見を入手した際には、遅滞なく厚生労働省医薬局食品保健部企画課新開発食品保健対策室まで報告すること。

Health, Labor and Welfare

Ministry Food Product Evaluation No. 1209004

Permit for labeling a product

as a Food for Specified Health Use

Name of applicant: Kao Corporation

With respect to "Healthya Water" for which application was made on December 22, 2004, I give the applicant permission to indicate "Food for Specified Health Use" on the label of the product concerned as described below, pursuant to Article 26, Paragraph 1 of the Health Promotion Law (Law No. 103 that was enacted in 2002).

December 9, 2005

Jiro Kawasaki

Minister of Health, Labor and Welfare

[Stamped with the Minister's seal]

Details

Permit number: No. 713

Content of labeling:

As this product contains a large amount of tea catechin and can facilitate the utilization of fat as energy, it is suitable for people who are concerned about body fat.

Others:

As for the rough standard amount of daily intake, the following labeling shall be made.

"Please drink one bottle per day as a rough standard"

As for the precautions to take in its intake, the following labeling shall be made.

" • The intake of a large amount of this drink neither cures disease nor promotes health.

• Excessive intake of this drink may lead to diarrhea depending on your physical constitution or current condition."

For the purpose of popularizing and encouraging a balanced diet, the following labeling shall be made.

"Take a balanced diet based on a combination of staple food, main dishes and side dishes."

If any new fact concerning the health care effect or safety of this food product is found, it shall be promptly reported to the Office of Health Policy on Newly Developed Foods in the Standards and Evaluation Division of the Food Safety, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labor and Welfare.

H

Appendix H
Safety Information on Tannase

I. Identify 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 addtive 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 evaluatino 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

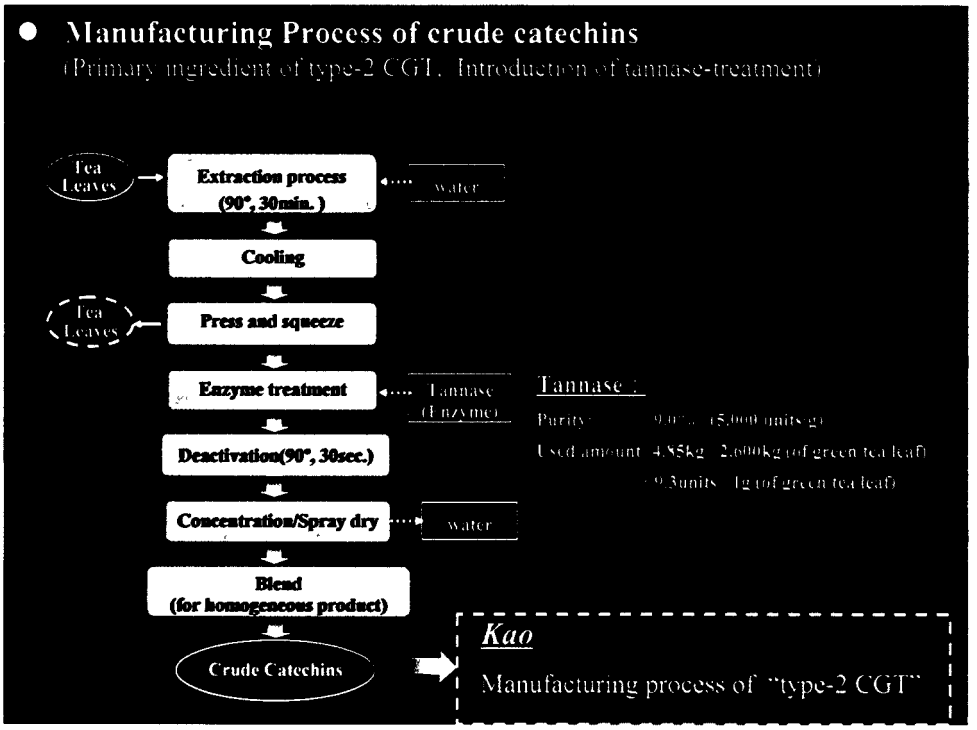
Kao is considering the introductino of tannase manufactured by Kikkoman. Kokkoman has sold tannase in the U.S. for 10 years (several tens of metric tons of tannase per year).

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 straing of *A. oryzaei* used to produce tannase is morphologically equivalent to other strains and is genetically stable.
- The tannase straing (Kikkoman Corporation) was originall 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. tannse 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**

BEST ORIGINAL COPY

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. GThese 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.)

I

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, ehtanolamine, 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

U.S. Pharmacopeia
The Standard of Quality

June 23, 2008

USP to Defer Decision on Green Tea Extract Cautionary Labeling

The United States Pharmacopeial (USP) Convention's Dietary Supplements Information Expert Committee in the Council of Experts has decided to defer its decision on whether a cautionary labeling statement should be included in the USP Powdered Decaffeinated Green Tea Extract monograph. This cautionary statement was originally proposed in *Pharmacopeial Forum* 33(6) (Nov-Dec 2007) and subsequently revised based on comments received on that proposal. The Committee's work on this matter, including the revised cautionary statement, is described in the recently published paper entitled Safety of Green Tea Extracts: A Systematic Review by the US Pharmacopeia, Drug Safety 2008; 31(6): 469-484.

The reason for the deferral is to allow the Committee time to review its safety classification policy for dietary supplements, which also is described in the above-referenced paper. The current policy specifies that an article determined to fall within "class 2" shall include a warning statement in the labeling section. The Committee intends to reexamine this policy and decide whether any modifications should be made to its classification system.

The Committee remains concerned about safety issues surrounding green tea extracts as used in dietary supplements. As it reviews its safety classification policy, it will also continue to monitor and evaluate clinical case reports and other information relating to the safety of dietary supplements containing these extracts.

Meanwhile, the USP Powdered Decaffeinated Green Tea Extract Monograph has been published without the cautionary labeling statement in the 2nd *Supplement* to *USP 31-NF 26* and will become official as scheduled on December 1, 2008. If the Committee determines after its further deliberations regarding its safety classification policy and the safety issues surrounding green tea extracts that a cautionary labeling statement for dietary supplements is warranted, the monograph may be revised to include such a requirement.

For more information, please contact mediarelations@usp.org.

Headquarters
12601 Twinbrook Parkway
Rockville Maryland 20852
+1 301 881 0666

Europe/Middle East/Africa
Munchensteinerstrasse 41
CH 4052 Basel Switzerland
+41 (0)61 316 30 10

USP-India Private Limited
ICICI Knowledge Park
Genome Valley
Labs 7 10, Phase III
Turkapally, Shameerpet
Ranga Reddy District
Hyderabad 500 078, A P, India
+91 40-2348-0088

USP-China
Building 1
Lane 67 Libing Road
Zhangjiang Hi Tech Park
Shanghai, 201203 China
+86 21 51370600

USP-Brazil
WTorre Technology Park
Avenida Ceci 1600
06460-905 Barueri SP Brazil
+55 11-4166-3300

www.usp.org

Pages 000270 - 000285 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Morgan Lewis
COUNSELORS AT LAW

Morgan, Lewis & Bockius, LLP
1111 Pennsylvania Avenue, N.W.
Washington, D.C. 20004
Tel: 202.739-3000
Fax: 202.739.3001
www.morganlewis.com

Stephen Paul Mahinka
Partner
202-739-5205
smahinka@morganlewis.com

Dr. Sharon A. Segal
Director of Regulatory Science
202-739-5427
ssegal@morganlewis.com

BY EMAIL AND MESSENGER

December 28, 2007

Dandapantula N. Sarma, R.Ph., Ph.D
Senior Scientist, Dietary Supplements
Standards Division
United States Pharmacopeia
12601 Twinbrook Parkway
Rockville, MD 20850

Re: Comments to USP's Proposed In-Process Revision: Powdered Decaffeinated Green Tea Extract Regarding Proposed Caution Statement

Dear Dr. Sarna:

Morgan, Lewis & Bockius LLP submits the following comments in response to the United States Pharmacopeia's (USP) publication, in 33(6) *Pharmacopeia Forum* (Nov.–Dec. 2007), of a proposed In-Process Revision of USP's proposed monograph on powdered decaffeinated green tea extract, published at 33(4) *Pharmacopeia Forum* 693 (July-Aug. 2007).

Based on the clinical and other data discussed below, and summarized in Attachment 2 of these comments, we are strongly of the view that the proposed caution statement included in the In-Process Revision is unwarranted and unsupported by relevant clinical and other data. Further, we believe that inclusion of the proposed caution statement will have unintended adverse consequences with respect to the regulatory status of current and

potential food and beverage products. Inclusion of the caution statement by USP for dietary supplements ineluctably will raise questions regarding the Food and Drug Administration (FDA) regulatory status of currently marketed food and beverage products containing green tea extracts, and will, as a practical matter, preclude consideration by FDA of any submissions requesting Agency concurrence as to the Generally-Recognized-as- Safe (GRAS) status of green tea extract substances, no matter how well-documented and supported

We commend the USP's Dietary Supplements Information Expert Committee (USP Committee) for its significant efforts in preparing the proposed monograph, and for its detailed review of the clinical studies and anecdotal case reports available at the time to the Committee for preparation of its draft manuscript on USP's safety review of green tea extracts, which you kindly provided to us for purposes of preparation of these comments.[1] In view of the current absence of an adequate and sufficient scientific basis to support inclusion of such a caution statement, however, we believe the USP Committee properly should not adopt any such caution statement unless and until adequate scientific evidence of adverse safety effects is developed. We believe that this course would more properly balance the Committee's interest in the presentation of potential public health concerns with the absence of an adequate and sufficient scientific evidentiary basis at present to warrant such a caution statement, particularly where inclusion of any such statement would unavoidably result in restriction of consumer availability of products containing green tea extract, with its recognized benefits.

As we demonstrate below, and as we believe the USP Committee understands, the evidence relied upon by the Committee is extremely limited and inadequate, consisting only of anecdotal case reports, which are replete with confounding additional factors. The toxicological mechanism is not clear, and liver damage was not observed in the clinical studies and animal reports reviewed by USP. Further, the USP Committee was not in a position to consider several recent clinical studies, as well as preclinical studies, which demonstrate no adverse health effects attributed to green tea extract, which we discuss below and in Attachment 2.

I. WELL-CONTROLLED CLINICAL AND ANIMAL STUDIES DO NOT SUPPORT A SAFETY CONCERN OR CAUTION STATEMENT FOR GREEN TEA EXTRACT

We have undertaken a detailed safety review of green tea catechin extracted from green tea when used as a food ingredient for the Committee's information. As you will note in the detailed summary of our safety review set out at Attachment 2, the safety of green tea catechin extracted from green tea is supported by a number of studies, including acute,

---

[1] Sarma, D.N., et al., 2007, US Pharmacopeia Safety Review of Green Tea Extracts. Manuscript under review.

subacute, subchronic, and chronic studies in rats, *in vitro* mutagenicity studies, and clinical studies that evaluated tolerance and nutritional effects. No hepatotoxic effects were observed in either the preclinical animal studies or the clinical studies. Further, a search of the published literature found no well-designed, placebo-controlled studies in humans or animals that demonstrates liver damage associated with the consumption of green tea extract.

We believe the several additional adequate and well-controlled clinical studies summarized in Attachment 2, which the Committee was not aware of and thus not in a position to have considered when deciding to suggest the proposed caution statement, provide a sufficient and proper basis for the Committee to now conclude that the inclusion of the proposed caution statement, based on limited anecdotal reports, is unwarranted and unsupported, and that any such statement should be not be adopted unless and until an adequate scientific basis for such a statement is developed.

II. THE ANECDOTAL CASE REPORTS THAT SERVE AS THE SOLE BASIS FOR INCLUSION OF THE PROPOSED CAUTION STATEMENT ARE INADEQUATE

A. Case Reports Generally Offer Limited Evidence in Assessment of Causality, and USP Relies Only on Such Anecdotal Case Reports in Its Causality Assessment of Green Tea Extract Safety

The USP Committee's manuscript indicates that the Committee relies exclusively on anecdotal case reports for its analysis of the safety of green tea extracts, even though it "encountered numerous clinical studies detailing the efficacy of green tea." [2] Although the Committee reviewed six clinical trials and found that "no adverse reactions were reported,"[3] it did not rely on them for the safety analysis, because "a major limitation" of these studies is the study design (open label, small scale) and the lack of statistical power associated with it, which does not allow the detection of any adverse effects other than those that are very common.[4] As noted above, the USP Committee not aware of and thus not in a position to consider several recent clinical studies, which were adequately sized and controlled, as well as certain preclinical studies, which demonstrate no adverse health effects attributed to green tea extract. These studies are noted and discussed in Attachment 2. We believe, as demonstrated below, that the anecdotal case reports relied on by the Committee are inadequate to establish causal relationships and that the proposed caution statement is thus without an adequate scientific basis.

---

[2] Manuscript at 8.

[3] Manuscript at 8.

[4] Manuscript at 13.

It is widely believed that case reports generally offer only limited evidence in the assessment of causality. In particular, case reports, such as those relied upon for the USP safety assessment, are often of inconsistent quality, and the interpretation of any findings is limited by confounding factors and missing data (*e.g.*, concurrent use of medications and other supplements; other ingredients in the supplements containing green tea extract; medical history). For example, one patient (case report #85756) consumed a polyherbal formulation that contained, in addition to green tea extract, *Cascara* sagrada and skullcap, and a contribution by either of those ingredients to the hepatotoxicity observed cannot be ruled out. Further as discussed below, such anecdotal case reports cannot substitute for well-designed clinical trials to assess causal relationships.

The 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. While recognizing that "the individual case reports were not strong," the Committee nonetheless proposed a caution label for green tea extracts, based on the "preponderance of the [case] reports," as well as "significant pharmacokinetic and toxicological data."[5] Although the Committee "deliberately used the term caution instead of warning" because no "certain causality" has been determined, nonetheless, the caution label reads "[i]n rare cases extracts from green tea have been reported to adversely affect the liver."[6] (original emphasis). The Committee cited no authority or rationale that consumers can or will be able to distinguish between a "caution" and a "warning." Indeed, as we discuss below in Section III, inclusion of unwarranted or inadequately supported caution statements can be expected to be deleterious to consumer understanding.

The USP Committee thus necessarily concluded that green tea extracts, at least probably or possibly, caused the liver injury in those cases. It is important to note, however, that the Committee determined that, when powdered decaffeinated green tea extract is used and formulated appropriately, it presents "no significant safety issues" of which the Committee is aware, or that would prohibit monograph development.[7] Notwithstanding this strong statement by the Committee, as we noted above, imposition of a caution statement will call into question the FDA regulatory basis for continued marketing of food and beverage products containing green tea extracts, and will preclude, as a practical matter,, FDA consideration of any GRAS notices submitted for review to the Agency regarding green tea extracts, consequences which we do not believe were either considered or intended by the Committee.

---

5 Manuscript at 16.

6 Manuscript at 16-17.

7 Manuscript at 5.

B. Case Reports Are of Limited Value for Causality Assessment

We concur with the Committee that anecdotal case reports of adverse drug experiences ("ADE") are an important source of information. They "call attention to potential adverse reactions or interactions, mechanisms, diagnostic techniques, or methods of management."[8] In addition, they can "generate or test hypotheses, and remind or educate."[9] However, as we believe the Committee also recognizes, by themselves, they are not a sufficient basis to impose cautions or warnings, because such case reports provide only limited evidence for the assessment of causality of adverse events, as described below:

- The quality of case reports varies significantly.[10] The quality of the case report is regarded as "critical for appropriate evaluation of the relationship between the product and adverse events."[11] The fact that guidelines have been issued by FDA relating to use of case reports so that clinicians can "obtain the information needed to assess whether an adverse event is actually an adverse drug reaction" further substantiates this point.[12]

- Case reports cannot substitute for a well-designed clinical trial to assess causal relationships. For example, a report released in 2003 by the Agency for Healthcare Research and Quality, of the Department of Health and Human Services, emphasized that "[c]ontinued analysis of case reports cannot substitute for a properly designed study to assess causality," and that "a hypothesis-testing study is needed" in order to assess causality between consumption and serious adverse events.[13]

- Thirty-four case reports from France, Spain, U.S., Canada, U.K., and Australia were relied upon by USP in its safety analysis of green tea extracts. However, it

---

8 Aronson, J.K., 2003. Anecdotes as evidence. BMJ. 326(7403):1346 (citing Jenicek, M. Clinical case reporting in evidence-based medicine. 2nd ed. London: Arnold, 1999.).

9 Aronson, J.K., 2003. Anecdotes as evidence. BMJ. 326(7403):1346 (citing Jenicek, M. Clinical case reporting in evidence-based medicine. 2nd ed. London: Arnold, 1999.).

10 Kelly, W.N., 2003. The quality of published adverse drug event reports. Ann Pharmacother. 37:1774-1778; Aronson, J.K., 2003. Anecdotes as evidence. BMJ. 326(7403):1346. Comment in: BMJ. 2003 Aug 2;327(7409):290.

11 FDA Guidance for Industry, Good Pharmacovigilance Practices and Pharmacoepidemiologic Assessment, March 2005. http://www.fda.gov/cder/guidance/6359OCC.pdf.

12 Aronson, J.K., 2003. Anecdotes as evidence. BMJ. 326(7403):1346

13 Ephedra and Ephedrine for Weight Loss and Athletic Performance Enhancement: Clinical Efficacy and Side Effects, a report prepared for the Agency for Healthcare Research and Quality, U.S. Department of Health and Human Services, AHRQ Publication No. 03-E022, February 2003. http://www.fda.gov/ohrms/dockets/98fr/95N-0304-bkg0003-reference07-01.pdf.

is questionable whether it is scientifically justified to combine these case reports from different countries, because, as has been observed, "there is no justification for combining or 'lumping' data reported from different countries: there were just too many differences."[14]

We have provided a summary table of all of the case reports relied on by the Committee, with further comments, at Attachment 1.

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

The algorithm the 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. Additionally, the Committee does not present a convincing scientific rationale for adopting the "Naranjo" algorithm here.

1. Causality Assessment Algorithms Are Not Generally Accepted

Various algorithms have been published since the 1970's that purport to assess the causality of adverse events. However, these algorithms are not generally accepted for two main reasons: lack of validity and lack of reliability.

(a) Lack of Validity

Validity is "a major requirement" of an algorithm that assesses causality.[15] Validity means that the "scores derived from the scale accurately reflect the phenomenon measured," and is usually assessed by comparing the results obtained from an algorithm with a conventional external standard (e.g., an expert panel).[16] Lack of validity appears to be a common problem for these causality assessment algorithms, as illustrated by the following examples:

- In a recent article, none of the 15 published algorithms, including Naranjo, Jones, and Kramer, "presented good sensitivity, good specificity and predictive values

---

[14] This conclusion was based on the information exchanged during a symposium in 1986 where a historical review of the international experience of ADR monitoring in 15 developed countries was presented Gharaibeh, M.N., et al., 1998. Adverse Drug Reactions: A Review. Drug Information J. 32:323–338.

[15] Busto, U., et al., 1982. Comparison of two recently published algorithms for assessing the probability of adverse drug reactions. Br J Pharmacol. 13:223-227

[16] Busto, U., et al., 1982. Comparison of two recently published algorithms for assessing the probability of adverse drug reactions. Br J Pharmacol. 13:223-227.

simultaneously" in the total sample and for serious and unexpected events.[17] For example, the Naranjo, Jones, and Kramer algorithms treated 71 non-drug related reports as adverse drug reactions (ADRs); as a result, all three methods had 0% specificity.[18]

- In another study, the algorithm described by Begaud, et al.,[19] was used to assess the probability of drug hypersensitivity reactions in 677 patients.[20] The results showed that this algorithm yielded a positive predictive value of 56.2%, meaning that of 100 reports that the algorithm identified as demonstrating drug hypersensitivity, only 56 of them were truly positive.[21] It should be noted that the authors asserted that the Begaud, et al., method is nonetheless "more efficient than the imputability methods of Jones and Naranjo with regard to the diagnosis of drug hypersensitivity reactions."[22]

(b) Lack of Reliability

Reliability of a causality assessment generally refers to the agreement among the results obtained from using different algorithms, and agreement among results obtained by different raters using the same algorithm. Current causality assessment algorithms have shown a lack of reliability. For example, compared to the Karch algorithm, the Kramer algorithm was more likely to assign a possible risk of ADE ($p = 0.0001$), while the Karch algorithm was more likely to assign a risk of unlikely ($p = 0.0001$).[23] The two algorithms agreed in just 41% of the cases.[24] Absolute agreement between the RUCAM algorithm and the clinical diagnostic scale (CDS) was found in only 18% of the cases.[25]

---

17 The authors used the Global Introspection (GI) method based on the WHO scale of imputation as the gold standard in the evaluation of the 15 algorithms. Macedo, A.F., et al., 2006. Can decisional algorithms replace global introspection in the individual causality assessment of spontaneously reported ADRs? Drug Saf. 29:697-702.

18 *Id.*

19 Begaud, B., et al., 1985. Imputabilité des effets inattendus ou toxiques des medicaments: actualisation de la méthode utilisée en France. Therapie. 40:111-118. (abstract); Moore, N., et al., 1985. Adverse drug reaction monitoring: doing it the French way. Lancet. 2:1056-1058.

20 Benahmed, S., et al., 2005. Accuracy of a pharmacovigilance algorithm in diagnosing drug hypersensitivity reactions. Arch Intern Med. 165:1500-1505.

21 *Id.*

22 *Id.*

23 Frick, P A., et al., 1997. Algorithms used in adverse drug event reports: a comparative study. Ann Pharmacother. 31(2):164-167.

24 *Id*

25 Freston, J., 2006. Presentation: Use and limitations of the RUCAM. AASLD-FDA-NIH-PhRMA Hepatotoxicity Steering Group Meeting, 2006 Presentations. http://www.fda.gov/cder/livertox/presentations2006/freston.htm.

Disagreement of one level (e.g., unlikely vs. possible) was reported in 47% of the cases, and the disagreement of two levels (e.g., unlikely vs. probable) in 31% of the cases.

2. USP Does Not Have a Convincing Scientific Rationale for Adopting the Naranjo Algorithm

The USP Committee adopted the Naranjo algorithm after it "debated the merits and limitations of using different causality algorithms."[26] Apparently, the Committee believed that the Naranjo scale is a "validated causality scale" that can "provide consistency in evaluations," "minimize biases," and facilitate "in accommodating the limitations of missing data."[27] However, it is not clear from the literature that the Naranjo algorithm is a validated approach, or that it is at least as scientifically robust as other algorithms, either in general or when applied to these specific case reports. The Committee instead appears to have relied on the perceived practical attributes of Naranjo, such as its being simple, fast, and flexible, in choosing it over other algorithms.

3. The Naranjo Scale Is inappropriate in Causality Assessment

In the original publication, Naranjo, et al., indicated that they used 63 ADRs to establish and validate their algorithm.[28] However, it has been shown that these 63 ADRs provided "a statistically inadequate sample size to support this conclusion [of Naranjo's validity and reliability]."[29] The validity of the algorithm was further weakened by the fact that those published ADRs used to evaluate the Naranjo algorithm "are not described other than journal of origin [where the Naranjo algorithm was first published]," raising the question of whether the information provided was sufficient to do a thorough evaluation.[30]

In addition, it has been reported that in patients admitted to intensive care units, the Naranjo algorithm showed substantial variability, as demonstrated by the low level of agreement between raters (who reviewed the 142 ADRs). Those who used the Naranjo scale had only "marginal reliability," as the average kappa value was 0.275 (kappa value

---

26 Manuscript at 8.

27 Manuscript at 8.

28 Naranjo, C., et al., 1981. A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther 30:239-245.

29 Kane-Gill, S.L., et al., 2005. Are the Naranjo criteria reliable and valid for determination of adverse drug reactions in the intensive care unit? Ann Pharmacother. 39:1823-1827.

30 Kane-Gill, S.L., et al., 2005. Are the Naranjo criteria reliable and valid for determination of adverse drug reactions in the intensive care unit? Ann Pharmacother. 39:1823-1827.

indicates the reproducibility between raters; a kappa value of 0.5 is considered good).[31] Therefore, it is quite possible that different raters, using the Naranjo scale, will derive different probability values from the same set of ADRs.

4. Use of the Naranjo Scale Is Improper, Particularly in the Assessment of Causality for Green Tea Extracts

(a) The Naranjo scale was improperly employed in the USP Committee's analysis because many of the cases had concurrent use of medications or polyherbal supplements in addition to green tea extracts.

Based on Table III-VI of the USP Committee's manuscript, 18 of the 27 cases that were categorized as "possible" causality, and one of the six "probable" causality cases, had either concurrent use of drugs/medications or polyherbal supplements. An example of such concurrent use in some of the case reports is shown in Table 1 below. Thus, it is possible that the drugs and ingredients in the poly-herbal formulation may interact with green tea extracts. To use the Naranjo scale in this context is inappropriate, because the Naranjo algorithm was "never intended to be used to evaluate adverse events resulting from the interaction of 2 drugs."[32] Instead, the scale is intended to assess the likelihood of an adverse drug event "associated with only one drug."[33]

Table 1. Example of concurrent use of drugs and poly-herbal formulations

Events that led to fulminant hepatitis (and ultimately liver transplantation) in a 48-year-old female who drank 30 g alcohol/day for the past 10 years (Gloro, et al., 2005, Case 0100091) are shown in the table below:

---

31 *Id.* (citing Streiner, D.L. and Norman, G.R. Reliability and validity. In: Streiner, D.L., Norman, G.R., eds. Health measurement scales – a practical guide to their development and use. 2nd ed. Oxford: Oxford University Press 1998:5-14, 62, 104-161).

32 Horn, J R., et al., 2007. Philip D Hansten, and Lingtak-Neander Chan Proposal for a New Tool to Evaluate Drug Interaction Cases. Ann Pharmacother 41:674-680.

33 *Id*

Day 1	2-36	Day 37	Day 38	39-42	Day 43	44-45	Day 46	Day 47
Exolise (green tea extract) for weight loss					Stops because epigastric pain and fatigue			
		Bronz'age (11 ingredients) for sunbathing	Stops because hearing voices and vertigo				**Jaundice, pale stool, dark urine appears**	
			nicergoline (30 mg/day) Piribedil (50 mg/day)		Stops because epigastric pain and fatigue			
								Paracetamol (1 g)

(b) <u>Some of the drugs or components of the poly-herbal formulations used in the case reports are known to cause liver injury.</u>

Below are four examples:

- Case LY0000143:

  Exolise (green tea extract) was given with a drug, chromocarb diethylamine, for 10 days. Symptoms appeared after 8 days of chromocarb diethylamine therapy and resolved when the drug was stopped.

- Stevens, T., 2005

  A poly-herbal formulation (Hydroxycut) contained 11 components in addition to green tea extract, including chromium polynicotinate. Together with other herbal extracts, chromium polynicotinate was noted to be "the ingredient likely to be responsible for the hepatotoxicity."[34]

[34] Manuscript, at 10 (citing Stevens, T., et al., 2005. Two patients with acute liver injury associated with use of the herbal weight-loss supplement hydroxycut. Ann. Intern. Med. 142:477-478).

- Case # 85756:

  Patient consumed a poly-herbal formulation containing *Cascara sagrada* and skullcap in addition to green tea, and liver injury has been reported with the use of *Cascara sagrada*.[35]

- CADRMP #30069:

  Patient had been taking a concomitant medication, i.e., medroxyprogesterone injections (150 mg every 3 months), which has a known serious adverse effect of abnormal liver function.[36]

Further, adverse events could occur after previously administered drugs were withdrawn. It has been reported that adverse response to drug withdrawal "may be delayed up to 1 year with cholestatic liver injury," and that antibiotics and NSAIDs are "the most common cause of DILI."[37]

(c) The Naranjo scale was improperly used in the USP Committee analysis because of a lack of critical information.

Among the 27 "possible" causality case reports reviewed by the USP Committee, seven of them (26%) either had "no information about other medications" or "no further information available" (see Attachment 1). However, the USP Committee nonetheless assigned "possible" causality to these cases.

Additionally, information on pre-existing conditions (e.g., liver disease) was included in only two of the case reports available for review. In an Agency for Healthcare Research and Quality report concerning ephedra, the following statements were made with regard to the absence of medical history data when assessing causality associations.

(1) "The absence of data could not be construed as a negative result." For example,

---

35 *Id* at 11 (citing Nadir, A., et al., 2000. Cascara sagrada-induced intrahepatic cholestasis causing portal hypertension: case report and review of herbal hepatotoxicity. Am J Gastroenterol. 95(12):3634-3637.).

36 *Id.* at 12.

37 Hussaini, S.H. and Farrington, E.A., 2007. Idiosyncratic drug-induced liver injury: an overview. Expert Opin Drug Saf 6:673-684.

"the absence of information about prior cardiac disease could not be construed as an absence of cardiac disease."[38]

(2) "Verbal histories indicating no prior history of serious conditions were not sufficient to rule out alternative explanations for the most serious adverse events, since unrecognized preexisting cardiac disease, . . . occur[s] with some frequency and are known to cause death, myocardial infarction, or stroke without warning in otherwise 'healthy' individuals."[39]

As another study concluded: "It is not possible to assess whether withdrawal of the suspect green tea extract dietary supplement is the cause of the resolution of symptoms in many of these cases, particularly if multiple medications/supplements were being ingested. If a manifestation remits or disappears with removal of a single drug, then the [causal] relationship becomes more suspect." [40] [However,] "if several suspected etiological factors are discontinued simultaneously, then unless 'rechallenge', . . . is carried out for *each* factor separately, the relationship can only be considered possible for *all* of the factors." [41] (emphasis in original).

The USP Committee is aware of the "challenges" in the analysis of adverse event reports concerning green tea extracts, including (at page 15 of the manuscript):

- Multiple ingredients;
- Unknown products;
- Incomplete case reports;
- Weak or missing information about prior medical history;
- Use of alcohol and other concurrent medications;
- Preexisting risk factors; and
- Other confounding variables.

Therefore, due to the limited validity of the Naranjo algorithm in this context, and the lack of information on concurrent medication and other attributes, all cases analyzed

---

38 Ephedra and Ephedrine for Weight Loss and Athletic Performance Enhancement: Clinical Efficacy and Side Effects, Chap. 2, at p.15. A report prepared for the Agency for Healthcare Research and Quality, U.S. Department of Health and Human Services, AHRQ Publication No. 03-E022, February 2003.

39 Ephedra and Ephedrine for Weight Loss and Athletic Performance Enhancement: Clinical Efficacy and Side Effects, Chap. 2, at p.15. A report prepared for the Agency for Healthcare Research and Quality, U.S. Department of Health and Human Services, AHRQ Publication No. 03-E022, February 2003.

40 Jones, J.K., 1982. Adverse drug reactions in the community health setting. approaches to recognizing, counseling, and reporting Fam and Comm Health. 5:58-67.

41 *Id* at 15.

using this algorithm will return a "possible" causality as long as green tea extract was consumed before the livery injury was reported. Such a result thus offers no adequate scientific basis for the Committee's conclusion that a caution statement is warranted.

D. USP Does Not Clearly Explain the Toxicological Mechanism of Liver Damage Associated with Consumption of Green Tea Extracts

The USP Committee proposes the following possible mechanism to explain the liver toxicity observed in the case reports: Green tea extracts administered under fasting conditions may lead to 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 and animal studies the Committee reviewed. Although the USP Committee relies 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 the 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 (8% 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. However, the symptoms of morbidity were vomiting and diarrhea, but nothing related to liver damage.

In addition, the Committee primarily relied on the pharmacokinetic profiles for its analysis. However, 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.

Finally, and most significantly, to the best of our knowledge, no well-designed placebo-controlled clinical studies in humans and animals is available that reports the occurrence of liver damage resulting from the consumption of green tea extract (see Attachment 2).

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.

E. 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 leverage products and as dietary supplements, and there are thus literally hundreds of products currently on the market containing green tea extract.[42] Because the reported liver toxicity was restricted primarily to two specific products, we believe that these

[42] 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)

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.

III. THE PROPOSED CAUTION STATEMENT SHOULD NOT BE ADOPTED UNLESS AND UNTIL AN ADEQUATE SCIENTIFIC BASIS OF SUPPORT IS DEVELOPED IN ORDER TO AVOID UNWARRANTED PROLIFERATION OF WARNING LABELING TO CONSUMERS

In view of the several additional clinical studies noted in Section I above and presented in detail in Attachment 2, and the severe deficiencies of the anecdotal case reports relied on by the Committee, as detailed in Section II above, we believe that inclusion of the proposed caution statement is not only unwarranted and unsupported, but also inadvisable from a consumer protection perspective. The adverse impact of proliferation of consumer warning labeling has long been a concern of both the Federal Trade Commission (FTC), which is the principal enforcement agency focused on advertising to consumers in the area of OTC drugs, as well as for foods and dietary supplements, and the FDA. The FTC Staff has noted such concerns on several occasions over the years to FDA, for example, in extensive comments on FDA's draft guidance documents regarding consumer-directed promotion of drugs. The FTC Staff observed that:

> As the amount of risk information increased, consumers who read and understand the information may benefit. On the other hand, the increased risk information may deter some consumers from reading information or may make it more difficult for them to comprehend.[43]

The FDA also has recognized on several occasions that imposition of unwarranted or weakly-supported caution and warning statements is counter-productive. In the preamble to its final rule on Warning and Notice Statements for Labeling of Juice Products, the Agency stated that: "FDA agrees that too many warning labels on foods could result in loss of consumer credibility and effectiveness." 63 Fed. Reg. 37029, 37035 (July 8, 1998). More recently, in its Final Guidance on Medical Device Patient Labeling, FDA concluded that:

---

[43] Coments of the FTC Bureau of Consumer Protection, Bureau of Economics, and Office of Policy Planning, FDA Request for Comments on Agency Draft Guidance Documents Regarding Consumer-Directed Promotion, Docket No. 2004D-0042 (May 10, 2004), at 9.

> Including too many warnings and precautions, over-warning, dilutes the strength of all the hazard alerts. We recommend that writers use care in what is designated as a warning or precaution. Careless designation can have the same diluting effect as over-warning . . . . Repeated exposure to unnecessary hazard alerts (not relevant or already known) reduces the effectiveness of the important warnings and precautions.[44]

Thus, while well-supported caution or warning statements in labeling are a necessary and proper consumer protection mechanism, proliferation of such statements by including tentative, preliminary, unsubstantiated, or weakly-supported warnings can be expected to be detrimental to consumer understanding and diminish consumer appreciation of other, well-founded, important warnings. Inclusion of the proposed caution statement by the Committee, therefore, is more likely to be counter-productive in view of the current absence of adequate scientific evidence with respect to any adverse safety effects of green tea extract. We are aware of no evidence to suggest that consumers will understand or appreciate the distinction the Committee attempts to draw between "caution" and "warning" statements. We concur instead in the Committee's final statements in the draft manuscript that, because the Committee "consistently monitors the current reports concerning the safety of supplements for which USP-NF monographs are developed, the safety classification may be reviewed as new information becomes available." The proper course, we submit, in these evidentiary circumstances, is for the Committee to not adopt any caution statement unless and until adequate scientific evidence is developed that properly supports such a statement.

We concur that the Committee's review of the case reports and other materials is useful to stimulate a dialogue and develop additional clinical evidence with respect to green tea extracts. However, in view of the current absence of an adequate scientific basis for the proposed caution statement, the Committee properly must delete from any published final version of the manuscript the conclusion that a caution statement should be included in the final monograph, and state instead that one will be considered in the future only in the event that an adequate scientific basis is developed that properly supports any such caution statement.

---

[44] FDA Center for Devices and Radiological health, *Final Guidance on Medical Device Patient Labeling*, at 42 (April 19, 2001).

IV. SUMMARY AND CONCLUSIONS

For the reasons set out above, and based on the several additional clinical trials and other evidence summarized at Attachment 2, as well as the severe limitations of the anecdotal case reports relied on by the Committee as the sole evidentiary basis for inclusion of the proposed caution statement, we believe that there is no adequate scientific basis on which the Committee can properly conclude that there is a hepatotoxic effect associated with the consumption of dietary supplements containing green tea extract. Consequently, we believe that the USP Committee properly should delete the proposed caution statement from the proposed draft manuscript for decaffeinated green tea extracts, and that the USP should not adopt any such caution statement in the final monograph for green tea extract, unless and until an adequate scientific basis for any such statement is developed.

We would be pleased to provide additional information with respect to the above comments and the Attachments.

Respectfully submitted,

Stephen Paul Mahinka, Esq.
Partner
Morgan, Lewis & Bockius LLP

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

ATTACHMENT 1

SUMMARY OF THE 34 CASE REPORTS REVIEWED BY THE USP COMMITTEE

Case ID	Patient (sex/age)	Naranjo score	Data source	Supplement intake; duration	Diagnosis	Outcome/day	Remarks
G293-1 1999	F/27	6 (probable)	French/Spanish Exolise recall	Exolise; 4 caps/day; 31 days	Cytolytic hepatitis	Recovered in 28 days	No alternative causality reported
H364-1 2000	F/46	6 (probable)	French/Spanish Exolise recall	Exolise; 3 caps/day; 1 month; then later 18 days	Cytolytic hepatitis	Recovered in 2 months	No alternative causality reported
J085-2 2000	F/38	5 (probable)	France – Tealine	Tealine; 6 caps/day; 20 days	Cytolytic hepatitis	Recovered	Reports positive rechallenge
Camiline Arkocaps tea left powder	F/19	6 (probable)	Published case reports				
Jimenez-Saenz and Martinez-Sanchez, 2006	M/45	5 (probable)	Published case reports	A green tea infusion (brand/quantity not reported) 6 cups/day; 4 months	Jaundice		Positive dechallenge and positive rechallenge. Case report does not include patient's medical history.
Bonkovsky HL, 2006	F/37	6 (probable)	Published case reports	TRA Complex; 2 g/day; 4 months	Increased liver enzyme values		Positive rechallenge. Confounding factors: multiplicity of the ingredients and co-ingestants
Molinari et al., 2006	F/44	5 (probable)	Published case reports	Green tea extract (720 mg/day); 6 months	Acute liver failure	Liver transplantation	Product information (brand, solvent of extraction, total catechins, and % EGCG) was not provided
BS0100614	F/47	4 (possible)	French/Spanish Exolise recall	Exolise; 5 caps/day; 5 months	Cytolytic + Cholestatic hepatitis	Recovered in 15 day	Patient had Haschimoto's thyroiditis
BS0100401	F/39	3 (possible)	French/Spanish Exolise recall	Not available	Cytolytic + Cholestatic hepatitis	Recovered in 2 months	Patient had hepatic adenoma
CN0100091	F/49	2 (possible)	French/Spanish Exolise recall	Exolise; 4 caps/day; 6 weeks	Cytolytic + Cholestatic hepatitis	Liver transplant	Patient taking several medications; reported alcohol use
LL0000241	F/50	3 (possible)	French/Spanish Exolise recall	Exolise: 4 caps/day; 41 days	Cytolytic + Cholestatic hepatitis	Recovered in 2 months	Omeprazole concurrent use
LY0000143	F/46	3 (possible)	French/Spanish Exolise recall	Exolise; 4 caps/day; 81 days	Cytolytic + Cholestatic hepatitis	Recovered in 15 days	Patient on several medications, rechallenge positive for Campel
H320-1 2000	F/50	4 (possible)	French/Spanish Exolise recall	Unknown intake; 1 month	Cytolytic + Cholestatic hepatitis	Recovered in 2 months	Patient taking several medications

000303

Case ID	Patient (sex/age)	Naranjo score	Data source	Supplement intake; duration	Diagnosis	Outcome/day	Remarks
K329-1 2002	F/42	4 (possible)	French/Spanish Exolise recall	Exolise; 4 caps/day; 3 months	Cytolytic + Cholestatic hepatitis	Recovered in 3 months	Patient taking several medications, including paracetamol
321530440	F/35	4 (possible)	French/Spanish Exolise recall	Exolise; 2 caps/day; 35 days	Cytolytic + Cholestatic hepatitis	Recovered in 2 months	No information about other medications
Case 4 Oct. 2001	M/32	3 (possible)	French/Spanish Exolise recall	Unknown intake; 3 months	Jaundice	No information	No further information available
Case 5 Oct. 2001	F/69	3 (possible)	French/Spanish Exolise recall	Exolise; 2 caps/day; 23 days	Hepatitis	No information	No further information available
Case 6 Sep. 2002	F/29	3 (possible)	French/Spanish Exolise recall	Exolise; 4 caps/day; 1.5 months	Cytolytic + Cholestatic	No information	No further information available
GR0000822	F/76	2 (possible)	France – Tealine	Tealine; Intake unknown; 31 days	Hepatitis	Recovered	Patient taking ciprofibrate for 10 years
PA9607529	F/47	3 (possible)	France – Tealine	Intake unknown, 90 days	Rise in transaminases	Recovered	Patient taking several medications, including piroxicam; had cholecystectomy 10 years ago
98.9155	F/49	3 (possible)	France – Tealine	Tealine; 6 caps/day; 3 weeks	Rise in transaminases	Recovered in 4 months	No other information
Gloro et al., 2005(Repeat of CN0100091)	F/49		Published case reports	Exolise; 4 caps/day; 6 weeks	Fulminant hepatitis	Liver transplantation	Confounding factors: alcohol, weight-loss program, acetaminophen, other poly-herbal formulation
Stevens et al., 2005	M/27	4 (possible)	Published case reports	Hydroxycut; 9 tabs/day; 5 weeks	Rise in transaminases	Recovered in 4 weeks	Poly-herbal formulation; no other medications reported (list)
Stevens et al , 2005	M/30	4 (possible)	Published case reports	Hydroxycut, 9 tabs/day; 5 days	Cholestatic hepatitis	Recovered in 2 months	Poly-herbal formulation, no other medications reported (list)
Porcel JM, 2005	F/53	4 (possible)	Published case reports	Fitofruit grasas acumuladas; 3 caps/day; 2 weeks	Elevated liver enzymes		Positive dechallenge. No other information was available
Javaid and Bonkovsky, 2006	F/46	3 (possible)	Published case reports	Chinese green tea; 7 months	Severe hepatocellular injury	Not known	No other information about patient history or concurrent medications was available
#15606 (Apr. 3, 2002)			FDA MedWatch		Acute, possible drug-induced hepatitis		Poly-herbal formulation; no further information reported
#15737 (Jun 11, 2002)			FDA MedWatch		Fulminant liver failure		Poly-herbal formulation

000304

Case ID	Patient (sex/age)	Naranjo score	Data source	Supplement intake; duration	Diagnosis	Outcome/day	Remarks
#62758 (Sep 8, 2003)			FDA MedWatch		Elevated liver function test values		Poly-herbal formulation
#80859 (Sep. 12, 2005)			FDA MedWatch		Reportedly "developed jaundice"	Recovered after 2 months	Patient took more than double recommended dose of poly-herbal formulation;
# 85756 (May 22, 2006)			FDA MedWatch		Reportedly "developed jaundice"		Patient increased alcohol to 5 times/week (5–6 drinks each time); poly-herbal formulation includes *Cascara sagrada* and skullcap
#180741 (Dec. 18, 2002)			Australian TGA report		Abnormal hepatic function		Used poly-herbal formulation (Jump Start for Women) for 7 days; recent acute renal failure; concurrent medications
#191840 (Date Unknown)			Australian TGA report		Abnormal liver function tests		Used poly-herbal formulation (Women's Ultivite containing 20 ingredients); needed visit to doctor's office
#210566 (Aug. 10, 2005)			Australian TGA report		Liver function tests abnormal; hepatitis		Positive dechallenge; Used poly-herbal formulation (4321 Slim & Detox containing 10 ingredients) for 4 days.
CADRMP #30069 (Oct. 24, 2005)	F/42	2 (possible)	Canadian CADRMP report	Green Lite (100 mg/cap, 6/day) 6 months	Liver transplant		Take concomitant medication – medroxyprogesterone injections (150 mg every 3 months) during previous years.

000305

ATTACHMENT 2

Safety Review of Green Tea Catechin Extracted from Green Tea as a Food Ingredient

Green Tea Catechin ("GTC") is a mixture of catechin monomers that is extracted from crude green tea extract. The safety of GTC is supported by a number of studies including acute, subacute, subchronic, and chronic studies in rats, *in vitro* mutagenicity studies, and several recent larger clinical studies of the tolerance and nutritional effects of GTC. Also, the safety of GTC was evaluated by the Ministry of Health and Welfare of Japan and was approved as safe for use in foods for specified health use ("FOSHU") in both a green tea beverage and a sports drink in Japan.

Contents:

1. **Prior and Current Use of Green Tea**
2. **Prior Use of Green Tea Extract**
3. **Daily Consumption of Green Tea in Japan**
4. **Daily Consumption of Green Tea in the United States**
5. **Absorption, Distribution, Metabolism and Excretion (ADME)**
6. **Toxicology Studies**
   - A. **Short-term Toxicity Studies of Green Tea Catechin (GTC)**
   - B. **28-day Toxicity Study of Green Tea Catechin (GTC) in Rats**
   - C. **Genotoxicity Studies of Green Tea Catechin (GTC)**
   - D. **6month Repeated dose oral Toxicity Studies of Green Tea Catechin (GTC)**
   - E. **Toxicology Studies on Substances Highly Similar in Composition to Green Tea Catechin (GTC)**
   - F. **Human Clinical Studies**

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 practice 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 more widely in civil society around the late 16th century. At present, the tea plant is cultivated in more than 20 countries of Asia, Africa, and South America as one of the most favorite horticultural plants, analogous in use to coffee and cocoa.[1]

Green tea is now considered one of the most popular drinks in the world.[2] Of the total amount of tea consumed in the world, approximately 20% is green tea,[3] 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]

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

---

1 Yamamoto, T., et. al. 1997. Chemistry and Applications of Green Tea. CRC Press (New York) p. 1.

2 Id. at p. 13.

3 Mukhtar H. and Ahmad N., 2000. Tea polyphenols: prevention of cancer and optimizing health. *Am. J. Clin. Nutr.* 71(suppl):1698S-1702S.

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)

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

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 also has 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, 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 extracted 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).[13] Green tea extract is approved for use as a food additive in many countries (e.g., Japan, South Korea) and has been consumed for

---

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.

13 See 21 C.F.R. § 182.20; Katsuo, K. 1987. Cultural history of Tea. *J Food Sci.* 117: 14-20.

many years in the context of a variety of applications, including, but not limited to, beverages and dietary supplements.

3. 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).[14]

4. Daily Consumption of Green Tea in the United States

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 1.

---

[14] Muramatsu, 1991 *Science of Tea*. Asakura Publishing Co.(Tokyo), p.153; Imai, K. 1997. Cancer Prevention Effects of Drinking Green Tea among a Japanese Population, *Preventive Medicine*. 26: 769-775; Suga et al. 1998; Sato et al. 1989. Possible contribution of green tea drinking habits to the prevention of stroke. Tohoku J.Exp.Med. 157:337-343.

Table 1. 2-Day Average Estimated Green Tea Consumption in the U.S.

Population	% Consumers	Unweighted number of consumers[15]	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.

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

Epigallocatechin-3-gallate (EGCg), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin are the major green tea catechins (GTC). EGCg comprises 50-60% of green tea catechins.[16]

Absorption of GTC into human plasma was investigated by chemiluminescence (CL)-HPLC in healthy subjects (n=3; 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

---

15 The estimates in this table 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 this table 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).

16 Miyazawa, T., et al., 2000. Absorption and metabolism of natural antioxidants. *Kagaku to Seibutu*. 38(2):104-114. [Article in Japanese]

mg EGC, respectively.[17] 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 intake, 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 were also 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 who had ingested green tea.[18] 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 healthy volunteers were measured 2 hours after ingestion of single doses of GTC in free or phospholipid complex form (equivalent to 400 mg EGCg), as determined by HPLC with coulometric detector.[19] 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 cathechins,

---

17 Nakagawa, K., et al., 1997. Dose-dependent incorporation of tea catechins, (-)-epigallocatechin-3-gallate and (-)-epigallocatechin into human plasma. *Biosci Biotech Biochem* 61(12):1981-1985.

18 Unno, T., et al., 1996. Analysis of (-)-epigallocatechin gallate in human serum obtained after ingesting green tea. *Biosci Biotech Biochem* 60(12):2066-2068.

19 Pietta, P., et al., 1998. Relationship between rate and extent of catechin absorption and plasma antioxidant status. *Biochem Mol Biol Int* 46(5):895-903.

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 phospholipid complex, although the percent absorption of EGCg did not exceed 2% of the given dose.

In a study using a HPLC-coulochem electrode array detection (CEAS) method, the limits of detection for EGCg, EGC, ECG, and epicatechin ranged from 0.5 ng/mL to 1.5 ng/mL of plasma or urine.[20] One hour after ingestion of 1.2 g of decaffeinated green tea (containing 88, 82, 32, and 33 mg of EGCg, EGC, epicatechin, and ECG, respectively), the plasma samples from four human volunteers contained 46-268 ng/mL (100-585 pmol/mL) EGCg, 82-206 ng/mL EGC, 48-80 ng/mL epicatechin. 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 epicatechin 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 epicatechin occurred at 3-6 hours after dosing. Most of the EGC and epicatechin 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 epicatechin. EGCg and ECG were not detected in the urine samples. The sulfate and glucuronide forms of both EGC and epicatechin were present at approximately a 2:1 ratio. Free EGC was present at <1% of the conjugated form, but free epicatechin 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 epicatechin 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 epicatechin 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.

---

[20] Lee, M., et al., 1995. Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiol Biomarkers Prev* 4(4):393-399.

The pharmacokinetic parameters of GTC have been investigated in several human studies. In one study, 18 healthy volunteers (six subjects per dose level) were given 1.5, 3, and 4.5 g of decaffeinated green tea solids dissolved in 500 mL of water.[21] The plasma concentrations of EGCg, EGC, and epicatechin 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 epicatechin (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 epicatechin (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 epicatechin (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 ($t_{1/2}$) values were approximately 5 hours and 2.7 hours for EGCg and EGC, respectively, and were not affected by the dose increase. For epicatechin, the mean $t_{1/2}$ 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 epicatechin was excreted within 8 hours. After 24 hours, EGC and epicatechin 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 GTC was determined after single oral dose administration of EGCg and Polyphenon E (decaffeinated mixture of EGCg,

---

21 Yang, C.S., et al., 1998. Blood and urine levels of tea catechins after ingestion of different amount of green tea by human volunteers. *Cancer Epidemiol Biomarkers Prev* 7(4):351-354.

EGC, epicatechin, and other tea polyphenols) by the HPLC-CEAS method.[22] Twenty healthy volunteers (five subjects per dose level) 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. After oral administration, 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 epicatechin were not detected in plasma after EGCg administration and were present at low/undetectable levels after Polyphenon E administration. High concentrations of EGC and epicatechin glucuronide/sulfate conjugates were found in plasma and urine samples after Polyphenon E administration. EGCg was not detectable in the urine. The systemic availability of EGCg increased at higher doses, possibly due to saturable presystemic elimination of orally administered green tea polyphenols.

In a third study, the pharmacokinetic parameters of GTC 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

---

22 Chow, H.-H.S., et al., 2001. Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and Polyphenol E. *Cancer Epidemiol Biomarkers*

epicatechin for an individual with a body weight of 70 kg) or EGCg (2 mg/kg) to eight healthy subjects.[23] The peak plasma concentrations of EGCg, EGC, and epicatechin 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 epicatechin 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 $ng \times hour \times mL^{-1}$, respectively. The T_{max} values were in the range of 1.3-1.6 hours. The elimination $t_{½}$ values of EGCg, EGC, and epicatechin 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 epicatechin 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 epicatechin were mostly in the conjugated form. Most of the conjugated catechins were in the glucuronide form. Over 90% of the total urinary EGC and epicatechin, almost all in the conjugated forms, were excreted during 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 $t_{1/2}$ 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

---

Prev 10(1):53-58.

23 Lee, M.-J., et al., 2002. Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. *Cancer Epidemiol Biomarkers Prev* 11(10 Pt 1):1025-1032.

methylation of EGC.[24,25] 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.[26] The levels of urinary M6 (5 ± 2 mg) accounted for 11.2% of the ingested epicatechin. 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 2 below.[12] All human studies demonstrated that only a small percentage of the orally-ingested catechins appears in the blood. EGC and epicatechin appear to be more bioavailable, but the fractions of these compounds that appeared in the plasma are also low, and only 3.3% and 8.9% of the ingested EGC and epicatechin were excreted in the urine. 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.

---

24 Weinshilboum, R.M., et al., 1999. Methylation pharmacogenetics: catechol *O*-methyltransferase, thiopurine methyltransferase, and histamine *N*-methyltransferase. Annu Rev Pharmacol Toxicol 39:19-52.

25 Zhu, B.T., et al., 2000. *O*-Methylation of tea polyphenols catalyzed by human placental cytosolic catechol-*O*-methyltransferase. Drug Meta. Dispos 28:1024-1030.

26 Lee, M.-J., et al., 2002. Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. *Cancer Epidemiol Biomarkers Prev* 11(10 Pt 1):1025-1032.

Table 2. Comparison of the EGCg pharmacokinetics

	LEE ET AL.[12,A]	YANG ET AL.[27,B]			CHOW ET AL.[28,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
$t_{1/2}$ (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.

Most of the ingested EGCg apparently does not get absorbed into the blood, and the absorbed EGCg is preferentially excreted through the bile to the colon in rats.[29] 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 epicatechin 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 epicatechin 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 epicatechin in

27 Yang, C.S., et al., 1998. Blood and urine levels of tea catechins after ingestion of different amount of green tea by human volunteers. *Cancer Epidemiol Biomarkers Prev* 7(4):351-354.

28 Chow, H.-H.S., et al., 2001. Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and Polyphenol E. *Cancer Epidemiol Biomarkers Prev* 10(1):53-58.

29 Chen, L., et al., 1997. Absorption, distribution, and elimination of tea polyphenols in rats. *Drug Metab Dispos* 25(9):1045-1050.

the intestine were similar to those in the kidney. These results suggest that EGCg is excreted mainly through the bile, while EGC and epicatechin are excreted through both the bile and urine. These results are in agreement with the observations in humans, when EGC and epicatechin, but not EGCg, were detected in human urine samples. Low levels of EGCg, EGC, and epicatechin were also detected in the liver. The liver and lung levels of EGCg, EGC, and epicatechin 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).[30] A very low amount 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.[31] 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 epicatechin, 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.[32] 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.[33]

---

30 Nakagawa, K. and T. Miyazawa, 1997. Absorption and distribution of tea catechin, (-)-epigallocatechin-3-gallate, in the rat. *J Nutr Sci Vitaminol* 43:679-684.

31 Miyazawa, T. and K. Nakagawa, 1998. Structure-related emission spectrometric analysis of the chemiluminescence of catechins, theaflavins and anthocyanins. *Biosci Biotech Biochem* 62(4):829-832.

32 Okushio, K., et al., 1996. Absorption of tea catechins into rat portal vein. *Biol Pharm Bull* 19(2):326-329.

33 Miyazawa, T., et al., 2000. Absorption and metabolism of natural antioxidants. *Kagaku to Seibutu*. 38(2):104-114. [Article in Japanese]

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 3 below.[34]

Table 3. Free EGCg concentration after drinking the green tea catechin 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, GTC ingested are mostly distributed in the gastrointestinal mucosa, and excreted in feces upon detachment of the mucosa.[35] Five to 8% of ingested GTC is absorbed within a relatively short time (probably from the stomach and upper small

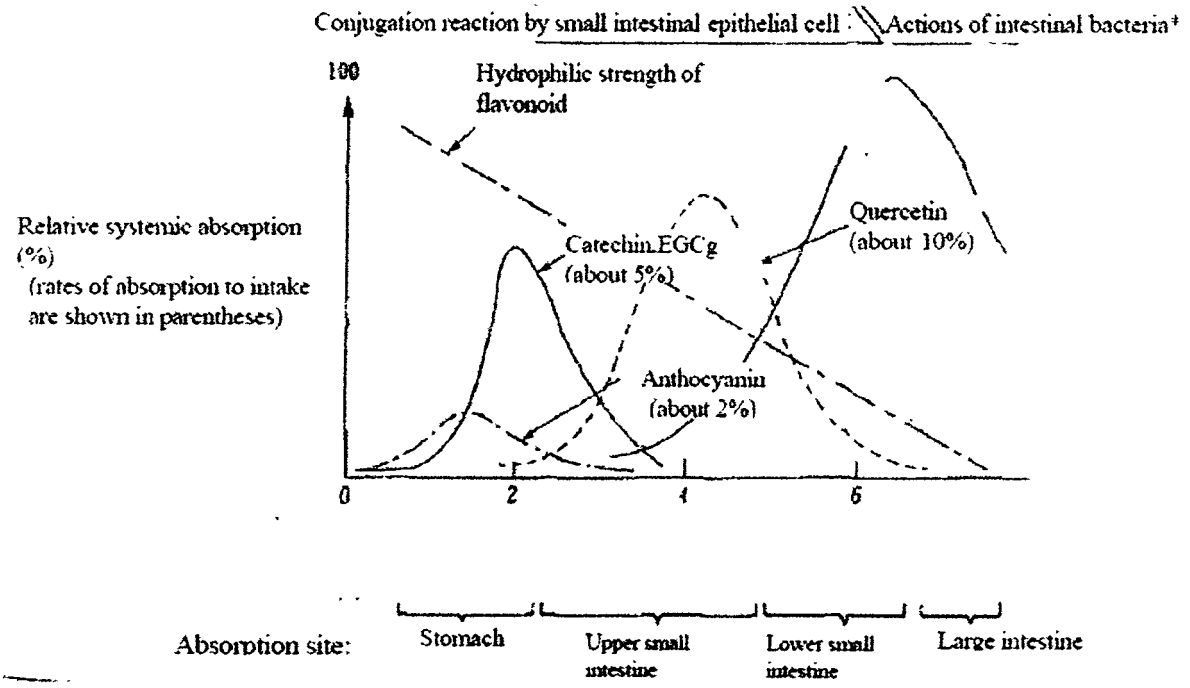
---

34 Miyazawa, T., et al., 2000. Absorption and metabolism of natural antioxidants. *Kagaku to Seibutu*. 38(2):104-114. [Article in Japanese]

35 Miyazawa, T., et al., 2000. Absorption and metabolism of natural antioxidants. *Kagaku to Seibutu*. 38(2):104-114. [Article in Japanese]

intestine), and partially conjugated with glucuronide and sulfate in mucoepithelial cells upon absorption. Figures 1 and 2 below provide graphical representations of the absorption and metabolic pathways for various flavonoids, including catechins, respectively.

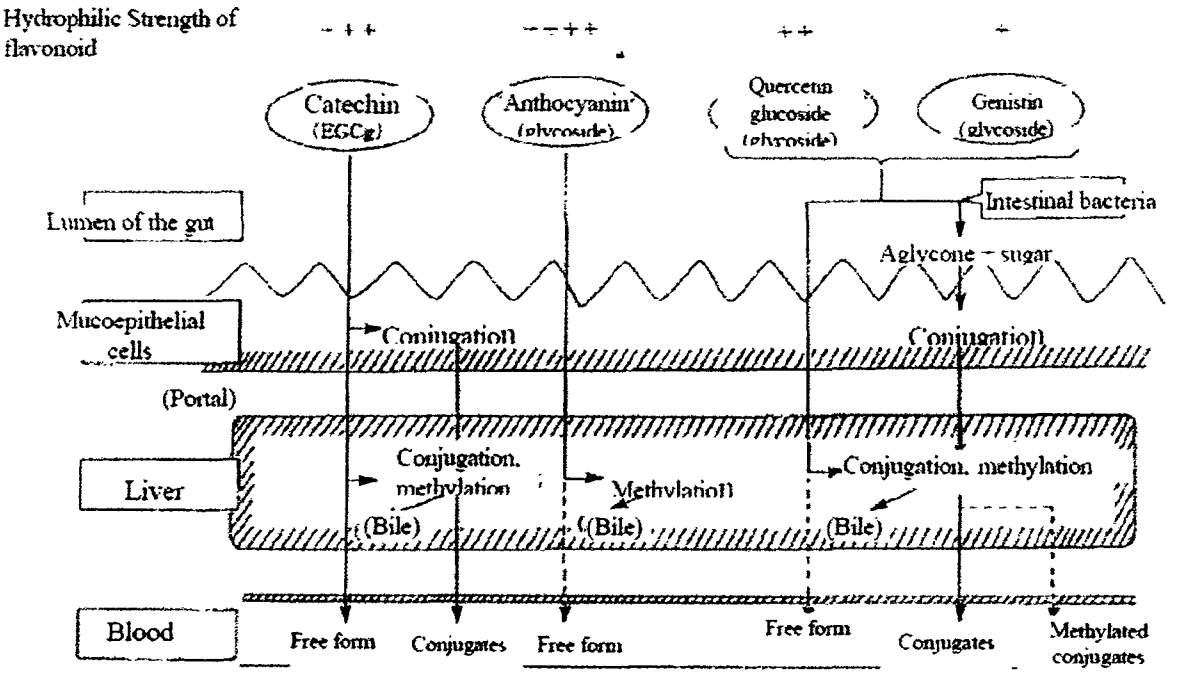
Figure 1. Absorption Site, Amount, and Maximum Absorption Time of Major Dietary Flavonoids



*Aglycone production, ring fission, and dehydration reaction by intestinal bacteria.

Source: Miyazawa et al. 2000.

Figure 2. Differences in Conjugation and Methylation due to Dietary Flavonoid Structure

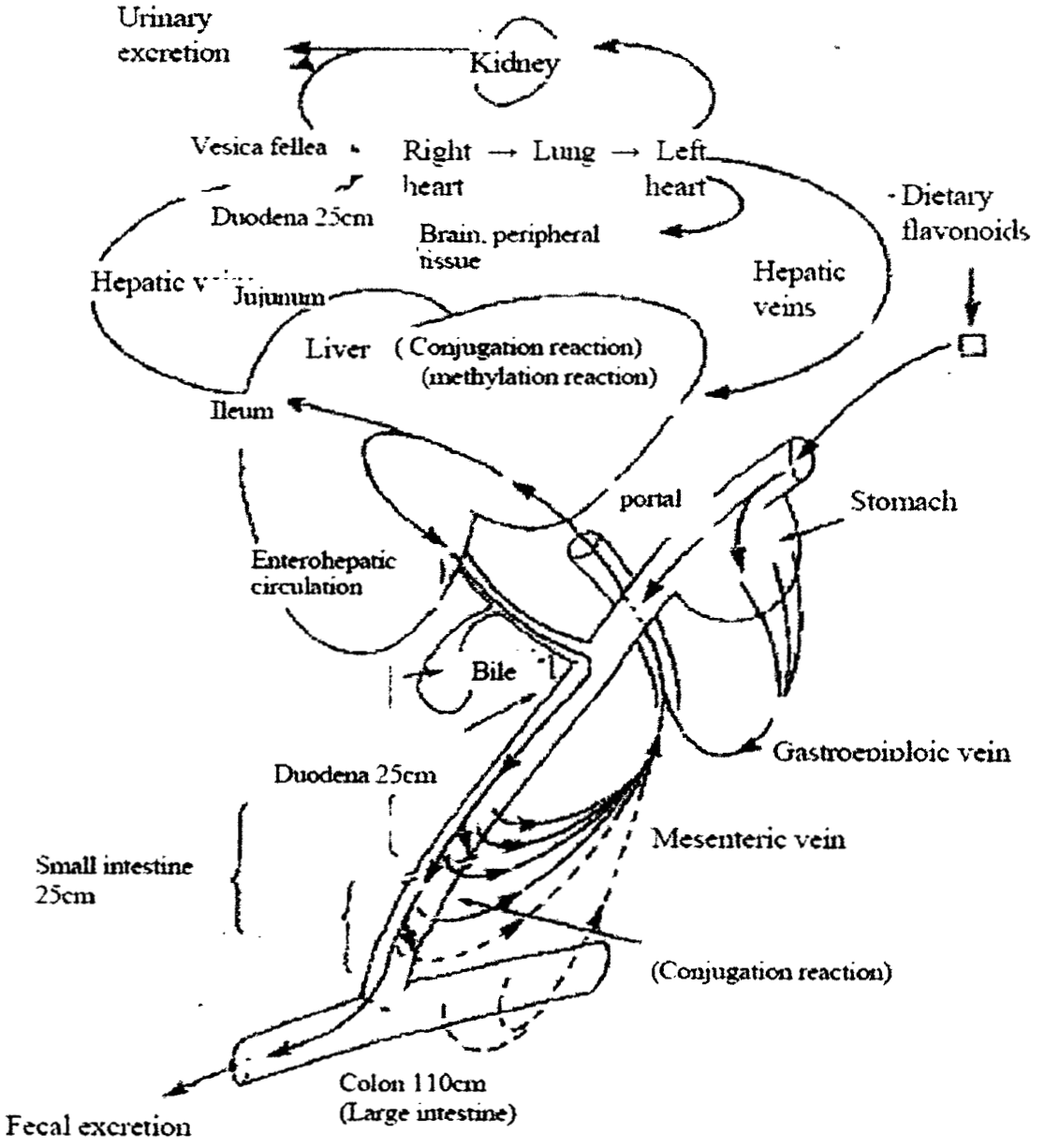


*As glucuronide and sulfate

Source: Miyazawa et al. 1000.

Following absorption, GTC 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 3 below provides a graphic representation of the absorption and metabolism of flavonoids, including catechins.

Figure 3. Absorption and Metabolic Pathway of Dietary Flavonoids



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.

6. Toxicology Studies

A. Short-term Toxicity Studies of Green Tea Catechin (GTC)

A number of short-term toxicity studies were conducted on GTC. Because of a heat-sterilization step in the manufacture of one type of Green Tea Beverage, many of these studies were conducted in parallel with both heat-treated ("HT-GTC") and non-heat-treated versions of Green Tea Catechin ("UT-GTC"). These unpublished studies are summarized in Table 4.

Table 4. Summary of Green Tea Catechin (GTC) Short-Term Toxicity Studies

Citation	Design/Duration	Test Substance	Test Animal	Endpoints	Results	Comments
Hurley J. WIL Research Lab Study WIL-101096. Acute Oral Toxicity Study of KH-1 (HFD) in Albino Rats	Heat-treated GTC 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-GTC	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	• Both animals died before day 3 following the first administration of 5000 mg/kg. • For the 2000 mg/kg/day dose, 2/6 females died and no male rats died.	• The LD_{50} for females was estimated to be between 2500-5000 mg/kg; the LD_{50} for males was estimated to be between 2000 mg/kg/day and 5000 mg/kg. • Study was performed according to U.S. GLP conditions.

000324

Citation	Design/Duration	Test Substance	Test Animal	Endpoints	Results	Comments
Kirkpatrick J et al. WIL Reseach 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 KH-1 (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-GTC	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, Ca2+, 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 weigh gain seen at 1000 and 1500 mg/kg/day. • This was a rang-finding study for a 6-month study in rats. • This testing was performed under OECD GLP conditions.

000325

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.	

000326

B. 28-day Toxicity Study of Green Tea Catechin (GTC) in Rats[36]

The potential toxicity of GTC was evaluated via daily administration to Crl:CD® (SD) rats of both sexes for 28 days. GTC (which, for the purposes of dose calculations, was considered to be 100% pure) was administered orally 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 GTC, an additional group (5 males and 5 females) was administered decaffeinated GTC (hereafter referred to as "D-GTC") in deionized water, orally 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,[37] serum chemistry,[38] and urinalysis[39]) were performed on all animals prior to the primary necropsy (i.e.,

---

36 Kirkpatrick, J.B. 2006. A 28-Day Repeated Dose Oral (Gavage) Toxicity Study of KH-4 (HFD) and KH-4 (Decaffeinated-HFD) in Rats (With Functional Observational Battery and Motor Activity Deterinations). WIL Research Laboratories, LLC. Study No. WIL-101112. Final Report dated June 1, 2006. (In this article, "HT-GTC" is described as "GTC type-1.")

37 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).

38 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;

study week 4). Complete necropsies were conducted on all animals, and selected organs[40] were weighed at the scheduled necropsy. Selected tissues[41] were examined microscopically from all animals.

No test article-related deaths or clinical observations were observed during the study. 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, serum chemistry, or urinalysis parameters. Macroscopic findings and 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 GTC group males. These findings correlated with lower food consumption in these groups in the first week of dosing. Body weights and food consumption were not affected in the 2000 mg/kg-bw/day GTC-DC groups. Minimal erosions of the glandular stomach were observed for one male and one female each in the 2000 mg/kg-bw/day group.

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 GTC animals) was derived from this study. The NOAEL

---

phosphorus; potassium; sodium; triglycerides (triglyceride).

39 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].

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

41 Adrenal glands (2); aorta; bone with marrow (femur, sternum); bone marrow smeara; brain (cerebrum level 1, cerebrum level 2, cerebellum with medulla/pons); epididymidesb; 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.)

for *systemic toxicity* was 2000 mg/kg-bw/day, the highest dose tested. Observations for the 2000 mg/kg/day GTC-DC-treated animals were similar to the 2000 mg/kg-bw/day GTC-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.

In a second study, the potential oral toxicity of the "Type 2" (sports drink) tea catechin preparation, which does not undergo heat sterilization, was studied in Crl:CD® (SD) rats of both sexes (5 animals/sex/group) for 28 days.[42] 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 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 meant 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.

---

42 Kirkpatrick, J.B. 2006. A 28-Day Repeated Dose Oral (Gavage) Toxicity Study of UHG in Rats (With Functional Observational Battery and Motor Activity Determinations). WIL Research Laboratories, LLC. W Ogura,R., et al. Genotoxicity studies on green tea catechin. Manuscript to be submitted

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, a NOAEL of 2000 mg/kg bw/day (the highest dose tested) was derived from this study.

Both 28-day toxicity studies were conducted in accordance with Good Laboratory Practices.

C. 6-month Repeated Dose Oral Toxicity Studies of Green Tea Catechin (GTC) [43]

The potential toxicity of HT-GTC was evaluated via daily administration to Crl:CD® (SD) rats of both sexes for 182 days. HT-GTC was administered orally by gavage in deionized water once daily for 182 consecutive days to 3 groups (Groups 2-4) of Crl:CD® (SD) rats. HT-GTC-DC was administered orally 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 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.

There were no test article-related effects on food consumption or clinical pathology parameters. There were no ophthalmic or macroscopic pathology findings. There were no test article-related deaths. 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-GTC groups. Lower body weight gains were noted in the 400 and 1200 mg/kg/day HT-GTC and 1200 mg/kg/day HT-GTC-DC group females. Based on these findings of reduced weight gain in females, a NOAEL of 400 mg/kg/day was estimated.

D. Genotoxicity Studies of Green Tea Catechin (GTC)

The following genotoxicity tests were conducted on HT-GTC and/or UT-GTC green tea catechin: bacterial reverse mutation assay (Ames test), chromosomal aberration assay in cultured Chinese

---

[43] Kirkpatrick, J.B. 2006. A 6-Month Repeated Dose Oral (Gavage) Toxicity Study of KH-4 and KH-4(decaffeinated-HFD) in Rats (With Functional Observational Battery and Motor Activity Determinations). WIL Research Laboratories, LLC. Final Report.(In this article, "HT-GTC" is described as "KH-4.")

hamster lung cells (CHL/IU), mouse lymphoma L5178Y/tk assay (MLA), and a bone marrow micronucleus assay in ICR CD mice. 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 GTC.

The results of these studies are summarized in Table 5. These tests were conducted in accordance with Good Laboratory Practice regulations.

Table 5. Summary of Genotoxicity Studies Conducted with Green Tea Catechin (GTC)

Test	GTC/Levels Tested/Design	Results
Bacterial revise mutation assay (Ames test)	• Heat-treated GTC(HT-GTC) 156-500 µg/plate (in dimethylsulfoxide (DMSO)) • Type 2 (UHG): 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. Nor more than a two-fold increase in the man 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-GTC in physiological saline: 56.3-900 µg/mL • UT-GTC 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-reduplicatio): 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-GTC: An increase of >10% was observed in the absence of metabolic activation in both the short-term and continuous treatments. UT-GTC: An increase of >10% was observed in the presence and absence of metabolic activation in the short-term treatment.

000332

Test	GTC/Levels Tested/Design	Results
		• The positive controls performed as expected. • The results of this study were considered positive for chromosomal aberrations.
Mouse Lymphoma L5178Y/tk assay	• HT-GTC in physiological saline: 52.7-3600 µg/mL • UT-GTC 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-GTC: 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-GTC: 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.

000333

Test	GTC/Levels Tested/Design	Results
Bone marrow micronucleus assay in mice	• HT-GTC and UT-GTC in physiological saline: 500, 1000, and 2000 mg/kg bw • Administered to 9-week-old male ICR (Crj: CD-1) mice in two oral 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 ration 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-GTC and UT-GTC 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.

000334

E. Toxicology Studies on Substances Highly Similar in Composition to Green Tea Catechin (GTC)

The medical literature contains toxicity studies performed on substances highly similar in composition to GTC (e.g., Polyphenon E, Polyphenon 70S, Polyphenon 30, THEA-FLAN-70A). The relative composition of these related substances are presented below in Table 6. Study results are summarized in Table 7.

Table 6. Catechin and Polyphenol Composition of Related Tea Catechin Products

	Polyphenon 70S	Polyphenon E	Polyphenon 30	Green Tea Catechin (GTC)
Total Catechin Monomers	78.7 %	87.3 %	34.2%	44.4%
Polyphenols	86.4 %	96.7 %	No data	52.9%
Caffeine	0 %	0 %	6.9%	2.6%
Relative Catechin Profile (% of Total Catechin Monomers)				
Gallocatechin (GC)	6.9 %	0.0 %	2.1%	5.4%
Epigallocatechin (EGC)	20.1 %	4.8 %	9.0%	29.8%
Catechin (C)	2.7 %	1.0 %	0.0%	2.0%
Epicatechin (EC)	8.5 %	10.2 %	2.3%	6.8%
Epigallocatechin gallate (EGCG)	42.9 %	74.8 %	17.0%	43.7%
Gallocatechin gallate (GCG)	4.7 %	4.0 %	0.7%	1.1%
Epicatechin gallate (ECG)	12.7 %	4.9 %	2.7%	10.4%
Catechin gallate (CG)	1.5 %	0.0 %	0.4%	1.0%

Table 7. Toxicology Studies on Substances Highly Similar in Composition to Green Tea Catechin (GTC)

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.
Faqi 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)	*Chang et al.* conducted mutagenic assays: 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
Matsumoto et al. 1999 (Polyphenon 30)	Single-dose oral toxicity of green tea extract was conducted in 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.
Kawakami et al. 1993 (THEA-FLAN-70A)	The single dose toxicity of THEA-FLAN-70A (tea extract) was assessed in rats. The test substance was administered orally to male and female rats at doses of 0, 1000, 1400, and 2000 mg/kg, and the animals were observed for 14 days. As a result of deaths observed at the two highest doses, the LD_{50}s were estimated to be more than 2000 mg/kg, and no apparent sex difference was observed in the toxicity.

F. Human Clinical Studies

A series of short- and long-term clinical investigations have been conducted with substances highly similar to GTC examining tolerability and nutritional effects in humans. In all cases, clinical studies have demonstrated that substances highly similar to GTC 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 weight/fat reducing effects of GTC.

1. Published Studies Assessing Safety-related Parameters After Consumption of Substances Highly Similar to Green Tea Catechin

Many of the studies assessing the safety of GTC or highly similar substances are published in peer-reviewed journals. Summaries of select published studies are provided in Table 8 below (see next page) and the safety findings from larger, controlled studies (especially as they relate to potential liver toxicity) are described in further detail below.

A recent double-blind, parallel group study was conducted involving 240 obese males and females who ingested one can of green tea containing 583 mg catechins per day or one can of green tea containing 96 mg catechins per day for 12 weeks.[44] Hematology and clinical chemistry parameters were assessed at baseline and every four weeks throughout the study period, including the following indices of liver function: glutamic-oxaloacetic transsaminase ("GOT"), glutamic-pyruvic transaminase ("GPT"), gamma glutamyl transferase ("γ-GTP"), and alkaline phosphatase ("ALP"). No differences were observed in these parameters between groups, suggesting that ingestion of GTC does not adversely affect liver function.

In a recently completed randomized, double-blind, controlled, parallel unpublished study conducted in the U.S. (not included in Table 8), 135 subjects ingested a beverage containing 1.54 mg/mL of tea catechins (in 350 mL) twice daily for a total of at least 1080 mg catechins per day, or

---

[44] Nagao, T., et al. 2007. High-dose catechins reduce body fat and the risk of obesity-related disease in humans. Obesity. 15(6):1473-1483 (see attached).

an identical volume of control beverage (n=67) for 12 weeks, followed by 12 weeks of additional follow-up.[45] Hematology and clinical chemistry were evaluated tthroughout the study, including the following indices of liver function: albumin, creatinine, total bilirubin, ALP, alanine aminotrasnferase, and aspartate aminotransferase. There were no statistically significant changes from baseline in any of these parameters for either the GTE or the control groups.

[45] A randomized, double-blind, controlled, parallel study to test the efficacy of tea catechins for enhancing loss of fat mass in overweight and obese men and women undergoing a mild energy restriction. Radiant Development, Inc. Report No. RD 05-1001 (2007).

Table 8. Summary of Published Studies Assessing Safety-related Parameters for GTC and Highly Similar Substances[46]

Study	Similarity of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
Nagao, T., et al. High-dose catechins reduce body fat and the risk of obesity-related disease in humans Obesity.2007.15(6): 1473-1483	Within 5% of GTC(heat treated) Specifications for all catechins	Double-blind, parallel Control group: 96.3 GTC mg/day (n=135) Test group: 583 GTC mg/day (n=135) 2-week run-in followed by 5-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, plast 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 hematology paramter. • No adverse effects were reported by the subjects in this study.

[46] Abbreviations Used in the Table: GTC = 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; FBG = fasting blood glucose; 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; 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.

BEST ORIGINAL COPY

000340

Study	Similarity of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
Nagao T., et al. Ingestion of tea rich in catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men. *Am J Clin Nutr* 2005. 81:1-8.	Within 3% of GTC(heat treated)specifications for all catechins	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 GTC mg per beverage (oolong tea) (n = 18) Test group: 689.9 mg GTC 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: Clinical chemistry: 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. Reduction in body fat in humans by long-term ingestion of catechins. *Progress in Medicine*. 2002. 26(11):1459-1464	Within 2% of GTC(heat treated)specifications for all catechins	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 GTC per beverage (green tea-like drink)	Control group: 41 male; 18 postmenopausal females Test group: 39 male, 19 postmenopausal females Mean age (male): 42.1 years (combined groups) Mean age	• 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 Clinical Chemistry: 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 Hematology: WBC, RBC, Hb,	• 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 group compared to baseline and control • No significant differences between

BEST ORIGINAL COPY

000341

Study	Similarity of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
		Test group: 588 mg GTC per beverage (green tea-like drink) Caffeine: 81-83 mg GTC in each beverage	(female): 54.8 years (combined groups) Mean BMI (male): 26.5 (combined groups) Mean BMI (female): 25.9 (combined groups)		Hct, PLT Urinalysis: glucose, protein, urobilinogen, ketone body, and protein; sediments if protein was positive Circulatory Pulse, diastolic blood pressure Daily diary	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 Effects of tea catechins on body fat metabolism in women. *Jap J Nutritional Assessment*. 2001. 19(3):365-76.	Within 2% of GTC(heat treated)specifications for all catechins	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 340 mL oolong tea beverage (562 mg TC/day)	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 Hematology: WBC, RBC, Hb, Hct, MCV, MCH,	• 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 • No clinically significant changes in blood parameters were observed between groups. • No "study-related poor physical condition" reported

BEST ORIGINAL COPY

000342

BEST ORIGINAL COPY

Study	Similarity of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
		Caffeine concentration kept constant			MCHC, PLT, UIBC	
Nagao T., et al. Tea catechins suppress accumulation of body fat in humans. *J Oleo Sci* 2001. 50(9):35.	Experiment 1: Within 7% of GTC(heat treated) specifications for all catechins Experiment 2: Within 13% of GTC(heat treated) specifications for all catechins Experiment 3: Within 3% of GTC(heat treated) specifications for all catechins	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, FBG, 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
Chow H.H., et al. Pharmacokinetics and	Refer to composition of	Five groups (8 subjects/group):	40 healthy subjects (8	• Adverse event reports	At weeks 0 and 4:	• All reported events (including excess gas, upset stomach, nausea,

000343

Study	Similarity of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
safety of green tea polyphenols after multiple-dose administration of epigaallocatchin gallate and polyphenon E in healthy individuals. *Clin Cancer Res* 2003 9:3312-19.	Polyphenon E in Table 6 above	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	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);	• Pharmacokinetics • Blood chemistry	Hematology WBC and RBC Clinical Chemistry 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	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 $t_{1/2}$ not significantly different after repeat administration

BEST ORIGINAL COPY

000344

Study	Similarity of Test Substance	Design/ Duration	Subjects	Primary Endpoints	Hematology/ Clinical Chemistry/Other	Results
			and 150 ± 19 pounds (Polyphenon E twice daily)			

BEST ORIGINAL COPY

000345