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March 29, 2007

Via Hand Delivery

Laura Tarantino, Ph.D.
Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety, HFS-200
5100 Paint Branch Parkway
College Park, Maryland 20740-3835

Re: GRAS Notification for alpha-Glycosyl isoquercitrin

Dear Dr. Tarantino:

Pursuant to proposed 21 C.F.R. § 170.36(c), on behalf of our client, San-Ei Gen F.F.I., Inc., we hereby notify the Agency of San-Ei Gen's determination, on the basis of scientific procedures in accordance with 21 C.F.R. § 170.30, that use of its alpha-Glycosyl isoquercitrin to protect flavors and colors in various specified foods is generally recognized as safe (GRAS) and therefore exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act. The reasons for San-Ei Gen's GRAS conclusion regarding its alpha-Glycosyl isoquercitrin are discussed in detail in the company's GRAS Notification, which is enclosed in triplicate.

We trust you will find the enclosed notification acceptable. Should any questions arise during the review process, please do not hesitate to contact us, preferably by telephone, so that we may respond as quickly as possible.

Very truly yours,

Melvin S. Drozen
John F. Foley

Before the
FOOD AND DRUG ADMINISTRATION
Department of Health and Human Services
Washington, D.C.

GRAS NOTIFICATION

Name of Notifier:	San-Ei Gen F.F.I., Inc.
Post Office Address:	All communications on this matter are to be sent in care of: Counsel for the Notifier Melvin S. Drozen John F. Foley Keller and Heckman LLP 1001 G Street, N.W., Suite 500 West Washington, D.C. 20001 Telephone: (202) 434-4222 (202) 434-4186.
Name of Substance and Intended Use:	*alpha*-Glycosyl isoquercitrin to protect flavors and colors in various specified foods.
Dated:	March 28, 2007

Melvin S. Drozen
John F. Foley
Counsel for San-Ei Gen F.F.I., Inc.

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I. CLAIM OF GRAS STATUS

A. Name and Address of Notifier

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All communications on this matter are to be sent in care of Counsel for the Notifier, Melvin S. Drozen and John F. Foley, Keller and Heckman LLP, 1001 G Street, N.W., Suite 500 West, Washington, D.C. 20001.
Telephone: (202) 434-4222, (202) 434-4186.

B. Common or Usual Name of the Notified Substance

alpha-Glycosyl isoquercitrin

Synonym: enzymatically modified isoquercitrin, EMIQ

Trade name: SANMELIN®

C. Applicable Conditions of Use

alpha-Glycosyl isoquercitrin is intended for use to protect flavors and colors in non-alcoholic beverages, including soft drinks, fruit juices, vegetable juices, as well as in flavored milk products. The antioxidant is also intended for use for the same purpose in wine coolers (<7% alcohol); shochu[1]-based flavored beverages (< 7% alcohol); frozen dairy products; gelatins and puddings; jams and jellies; soft candy; cakes, cookies, pastries and pies; powdery or canned soup; and chewing gum. For all of the foods identified above in this paragraph, with the exception

[1] Shochu is a distilled alcoholic beverage similar to vodka, which is indigenous to Japan. It usually contains about 25% alcohol, and is often mixed with plum or other fruit juices to make a cocktail-type beverage that would contain less than 7% alcohol in the United States.

of chewing gum, *alpha*-glycosyl isoquercitrin will be used at a maximum of 150 parts per million (ppm) in the finished food. The maximum use level in chewing gum is 1500 ppm.

D. Basis for GRAS Determination

The described use of *alpha*-glycosyl isoquercitrin has been shown to be generally recognized as safe (GRAS) on the basis of scientific procedures, in accordance with 21 C.F.R. § 170.30, as discussed more fully in the accompanying summary of the basis for the GRAS determination. This determination is supported by an expert panel review of the relevant toxicological data, as evidenced by the conclusions set forth in Appendix 1.

E. Statement of Availability of Data

The data and information that are the basis for the GRAS determination are available for the Food and Drug Administration's review and copying or will be sent to FDA upon request.

* * *

The foregoing and accompanying information considered, San-Ei Gen F.F.I., Inc. hereby notifies the Agency through its attorneys that its *alpha*-glycosyl isoquercitrin, as described below, is GRAS when used to protect flavors and colors in the foods described in foregoing Section C, such that the level of *alpha*-glycosyl isoquercitrin in the finished food will be no greater than 150 ppm, with the exception of chewing gum, in which the antioxidant may be present at up to 1500 ppm. Accordingly, *alpha*-glycosyl isoquercitrin is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

Respectfully submitted,

San-Ei Gen F.F.I., Inc.

By:

Melvin S. Drozen

John F. Foley

Keller and Heckman LLP

COUNSEL FOR THE NOTIFIER

II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE NOTIFIED SUBSTANCE

A. Name

1. Chemical Name

alpha-Glycosyl isoquercitrin.

2. Other Names

alpha-Glycosyl isoquercitrin is also sometimes referred to as enzymatically modified isoquercitrin or EMIQ. It will be sold under the trade name SANMELIN®. *alpha*-Glycosyl isoquercitrin is referred to as EMIQ hereafter in this notification.

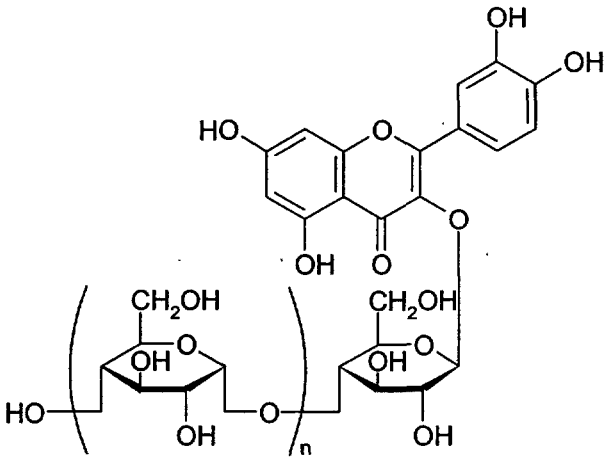
B. Chemical Description

1. Structural Formula

EMIQ has the general structure shown in Figure 1.

Figure 1

EMIQ



The number of glucose units may vary from 1 to 11 (n=0 to 10). (See Appendices 2 and 3.)

2. CAS Registry Number

None.

3. Molecular Weight

The average molecular weight of EMIQ is 800 Daltons.

C. Physical Description

EMIQ is a yellow to yellow-brown powder having a distinctive odor. It is soluble in water.

D. Method of Manufacture

EMIQ is manufactured from rutin, quercetin-3-rhamnoglucoside, which is isolated from *Sophora japonica*. Isolated rutin is converted enzymatically to isoquercitrin using rhamnosidase (EC. 3.2.1.40). An aqueous solution of isoquercitrin and dextrin is treated with cyclodextrin glucanotransferase (EC.2.4.1.19), which adds glucose units to form EMIQ. The solution is heated to inactivate the enzymes, then filtered and evaporated under vacuum. The residue is dissolved in ethanol, and impurities are removed by crystallization. Finally, the ethanol solution is evaporated under vacuum to yield EMIQ. Analyses confirm that there is no enzyme activity in the finished EMIQ under the conditions of processing (*see* Appendix 4a). All enzymes used in the production of EMIQ are on the List of Existing Food Additives in Japan's Food Sanitation Law and are produced under food additive good manufacturing practices (GMP) in Japan (*see* Appendix 4b). The quality and safety of the enzymes are shown by manufacturing flow charts provided by San-Ei Gen F.F.I., with accompanying product specifications from the enzyme manufacturers (*see* Appendix 4c). The notifier intends to sell EMIQ in a liquid formula under the trade name SANMELIN®. SANMELIN® is a 15% EMIQ solution in ethanol (20%), glycerol and water.

E. Specifications/Quantitative Composition

The substance meets the specifications set forth in Table 1, below, which were established by San-Ei Gen F.F.I., Inc. for food-grade EMIQ. *See* Appendix 5.

Table 1

Content	Not less than 90%, calculated on a dried basis.
Identification	As flavonoids and quercetin. Tests specified in Appendix 5, Section 4.2
Lead	Not more than 2 ppm
Arsenic (as As_2O_3)	Not more than 2 ppm
Quercetin	Not more than 1%

Analyses of five batches of dried EMIQ showed a mean percent purity of 94.7%, recognizing EMIQ as the sum of isoquercitrin to isoquercitrin + 7 units of glucose (*see* Appendix 6). Minor constituents include quercetin, sodium sulfate, and higher glycosides of isoquercitrin. The complete analyses for these five batches EMIQ are also provided in a supplement to Appendix 6. The analysis of quercetin in these batches is shown in Appendix 7.

III. INFORMATION ON SELF-LIMITING LEVELS OF USE

This notification states San-Ei Gen F.F.I., Inc.'s conclusion that its EMIQ is GRAS for use in food to protect flavors and colors subject to the maximum concentrations shown in Table 3, set forth below in Section IV.B ("Intake Estimate"). These concentrations are self-limiting in the sense that the antioxidant is fully functional at the specified levels and higher levels will not provide any additional practical benefit. Results of stability tests, in which flavored beverages were exposed to sunlight for 36 hours, with or without 150 ppm EMIQ, are provided in Appendix 8a. Similar experiments, in which synthetic colorants in model beverages were exposed to radiation equivalent to one day of sunlight, with or without 75 ppm EMIQ, are reported in Appendix 8b.

IV. DETAILED SUMMARY OF BASIS FOR NOTIFIER'S GRAS DETERMINATION

A. Data and Information Relied on to Establish Safety

General recognition of safety for the intended use of EMIQ is based on scientific procedures, in keeping with 21 C.F.R. § 170.30(b), which consist of published and unpublished data relating to the safety, identity, characteristic properties, intended use, and estimated dietary exposure to the antioxidant. As explained in further detail below, EMIQ should be considered safe for the intended use based on:

(1) toxicity studies on EMIQ, *per se* (which include a 13-week rat study and a chronic toxicity/carcinogenicity study, both of which are published in peer-reviewed journals and which are adequate to support the safety of EMIQ at the anticipated dietary level);

(2) the metabolic similarity of EMIQ to natural plant constituents that have always been present in the human diet; and

(3) the body of toxicological data supporting the safety of the intake of quercetin, the aglycone of EMIQ, that may arise from the intended use of EMIQ.

B. Intake Estimate

The foods and the maximum EMIQ use levels are shown in Table 2, below.

Table 2

Foods to Which EMIQ May Be Added

Typical and Maximum Use Levels

Food	Typical Use Level (ppm)	Maximum Use Level (ppm)
Non-alcoholic beverages, including soft drinks, fruit and vegetable juices	75	150
Wine coolers (< 7% alcohol)	75	150
Shochu-based flavored beverages (< 7% alcohol)	75	150
Chewing gum	750	1500
Frozen dairy products	75	150
Flavored milk products	75	150
Gelatins and puddings	75	150
Jams and jellies	75	150
Soft candy	75	150
Cakes, cookies, pastries, pies	75	150
Soups	75	150

The most accurate estimate of the average chronic daily exposure to EMIQ would be one based on data from a food consumption survey of significant duration, *e.g.*, months to years. Because such data are not available, a common alternative approach is to combine amounts consumed per eating occasion from a briefer food consumption survey (*e.g.*, USDA's 1994-96, 1998 two-day *Continuing Survey of Food Intakes by Individuals* (CSFII)), with consumption frequency data such as that developed by the National Health and Nutrition Examination Survey (NHANES) conducted

by the Centers for Disease Control. A second alternative approach is available, however, for ingredients such as EMIQ with multiple intended uses. For example, a reasonable estimate of long-term average daily intake can be determined for ingredients with multiple uses based on the average daily intakes for the total survey population in the two-day CSFII. In the absence of either a consumption survey of significant length or frequency data, and in the absence of appropriate NHANES frequency of use categories, this is the approach that the notifier has used to estimate long-term exposure to EMIQ from its various intended uses, with the notable exception of soft drinks.

An intake class that should be addressed separately is that of soft drinks. Soft drinks, particularly carbonated soft drinks, may be consumed at high levels by a large segment of the population.[2] Intake reaches its maximum at adolescence and only slightly declines thereafter.[3] Therefore, it is prudent to assume potentially high uses over an extended time period. We understand that FDA estimates that the mean *users only* intake of "carbonated beverages" is 502.5 grams per person per day (g/p/d), and the 90th percentile users only intake is 1016.1 g/p/d.[4] These intakes were obtained from the 1994-96, 1998 USDA CSFII, and are properly for carbonated beverages, a subset of "soft drinks." Mean and 90th percentile intakes for "soft drinks"

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[2] In this respect, mean long term intake estimates would fail to account for long term consumption patterns by high users, because as the survey lengthens, more and more users are brought into the pool, whereas a true measure of chronic consumption would be to follow the specific population of high users over an extended time period.

[3] Mean intakes of soft drinks, as listed in Table 2.064 of USDA's *Foods Commonly Eaten in the U.S.*, a subset of CSFII, are essentially unchanged in ages 12-39 (814 g males, 12-19; 804 g males, 20-39) and decline slightly in age groups 40-59 (642 g males). Corresponding female intakes are lower (590 g, 12-19; 630 g, 20-39) but are quite similar when expressed as intakes per unit body weights. (Mean intakes in this table are intakes for a single day over a 2 day sampling time, and are not averages over 2 days.)

[4] Communication from Dr. Paul Kuznesof, Senior Chemist at FDA's Center for Food Safety & Applied Nutrition, Office of Food Safety, to Dr. Michael T. Flood, Keller and Heckman LLP, November 16, 2005. The data reflect consumption averaged over a two day period.

are lower than for "carbonated beverages" because of inclusion of other beverages whose daily consumption is significantly lower than that of carbonated beverages.

As a conservatism, we use the 90th percentile users only intake of 1016.1 g/p/d for "carbonated beverages" in estimating intake for "soft drinks." However, a high intake user of carbonated beverages is not likely to be a regular user of fruit drinks, vegetable juices, wine coolers or flavored milk products. Therefore, the value of 1016.1 g/p/d for carbonated beverages will encompass these other relatively minor uses. A highly conservative intake estimate is provided in Table 3.

The foods in which EMIQ may be used as a GRAS ingredient, typical use levels, and estimated intakes of the ingredient for specific foods and categories (based primarily on average daily intakes for the total survey population in the CSFII 1994-96, 1998 survey) are provided in Table 3, below. Estimated intakes for specific food products or food categories were obtained from the USDA reports as indicated by the notes to the table, and in the subsequent section on methodology.

Table 3

Foods to Which EMIQ May Be Added

Estimated Intakes

Food [a]	**Maximum Use Level** ppm, mcg/g food (mg/g food)	**Estimated Intake of Food Class** [b] g/day	**Estimated EMIQ Intake** [c] mg/day
Beverages, non-alcoholic soft drinks, includes carbonated beverages, fruit drinks, flavored milk products, wine coolers, and shochu-based flavored beverages (<7% alcohol)	150 (0.15)	1016.1	152.4

Food [a]	Maximum Use Level ppm, mcg/g food (mg/g food)	Estimated Intake of Food Class [b] g/day	Estimated EMIQ Intake [c] mg/day
Candy and Gelatins	150 (0.15)	7●	1.05
Chewing gum	1500 (1.5)	1.5	2.25
Cakes	150 (0.15)	9■	1.35
Cookies	150 (0.15)	8■	1.2
Jams and Jellies	150 (0.15)	12	1.8
Pies	150 (0.15)	7■	1.05
Frozen Desserts (USDA survey category: milk desserts)	150 (0.15)	27●	4.05
Soups	150 (0.15)	52■	7.8
Estimated Intake From Food Uses			173 mg/day

Notes:

a. Food classes are indicated as proposed by the notifier. The food categories used to estimate daily intakes were obtained primarily from USDA's CSFII 1994-96 survey (details provided below) as presented and published in Table Set 10 (denoted by a black circle ●), February 1999, and in *Foods Commonly Eaten in the United States* (FCEUS), Appendix B (denoted by a black square ■), January 2002.[5] One exception is the value for jams and jellies taken from the Nationwide Food Consumption Survey of 1977-78 (*Foods Commonly Eaten by Individuals*, February 1990). The chewing gum use rate, representing the average use of stick chewing gum, was obtained from a proprietary survey provided to the National Chewing Gum Association by The NPD Group, Inc., Rosemont, IL. There are no current intake data available for "Shochu-based flavored beverages" in the United States. As noted in footnote 1, shochu is a distilled alcoholic

[5] The USDA CSFII 1994-96 survey was modified by the 1998 CSFII survey, which focused on children from birth to 9 years old. Results from the 1998 survey were combined with the 1994-96 survey. The combined survey is referred to as 1994-96, 1998.

beverage, indigenous to and popular in Japan, that usually contains about 25% alcohol. The beverage proposed for the sale in the U.S. market contains shochu and other ingredients, such as fruit juices, and has an alcohol content of less than 7 percent. In the absence of specific intake data for shochu-based flavored beverages, the notifier submits that intake data for "wine coolers" is comparable and representative of the likely intake. The intake of "wine coolers" is calculated as 10% of wine intake, as reported in CSFII 1994-96, 1998.

b. Total population intakes of food products and food categories were obtained from the USDA surveys mentioned. Categories most closely corresponding to the intended use of the ingredient were used whenever possible. When specific applications corresponded to a small proportion of the USDA category, the intake of that food was adjusted by estimation. As discussed above, beverage intake is not the mean total population intake, but 90th percentile, users only intake of carbonated beverages.

c. The estimated daily intake (EDI) of the ingredient was calculated as:

EDI = maximum use level (mg/g food) x estimated intake of food class (g/day)

1. Methodology

As discussed at the beginning of this Section B, in the absence of access to longer-term surveys or consumption frequency data, USDA's series of two-day food consumption surveys offer a reasonable way to estimate average long-term intakes for multiple-use additives, such as EMIQ. The notifier's intake estimate for EMIQ relies primarily on data contained in USDA's 1994-96, 1998 CSFII survey and information on the average intake of foods in the United States (calculated on the total-population basis) found in USDA's FCEUS. In Table 3, above, with the exception of soft drink consumption, the values provided for "Mean Population Intake of Food Class" are the mean quantities consumed per person per day for the food classes identified, as reported in Appendix B of FCEUS. These mean quantities consumed provide an approximation of long-term average daily intake by consumers. Soft drink consumption is considered a special case, due to the high use by large segments of the population. The 90th percentile users only intake of 1016 g/p/d for carbonated beverages is used for soft drinks. It then is unrealistic to consider other beverage intakes independently of soft drink consumption, as a high percentile user of soft drinks is not likely to consume other beverages in significant quantities. Using this methodology, Table 34 shows that the EDI for EMIQ is 173 mg/p/d, and is driven almost entirely by soft drink consumption. For this reason, no attempt was made to perform a more sophisticated analysis, such as Monte Carlo modeling. The intake estimate is highly conservative. The maximum EMIQ use level, as opposed to typical use level, was used in the estimate, use of 2-day survey intakes overestimates chronic consumption, and EMIQ is not likely to be used in all or even most beverages and other foods for which an intake was calculated. As discussed in Section IV.D.1.b.iv, below, based on the "no observed adverse effect level" (NOAEL) established from a two-year carcinogenicity study of EMIQ in rats, the acceptable daily intake (ADI) is 293 mg/p/d.

C. ADME of EMIQ, Quercetin, and Quercetin Glycosides

The absorption, distribution, metabolism, and excretion (ADME) of EMIQ are relevant to the aglycone, quercetin, which showed some evidence of carcinogenicity in a study sponsored by

the National Toxicology Program (NTP), as discussed in Section D. Although an *in vivo* ADME study of EMIQ has not been carried out, many metabolism and pharmacokinetics studies of quercetin and quercetin glycosides have been reported.

The metabolism of quercetin and quercetin glycosides has not been completely elucidated, but the following is known:

1. *In vitro* metabolic study of EMIQ.

   EMIQ was found to degrade *in vitro* in the presence of salivary and pancreatic *alpha*-amylase. As shown by HPLC, isoquercitrin glycosides IG_3-IG_7 (*see* Figure 1) were converted to isoquercitrin (IQC), IG_1, and IG_2 by action of the amylases over the time period that the enzymes are expected to be in contact with EMIQ after ingestion. IQC, IG_1, and IG_2 were not hydrolyzed by these enzymes. No degradation of EMIQ was observed in simulated gastric juice. (Tamura *et al.*, 2005.)

   Although an *in vitro* study, the results strongly suggest that, by the time EMIQ enters the small intestine, only IQC, IG_1, and IG_2 are present.

2. Quercetin and quercetin glycosides: probable human metabolism.

   Quercetin glycosides or plant extracts containing an equivalent concentration of these glycosides were respectively administered to healthy human volunteers. Only quercetin glucuronides, but no free quercetin, were detected in plasma by HPLC analysis. This study demonstrated that quercetin glycosides produce the same metabolites, and one metabolite is not transformed into another. Isorhamnetin was the only detectable phase-I metabolite, and only conjugated forms of isorhamnetin and quercetin were present in human plasma and urine. (Graefe *et al.*, 2001.)

EMIQ not absorbed in the small intestine may be metabolized and absorbed in the large intestine as phenolic acids or as quercetin, *per se*.

Two types of human anaerobic bacteria which can utilize quercetin-3-glycoside were isolated from human feces. One utilizes the glucose moiety of glycosides as a carbon and energy source; the other degrades the aromatic ring system. The following metabolites were detected: acetate, butyrate, ethanol, hydrogen, and 3,4-dihydroxyphenylacetic acid. (Schneider *et al.*, 1999.)

A suggested metabolic pathway of EMIQ in humans is provided in Appendix 9, based on Tamura *et al.* (2005), Terao (2005), Choudhury *et al.* (1999), and Schneider *et al.* (1999).

D. Toxicological Studies

1. Toxicity Data on EMIQ, *per se*

a. Genotoxicity

EMIQ tested negative for mutagenic activity in the bacterial reverse mutation assay using *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, TA1538, and *E. coli* strain WP-*uvr*A. (*See* Appendix 10, San-Ei Gen F.F.I., Inc. Report.)

b. Oral Toxicity

i. Acute Toxicity Study

The oral LD_{50} in Sprague-Dawley rats is >25 g/kg. (*See* Appendix 11, San-Ei Gen F.F.I., Inc. Report.)

ii. 4-Week Toxicity Study

In a range-finding test prior to the 90-day study, F344/DuCrj rats received EMIQ at 0, 0.625, 1.25, 2.5, or 5.0% in their diets for 4-weeks. No clinical signs or mortality related to treatment were found during the study. A tendency for lower mean body weights was observed in both sexes of the 2.5% and 5% groups. On macroscopic examination, yellow discoloration of the cranium and fore- and hind-limb bones was found in both sexes receiving 1.25% or more of the test substance, which is yellow to yellow-orange in color. The bone discoloration was unaccompanied by any histopathological changes. Organ weights did not differ significantly from those of the controls. (*See* Appendix 12, San-Ei Gen F.F.I., Inc. Report.)

iii. 13-Week Toxicity Study

Ten F344/DuCrj rats per sex, per group received 0, 0.3, 0.625, 1.25 or 2.5% EMIQ in their diets. An additional 10 rats/sex/group received 0 or 2.5% EMIQ for 13-week, followed by a 4-week recovery period. Yellow discoloration of the cranium and femoral bone was seen in females in the 1.25% and 2.5% groups and in males in the 0.625% and higher groups, and yellow or yellow-brown discoloration of the urine was observed in all treated groups. The discoloration was still observed in animals after the 4-week recovery period. Decreased body weight gain in both sexes was observed in the 2.5% group. (Tamano *et al.*, 2001; *see* Appendix 13a.)

After the study was published, the authors revised the NOAEL from 0.3% to 1.25%, due to conclusions from an external peer review. The peer reviewer concluded that, although the observed bone discoloration was treatment related, the absence of any histopathological correlations indicates that the effect was not toxicologically significant (M. Takahashi, 2002; *see* Appendix 13b). The principal investigator concurred that the NOAEL should be 1.25% (S. Tamano, 2002, Appendix 13c). A NOAEL of 1.25% corresponds to a dietary intake of about 800 mg/kg bw/day.

iv. Chronic Toxicity/Carcinogenicity Study

Groups of 50 male and 50 female F344/DuCrj rats received 0, 0.5 or 1.5% EMIQ in their diets for 104 weeks. The doses were set from the results of the 13-week study. There were no treatment-related clinical signs of toxicity in the treated groups. Body weights, feed consumption, survival rates, and blood and clinical chemistry did not differ from controls. There was a slight, but significant decrease in relative spleen weights in all treated groups, with no histopathological correlate. An increase in the incidence of pituitary adenomas in the high-dose females was observed, which was not judged to be treatment-related. There were no apparent effects of the test substance on development of kidney neoplasms, hyperplasias, or chronic nephropathy. Bone discoloration was not reported. (Salim *et al.*, 2004; *see* Appendix 14a)

Based on the decrease in spleen weights, Salim *et al.* concluded that "the lowest non-effective dose level was less than 0.5% in the diet . . . ." The principal investigator recently re-reviewed the haematoxylin and eosin-stained spleen sections from all dosage groups together with the controls and concluded that the decrease in spleen weights was not toxicologically significant due to the absence of accompanying microscopic lesions (January 5, 2006 letter from Professor Shoji Fukushima to Professor Joseph F. Borzelleca. *See* Appendix 14b). Based on this conclusion, the NOAEL for EMIQ is at least 1.5% in the diet (the highest dose level), corresponding to 598 mg/kg/day for females and 489 mg/kg/day for males. Using a 100-fold safety factor, the lower NOAEL converts to an acceptable daily intake (ADI) of 4.89 mg/kg/day, or 293 mg/person/day (mg/p/d), (60 kg/p).

2. Toxicity Studies on Related Flavonoids

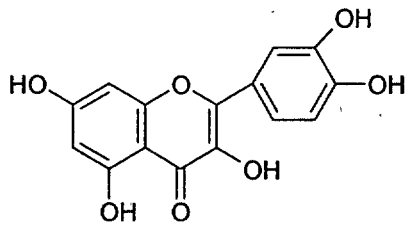
a. Enzymatically Decomposed Rutin (Isoquercitrin)

Enzymatically decomposed rutin is primarily (>95%) isoquercitrin, a major component of EMIQ and the one likely to be absorbed in the small intestine at the highest concentration. Ten male and 10 female Wistar rats received 0, 0.2, 1 or 5% of the test substance in their diets for 13 weeks. No mortality or obvious clinical signs, except for yellow urine, were observed throughout the treatment period. A slight reduction in body weight was observed in the 5% male group. There was no treatment-related effect on absolute or relative spleen weights. There were no obvious macroscopic findings in any group. Bone discoloration was not mentioned. No renal toxicity was observed. Based on slightly reduced body weight gains, which were not statistically significant, and changes in certain blood and clinical chemistry parameters in males, the NOAEL was set at 1% for males (539 mg/kg bw/day) and 5% for females (3227 mg/kg bw/day). (Hasumura *et al.*, 2004.)

b. Quercetin

Quercetin is the aglycone of EMIQ. Quercetin is widely distributed in fruits and vegetables in the form of glycoside conjugates, not as the aglycone itself. The structure is the following:

Quercetin



Genotoxicity data have been summarized by the International Agency for Research on Cancer (IARC) (1983, 1999). Quercetin has tested positive for genotoxicity in most *in vitro* assays. It has induced gene mutations in base substitution as well as frameshift strains of *Salmonella typhimurium* with and without exogenous metabolic activation. It also has tested positive in mouse lymphoma assays, chromosomal aberration assays, and sister chromatid exchange assays in various cell lines. In contrast to the *in vitro* assays on quercetin, most of the *in vivo* tests have been negative, including mouse and rat micronucleus assays, a rat chromosomal aberration assay, and dominant lethal mutation assays in rats and mice.

Quercetin was tested in several carcinogenicity studies in rats by oral administration in the diet and by topical application in mice. These studies are summarized in Table 4.[6]

Table 4

Animal	No. Animals	Dose	Length	Response	Reference
DdY Mice	38M/35F	2%	842 d	negative	Saito (1980)
A/JJms Mice	23M/24F	5%	23 wk	negative	Hosaka (1981)
Albino Rats	7M/18F	0.1%	58 wk	increase in intestinal and bladder tumors	Pamukcu (1980)
ACI Rats	10M/10F	1%	540 d	negative	Hirono (1981)
	8M/9F	5%	540 d		

[6] Of the nine studies summarized in the table in the text, only the last two, Ito (1989) and National Toxicology Program (NTP) (1992), were conducted according to current standards. Nevertheless, in aggregate, the seven studies conducted between 1980 and 1984 present a strong indication that quercetin is not carcinogenic. The results of the one study conducted during this period that showed carcinogenic activity (Pamukcu *et al.*, 1980) could not be repeated in other laboratories, suggesting that they could be due to the specific strain of rat or the diet.

Animal	No. Animals	Dose	Length	Response	Reference
	20M/20F	10%	850 d		
F344 Rats	15M/15F	0.1%	540 d	negative	Takanashi (1983)
F344 Rats	9M/11F	0.1%	64 wk	negative	Stoewsand (1984)
	8M/8F	0.2%			
Golden Hamsters	20M/20F	10%	735 d	negative	Morino (1982)
	15M/15F	4%	709 d		
F344/DuCrj rats	50M/50F	1.25%	104 wk	negative	Ito (1989)
		5.0%			
F344/N rats	50M/50F	0.1%	104 wk	increase in renal tubular tumors	NTP (1992)
		1%			
		4%			

Evidence of carcinogenic activity was seen in only two studies in rats. Quercetin increased the incidences of intestinal and urinary bladder tumors in one study (Pamukcu, 1980), but this effect was not seen in subsequent studies. In the other study (NTP, 1992) quercetin produced a low, but statistically significant, increase in the incidence of renal tubular neoplasms, primarily adenomas, in male rats. The increase reached statistical significance only after step-sectioning of renal tissue. Moreover, all kidney neoplasms were adenomas with the exception of a single adenocarcinoma in one high-dose male. The study authors concluded that these results provided "some evidence of carcinogenic activity" in male rats and "no evidence of carcinogenic activity" in female rats. Carcinogenicity in the kidney has not been found in other studies of quercetin in rats, mice, or hamsters. IARC (1999) has concluded that there is limited evidence in experimental animals for the carcinogenicity of quercetin, and that the substance is not classifiable as to its carcinogenicity to humans (Group 3).

The issue of potential quercetin carcinogenicity is relevant to EMIQ not only because quercetin is a minor impurity in EMIQ, but also because of the likelihood that EMIQ is metabolized to quercetin, or more accurately, because the metabolites of quercetin and EMIQ present in plasma may be identical. Therefore, in an effort to more clearly elucidate the mechanism of tumor formation in the kidney, San-Ei Gen F.F.I., Inc. sponsored a study by Dr. Gordon C. Hard, a recognized expert on renal pathology. Dr. Hard reviewed the histology slides and the pathology data at the NTP archives. His complete report is provided in Appendix 15.[7]

Dr. Hard reached the following conclusions:

1. There was a modest increase in renal tubule tumors in the mid- and high-dose male rats, but because of down grading of some adenomas to atypical tubule hyperplasia (ATH) the counts of these lesions were lower than reported in the NTP study.

2. Spontaneous chronic progressive nephropathy (CPN) was exacerbated by chemical treatment in the high-dose males, and to a lesser extent in the mid-dose males. Thus, 20% of the high-dose males and 12% of the mid-dose males had end-stage CPN compared to only 2% in the control and low-dose males. There was no exacerbation of CPN in the high-dose females, correlating with no increase in renal tubule tumors.

3. The adenomas were small and borderline basophilic lesions, and along with basophilic foci of ATH, they occurred predominantly in rats having advanced CPN. Dr. Hard concludes that the neoplastic response is linked to exacerbated CPN.

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[7] This report was presented at the 25th Annual Meeting of *the Society of Toxicologic Pathology* in Vancouver, Canada, in June 18-22, 2006, *in press*: Hard, G.C. *et al.*, Re-evaluation

4. Based on careful microscopic examination of the relevant lesions, and their distinctive morphology, the single carcinoma, observed in a high-dose male, and 4 other lesions ranging from ATH to adenoma present in either males or females, are considered to be of spontaneous (possibly familial) origin, unrelated to quercetin treatment.

5. Examination of renal tissue not involved in the CPN process at 6 months, 15 months, and 2 years showed no evidence of any tubule alterations that would be indicative of chemical-associated toxicity. Therefore, modes of action involving direct or indirect DNA reactivity, sustained cytotoxicity and regeneration, or *alpha*-2μ-globulin nephropathy are excluded.

6. The most likely mechanism underlying tumor development in the NTP study is interaction of quercetin with CPN. This mode of action is believed to have no relevance for species extrapolation in risk assessment, as the syndrome of rat CPN appears to have no strict counterpart in humans.

Therefore, the weight of evidence from all animal studies indicates that quercetin is not of carcinogenic risk to humans.

c. Rutin

Rutin, a quercetin glycoside, has been tested for carcinogenicity in rats and hamsters, but the studies were not run under current test protocols. As summarized in IARC (1983), a group of 11 male and 10 female ACI rats received 5% rutin in their diets for 540 days. A second group of 20 males and 20 females received a diet containing 10% rutin for up to 850 days. Groups of

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of the kidney tumors and renal histopathology . . . ., *Food Chem. Toxicol.* (2006), doi:10.1016/j.fct.2006.10.018

30 males and 22 females and of 33 males and 33 females, respectively, were fed a normal diet. No significant increase in the incidence of tumors was observed (Hirono *et al.*, 1981). Groups of 20 male and 20 female golden hamsters were fed 0 or 10% rutin for up to 735 days. There was no significant increase in the incidence of tumors in the treated animals (Morino *et al.*, 1982).

E. Information Unfavorable to the GRAS Determination

The fact that quercetin has tested positive for genotoxicity in *in vitro* assays and that evidence of carcinogenic activity was found in two studies of quercetin in rats, on its face, obviously could be viewed as unfavorable information *vis-à-vis* the GRAS determination since quercetin is an impurity in EMIQ and it is likely that the metabolites of quercetin and EMIQ present in plasma may be identical. The issue of potential quercetin carcinogenicity is discussed thoroughly in Section IV.D.2.b, above, in which it is concluded that the weight of evidence from all animal studies indicates that quercetin is not of carcinogenic concern to humans.

F. Basis for Concluding that the Notified Use of EMIQ Is GRAS

San-Ei Gen F.F.I., Inc. has concluded that the above-described use of EMIQ has been shown to be GRAS for the intended use on the basis of scientific procedures, in accordance with 21 C.F.R. § 170.30. This conclusion is supported by the determinations of an independent panel of qualified experts[8], which evaluated the safety of EMIQ and determined the general recognition of safety of the substance under its intended conditions of use in food.

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[8] The Panel consisted of Professor Joseph F. Borzelleca, Ph.D., Department of Pharmacology and Toxicology of the Medical College of Virginia, Virginia Commonwealth University; Walter Glinsmann, M.D., Glinsmann, Inc.; and Professor Jesse Gregory, Ph.D., University of Florida Food and Agricultural Sciences. The Panel's report is attached as Appendix 1.

G. Conclusion

Considering the foregoing, we respectfully submit that all criteria for general recognition of safety based on scientific procedures are met and, thus, that EMIQ is generally recognized as safe for addition at the specified levels to the above-described foods to protect flavors and colors.

V. LIST OF APPENDICES

1. Conclusions of Expert Panel
2. Journal of Chromatography A, 895, 279-283, (2000)
3. J. Food Hyg. Soc. JAPAN 41(1), 54-60, (2000)

4a. Analysis of Enzymatically Modified Isoquercitrin for Enzyme Activity, San-Ei Gen F.F.I., Inc., September 15, 2006

4b. Safety of enzymes used in making EMIQ, San-Ei Gen F.F.I., Inc., August 1, 2006

4c. Enzyme Specifications, San-Ei Gen F.F.I., Inc., September 15, 2006

5. Analysis of specifications on EMIQ, San-Ei Gen F.F.I., Inc., August 1, 2006
6. Composition Analysis of EMIQ, San-Ei Gen F.F.I., Inc., July 18, 2003; Supplement
7. Analysis of quercetin in EMIQ, San-Ei Gen F.F.I., Inc., May 15, 2003

8a. Stabilization effect of EMIQ on flavors, San-Ei Gen F.F.I., Inc., June 25, 1993

8b. Preventive effect of EMIQ on food colorants' deterioration, San-Ei Gen F.F.I., Inc., November 6, 2006

9. Probable metabolic pathway of EMIQ.

10. Bacterial reverse mutation study of enzymatically modified isoquercitrin (SANMELIN®), San-Ei Gen F.F.I., Inc., December 19, 1986

11. Acute oral toxicity study of SANMELIN® 11130 in rats, San-Ei Gen F.F.I., January 10, 1987

12. Preliminary study for 90-day oral toxicity of enzymatically modified isoquercitrin in F344/DuCrj rats, San-Ei Gen F.F.I., Inc., January 28, 2003

13a. Tamano S., Hatahara Y., Sano M., Hagiwara A., Nakamura M., Washino T. and Imaida K. (2001). 13-Week oral toxicity and 4-week recovery study of enzymatically modified isoquercitrin in F344/DuCrj rats. *Jpn. J. Food Chem.* **8(3),** 161-167

13b. Results of peer review on the final report of "Ninety-Day Repeated Oral Toxicity Study and 28-Day recovery study of enzymatically modified isoquercitrin in rats," reviewed by Dr. Michihito Takahashi, October 8, 2002

13c. Answer for the peer review, Dr. Seiko Tamano, November 14, 2002

14a. Salim E., Kaneko M., Wanibuchi H., Morimura K. and Fukushima S. (2004). Lack of carcinogenicity of enzymatically modified isoquercitrin in F344/DuCrj rats. *Food Chem. Toxicol.* **42(12),** 1949-1969

14b. January 5, 2006 letter from Professor Shoji Fukushima to Professor Joseph F. Borzelleca

15. Expert report on the re-evaluation of renal histopathology in the NTP 2-year carcinogenicity bioassay of quercetin, reviewed by Dr. Gordon C. Hard; *in press*: Hard, G.C. *et al.*, Re-evaluation of the kidney tumors and renal histopathology . . . ., *Food Chem. Toxicol.* (2006), doi:10.1016/j.fct.2006.10.018

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Appendix 1

REPORT OF THE EXPERT PANEL ON THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF ALPHA-GLYCOSYL ISOQUERCITRIN (EMIQ)

INTRODUCTION

San-Ei Gen F.F.I., Inc. (San-Ei Gen), convened an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, to conduct a critical and comprehensive evaluation of the available pertinent data and information on alpha-glycosyl isoquercitrin (EMIQ), and determine whether, under the conditions of intended use as an antioxidant to protect flavors and colors in non-alcoholic beverages, including soft drinks, fruit juices, vegetable juices, flavored milk products, wine coolers, shochu-based flavored beverages, frozen dairy products, gelatins and puddings, jams and jellies, soft candy, cakes, cookies, pastries, pies, powdered or canned soups, and chewing gum, it is safe and would be Generally Recognized As Safe (GRAS) based on scientific procedures. The Panel consisted of the following qualified scientific experts: Prof. Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University, Medical College of Virginia), Walter Glinsmann, M.D. (President, Glinsmann, Inc.) and Prof. Jesse F. Gregory III, Ph.D. (University of Florida). *Curricula vitae* of the Panel are provided in Attachment 1.

The Panel, independently and collectively, critically examined a comprehensive package of data and information compiled from the literature and other published sources through June 2006 ("GRAS dossier") prepared by Dr. Michael Flood, staff toxicologist at Keller and Heckman, and organized in the form of a GRAS notification (GRN). The Panel also evaluated other information deemed appropriate or necessary. The Panel evaluated information pertaining to the method of manufacture and product specifications, supporting analytical data, intended use levels in specified food products, consumption estimates for all intended uses, and a comprehensive assessment of the available scientific literature pertaining to the safety of EMIQ. Following independent, critical evaluation of such data and information, the Panel unanimously concluded that, under the conditions of intended use in foods described herein, EMIQ meeting appropriate food-grade specifications and manufactured and used consistent with current Good Manufacturing Practice (cGMP) is safe and GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below.

MANUFACTURE

EMIQ is manufactured from rutin, quercetin-3-rhamnoglucoside, in two principal steps: rutin is isolated from *Sophora japonica* and converted enzymatically to isoquercitrin using rhamnosidase (EC. 3.2.1.40); and an aqueous solution of isoquercitrin and dextrin is treated with cyclodextrin glucanotransferase (EC. 2.4.1.19), which adds glucose units to form EMIQ, which has an average molecular weight of about 800 daltons. The solution is then heated to inactivate the enzymes and filtered and evaporated under

vacuum. The residue is dissolved in ethanol and the impurities removed by crystallization. The ethanol solution is evaporated under vacuum to yield EMIQ. Analyses confirm that there is no enzymatic activity in the finished EMIQ. All enzymes used in the production of EMIQ are on the List of Existing Food Additives in Japan's Food Sanitation Law and are produced under food additive GMP in Japan.

San-Ei Gen's product is similar to a preparation known as "alpha-G-rutin." This latter compound is prepared by enzymatically adding glucose units to the glucose moiety of rutin, without first cleaving off the rhamnose group. Both EMIQ and alpha-G-rutin are water soluble, in contrast to quercetin or rutin, and this property facilitates their use in beverages.

EMIQ consists of isoquercitrin (quercetin-3-glucoside) and up to 10 additional glucose units in the readily digestible alpha-1,4 linkage. The relative percentages of the individual glucosides decline with increasing chain length from approximately 20% for isoquercitrin with an additional one glucose to approximately 1% for isoquercitrin with 10 additional glucose units. There is no evidence of residual rutin in analysis by high performance liquid chromatography or capillary electrophoresis. Quercetin is reportedly present at less than 1%.

SPECIFICATIONS/QUANTITATIVE COMPOSITION

Specifications have been established and these appear in Appendix 5 of the GRAS dossier. Five different lots were analyzed and consistency was clearly demonstrated (Appendices 6 and 7 of GRAS Notification). Some of the minor constituents include quercetin, sodium sulfate and higher glycosides of isoquercitrin.

EMIQ is recommended for use at levels consistent with cGMP, that is, at levels that will provide the desired antioxidant effect; higher levels will not provide any additional practical benefit. Technical effect data are provided in Appendix 8 of the GRAS Notification. When bottles containing a flavored cider preparation and 150 ppm EMIQ were exposed to sunlight for 36 hours, the profile of 19 flavor components, as determined by gas chromatography, remained essentially unchanged. In another experiment, three synthetic FD&C food colorants in dilute solutions containing 75 ppm EMIQ were stable when irradiated with a xenon lamp for 1 and 2 hours, equivalent to one day of sunlight in midsummer.

EMIQ was stable when stored at 60°C over a 5 week period.

PROPOSED USES/ESTIMATED EXPOSURES

EMIQ is used as an antioxidant in various foods. The proposed maximum use levels are 150 ppm for beverages, desserts, ice cream, bakery products (cakes, cookies) and 1500 ppm in chewing gum. Keller and Heckman estimated the intake from proposed uses using data from USDA's 1994-1996 Continuing Survey of Food Intakes by Individuals and maximum use levels of EMIQ and reported an estimated mean daily intake (EDI) of 173 mg/person/day (*cf.* Section C and Tables 3 and 4 of the GRAS dossier). Intake of carbonated beverages is estimated as the 90th percentile users-only

intake, a highly conservative assumption made because of potential high intakes of soft drinks in certain subpopulations, such as young adults. Consumption of "carbonated beverages" exceeds that of "soft drinks," so use of carbonated beverage intake is especially conservative. Intake of other foods is based on the average intakes for the entire population, not users-only intakes. This procedure provides a more realistic estimate of chronic exposure than more conservative estimates, such as summation of users-only average or 90th percentile intakes.

Exposure to quercetin due to its presence in fruits and vegetables has been estimated at about 25 mg/person/day. Most of the quercetin naturally occurring in food is present as glycoside conjugates and this form of quercetin more nearly approximates that of EMIQ.

SAFETY ASSESSMENT OF EMIQ

Absorption, Distribution, Metabolism, Excretion Studies

Studies in animals or humans are not available on EMIQ, *per se*, but numerous studies are available on related compounds including quercetin and quercetin glycosides. It is likely that EMIQ may be biotransformed and absorbed in the large intestine as phenolic acids or as quercetin, *per se*. A metabolic pathway for EMIQ is presented in Appendix 9 of the GRAS Notification.

In vitro, isoquercitrin glycosides IG_3-IG_7 were reported to degrade in the presence of salivary and pancreatic alpha-amylase to isoquercitrin (IQC), IG_1 and IG_2. EMIQ was not degraded in simulated gastric juice.

When quercetin glycosides were administered to healthy human volunteers, only quercetin glucuronides, but no free quercetin, were detected in plasma. This study demonstrated that quercetin glycosides produce the same metabolites and one metabolite is not transformed into another. Quercetin and isoquercitrin exhibit similar bioavailability when administered orally in human and animal (rat and pig) pharmacokinetic studies.

Acute Toxicity

The acute oral toxicity of EMIQ in rats is > 25 g/kg bw.

Subacute Toxicity

In a range-finding test (4 weeks) prior to the 90-day study, F344/DuCrj rats received EMIQ at 0, 0.625, 1.25, 2.5, or 5.0% in their diets. No clinical signs or mortality related to treatment were reported. Macroscopic examination revealed yellow coloration of the cranium and fore- and hind-limb bone in all animals receiving 1.25% or more of the test substance.

Subchronic Toxicity

F344/DuCrj rats received 0, 0.3, 0.625, 1.25 or 2.5% EMIQ in their diets for 13 weeks. There were no consistent, treatment-related, dose-dependent adverse effects reported. Yellow coloration of the cranium and femoral bone was seen in the females at the 1.25% and higher and in the males at 0.625% and higher. These were unaccompanied by any histopathological change. The no observed adverse effect level (NOAEL) was reported to be 1.25%, which corresponds to about 800 mg/kg bw./day. This is a conservative NOAEL. If the bone coloration is not biologically/toxicologically significant, then the NOAEL should be 2.5%.

Chronic Toxicity

In a 24-month carcinogenicity study, F344 male and female rats received 0, 0.5, or 1.5% EMIQ in their diets. There were no consistent, treatment-related, dose-dependent adverse effects reported in clinical signs, body weights, food intake, hematology, clinical chemistry, and histopathology. No significant increases in neoplasms were observed upon gross examination. Histological examination, performed on the control and 1.5% groups, revealed an increased incidence of adenomas in the anterior lobe of the pituitary gland in the 1.5% group, but the increase was not statistically significant. No other significant increases in neoplasms were observed. The NOAEL was determined to be the highest level tested, which corresponded to 489 mg/kg bw./day for males and 597 mg/kg bw./day for females. There was no mention of bone discoloration that was observed in the 28-day and 90-day studies.

Genotoxicity

EMIQ tested negative in the bacterial reverse mutation assay using *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, TA1538, and *E. coli* strain WP-uvrA.

Toxicity Studies on Related Flavonoids

Enzymatically Decomposed Rutin (Isoquercitrin)

Enzymatically decomposed rutin is primarily (>95%) isoquercitrin, the major component of EMIQ, and is readily absorbed in the small intestine. Male and female Wistar rats received 0, 0.2, 1 or 5% of the test substance in the diet for 13 weeks. The NOAEL was determined to be 1% in males (based on changes in hematology and clinical chemistry) and 5% for females. Equivalent intakes at these levels were 539 mg/kg bw./day for males and 3227 mg/kg bw./day for females.

Quercetin

Quercetin is the aglycone of EMIQ, and is widely distributed in fruits and vegetables in the form of glycoside conjugates, not as the aglycone itself. Nine studies on the potential carcinogenicity of quercetin were reviewed but only two meet current standards. One study (Ito, 1989) reported no evidence of carcinogenicity in F344/DuCrj male and female rats at levels of 1.25% and 5.0% for 104 weeks. The NTP study (1992) conducted in male and female F344/N rats revealed renal tumors in male rats only and the authors concluded that there was some evidence of carcinogenic activity in male rats and no evidence of carcinogenic activity in female rats. IARC (1999) concluded that there is limited evidence in experimental animals for the carcinogenicity of quercetin, and that the substance is not classifiable as to its carcinogenicity to humans. Dr. Gordon Hard, an internationally recognized expert in renal pathology, read the NTP slides and concluded that the tumors observed in the male rats involved spontaneous chronic progressive nephropathy (CPN) and therefore have no relevance for species extrapolation since rat CPN has no human counterpart.

Rutin

Rutin is a quercetin glycoside. The available information on the potential carcinogenicity of rutin, which includes dietary studies in ACI rats and golden hamsters, suggests that rutin is not carcinogenic in the animals tested.

Summary

The weight of available evidence of the safety data for isoquercitrin (EMIQ), quercetin, and rutin suggests that these substances are not carcinogenic for humans and possess a low order of toxicity. The NOAEL for EMIQ is approximately 500 mg/kg bw./day.

CONCLUSION

We, the Expert Panel, have, independently and collectively, critically evaluated the data and information summarized in the GRAS dossier for alpha-glycosyl isoquercitrin (EMIQ) and conclude that the use of EMIQ, meeting appropriate food grade specifications described herein and manufactured consistent with cGMP, as an antioxidant to protect colors and flavors in foods and beverages at levels presented herein (consistent with current GMP), is safe and Generally Recognized As Safe (GRAS) based on scientific procedures.

Professor Joseph F. Borzelleca, Ph.D.

Medical College of Virginia/VCU

Professor Jesse F. Gregory III, Ph.D.

University of Florida

Walter Glinsmann, M.D.

Glinsmann, Inc.

Attachment 1

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American Association for the Advancement of Science
American Chemical Society
American College of Toxicology*
American Society of Pharmacology and Experimental Therapeutics**
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Institute of Food Technologists (Professional Member)

International Society of Regulatory Toxicology and Pharmacology*
(Councilor
Sigma XI
Society of Experimental Biology and Medicine*
(Councilor; Program Chairman of Southeastern Section)
Society for RiskAnalysis
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Board of Scientific and Policy Advisors

American Council on Science and Health

Journals

Editor, Food Chemical Toxicology, 1992-

Editorial Board
Environmental Carcinogenesis Reviews
Journal of Environmental Pathology, Toxicology Oncology
Journal of Environmental Science and Health,
Journal of the American College of Toxicology
Journal of Toxicology: Cutaneous and Ocular Toxicology
Journal of Applied Toxicology,
Pharmacology,
Pharmacology and Drug Development,
Toxicology and Applied Pharmacology,

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

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)
Introduced the use of closed circuit TV and TV tapes in pharmacology
Introduced clinical pharmacological experiments into the medical and dental programs
Planning and participation in continuing education program (Schools of Dentistry, Medicine and Pharmacy)
Planning and administering each of the three major efforts in pharmacology (dental, medical, pharmacy) since
Graduate Program - assisted in developing graduate training program in toxicology

Current Teaching Activities

Presents 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,

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

Thomas Jefferson University
Distinguished Alumnus Award,

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

Virginia Commonwealth University - School of Basic Health Sciences, Dept. of Pharmacology and Toxicology
Professor of the Year-

American College of Toxicology
Distinguished Service Award-

Awards

Virginia's Life Achievement in Science Award- April

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

International Society for Regulatory Toxicology and Pharmacology's International Achievement Award for

Society of Toxicology- Education Award-

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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.

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

ABSTRACTS

A Subchronic Toxicity Study In Rats And Genotoxicity Tests With Surelease® Aqueous Ethylcellulose Dispersion, ACT & SOT Annual Meetings, March 2003

BOOKS and BOOK CHAPTERS

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

BOOKS and BOOK CHAPTERS

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

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

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

Research Office, Federation of American Societies of Experimental Biology (FASEB):

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

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

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

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

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

Evaluation of the health aspects of alginates as food ingredients.

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

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

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

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

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

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

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

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

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

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

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

Evaluation of the health aspects of dextran as food ingredients.

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

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

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 certain calcium salts as food ingredients.

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

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

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

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

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

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

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

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

Evaluation of the health aspects of phosphates as food ingredients.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Evaluation of the health aspects of glycerophosphates as food ingredients.

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

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.

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.

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

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

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

Evaluation of the health aspects of hypophosphites as food ingredients.

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.

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

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

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

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

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

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 corn silk as a food ingredient.

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

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.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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 soy protein isolates as food ingredients.

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

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

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

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

Evaluation of the health aspects of starch and modified starches as food ingredients.
Evaluation of the health aspects of carbon dioxide as a food ingredient.

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

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

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

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

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

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

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

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

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

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

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

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

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

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 soya fatty acid amines as food ingredients.

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

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

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

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.

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

Evaluation of the health aspects of peptones as food ingredients.

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

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

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

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.

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, Volume 2, Volume 3,
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.

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

CURRICULUM VITAE

JESSE F. GREGORY, III

Rm 208, Food Science & Human Nutrition Building
Food Science & Human Nutrition Department
Institute of Food and Agricultural Sciences
University of Florida
Gainesville, FL 32611-0370

EDUCATION

Cornell University, Food Science, B.S.,
Cornell University, Food Science, M.S.
Michigan State University, Food Science, Ph.D.,

EMPLOYMENT EXPERIENCE

Professor, Food Science and Human Nutrition Department
University of Florida
Associate Professor, Food Science and Human Nutrition Department, University of Florida
Assistant Professor, Food Science and Human Nutrition Department, University of Florida
Graduate Research Assistant, Department of Food Science and Human Nutrition, Michigan State University
Graduate Research Assistant, Department of Food Science
Cornell University

PROFESSIONAL ORGANIZATIONS

American Society for Nutritional Sciences
American Society for Biochemistry and Molecular Biology
American Chemical Society
Institute of Food Technologists

HONORS

Cornell National Scholarship,
NIH Nutrition Traineeship, Michigan State University,
Great Lakes Section IFT Graduate Achievement Award,
Nutrition Foundation Future Leaders Award,
Recognition for directing the research of students who received the "IFAS Award for Excellence in Graduate Research-Best Master's Thesis": Brian P. F. Day, 1982; Ana R. Abad, 1986; Rene Engelhardt, 1988; Lisa M. Rogers,
Samuel Cate Prescott Award for Research, Institute of Food Technologists,
Gamma Sigma Delta (Agricultural Honorary),
USDA Secretary's Award for Superior Service - (for folate research)
University of Florida - Professorial Excellence Award
G. Malcolm Trout Visiting Scholar, Food Science & Human Nutrition Dept., Michigan State Univ
Outstanding Alumni Award, College of Agriculture and Natural Resources, Food Science and Human Nutrition, Department, Michigan State University,
Designated by Institute of Scientific Information (ISIhighlycited.com) as a "Highly Cited Researcher,"
(at that time, only 8 UF faculty members had received this recognition)

University of Florida Research Foundation Professorship,
Centrum Award for Human Nutrition Research Am. Society for Nutritional Sciences.
UF Gamma Sigma Delta, Senior Faculty Award of Merit,

SELECTED PUBLIC SERVICE AND RELATED ACTIVITIES

National Academy of Sciences, National Research Council and Institute of Medicine Committees:

Committee on Food Protection,
Committee on Nutrition Components of Food Labeling,
Committee on State Food Labeling,
Institute of Medicine, Food and Nutrition Board. Invited presentations and written reviews regarding formulation of Dietary Reference Intake values for vitamin B6 and folate,
Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health.

National Institutes of Health and US Dept of Health and Human Services

Invited panel member at NIH/CDC workshop to evaluate methodological issues related to biochemical measures of folate and vitamin B-12 in National Health and Nutrition Examination Survey,
NIH Nutrition Study Section, Reviewer, Oct. 1992; Study Section Member Additional periodic service as needed as reviewer on Special Emphasis Panels
NIH Integrated Nutrition and Metabolic Processes Study Section, Temporary member
Special Review Panel for NIH Molecular and Cellular Endocrinology (MCE) Study Section,
Special Review Panel. National Institute for Dental and Craniofacial Research, and (review of an international nutrition/supplementation project).
Special Review Panel for review of an interagency research agreement on food composition research. National Heart, Lung, and Blood Institute,
Special Review Panel for review of a national multi-site research program on food composition and cardiovascular health. National Heart, Lung, and Blood Institute,
NIH, Office of Dietary Supplements, Member of Advisory Committee on Bioavailability,
NIH, Center for Scientific Review, Advisory Committee on Peer Review within the Endocrinology and Reproductive Sciences/Nutritional and Metabolic Sciences Integrated Review Groups,
Centers for Disease Control and Prevention, Review Committee on National Health and Nutrition Examination Survey 99+,
Food and Drug Administration, Food Advisory Committee, Folic Acid Subcommittee. Invited written and oral testimony regarding stability and bioavailability of folic acid during deliberations on cereal-grain fortification,

USDA Competitive Grants Human Nutrition Review Panel, Human Nutrition Program,

Editorial boards and related activities:

Board of Contributing Editors, *Nutrition Reviews*,
Academic Press Inc., Food and Nutrition Advisory Panel,
Smithsonian Inst.-National Academy of Sciences, National Science Resources Center. Served as consultant in development of "Food Chemistry," a student activity book and teacher's guide for grades 4-5
Encyclopedia of Food Sciences and Nutrition Editorial Advisory Board,
Journal of Nutrition: Editorial Board, 1991-2002. Associate Editor,
Journal of Food Composition and Analysis, Editorial Board,
Journal of Food Science, Editorial Board,

Activities in professional societies:

Institute of Food Technologists Committees:

Nutrition Division Executive Committee, [redacted]
Program Committee, [redacted]
Many award juries
Nutrition Division Education Committee, Chair, [redacted]

American Society for Nutritional Sciences (recently renamed American Society for Nutrition) Committees:
Publications Management Committee, [redacted]
Nomenclature Committee, [redacted]
Workshop on Recommendations for Rodent Diets, Chaired committee on water-soluble vitamins, [redacted]
Nominating Committee for Elected Officers, [redacted]
Program Planning Committee, [redacted] Chair [redacted]
Finance Committee, [redacted] Many award juries [redacted]
Public Policy Committee, [redacted]
Chair, ASNS Vitamin and Mineral Research Interest Section, [redacted]
ASNS representative at FASEB Consensus Conference on Federal Research Funding, [redacted] Chaired subcommittee on USDA funding, [redacted]

SUMMARY OF PROFESSIONAL EXPERIENCE

Research and teaching pertaining to nutritional biochemistry of B-vitamins, food chemistry, and nutritional quality of foods. Particular areas of research include: bioavailability, metabolism, function and analysis of B vitamins (especially folate and B6); stable-isotopic methods of nutrition research; folate biosynthesis in plants.

DEPARTMENTAL AND UNIVERSITY SERVICE

Food Science and Human Nutrition Department:

Served as acting department chair [redacted]
Assorted departmental committees:
- Undergraduate committee [redacted]
- Graduate Committee ([redacted]
- Animal Care and Use [redacted]
- Committee to Review Possible Departmental Restructure [redacted]

Search and Screen Committees
- Food Chemistry Assistant Professor (chaired), [redacted]
- Food Chemistry Assistant Professor (chaired), [redacted]
- Department Chair (chaired), [redacted]
- Department Chair, [redacted]
- Food Microbiology Assistant Professor, [redacted]
- Nutritional Biochemistry Assistant Professor, [redacted]
- Nutritional Biochemistry Assistant Professor, [redacted]
- Nutritional Genomics Assistant Professor, [redacted]

Co-chaired (with C. Sims) revision of Food Science graduate curriculum, [redacted]
FSHN departmental strategic planning: Research Committee chair. [redacted]

IFAS and University Committees:

Center for Nutritional Sciences, University of Florida. Member of Board of Directors [redacted] Council on General Education, University of Florida [redacted]
Search and Screen Committee; IFAS Dean for Academic Programs & Dean, College of Agriculture, [redacted]
University Senate, [redacted]
Tenure and Promotion Committee, Institute of Food and Agricultural Sciences, [redacted]
Periodic ad hoc service on IFAS Project Review Committee
UF Research Foundation Professorship – IFAS Advisory Committee, [redacted]
College of Agricultural and Life Sciences Graduate Awards Committee, [redacted]

UF General Clinical Research Center: Scientific Advisory Committee member,
UF General Clinical Research Center: Core Lab Subcommittee,
Faculty Senate Committee on Faculty Compensation,

TEACHING

Courses:

FOS 4311 "Food Chemistry" (4 credits),
FOS 6646 "Proteins and Enzymes in Food Systems"
(50-60% of 4 credit course)
FOS 4321 "Food Analysis", (4 credits),
FOS 6315 "Advanced Food Chemistry" (4 credits),
Teaching evaluations for course and instructor (JFG) exceed departmental and college means in approximately 95% of these course offerings.

Assorted guest lectures: FOS 4311 (Food Chemistry), HUN 6812 (Analytical Techniques in Nutritional Biochemistry)

INTERNATIONAL ACTIVITIES

Invited presentation entitled "The Bioavailability of Vitamin B6 - Recent Findings" at the International Multidisciplinary Conference on Vitamin B6, Philadelphia, PA

Invited presentation at the international conference entitled, "Bioavailability '93: Nutritional, Chemical and Food Processing Implications of Nutrient Bioavailability." Presentation title: "Benefits and problems of radioactive and stable isotopes in studying the bioavailability of vitamins," Ettlingen, Germany,

Invited keynote lecture entitled "The use of stable isotopes in studies of folate absorption and metabolism." Meeting of the European Community Food Linked Agro-Industrial Research Group (Concerted Action No. 10, on The Measurement of Micronutrient Absorption and Status), Ettlingen, Germany,

Invited member of B-vitamin planning committee, Project on the Bioavailability of Nutrients, International Life Science Institute-European Branch,

Invited presentations at the meeting of the Project on the Bioavailability of Nutrients, International Life Science Institute-European Branch. Presentations entitled: "Bioavailability of Folate," "Bioavailability of Vitamin B6," and "Bioavailability of Thiamin," Norwich, UK,

Convened and chaired a workshop entitled "Folate from Foods or Supplements" and presented opening paper at the Bioavailability '97 conference, Wageningen, The Netherlands,

Served as invited chair of a workshop organized by the UK Food Standards Agency to determine research priorities on folate bioavailability I presented the opening lecture entitled "Folate Bioavailability: Issues and Priorities," moderated discussion, and led formulation of conclusions & research priorities. London, UK.

Served as the invited outside examiner ("opponent") in the Ph.D. exam of Lena Strålsjö, Department of Food Science, Swedish Agricultural University, Uppsala, Sweden,

Served as a scientific consultant on multi-institution European Union research project entitled "Folate: From Food To Functionality and Optimal Health."

Served as an invited outside examiner ("opponent") in the Ph.D. exam of Alida Melse-Boonstra, Department of Nutrition, Wageningen University, the Netherlands,

Presented keynote lecture entitled "Integrating the issues: folate intake, bioavailability and metabolism in the era of fortification" at EuroFoodFolate 2004 (First International Conference on Food Folate: Analysis, Bioavailability and Health), Warsaw, Poland,

Served on Scientific Advisory Committee for 13th Int. Symposium on Chemistry & Biology of Pteridines & Folates, Egmond aan Zee, The Netherlands. Presented invited lecture entitled: "Stable Isotopic Tracers for Studies of Folate Bioavailability and Metabolism: Development, Principles and Applications."

LECTURES, SPEECHES OR POSTERS PRESENTED AT PROFESSIONAL CONFERENCES OR MEETINGS

(does not include assorted volunteered oral or poster research presentations published as abstracts during this period)

A. International

Invited presentation entitled "The Bioavailability of Vitamin B6 - Recent Findings" at the International Multidisciplinary Conference on Vitamin B6, Philadelphia, PA

Invited presentation at the international conference entitled, "Bioavailability '93: Nutritional, Chemical and Food Processing Implications of Nutrient Bioavailability.". Presentation title: "Benefits and problems of radioactive and stable isotopes in studying the bioavailability of vitamins," Ettlingen, Germany,

Invited keynote lecture entitled "The use of stable isotopes in studies of folate absorption and metabolism." Meeting of the European Community Food Linked Agro-Industrial Research Group (Concerted Action No. 10, on The Measurement of Micronutrient Absorption and Status), Ettlingen, Germany,

Invited presentations at the meeting of the Project on the Bioavailability of Nutrients, International Life Science Institute-European Branch. Presentations entitled: "Bioavailability of Folate," "Bioavailability of Vitamin B6," and "Bioavailability of Thiamin," Norwich, UK,

Convened and chaired a workshop entitled "Folate from Foods or Supplements" and present opening paper at the Bioavailability '97 conference, Wageningen, The Netherlands,

Invited to chair a workshop to define research priorities regarding folate bioavailability and present opening lecture entitled "Folate Bioavailability: Issues and Priorities." UK Food Standards Agency, London,

Invited seminar entitled "Nutritional and Genetic Effects on Human Folate and One-Carbon Metabolism," Department of Food Science, Swedish University of Agricultural Sciences, Uppsala, Sweden,

Invited seminar entitled "Measuring In Vivo Rates of Human One-Carbon Metabolic Reactions Using Stable Isotopes." Department of Nutrition, Wageningen University, the Netherlands,

EuroFoodFolate 2004, First International Conference on Folates: Analysis, Bioavailability and Health; Warsaw, Poland; presented keynote lecture entitled "Integrating the issues: folate intake, bioavailability, and metabolism in the era of fortification."

Invited presentation entitled "Using Tracers to Probe the Effects of Genetics and Vitamin Nutrition on Human One-Carbon Metabolism." Canadian Conference entitled Metabolomic and Proteomic Advances in Nutrition Research, Nutrition Research Program - University of British Columbia and British Columbia Research Institute For Children's & Women's Health, Vancouver, BC, Canada,

Presented invited lecture entitled: "Stable Isotopic Tracers for Studies of Folate Bioavailability and Metabolism: Development, Principles and Applications." 13th Int. Symposium on Chemistry & Biology of Pteridines & Folates, Egmond aan Zee, The Netherlands,

B. Representative Major National Presentations

Invited presentation on measurement of food folate at Workshop on Folates, Methylation, and Cancer Prevention, National Cancer Institute, Bethesda, MD,

Opening presentation entitled "B-vitamins: problems and advances in assessing their bioavailability," at minisymposium entitled "B Vitamins: Bioavailability and Metabolism," FASEB Annual Meeting, Las Vegas, NV.

Invited presentation on "Nutritional Requirements for Water-Soluble Vitamins of Laboratory Animals," American Institute of Nutrition, Workshop on Laboratory Animal Diets, New Orleans, LA, (chaired committee on water-soluble vitamins)

Invited opening presentation of minisymposium entitled "Relationships between dietary forms of vitamin B6, bioavailability and vitamin B6 requirements" at FASEB Annual Meeting, Washington, D.C.,

Invited presentation on analytical aspects of food labeling. Report of the National Academy of Sciences Committee on Nutrition Components of Food Labeling, Washington, D.C.,

Invited presentation on "Nutrition and other labeling issues related to shrimp," National Shrimp Processors Association, Orlando, FL

Invited presentation entitled "The nutritional properties of pyridoxine-ß-D-glucosides" at symposium on Biochemistry and Molecular Biology of β-Glucosidases, American Chemical Society Meeting, Washington, D.C.,

Invited presentation on "Technological aspects of food fortification with folic acid," U.S. Food and Drug

Administration, Food Advisory Committee, hearing on issues concerning neural tube defects and maternal folate nutrition, Washington, D.C.

Conference entitled Mathematical Modeling in Experimental Nutrition V. Fort Wayne, IN, Presented invited paper on "Modeling of folate metabolism."

Institute of Food Technologists, Atlanta, GA, June 94. Presented invited paper entitled "Issues of Nutrient Bioavailability" at symposium entitled "Should the RDAs be Revised?"

American Cereal Chemists Soc. Annual Meeting. Presentation entitled "Folate enrichment of cereal products - scientific background." Invited speaker in symposium entitled "Folate Enrichment of Cereal Products," Nashville, TN,

Invited presentation: "Determination of Folate in Food Products." American Institute of Baking Workshop entitled "Adding Folic Acid to Grain-Based Foods." Kansas City, MO.

Invited presentation entitled "Bioavailability of Vitamin B6" at National Academy of Sciences, Institute of Medicine Workshop on Selected B Vitamins: B1, B2, Niacin, B6, Pantothenic Acid, and Biotin, Panel on Folate and Other B Vitamins, Food and Nutrition Board. Irvine, CA,

Invited presentation, "Do Available Food Composition Data for Folic Acid Meet Current Research Needs?" National Nutrient Databank Conference. San Francisco, CA,

National High Field Magnet Laboratory, invited presentation entitled "Isotopic Applications in Studies of Folate Metabolism." Workshop on Biomedical Applications of Isotopes. Tallahassee, FL,

Invited presentation on "Kinetics of Folate Metabolism" at "The Current Status of Knowledge on Folate and Human Development - A Symposium and Workshop," U.S. Environmental Protection Agency, Research Triangle Park, NC. (invited participation in conference with main focus on status of folate and birth defects).

Invited presentation "Case Study: Bioavailability of Folate" at Conference/ Workshop on the Bioavailability of Nutrients and Other Bioactive Components of Dietary Supplements: Defining the Research Agenda. National Institutes of Health, Bethesda, Maryland.

Invited presentation to U.S. Food and Drug Administration on nutritional and technological issues pertaining to their consideration of a new form of folate supplement, Washington, D.C.

Invited presentation "Kinetics of folate and one-carbon metabolism in human subjects." (also chaired session) FASEB Summer Research Conference on Folate, B12 and One-Carbon Metabolism, Snowmass Village, CO.

"Dietary Folate in a Changing Environment: Bioavailability, Fortification and Requirements." Presented in symposium "Folate: Health Problems and Solutions" at IUFOST meeting, Chicago, IL,

Invited presentation "Dissecting the components of human one-carbon metabolism with stable isotope tracers - effects of vitamin nutrition and genetic polymorphisms." (also chaired session) FASEB Summer Research Conference on Folate, B12 and One-Carbon Metabolism, Indian Wells, CA.

C. <u>Assorted Invited State and Local Presentations</u>

Florida Section, Institute of Food Technologists Campus Meeting presentations:

"Stable isotopes in vitamin research,"

"Recent applications of stable isotopes in vitamin research,"

University of Florida, National Dairy Council Nutrition Symposium, "Applications of stable-isotopic methods in human nutrition research,"

University of Florida, Food Science and Human Nutrition Seminar, "Nutrition labeling for the 1990s,"

University of Florida, Food Science and Technology Seminar, "Folic acid: a rationale for its addition to food,"

University of Florida, Nutritional Sciences Seminar, "Nutritional properties of vitamin B6 glucosides,"

University of Florida, Food Science and Human Nutrition Department and Gamma Sigma Delta Senior Faculty Award Seminar, "Evolving folate research: a better knowledge of nutrition through chemistry,"

D. <u>Invited Research Seminars</u>

University of Florida, Gastroenterology Interdepartmental Research Conference, "Bioavailability of folate,"
University of Florida, Gastroenterology Interdepartmental Research Conference, "Bioavailability of vitamin B6,"

University of Florida, Gastroenterology Interdepartmental Research Conference, "Bioavailability of dietary folate,"

Michigan State University, Food Science and Human Nutrition Department, "The Bioavailability of vitamin B6,"

University of Florida, Horticultural Sciences Seminar, "Bioavailability of vitamin B6 in fruits and vegetables,"
University of Florida, Department of Microbiology and Cell Science, "The nutritional significance of vitamin B6-ß-glucosides,"
University of Alabama-Birmingham, "The nutritional properties of glycosylated forms of vitamin B6,'
Oregon State University, Nutrition Research Institute, "Isotopic and enzymatic studies of folate bioavailability" and "The nutritional properties of pyridoxine glucosides,"
University of Illinois, Division of Nutritional Sciences, "Isotopic and enzymatic studies of folate bioavailability,"

University of Minnesota, Department of Food Science and Nutrition, "Folate bioavailability and in vivo kinetics,"

University of Guelph (Ontario), Department of Nutritional Sciences. Invited seminar entitled "Folate absorption and metabolism: applications of stable isotopes."
Louisiana State University Medical Center at Shreveport, Dept. of Pharmacology and Therapeutics, "Nutritional and Metabolic Function of Naturally Occurring Vitamin B6 ß-D-Glucosides,"
University of Florida, Gastroenterology Interdepartmental Research Conference, "Nutritional and Metabolic Function of Naturally Occurring Vitamin B6 ß-D-Glucosides,"
Seminar entitled "Do we need more folate? Applications of stable isotopes in studies of folate absorption and metabolism." Dept. of Food Science and Human Nutrition, Michigan State University, as G. M. Trout Annual Lecture and Visiting Scholar.
Seminar entitled "Developments in Folate and One-Carbon Metabolism: Kinetic Studies Using Stable-Isotopic Protocols in Humans." Gastroenterology Research Series, University of Florida,
Seminar entitled "Applications of Stable Isotopes to Determine the Kinetics of Folate Turnover and Metabolic Function in Human Subjects," USDA Human Nutrition Research Center on Aging, Boston, MA
Clinical Nutrition Research Unit seminar, Vanderbilt University, Nashville, TN. "Stable isotopic applications in folate and homocysteine metabolism."
Seminar entitled "Stable Isotopic Applications in Folate and Homocysteine Metabolism," Division of Nutritional Sciences, Cornell University, Ithaca, NY,
Seminar entitled "Stable Isotopic Applications in Folate and Homocysteine Metabolism," Departments of Nutritional Sciences and Food Science, Ohio State University, Columbus, OH,
Seminar entitled "Nutritional and Genetic Effects on Human Folate and One-Carbon Metabolism." Department of Food Science, Swedish University of Agricultural Sciences, Uppsala, Sweden,
Seminar entitled "Measuring In Vivo Rates of Human One-Carbon Metabolic Reactions Using Stable Isotopes." Department of Nutrition, Wageningen University, the Netherlands,
Seminar entitled "Nucleotide Synthesis in Humans: Relationships between Folate Status and Methylenetetrahydrofolate Reductase 677 C→T Genotype." Nutritional Sciences Program, University of Washington and Fred Hutchinson Cancer Research Center, Seattle, WA,

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"Identification and biological characterization of vitamin B6 thermal degradation products." Nutrition Foundation Grant #564 (Future Leaders Award). J. F. Gregory, PI.

"The bioavailability of folacin in foods." USDA Competitive Grants Office #50-2121-1-766-0. J. F. Gregory, PI.

"Special equipment proposal: Spectropolarimeter for circular dichroism." Biomedical Research Support Grant (NIH). R. D. Brown, PI; J. F. Gregory and six others as Co-PIs.

"Investigations into enzyme(s) responsible for melanosis in deep sea crab." Gulf and South Atlantic Fisheries Development Foundation, Inc. M. R. Marshall, W. S. Otwell, and J. F. Gregory, PIs.

"Determination of vitamin B6 and folacin bioavailability using isotopic enrichment methods." USDA Competitive Grants Office #83-CRCR-1-1240. J. F. Gregory, PI.

"Ion chromatography for determining residual sulfite in shrimp." Florida Seagrant Project #83-5. M. R. Marshall, J. F. Gregory, and W. S. Otwell, Co-PIs.

"Investigate Control of Melanosis in Shrimp." Gulf and South Atlantic Fisheries Development Foundation, Inc. Grant #27-01-49373/1000. M. R. Marshall, J. F. Gregory and W. S. Otwell, Co-PIs.

"Determination of Folacin Compounds in Infant Formula." Ross Laboratories Inc. J. F. Gregory, PI.

"Analysis of Infant Formulas." Department of Food and Nutrition Services, Shands Hospital, University of Florida. J. F. Gregory, PI.

"Stable-isotopic and Enzymatic Investigation of Folacin Bioavailability." USDA Competitive Grants Office #86-CRCR-1-1938. J. F. Gregory, PI.

"The Nutritional Properties of Pyridoxine Beta-Glucoside." NIH Grant #DK37481: J. F. Gregory, PI.

"Stable-isotopic and Radioisotopic Investigation of Folate Bioavailability." USDA Competitive Grants Office #88-37200-3620. J. F. Gregory, PI.

"The Nutritional Properties of Pyridoxine Beta-Glucoside." NIH Grant #DK37481: J. F. Gregory, PI.

"Stable-isotopic Investigation of Folate Bioavailability and Nutritional Status." USDA National Research Initiative-Competitive Grants Program #91-37200-6305. J. F. Gregory, PI.

"Folate Nutritional Status and In Vivo Kinetics." USDA National Research Initiative -Competitive Grants Program #92-37200-7466. J. F. Gregory, PI.

"The Nutritional Properties of Pyridoxine Beta-Glucoside." NIH Grant #DK37481: J. F. Gregory, PI.

"Research Training in Nutrition." Institutional National Research Service Award. NIH Grant #1 T32 DK07667.

[redacted] total direct costs awarded. R.J. Cousins, PI; J.F. Gregory and 5 others as participating research preceptors. (not counted in funding summary shown below)

"The Bioavailability of Folate." USDA National Research Initiative -Competitive Grants Program #94-37200-0604. [redacted] J.F. Gregory, P.I.

"Folate and Vitamin B6 Dependence of One-Carbon Metabolism." USDA National Research Initiative -Competitive Grants Program #96-35200-3210. [redacted] J.F. Gregory, P.I.

"Postdoctoral Fellowship: Folate and Vitamin B6 Dependence of One-Carbon Metabolism." American Heart Association, Florida Affiliate # 9840016FL. [redacted] Postdoctoral support for Dr. Geraldine Cuskelly (J. F. Gregory is PI and mentor).

"The Nutritional Properties of Pyridoxine-β-D-Glucoside." NIH Grant #DK37481. Bridging funds [redacted] J.F. Gregory, P.I.

"The Nutritional Properties of Pyridoxine-β-D-Glucoside." NIH Grant #DK37481. [redacted] J.F. Gregory, P.I.

"Vitamin B6 Dependence of Homocysteine Metabolism" USDA-NRICGP Grant 00-35200-9113. [redacted] J.F. Gregory, P.I.

"Genetic Effects on Folate-Dependent One-Carbon Metabolism" NIH Grant 1 R01 DK56274. [redacted] J.F. Gregory, P.I.

"Genetic Effects on Folate-Dependent One-Carbon Metabolism" NIH Grant 1 R01 DK56274, supplemental award. [redacted] J.F. Gregory, P.I.

"Folate Synthesis, Catabolism and Engineering In Plants" NSF Grant # 0129944. [redacted] award (direct costs). A. D. Hanson, P.I., J.F. Gregory, Co-P.I.

"Transport of Folates and their Precursors in Plants" NIH/NIGMS grant # R01 GM071382. [redacted] A.D. Hanson, P.I., J.F. Gregory, Co-investigator.

"Folate Synthesis, Catabolism and Engineering In Plants" NSF Grant MCB-0443709. [redacted] A. D. Hanson, P.I., J.F. Gregory, Co-P.I.

"Vitamin B6 Effects on One-Carbon Metabolism." NIH Grant #1 R01 DK072398. [redacted] J.F. Gregory, P.I.

"The response of choline and folate status to controlled levels of choline intake." NIH Grant # S06 GM53933, Marie Caudill P.I., JFG co-investigator Total direct [redacted]

"Folate Absorption Across the Large Intestine." Natural Sciences and Engineering Research Council of Canada Grant #RGPIN 46513-05 Deborah L. O'Connor, P.I., JFG co-investigator. [redacted]

WALTER H. GLINSMANN, M.D.

EDUCATION:

B.A. - Columbia College, New York, NY
M.D. - Columbia College of Physicians and Surgeons, New York, NY
Internship and Residency in Medicine - New York Hospital (Cornell Medical School), New York, NY
Service and Training in Endocrinology and Metabolism at Walter Reed Army Medical Center, Department of Metabolism, Army Medical Center and Army Institute of Research, Washington, DC

MEDICAL LICENSE:

New York State Physician, No. 086359.

EMPLOYMENT HISTORY:

t Principal activities are as President, Glinsmann, Inc.

As below, with change in academic affiliation to Fellow, Center for Food Safety and Nutrition Policy, re-located to Virginia Polytechnic Institute and State University (Virginia Tech), Alexandria, VA.
(Completed term as Scientific Advisor to Technical Committee, International Life Sciences Institute.)
Appointed as Fellow, American Society for Nutrition.

President, Glinsmann, Inc. Evaluations of safety and health effects of foods, the development of nutritional products, and food-related claims.
Fellow and Adjunct Professor, Georgetown Center for Food and Nutrition Policy, Medical College and Graduate School of Public Policy, Georgetown University, Washington, DC
Member, Expert Panel on Nutrition and Electrolytes, United States Pharmacopeial Convention, Inc.
Scientific Advisor to Technical Committee on Food Components for Health Promotion ("Functional Foods" or "Nutraceuticals"), International Life Sciences Institute, North America; to the International Food Information Council; and to U.S. and International Food Companies.

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Advisor to the Committee on Nutrition and Electrolytes, United States Pharmacopeial Convention.
Scientific Advisor to the Technical Committee on Food Components for Health Promotion, International Life Sciences Institute, North America.
Expert Consultant, Office of the Deputy Director for Programs, Center for Food Safety and Applied Nutrition (CFSAN or the Center), U.S. Food and Drug Administration (FDA).

Expert in Nutrition, Office of Disease Prevention and Health Promotion, Office of the Assistant Secretary for Health, Department of Health and Human Services (DHHS). Provided management/technical support and coordination for nutrition and dietary guidance-related activities for DHHS among U.S. government agencies, e.g., USDA and National Institute of Health Components.

Visiting Fellow, Georgetown Center for Food and Nutrition Policy and Ceres Forum®, Georgetown Medical School.
Director, Nutrition Policy Staff, Office of Disease Prevention and Health Promotion, Office of the Assistant Secretary for Health, Public Health Service (PHS), DHHS. Provided leadership and coordination among DHHS agencies for Nutrition Policy Board functions; implementation of year 2000 nutrition objectives; development of dietary guidance with U.S. Department of Agriculture; and initiatives in food labeling, safety, and fortification: included program coordination for nutrition monitoring and related research; DHHS representative at international nutrition conferences and committees on interagency nutrition research initiatives; preparation of nutrition and health reports and briefing materials for Congress.

Associate Director for Clinical Nutrition, Division of Nutrition, CFSAN, FDA. Provided program direction for human clinical and population-based research on: food safety; special dietary use and medical food products; novel food ingredients; health impacts of dietary behaviors of select populations; nutrient imbalances that relate to human morbidity and mortality; and techniques for assessing and monitoring nutritional status and adverse reactions to foods, food additives, and contaminants. Chaired the Center's Health Hazard Evaluation Board and Task Force for developing Guidelines for Clinical Testing for Food Additives and served as Senior Center Medical Officer for developing Medical Foods Regulations. Represented Center or Agency interests and positions and provided guidance on human food safety evaluations to industry.

Chief, Clinical Nutrition Branch, Division of Nutrition, CFSAN, FDA. Developed and managed clinical nutrition activities, human food safety assessment programs, and supervised the revision of Nutrition Monitoring Programs. Special assignments included: FDA Research in Human Subjects Committee; CFSAN Health Hazards Evaluation Board;

primary responsibility for Center programs dealing with Clinical Nutrition, Clinical Investigations, Foods for Special Dietary Use, and Health-and Injury-Related Surveillance; Chairperson, Task Forces on Medical Foods Regulation and Evaluation of Health Aspects of Sugars Contained in Carbohydrate Sweeteners; and member Task Forces on Research Priorities in Biotechnology, Food Monitoring, Nutrition, and Toxicology.

Chief, Experimental Nutritional Branch and Director of Research, Division of Nutrition, CFSAN, FDA. Developed, managed, and conducted research based on CFSAN priorities; Special assignments included: FDA Research Involving Human Subjects Committee; Center/FDA Science Liaison; Center Health Hazards Evaluation Committee; and Task Forces on Nitrates-Nitrosamines, Research and Research Facilities Plans, Medical Foods Regulations, and the Coordinated Use of Databases to Estimate Exposures.

Chief, Section on Physiological Controls, Laboratory of Biomedical Sciences, National Institute of Child Health and Human Development (NICHHD), National Institutes of Health (NIH), Bethesda, Maryland. Developed and managed research; Chief Intramural Research Contracts Officer (NICHHD); Institute Safety Officer; Member/Chair of Clinical Research Review and Grants and Contracts Review Committees.

Senior Research Investigator, Laboratory of Biomedical Sciences, NICHHD, NIH.

Guest Investigator, Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, NIH.

Medical Officer, Research Planning and Program Development, Growth and Development Program, NICHHD, NIH.

Assistant Chief, Department of Metabolism and Attending Physician in Medicine, Walter Reed Army Hospital, and Senior Investigator, Walter Reed Army Institute of Research, and Institute of Pathology, Washington, DC.

PROFESSIONAL MEMBERSHIPS

American Institute of Nutrition, renamed American Society for Nutritional Sciences (Membership Committee,
American Society for Clinical Nutrition (Membership Committee, Chair.
Publications Management Committee, American Journal of Clinical Nutrition,
American Physiological Society
Institute of Food Technologists
American Association for the Advancement of Science
American Diabetes Association
New York Academy of Sciences

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AWARDS

Fellow, American Society for Nutritional Sciences,

FDA Commendable Service Award: assessing safety and labeling of monosodium glutamate,

DHHS Secretary's Award for Excellence in Public Service, Implementing the Food Labeling Initiative,

PHS Citation for exemplary and creative work as a management clinician in nutrition research and investigative matters,

FDA Award of Merit for Leadership, Health Hazard Evaluations,

PHS Unit Commendation, Food Irradiation Safety Evaluation

PHS Citations for Outstanding Management Performance in Clinical Nutrition and Food Safety Evaluations,

PHS Meritorious Service Medal for sustained high quality leadership and outstanding contributions to Nutrition and Food Safety,

FDA Award of Merit for Leadership, Aspartame Clinical Investigation Team,

FDA Commissioner's Special Citation to the Research Involving Human Subjects Committee for unique and outstanding performance to the Food and Drug Administration by promoting research while ensuring the protection of the human subjects involved,

PHS Commendation Medal for sustained high quality leadership and professional accomplishments in planning, implementing, and evaluating nutrition research at the FDA,

EXAMPLES OF SEMINARS, INVITED LECTURES

Clinical Investigations for Establishing Safety and Efficacy for Food, Annual Meeting of Institute of Food Technologists, Chicago, IL

Is There a Need for Cyclical Third Party Reviews? An Affirmative Assessment,
10th Annual Conference, Functional Foods for Health, U. Illinois, Chicago, IL

Orientation to Functional Foods, Pediatric Academic Societies' Annual Meeting, Baltimore, MD

Safety Regulation for Dietary Supplements: The System Needs a Significant Change - Toxicology Roundtable, Experimental Biology, Orlando, FL

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Communicating Benefits to the Consumer: FTC, FDA, and Claim Substantiation. Warner-Lambert-Pfizer Nutrition Symposium, Morris Plains, NJ

Concepts, Standards, and Substantiation of Effects of Functional Foods
Workshop on Functional Foods, Kappel am Albis, Switzerland

Scientific Concepts, Standards, and Substantiation
American Association of Cereal Chemists Annual Meeting, Functional Foods – Strategies for the Food Industry, Newport Beach, CA

Distinguishing a Nutrient from a Drug
Ceres Forum® on What is a Nutrient? Georgetown University Conference Center, Washington, DC

The Role of Clinical Investigations in Establishing Safety and Efficacy for Functional Foods
Course: A Global Perspective on Regulatory Approval for Food Ingredients, Nutraceuticals, and Dietary Supplements. Institute of Food Technologists Annual Meeting '98, Atlanta, GA

Functional Foods: Regulatory Considerations
Symposium: Functional Foods for Health Promotion: A Public Health Opportunity? Experimental Biology '98, San Francisco, CA

Functional Foods: Regulatory Considerations
Panel: Are Consumers Ready for Functional Foods? American Dietetic Association Annual Meeting, Boston, MA

Overview of Research on Health Benefits from Specific Phytochemicals
Panel on Demystifying Functional Foods, Public Voice 20th Annual National Food Policy Conference, Washington, DC

Dietary Guidance and the U.S. Food Supply: Focus on Infants and Young Children
Health Visitors Association Centenary Conference, Harrogate, England

U.S. Health Claims: Rational and Lessons Learned
Symposium on Health Claims for Foods in Canada, U. Toronto, Ontario, Canada

Statements of Nutritional Support: Claim Substantiation
Symposium on the New Structure/Function Claims Frontier Under DSHEA, National Nutritional Foods Association, Washington, DC

Regulatory Framework: United States
Workshop on Antioxidants, ILSI-Europe, Brussels, Belgium

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Perspective on the Future of Designer and Functional Foods: Definitions, Regulatory Perspective, and Health Claims
Annual Meeting, ILSI, Cancun, Mexico

Food Labeling and Food Safety Requirements in the United States and *International Developments in Food Fortification, Foods for Special Dietary Use, and Functional Foods*, Seminars, Doosan Technical Center, Seoul, Korea

Functional Foods in North America and Regulatory Aspects of Functional Foods
First International Conference on East-West Perspectives on Functional Foods, ILSI-Southeast Asia, Singapore

PUBLICATIONS

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Glinsmann, W.H., Dennis, D.A. Regulation of nonnutritive sweeteners and other sugar substitutes. In *Sweeteners*, Eds. N. Kretchmer and C. Hollenbeck, p. 257-85, CRC Press, Boca Raton, Florida, 1991.

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000101

Appendix 2

Pages 000103 - 000107 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Appendix 3

Pages 000109 - 000116 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Appendix 4a

Analysis of Enzymatically Modified Isoquercitrin for Enzyme Activity

September 15, 2006

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000118

Laboratory:

Name: Research & Development Group,
Fundamental Research Division,
San-Ei Gen F.F.I., Inc.

Address: 1-1-11, Sanwa-cho, Toyonaka, Osaka

Study period:

September 1, 2006 to September 15, 2006

Analysis date:

September 1, 2006 to September 6, 2006

Study coordinator:

Name: Masamitsu Moriwaki
Fundamental Research Division,

Address: 1-1-11, Sanwa-cho, Toyonaka, Osaka

Study operator:

Name: Kazuhiro Emura
Research & Development Group,
Fundamental Research Division,

Address: 1-1-11, Sanwa-cho, Toyonaka, Osaka

1. Study title:
   Analysis of Enzymatically Modified Isoquercitrin for enzyme activity

2. Study purpose:
   Confirm that the step to incubating at 98 degrees C for 5 minutes at pH4.2 inactivates the both enzymes of *Naringinase* and *CGTase* completely.

3. Tested material:
   Isoquercitrin product: (lot # 060830, San-Ei Gen F.F.I., Inc.)
   Rutin: Rutin D-10 (lot # 050804, content: 99.7%, Matsuura-Yakugyou Co., Ltd.), it is in accordance with the 3rd edition of Standard for Exiting Food Additives authorized by Japan Food Additives Association.
   Naringinase: (*Penicillium decumbens*, Amano Enzyme Inc.)
   CGTase: Cyclodextrin glucanotransferase (*Bacillus pseudalcaliphilus*, San-Ei Gen F.F.I., Inc.)

4. Study method

1) Inactivation of enzymes

Dissolve about 20mg of *Naringinase* in about 2ml of 50mM citrate buffer solution (pH4.2), and use this solution as control solution A. And then, inactivate 1ml of control solution A by incubating at 98 degrees C for 5 minutes, and use this solution as sample solution A. Separately, dissolve about 20 mg of *CGTase* in about 2ml of 50mM citrate buffer solution (pH4.2), and use this solution as control solution B. And then, inactivate 1ml of control solution B by incubating at 98 degrees C for 5 minutes, and use this solution as sample solution B.

2) Assay for Rutin-hydrolyzing activity

Naringinase catalyzes hydrolysis of Rutin to Isoquercitrin (hereinafter called as IQC).

(1) To the reaction mixture containing 50mg of polyphosphoric acid, 32mg of emulsifier, 6.8mg of Rutin, and 6.5mg of sodium hydroxide in 100 ml of water, add sample solution A, and incubate at 70 degrees C for 24 hours at pH 4.9 with mix. Separately, to the reaction mixture containing the same components, add the control solution A, and incubate at 70 degrees C for 24 hours at pH 4.9 with mix.

(2) To each 10 micro L of these reaction mixtures at 2, 6, 24 hours, add 1ml of 0.085% phosphoric acid, and use these solutions as the test solutions. Using 20 micro L of the test solutions, perform HPLC under the operating conditions given below. Measure the peak areas of Rutin and IQC in triplicate for accuracy. Calculate the reaction rate by the following formula.

Reaction rate =IQC peak area / (Rutin peak area + IQC peak area) X 100 (%)

(3) *Apparatus and Operation conditions*

HPLC: Shimadzu Corp.
Detection: 351nm
Column: ODS, 4.6 mm i.d. x 150 mm (Imtakt Cadenza C18)
Column temperature: 40 degrees C
Mobile phase: A mixture of 0.085% phosphoric acid - acetonitrile (83:17)
Flow rate: 0.8 ml/min

3) Assay for transglycosylating activity

CGTase catalyzes transglycosilation to IQC.

(1) To the reaction mixture containing 6.0g of dextrin, 1.0g of IQC, 0.3g of 48% sodium hydroxide, and 32mg of calcium chloride dihydrate in 100 ml of water, add sample solution B, and incubate at 60 degrees C for 24 hours at pH 7.25 with mix. Separately, to the reaction mixture containing the same components, add control solution B, and incubate at 60 degrees C for 24 hours at pH 7.25 with mix.

(2) To each 10 micro L of these reaction mixtures at 2, 18, 24 hours, add 1ml of 0.085% phosphoric acid, use these solution as the test solutions. Using 20 micro L of the test solutions, perform HPLC under the operating conditions given above.

Measure each peak area of constituents of EMIQ in triplicate for accuracy, calculate the ratio of the peak areas, and express as IQC, IQC+Glucose(Gl), IQC+G2, IQC+G3, IQC+G4, IQC+G5, IQC+G6, and IQC+G7.

5. Results

These results showed that the reaction mixtures didn't react with inactivated enzyme solutions at all, comparing with control solutions.

The step to incubating at 98 degrees C for 5 minutes at pH4.2 on manufacturing process was confirmed that the both of *Naringinase* and *CGTase* were inactivated completely.

(1) Reaction rate of *Naringinase*, three times analyzed.

<Reaction rate (%) >

	Control solution A			Sample solution A (Inactivated *Naringinase*)		
No.	2h	6h	24h	2h	6h	24h
1	30.0	43.9	59.3	0.2	0.2	0.2
2	29.3	43.0	59.9	0.2	0.2	0.2
3	30.0	43.2	58.0	0.1	0.2	0.2

(2) Composition of EMIQ, three times analyzed.

< HPLC peak area at 2 hours> (Area %)

	IQC without *CGTase*	Control solution B			Sample solution B (Inactivated *CGTase*)		
		1	2	3	1	2	3
IQC	98.3	61.6	61.2	62.1	99.0	99.0	98.9
IQC+G1	0.2	14.6	14.7	14.4	0.1	0.1	0.1
IQC+G2	0.9	6.7	6.8	6.6	0.8	0.8	0.9
IQC+G3	0.6	3.0	3.0	2.9	0.1	0.1	0.1
IQC+G4	ND	4.2	4.3	4.2	ND	ND	ND
IQC+G5	ND	4.1	4.1	4.0	ND	ND	ND
IQC+G6	ND	3.2	3.3	3.2	ND	ND	ND
IQC+G7	ND	2.6	2.6	2.6	ND	ND	ND

ND: not detected

< HPLC peak area at 18 hours> (Area %)

	IQC without *CGTase*	Control solution B			Sample solution B (Inactivated *CGTase*)		
		1	2	3	1	2	3
IQC	98.3	28.6	28.7	28.3	98.0	98.1	98.2
IQC+G1	0.2	20.4	20.4	20.5	0.4	0.3	0.3
IQC+G2	0.9	15.4	15.3	15.5	1.1	1.1	1.1
IQC+G3	0.6	10.5	10.4	10.8	0.5	0.5	0.4
IQC+G4	ND	8.9	8.9	8.9	ND	ND	ND
IQC+G5	ND	7.0	7.1	7.0	ND	ND	ND
IQC+G6	ND	5.3	5.3	5.2	ND	ND	ND
IQC+G7	ND	3.9	3.9	3.8	ND	ND	ND

ND: not detected

< HPLC peak area at 24 hours> (Area %)

	IQC without *CGTase*	Control solution B			Sample solution B (Inactivated *CGTase*)		
		1	2	3	1	2	3
IQC	98.3	27.4	26.9	27.3	97.0	97.3	97.6
IQC+G1	0.2	20.3	20.5	20.3	0.6	0.5	0.4
IQC+G2	0.9	15.7	15.8	15.6	1.5	1.3	1.2
IQC+G3	0.6	11.0	11.2	10.9	0.9	0.9	0.8
IQC+G4	ND	9.0	9.1	9.1	ND	ND	ND
IQC+G5	ND	7.2	7.0	7.3	ND	ND	ND
IQC+G6	ND	5.5	5.5	5.5	ND	ND	ND
IQC+G7	ND	3.9	4.0	4.0	ND	ND	ND

ND: not detected

Appendix 4b

Safety of enzymes used in making *alpha*-glycosyl isoquercitrin

	rhamnosidase	cyclomaltodextrin glucanotransferase	
IUBMB	EC.3.2.1.40	EC.2.4.1.19	
Source	*Penicillium decumbens*	*Bacillus pseudalcaliphilus*	*Bacillus macerans*
DNA technology	Non GMO		
GMP	Both enzymes are produced under food additives good manufacturing practice authorized by Japan Food Additives Association.		
Impurities	It is registered as No.292 on the List of Existing Food Additives in Japan Food Sanitation Law.	It is registered as No.193 on the List of Existing Food Additives in Japan Food Sanitation Law.	
Deactivating step	Both enzymes are deactivated by heating at 98 drgree C for 5 minutes.		
Amount	4755mg/kg(IQC) (5300U/g)	7143mg/kg(EMIQ) (1000U/g)	undecided

Appendix 4C

Certification Sheet, especially for Manufacturing Flow Chart of Enzymes

1. *Naringinase* (Product Name: Naringinase Amano Powder)

Culturing(*Penicillium decumbens*) → Water Extraction → Sterile filtration → Purification → Drying → Grinding → Sieving → Formulation → Filling → Weighing → Product (QA.testing)

2. *CGTase* (Product Name: Contizyme)

Culturing(*Bacillus macerans*) → Filtration → Concentration → Sterile filtration → Addition of stabilizer → (Stock solution) → Formulation (stock solution + stabilizer) → Filling (QA.testing) → Weighing → Product

Signature: _____

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Phone: 81 6 6333 0521
Dated: September 15, 2006

Products specification

Contizyme

Article	Method	Specification
Properties	Visual test	Brown, liquid
pH	-	6.0 - 10.0
Aresenic	※1	≦2 μg/mL
Lead	※1	≦5 μg/mL
Activity	BV method, pH5.5	≧600 units/mL
Activity	Tilden-hudson method, pH5.5	≧600 units/mL
Viable count	SCD agar plate dilution method	≦10,000/mL
E. Coli	※1	Negative

Naringinase Amano Powder

Article	Method	Specification
Properties	Visual test	White or light brown, powder
Loss on drying	1g, 105℃, 4h	≦10%
Aresenic	※1	≦2 μg/g
Lead	※1	≦5 μg/g
Activity	Amano method, pH 3.5	Observed value
Viable count	SCD agar plate dilution method	≦1,000/g
E. Coli	※1	Negative

※1 The method is defined in "Japan's Specifications and Standard for Food Additives".

Appendix 5

Analysis of specifications on *alpha*-glycosyl isoquercitrin

August 1, 2006

Fundamental Research Division
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Phone: 81 6 6333 0521

Laboratory:

Name: Fundamental Research Division
Research & Development Group
San-Ei Gen F.F.I., Inc.

Address: 1-1-11, Sanwa-cho, Toyonaka, Osaka 561-8588

Study period: July 19, 2006 to August 1, 2006

Analysis date: November 21, 2002, to June 16, 2004

Study coordinator:

Name: Hisashi Tanaka
Fundamental Research Division
Research & Development Group

Study operator:

Name: Kazuhiro Emura
Fundamental Research Division
Research & Development Group

1. Study Title

Analysis of Specifications on enzymatically modified isoquercitrin

2. Study Purpose

To confirm the accuracy and reproducibility of analysis methods on some specifications designated for *alpha*-glycosyl isoquercitrin.

3. Materials

Alpha-glycosyl isoquercitrin	Lot. 970407
	Lot. 991004
	Lot. 020329
	Lot. 021101
	Lot. 021105

4. Methods

Every 5 lots were analyzed for the following items in triplicate.

4.1 Assay

Dry up *alpha*-glycosyl isoquercitrin, weigh accurately about 0.2 g, add 50 ml of water to make a solution, filtrate through a glass filter it if necessary, wash it with water, and make an accurate 100 ml of solution with the filtrate and the filtrated solution.

Weigh 1 ml of this solution accurately, add phosphoric acid solution (1 -> 1000) to make an

accurate 100 ml of the solution, then the test solution is prepared.

Separately, dry up rutin (for measurement) at 135 degree C for 2 hours, weigh accurately about 0.2 g of the rutin, add 80 ml of methanol, warm to make a solution, then add methanol to make an accurate 100 ml solution.

Weigh 1 ml of this solution accurately, add phosphoric acid solution (1 -> 1000) to make an accurate 100 ml of solution.

The standard solution is prepared. Measure the absorbance of the test solution and the standard solution at a wavelength of 351 nm to a control of a phosphoric acid solution (1 -> 1000) and determine the content of *alpha*-glycosyl isoquercitrin based on the following equation.

The rutin equivalent content is regarded as a content of *alpha*-glycosyl isoquercitrin.

Content = A1 x R/ (A2 x S) x 100 (%)

Where A1: the absorbance degree of the test solution

A2: the absorbance degree of rutin solution

S: Sample weight used (mg)

R: Rutin weight used (mg)

* rutin: $C_{27}H_{30}O_{16}.3H_2O$

4.2 Identification

As flavonoids (1) To 5 mg of *alpha*-glycosyl isoquercitrin, add 10 ml of water, and make a solution, then add one or two droplets of ferric chloride solution (1 -> 50). The solution appears to be black-brown.

As flavonoids (2) To 5 mg of *alpha*-glycosyl isoquercitrin, add 10 ml of sodium hydroxide solution (1 -> 1000), and make a solution. The solution appears to be orange.

As flavonoids (3) To 5 mg of *alpha*-glycosyl isoquercitrin, add 5 ml of water, warm to make a solution, then add 2 ml of hydrochloride and 0.05 g magnesium powder. The solution slowly becomes to be orange.

As flavonoids (4) To 100 mg of *alpha*-glycosyl isoquercitrin, add 100 ml of 0.5 mol/l sulfuric acid, and make a solution, then cool down after boiling the solution for 2 hours. The solution has a yellow precipitate.

As quercetin (5) To 10 mg of *alpha*-glycosyl isoquercitrin, add a phosphoric acid solution (1 -> 1000), and make a solution. The solution appears to be yellow and has two maximum absorption at wavelength of 255 to 260 and 348 to 353 nm.

4.3 Purity

4.3.1 Lead (Method 1, Lead Limit Test, Japan's Specifications and Standards for Food Additives 7th Edition)

Weigh the specified quantity of the sample, place into a platinum or quartz crucible, moisten with a small amount of sulfuric acid, and ignite slowly at a temperature as low as possible until the sample is almost incinerated. Cool, add 1 ml of sulfuric acid, heat slowly, and ignite at 450 degree C to 550 degree C to incinerate. Dissolve the residue in a small amount of diluted nitric acid (1 -> 150), add diluted nitric acid (1 -> 150) to make 10 ml.

Measure 1.0 ml of Lead Standard Solution, add diluted nitric acid (1 -> 150) to make 10 ml.

Measure the atomic absorbances in the flame type method for the test solution and the control solution under the conditions given below. The absorbance of the test solution does not exceed that of control soluiton.

Operating Condition

Light source: Lead hollow cathode lamp

Analytical line wavelength: 283.3 nm

Supporting gas: Air

Inflammable gas: Acetylene

4.3.2 Arsenic (Method 3, Apparatus C, Aresnic Limit Test, Japan's Specifications and Standards for Food Additives 7th Edition)

Weigh the specified amount of the sample, and place it in a crucible of platinum, quartz, or porcelain.

Add 10 ml of a solution of magnesium nitrate in ethanol (1 -> 50), ignite ethanol, and heat gradually at 450 degree C to 550 degree C to incinerate.

If the carbonized material still remains, moisten with a small quantity of a solution of magnesium nitrate in ethanol (1 -> 50), and ignite again at 450 degree C to 550 degree C to incinerate.

After coolong, add 3 ml of hydrochloric acid, heat on a water bath to dissolve the residue, and use it as the test solution.

To 4 ml of the test solution, add 1 ml of hydrochloric acid and 1 ml of potassium iodide solution (1 -> 10), heat on a water bath at 70 degree C for 4 minutes, and add water to make 20 ml.

Flowing argon gas through the apparatus, introduce the test sample, an appropriate concentration of hydrochloric acid (1 - 6 mol/l), and sodium tetrahydroborate TS into the apparatus continuously at flow rate of 1 - 10 ml/min with pump A and mix successively in the apparatus to form arsenic hydride.

The method that potassium iodide solution (1 -> 10) is also introduced continuously into the apparatus, introduce the test solution (if necessary, after dilution with water), an appropriate concentration of hydrochloric acid (1 - 6 mol/l), potassium iodide solution (1 -> 10), and sodium tetrahydroborate TS successively into the apparatus in the same procedure as described above.

Arsenic hydride formed is separated from the waste liquid by gas liquid separator F, and gas containing arsenic hydride is introduced into an atomic absorption spectrophotometer (flame type)

equipped with an absorption cell.

Measure the atomic absorbance of the test solution at 193.7 nm.

The absorbance of the test solution does not exceed that of the following control solution.

Preparation of the control solution is carried out at the same time as the test solution.

Prepare the control in the same manner as for the test solution, using the specified Arsenic Standard Solution.

4.4 Content of quercetin

About 10 mg of each *alpha*-glycosyl isoquercitrin product is accurately weighed, solved in a precise 100 ml methanol. An accurate 1 ml methanol solution is accurately diluted into 100 ml with methanol, sample solution.

About 10 mg of quercetin is accurately weighed, solved in an accuratae100 ml methanol, standard solution.

10 micro litter of sample and standard solutions are analyzed in the following HPLC conditions. Measurement was done in triplicate for accuracy.

Apparatus and Operation conditions:

HPLC (JASCO Corp.)

Column: YMC ODS A-302 φ4.6 x 150 mm

Effluent: Acetonitril + water + phosphoric acid(250 + 750 + 2)

Detect: 254nm

Column temperature: 45 degree C

Flow rate: 1 ml/min (for quercetin)

Flow rate: 0.5 ml/min (for isoquercitrin)

5. Results

5.1 Assay (As Rutin content)

	Rutin content (%)			
Lot	1	2	3	Averaged
970407	70.6	70.4	70.6	70.5
970407	67.0	67.2	67.3	67.2
020329	76.0	76.0	76.1	76.0
021101	74.2	74.4	73.9	74.2
021105	73.0	72.7	72.8	72.8

5.2 Identity

5.2.1 Identity (1)

Lot	Identity (1)		
	1	2	3
970407	Pass	Pass	Pass
970407	Pass	Pass	Pass
020329	Pass	Pass	Pass
021101	Pass	Pass	Pass
021105	Pass	Pass	Pass

5.2.2 Identity (2)

Lot	Identity (2)		
	1	2	3
970407	Pass	Pass	Pass
970407	Pass	Pass	Pass
020329	Pass	Pass	Pass
021101	Pass	Pass	Pass
021105	Pass	Pass	Pass

5.2.3 Identity (3)

Lot	Identity (3)		
	1	2	3
970407	Pass	Pass	Pass
970407	Pass	Pass	Pass
020329	Pass	Pass	Pass
021101	Pass	Pass	Pass
021105	Pass	Pass	Pass

5.2.4 Identity (4)

Lot	Identity (4)		
	1	2	3
970407	Pass	Pass	Pass
970407	Pass	Pass	Pass
020329	Pass	Pass	Pass
021101	Pass	Pass	Pass
021105	Pass	Pass	Pass

5.2.5 Identity (5)

Lot	Maximum absorption wavelength		
	1	2	3
970407	Pass	Pass	Pass
970407	Pass	Pass	Pass
020329	Pass	Pass	Pass
021101	Pass	Pass	Pass
021105	Pass	Pass	Pass

5.3 Purity

5.3.1 Lead

Lot	Lead (ppm)		
	1	2	3
970407	2 >	2 >	2 >
970407	2 >	2 >	2 >
020329	2 >	2 >	2 >
021101	2 >	2 >	2 >
021105	2 >	2 >	2 >

5.3.2 Arsenic

Lot	AS_2O_3 (ppm)		
	1	2	3
970407	0.5 >	0.5 >	0.5 >
970407	0.5 >	0.5 >	0.5 >
020329	0.5 >	0.5 >	0.5 >
021101	0.5 >	0.5 >	0.5 >
021105	0.5 >	0.5 >	0.5 >

5.4 Content of quercetin

Lot	Quercetin content (%)			
	1	2	3	Averaged
970407	0.06	0.05	0.06	0.06
970407	0.13	0.13	0.13	0.13
020329	0.24	0.23	0.24	0.24
021101	0.18	0.18	0.18	0.18
021105	0.18	0.18	0.18	0.18

000137

Appendix 6

Composition Analysis of EMIQ

July 18, 2003

Fundamental Research Division
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1-1-11, Sanwa-cho, Toyonaka, Osaka 561-8588 JAPAN
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000139

Laboratory:

Name: Fundamental Research Division
Research & Developmet Group
San-Ei Gen F.F.I., Inc.

Address: 1-1-11, Sanwa-cho, Toyonaka, Osaka 561-8588

Study Period: July 2, 2003 to July 8, 2003

Study director: Hisashi Tanaka
Fundamental Research Division
Research & Development Group

Study coordinator: Kazuhiro Emura
Fundamental Research Division
Research & Development Group

1. Study Title:

Analysis of composition of EMIQ

2. Study Purpose:

A weight composition of EMIQ is analyzed with a HPLC as an indicator of a stable manufacturing process.

3. Tested materials

5 EMIQ products were used and their Lot. No.s are 970407, 991004, 020329, 021101, 021105.

Rutin (standard): Rutin D-10 (lot 020414, content: 99.7%, Matsuura-Yakugyou Co., Ltd.), it is in accordance with the 3rd edition of the Japanese de facto standards for naturally existing food additives.

4. Analysis methods

1) Production of Rutin calibration curve

(1) Rutin is dried at 135 degree C for 2 hours.

(2) Rutin 0.1 g accurately weighed is solved in a 5mL methanol and messed up to 100mL accurately with a deionized water. In the same procedure, two other solutions were prepared. (Three solutions)

(3) The above each Rutin solution is diluted to be 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0%(V/V) with deionized water.

(4) The six Rutin solutions are analyzed by HPLC to produce a calibration curve with each peak chart area.

000140

2) HPLC analysis of enzymatically modified isoquercitrin (Refer to the chromatogram on the last page)

(1) EMIQ is dried at 135 degree C for 2 hours.

(2) EMIQ about 0.1 gram weighed accurately, added with a 5ml methanol to make a suspension, and then added with a deionized water to make an accurate100ml solution. An accurate 5ml of this solution is added with a deionized water to make an accurate 100ml solution (sample solution).

(3) Sample solutions are analyzed by HPLC and determined on Rutin equivalent concentrations comparing peak areas of samples and Rutin calibration curve.

(4) The constituent ratios in enzymatically modified isoquercitrin samples were calculated comparing molecular weight to Rutin MW by the following formula.

(5) Each constituent consists of Isoquercitrin (IQC) and esterified with additional glucoses. Accordingly, a constituent is expressed in IQC+Gn and its MW, 464.37+162.14n and Rutin's MW is 610.51. Then, a weight composition of IQC+Gn = Rutin equivalent concentration (weight) X (464.37 + 162.14n)/610.51.

[NOTE]

HPLC areas are detected at a wavelength of maximum absorbance of 351 nm due to a flavonoid structure and each peak area ratio means a ratio of molar concentration, accordingly, at first a Rutin equivalent concentration (weight) is determined and converted to a constituent weight by the above formula.

3) Apparatus

HPLC: Agilent Technologies Inc. Series 1100

4) Operation condition

Column: ODS φ4.6 × 250mm (Waters Corporation: Symmetry C18)

Fluent: 0.085% phosphoric acid + 15% acetonitrile

Flow rate: 0.8ml/min

Detect : 351nm

Column Temp.: 40 degree C

Injection volume: 20 micro L

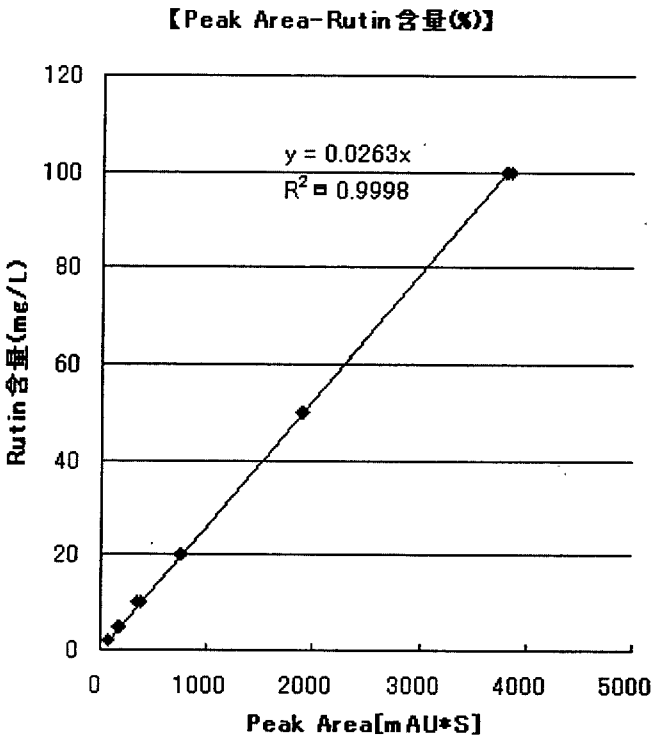
000141

5. Results

1) Rutin calibration curve

<Rutin Calibration>

mg/L	Peak Area(mAU*s)
100	3779.1227
100	3797.4385
100	3842.0645
50	1876.5491
50	1892.5281
50	1902.3018
20	752.1540
20	733.9498
20	742.0956
10	372.2253
10	348.1959
10	343.8056
5	187.0789
5	174.0171
5	165.3446
2	70.8327
2	67.5496
2	65.0992



(1) Composition data on 5 samples, three times analyzed

<1st Analysis, HPLC Peak Area> (mAU*s)

Lot.No.	970407	991004	020329	021101	021105
Conc. (mg/L)	192.8	201.6	195.2	200	190.8
IQC (IQC+G0)	1041.392	786.869	1865.976	1633.050	1291.592
IQC+G1	1282.523	1160.949	1286.023	1330.436	1189.791
IQC+G2	1190.752	1235.372	1118.862	1193.352	1151.615
IQC+G3	648.503	707.535	532.006	590.602	592.889
IQC+G4	484.149	573.303	414.131	451.097	471.840
IQC+G5	317.173	404.948	275.988	298.060	325.437
IQC+G6	193.419	253.790	172.964	184.149	203.938

IQC+G7	96.656	124.993	90.441	94.495	102.705
IQC+G8	37.218	45.320	35.688	37.422	38.727
IQC+G9	18.711	20.564	18.368	18.991	18.738
IQC+G10	21.722	16.360	13.886	17.059	14.455

<2nd Analysis HPLC Peak Area> (mAU*s)

Lot.No.	970407	991004	020329	021101	021105
Conc. (mg/L)	199	198.6	211	193	194
IQC (IQC+G0)	1078.157	800.227	2018.563	1570.353	1323.058
IQC+G1	1324.269	1168.924	1384.410	1273.112	1206.193
IQC+G2	1230.147	1203.739	1207.511	1139.345	1185.759
IQC+G3	670.287	698.555	576.431	569.401	610.382
IQC+G4	500.295	565.031	447.761	436.187	483.815
IQC+G5	328.658	400.941	298.893	289.755	333.946
IQC+G6	200.844	251.495	186.313	179.691	209.035
IQC+G7	100.800	123.581	97.202	92.580	106.100
IQC+G8	38.378	44.863	37.948	35.788	39.414
IQC+G9	21.575	22.466	20.672	21.275	21.407
IQC+G10	24.082	16.618	15.233	17.109	15.480

<3rd Analysis HPLC Peak Area> (mAU*s)

Lot.No.	970407	991004	020329	021101	021105
Conc. (mg/L)	196.2	200	197.4	193.8	199
IQC (IQC+G0)	1055.130	785.277	1896.264	1564.404	1346.952
IQC+G1	1298.432	1152.945	1297.655	1265.060	1227.177
IQC+G2	1206.778	1227.928	1122.146	1125.160	1196.121
IQC+G3	657.082	703.820	540.028	566.979	624.869
IQC+G4	491.092	569.801	418.358	434.670	494.337
IQC+G5	322.613	403.224	279.464	288.694	341.405
IQC+G6	196.572	253.577	174.391	179.272	214.282
IQC+G7	99.036	125.540	91.078	92.391	108.451
IQC+G8	36.907	44.733	35.405	35.492	39.843
IQC+G9	21.665	24.292	20.033	20.930	22.004
IQC+G10	23.482	17.069	14.294	17.331	15.974

3) EMIQ's Rutin equivalent weight ratio on 5 lots, averaged on 3 figures

Shown as relative ratios of constituents on Rutin equivalent weight.

< EMIQ weight composition as a Rutin equivalent (%)>

	970407	991004	020329	021101	021105
IQC(IQC+G0)	14.199	10.396	25.189	21.368	17.847
IQC+G1	17.467	15.262	17.291	17.337	16.324
IQC+G2	16.226	16.068	15.025	15.496	15.919
IQC+G3	8.838	9.245	7.183	7.740	8.235
IQC+G4	6.600	7.485	5.578	5.925	6.532
IQC+G5	4.332	5.298	3.722	3.929	4.508
IQC+G6	2.643	3.325	2.325	2.434	2.826
IQC+G7	1.326	1.639	1.215	1.253	1.429
IQC+G8	0.503	0.591	0.475	0.487	0.532
IQC+G9	0.277	0.295	0.257	0.275	0.280
IQC+G10	0.310	0.219	0.189	0.231	0.207
total	72.720	69.825	78.449	76.474	74.638

4) EMIQ weight composition ratio, averaged on 3 values

Use values in result 3) table, calculate according to the above formula.

< EMIQ weight composition (%)>

	M.W.	970407	991004	020329	021101	021105
IQC (IQC+G0)	464.37	10.80	7.91	19.16	16.25	13.57
IQC+G1	626.51	17.93	15.66	17.74	17.79	16.75
IQC+G2	788.65	20.96	20.76	19.41	20.02	20.56
IQC+G3	950.79	13.76	14.40	11.19	12.05	12.83
IQC+G4	1112.93	12.03	13.64	10.17	10.80	11.91
IQC+G5	1275.07	9.05	11.07	7.77	8.20	9.42
IQC+G6	1437.21	6.22	7.83	5.47	5.73	6.65
IQC+G7	1599.35	3.47	4.29	3.18	3.28	3.74
IQC+G0-7total		94.22	95.56	94.10	94.13	95.43
IQC+G8	1761.49	1.45	1.71	1.37	1.41	1.53
IQC+G9	1923.63	0.87	0.93	0.81	0.87	0.88
IQC+G10	2085.77	1.06	0.75	0.65	0.79	0.71

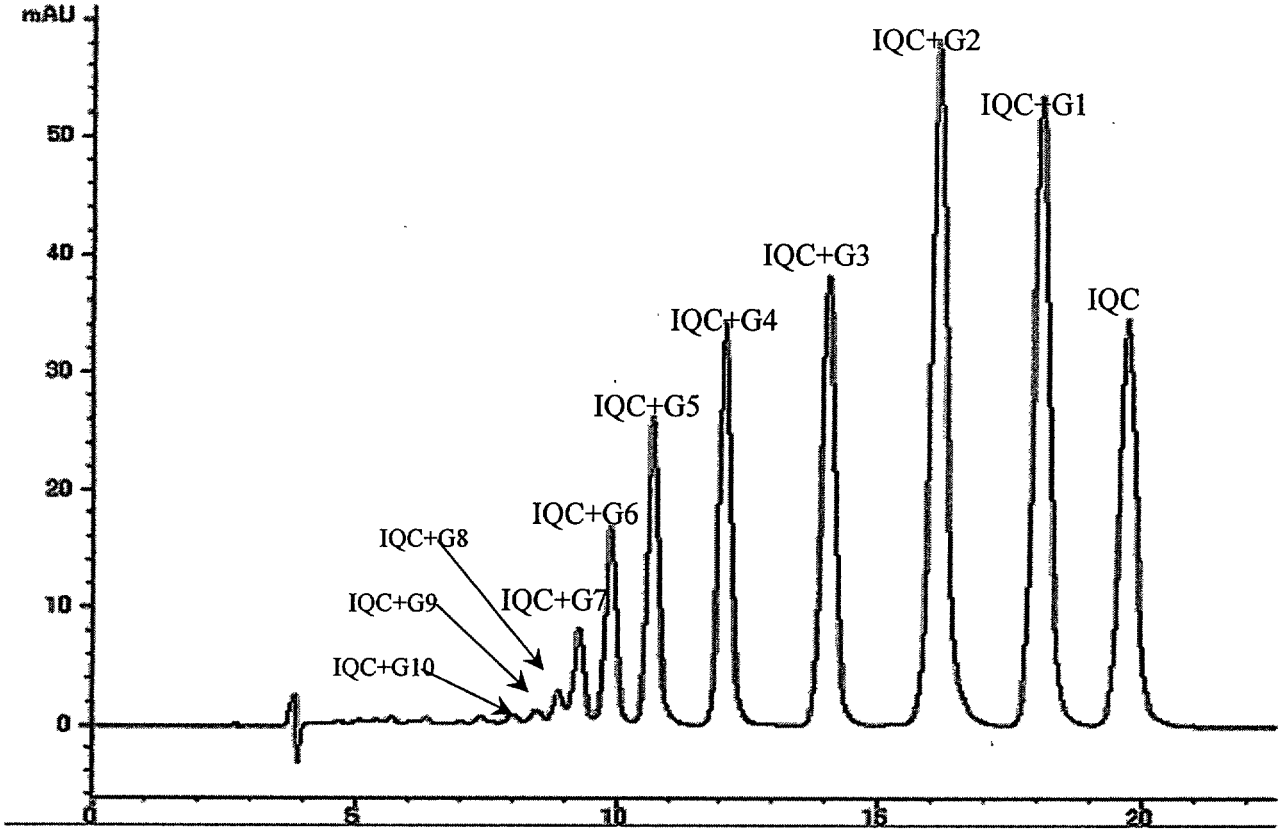
IQC+G0-10total		97.60	98.94	96.93	97.19	98.56

5. Considerations

An accumulated weight % of IQC to IQC+G7 is 94.10 to 95.43%. The accumulated weight % of IQC to IQC+G10 is 96.93 to 98.94%, where three peaks detected next before IQC+G7 are estimated to be IQC+G8, IQC+G9 and IQC+G10 although not confirmed.

Appendix

Constituent names of peak areas by HPLC chromatogram on LOT No.991004 is shown.



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Supplement Sheet of Appendix 6

The following table shows the compositions including water, quercetin and sodium sulfate of five batches of EMIQ .

We couldn't detect the residues of 0.75-2.46 % as longer chained glycosides than isoquercitrin + 10 units of glucose by HPLC method because of low separation and small areas. Water (loss on drying) and sodium sulfate were analyzed in accordance with Japan's Standards for Food Additives 7th Edition.

Table Compositions of EMIQ (w/w(%))

Compound s	Weight compositions (%)				
	970407	991004	020329	021101	021105
Isoquercitrin (IQC)	10.12	7.33	17.99	15.26	12.69
IQC + G1	16.80	14.52	16.66	16.70	15.66
IQC + G2	19.64	19.24	18.22	18.79	19.23
IQC + G3	12.90	13.34	10.50	11.32	11.99
IQC + G4	11.27	12.64	9.54	10.14	11.13
IQC + G5	8.48	10.25	7.30	7.70	8.80
IQC + G6	5.83	7.25	5.14	5.38	6.22
IQC + G7	3.25	3.98	2.99	3.08	3.50
IQC + G8	1.36	1.58	1.29	1.32	1.43
IQC + G9	0.82	0.86	0.76	0.81	0.82
IQC + G10	0.99	0.70	0.61	0.74	0.66
Water (loss on drying)	6.29	7.33	6.13	6.12	6.52
Quercetin	0.06	0.13	0.24	0.18	0.18
Sodium sulfate	0.09	0.10	0.17	0.14	0.13
IQC + Gn (n>10)	2.10	0.75	2.46	2.32	1.04
Total	100.00	100.00	100.00	100.00	100.00

Appendix 7

Analysis of quercetin in EMIQ

May 15, 2003

Analysis Group
Quality Assurance Division
San-Eigen F.F.I., Inc.
1-1-11, Sanwa-cho, Toyonaka, Osaka 561-8588 JAPAN
Phone: 816 6333 0521

000147.001

Laboratory:

Name: Fundamental Research Division, Research & Development Laboratory, San-Ei Gen F.F.I., Inc.

Address: 1-1-11, Sanwa-cho, Toyonaka, Osaka

Study period:

April 1, 2003 to April 4, 2003

Analysis date:

April 1, 2003 to April 4, 2003

Study coordinator:

Name: Takashi Morimoto
Analysis Group, Quality Assurance Division

Address: 1-1-11, Sanwa-cho, Toyonaka, Osaka

Study operator:

Name: Sakiko Ooishi
Analysis Group, Quality Assurance Division

Address: 1-1-11, Sanwa-cho, Toyonaka, Osaka

1. Study title:
   Analysis of quercetin in the products

2. Study purpose:
   A content of quercetin in a product is checked for a stable manufacturing procedure.

3. Tested material:
   Five(5) EMIQ products(lot # 970407, 991004, 020329, 021101, 021105) are used.
   Dehydrated quercetin (Funakoshi Co., Ltd.) is used as the standard.

4. Study method:
   Content of quercetin
   (1) EMIQ products and Quercetin standard are dried at 135 degree C for 2 hours.
   (2) About 10 mg of each EMIQ product is precisely weighed, solved in a precise 100 ml methanol. A precise 1 ml methanol solution is precisely diluted into 100 ml with methanol, sample solution.
   (3) About 10 mg of quercetin is precisely weighed, solved in a preicse100 ml methanol, standard solution.
   (4) 10 micro litter of sample and standard solutions are analyzed in the following HPLC

conditions. Measurement was done in triplicate for accuracy.

(5) Apparatus and Operation conditions:

HPLC (JASCO Corp.)

Column: YMC ODS A-302 ϕ 4.6 x 150 mm

Effluent: Acetonitril + water + phosphoric acid(250 + 750 + 2)

Detect: 254nm

Column temperature: 45 degree C

Flow rate: 1 ml/min (for quercetin)

Flow rate: 0.5 ml/min (for isoquercitrin)

5. Results

On the 5 samples analyzed, the maximum content of quercetin was 0.24% and the minimum, 0.05%. (Quercetin is one of decomposed derivatives from EMIQ and is regarded as an indicator of manufacturing conditions. One (1) % is the maximum limit as a normal condition and enzymatic glucosidation is on until the quercetin concentration becomes under 1 %.)

Fig. 1 Calibration curve

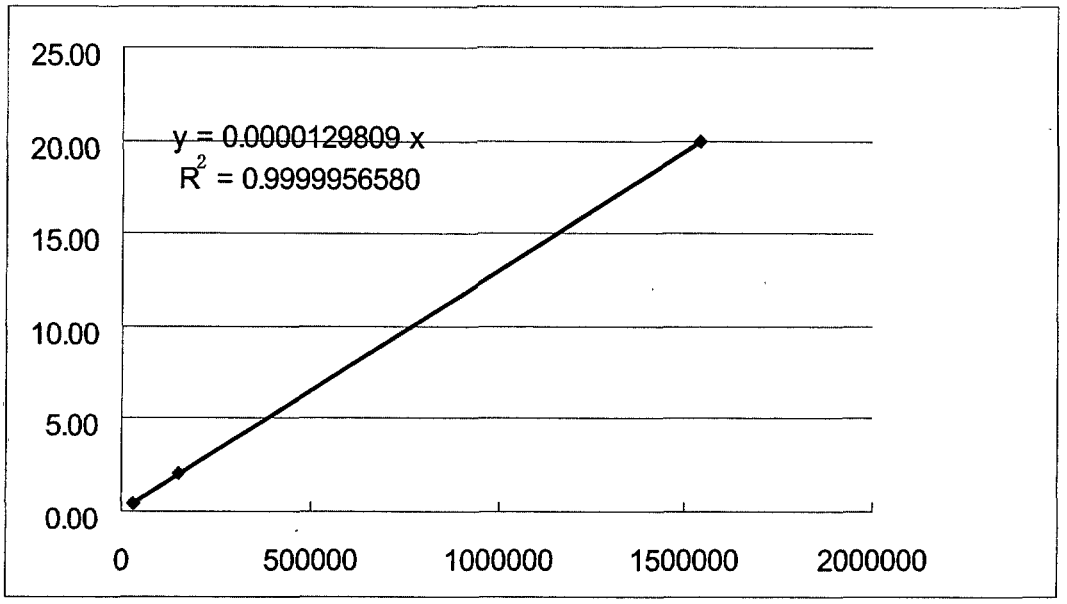

Table 1. Concentrations of Quercetin in the 5 products

	Area	Conc. (μg/ml)	Sample weight(g)	Quercetin (%)	Mean
	48289	0.62683	0.1013	0.06	
	50061	0.64984	0.1279	0.05	0.06
Lot.970407	50126	0.65068	0.1050	0.06	
	100825	1.30880	0.1025	0.13	
	103286	1.34075	0.1062	0.13	0.13
Lot.991004	110761	1.43778	0.1147	0.13	
	201531	2.61605	0.1110	0.24	
	184668	2.39716	0.1030	0.23	0.24
Lot.020329	184987	2.40130	0.1004	0.24	
	175922	2.28363	0.1281	0.18	
	141299	1.83419	0.1047	0.18	0.18
Lot.021101	149020	1.93441	0.1064	0.18	
	147409	1.91350	0.1042	0.18	
	186549	2.42157	0.1341	0.18	0.18
Lot.021105	166350	2.15937	0.1194	0.18	

Appendix 8a

Stabilization effect of EMIQ on flavors

June 25, 1993

Fundamental Research Division
San-Ei Gen F.F.I., Inc.
1-1-11, Sanwa-cho, Toyonaka, Osaka 561-8588 JAPAN
Phone: 81 6 6333 0521

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Laboratory:

Name:	Fundamental Research Division Research & Developmet Group San-Ei Gen F.F.I., Inc.
Address:	1-1-11, Sanwa-cho, Toyonaka, Osaka 561-8588, Japan

Study Period:	June 14, 1993 to June 21, 1993
Study director:	Tsutomu Wasino, Ph.D Fundamental Research Division
Study coordinator:	Hisashi Tanaka Fundamental Research Division Research & Development Group

1. Study Title:

   Stabilization effect of EMIQ on flavors

2. Study Purpose:

   1) In order to identify the effect of EMIQ as antioxidant on the deterioration of some flavors caused by sunlight, a flavored beverage with or without EMIQ in a glass bottle was kept under sunlight exposure for several days. The stability of flavors was checked by GC/MS analysis before and after the sunlight exposure.

   2) An additional sensory test was performed to check the effect of EMIQ and to confirm the taste improvement.

3. Materials and Methods

   1) GC/MS analysis

- Antioxidant

  EMIQ preparation: Sanmelin® AO-1007 (EMIQ 15%, ethanol 20%, glycerol 20%, water 45%)

- Study procedure

  Preparation of beverages

Sucrose	9 % (W/V)	
Citric acid (crystal)	0.09 % (W/V)	
Trisodium citrate	0.01 % (W/V)	
Champaign beverage essence M	0.1 % (W/V)	
Sanmelin® AO-1007	0.1 % (W/V)	or not added (0 %)
Ion exchanged water	200 ml	

The above preparation was poured in a transparent, colorless glass bottle with 15 ml headspace, and sealed. It was sterilized at 85 degrees C for 30 minutes and cooled to a room temperature.

- Acceleration study

  The bottles containing the flavored beverage with or without EMIQ (prepared as per the above) were exposed to sunlight for 36 hours. The accumulated irradiation energy measured was 36.8 Langley (300-400nm) at average temperature of 24 degrees C from 17:50, June 14 to 13:00, June 16. Besides the above bottles, some bottles containing only the flavored beverage without EMIQ were set aside as a control. They were not exposed to sunlight and kept in the dark at an average temperature of 4 degrees C.

- Flavor analysis

  0.5 ml of Internal standard solution (0.1 % Ethyl heptanoate in acetone) was added to the beverage 600 ml of the above mentioned recipe. And it was extracted with 200 ml diethyl ether and washed with 200 ml ion exchanged water. Then, it was dehydrated with anhydrous sodium sulfate, and condensed to 0.5 ml volume in Kuderna-Danish Evaporative Concentrator, then analyzed by GC/MS.

- Apparatus and conditions applied for analysis

Gaschromatograph :	HEWLETT-PACKARD 5890A
Column :	J&W DB-WAX (0.32 mm i.d. × 60 m)
Column temperature :	60 degrees C (5 min.hold)---220 degrees C, 3 degrees C/min
Injection temperature :	250 degrees C
Detector temperature :	250 degrees C
Injection volume :	1μl (split ratio 70:1)
Mass spectrometer :	Hitachi M-80B
Ionization method :	electron shock (20 eV)

2) Sensory Test

10 panelists tested the samples. Sample beverages were already refrigerated at 4 degrees C. A panelist drank beverages with or without EMIQ exposed to sunlight and beverages with EMIQ unexposed. These samples were kept in the same stressed conditions as those for a GC/MS analysis. He/she evaluated the tastes and flavors.

4. Results

1) GC/MS analysis

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Among flavor components detected and determined by GC/MS, the components that were mostly susceptible to sunlight irradiation are listed in the following table. The peak area under unirradiated condition was set as 100.0, and the corresponding areas of beverages with or without EMIQ were calculated accordingly. Only *trans*-linalool oxide (furanoid) was not detected in an

unirradiated bottle. It was detected in a beverage without EMIQ, but not detected again in a beverage with EMIQ.

Flavor components	Unirradiated	Not added	+EMIQ
Limonene	100.0	9.8	50.1
cis-Linalool oxide (furanoid)	100.0	606.9	145.2
trans-Linalool oxide (furanoid)	N.D.	detected	N.D.
Linalool	100.0	30.2	142.2
1-Terpinenol	100.0	80.3	116.1
Terpinen-4-ol	100.0	48.2	127.5
Heptanol	100.0	85.0	108.6
Neral	100.0	0.0	73.6
α-Terpineol	100.0	19.7	121.7
Borneol	100.0	28.5	103.2
Geranial	100.0	0.0	54.4
Carvone	100.0	29.0	114.1
p-Methyl acetophenone	100.0	37.1	105.4
Nerol	100.0	22.7	113.6
trans-Carveol	100.0	82.0	108.3
cis-Carveol	100.0	68.5	124.8
Limonen-10-ol	100.0	26.8	120.1
Limonen-1,2-diol	100.0	374.5	115.9
Vanillin	100.0	37.5	111.9

2) Sensory Test

All the 10 panelists evaluated that unexposed beverages tasted better than exposed ones. Also, they evaluated tasted beverages with or without EMIQ, which was better and tasted more similar to unexposed one.

Panelist number who evaluates the beverage tastes better

Not added beverages	EMIQ added bevergaes
0	10

All the panelists unanimously evaluated that beverages with EMIQ tasted better than those without EMIQ and those with EMIQ tasted similar to those unexposed than those without EMIQ. Beverages with EMIQ didn't give deteriorated flavors and were preferred. Many panelists

evaluated that the beverages without EMIQ gave deteriorated flavors and were not smoothly to drink. This sensory test confirmed the favorable effect of EMIQ on flavors.

- Conclusion

The result shows that sunlight irradiation generally decreases the concentration of flavor components. However, Sanmelin® AO-1007 prevented it from the deterioration, and kept the concentration similar to the unirradiated one. Especially, neral and geranial, which are the significant compounds for constituting a lemon-like flavor, almost completely disappeared in an irradiated one, but beverages added Sanmelin® AO-1007 maintained the concentration higher 50% than the original. Furthermore, the oxidation product like linalool oxide (furnaoid) tends to increase by irradiation, but Sanmelin® AO-1007 prevented its increase as well.

The sensory test also shows that EMIQ (Sanmelin® AO-1007) can keep the flavor quality almost unchanged after irradiation. The beverages without EMIQ were not suitable to drink due to their deteriorated flavor.

Appendix 8b

Preventive effect of EMIQ on food colorants' deterioration

November 6, 2006

Fundamental Research Division
San-Ei Gen F.F.I., Inc.
1-1-11, Sanwa-cho, Toyonaka, Osaka 561-8588 JAPAN
Phone: 81 6 6333 0521

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Laboratory:

Name: Research & Development Group
Fundamental Research Division
San-Ei Gen F.F.I., Inc.

Address: 1-1-11, Sanwa-cho, Toyonaka, Osaka 561-8588, Japan

Study period: October 25, 2006, to November 2, 2006

Analysis date: October 25, 2006, to November 6, 2006

Study director:

Name: Msamitsu Moriwaki, Ph.D.
Research & Development Group

Study coordinator:

Name: Kazuhiro Emura
Fundamental Research Division
Research & Development Group

1. Study Title

Preventive effect of EMIQ on food colorants' deterioration

2. Study Purpose

In order to identify the effect of EMIQ as antioxidant on the deterioration of some food colorants caused by light irradiation, colored model beverages added a food colorant with or without EMIQ in a glass vessel were kept under sunlight exposure. The stability of food colorants was checked before and after the sunlight exposure.

3. Materials and Methods

3-1. Materials

3-1-1. Food colorants

The following synthetic food colorants were used, FD & C Red No.40 (Allura Red AC), FD & C Yellow No.5 (Tartrazine) and FD & C Blue No.1 (Brilliant Blue FCF) , commercial products by San-Ei Gen F.F.I., Inc.

3-1-2. Antioxidant

EMIQ preparation

Trade name: Sanmelin® AO-3000

Composition: EMIQ 15 %, ethanol 20 %, glycerol 35 %, water 30 %(w/w)

3-2. Methods

3-2-1. Test solutions

Colorants concentration

FD & C Red No.40	0.001 w/w%
FD & C Yellow No.5	0.001 w/w%
FD & C Blue No.1	0.0003 w/w%

Antioxidant concentration

Sanmelin® AO-3000	0.05 w/w% (75 ppm as EMIQ)

The above colorants were dissolved in 0.2 w/w% citric acid aqueous solution containing 13.3 w/w% high fructose corn syrup (Brix 10°) with or without 0.0075 w/w% EMIQ. The colorants solutions were adjusted to pH 3.0 by addition of trisodium citrate. The solutions were sterilized by 95 degrees C for 1 sec, glass vessels (110 ml) were filled with the solutions, and then cooled in a water bath to room temperature.

3-2-2. Acceleration test

For light stability test, the solutions were irradiated using a Xenon long life fade meter (XWL-75R, 50 Langley/h, Suga Test Instruments Co., Ltd., Japan). On the basis of 100 Langley (Ly) (300-400 nm) corresponding to sunlight on a day in midsummer, the solutions were irradiated for 50 Ly and 100 Ly at average temperature of 24 degrees C, which irradiation time was 1 hr and 2 hr respectively. After the treatment, the absorbance strength (At) was measured at each wavelength of maximum absorption of food colorants with a path length of 1.0 cm and changes of color were determined from the ratio of the absorbance strength after irradiation to the initial absorbance strength (Ai). The initial color residue (0 Ly) was set as 100%. Color residue was calculated with the following equation:

Color residue (%)

= Absorbance strength after irradiation (At) / Initial absorbance strength (Ai) × 100

4. Results

Table 1 shows the result of light stability test.

Table 1 Light stability of colorants in a model beverage (pH 3.0) with or without EMIQ

Integrated irradiance (Ly)	FD & C Red No.40 (%)		FD & C Yellow No.5 (%)		FD & C Blue No.1 (%)	
	-EMIQ	+EMIQ	-EMIQ	+EMIQ	-EMIQ	+EMIQ
0	100	100	100	100	100	100
50	69.9	97.1	15.3	92.2	10.4	99.2
100	3.8	94.9	0.9	84.4	0.1	98.7

5. Conclusion

The result shows that the food colorants in beverages without EMIQ were decreased by sunlight irradiation more as the irradiation time was longer. However, EMIQ (Sanmelin® AO-3000) significantly prevents the deterioration of colorants comparison with controls. EMIQ (Sanmelin® AO-3000) can keep food colorants under sunlight irradiation.

Appendix 9

Appendix 9

Probable Metabolic Pathway of EMIQ

1. EMIQ can be partially deglycosylated by salivary and pancreatic amylases. Only isoquercitrin, IG_1 and IG_2 are likely to enter the small intestine intact (Tamura *et al.*, 2005).

2. EMIQ that does enter the small intestine is partially absorbed. Likely metabolites found in plasma from absorption in the small intestine are glucuronides and sulfates of 4'-*O*-methylquercetin (isorhamnetin) and 3'-*O*-methylquercetin (tamarixetin) (Terao, 2005; Choudhury, et al., 1999).

3. EMIQ that is not absorbed in the small intestine passes into the large intestine, where it is metabolized by resident enterobacteria. Bacteria possessing glycosidase enzymes will convert EMIQ to quercetin, which can then be absorbed into the general circulation and be present as glucuronides and sulfates. Other bacteria are known to act on the aromatic rings of quercetin, forming small chain fatty acids and phenyl and hydroxyphenylacetic acids all of which could be absorbed and enter the circulation. (Choudhury, *et al.*, 1999; Schneider *et al.*, 1999).

Appendix 10

Appendix 10

Bacterial Reverse Mutation Study of Enzymatically Modified Isoquercitlin (SANMELIN®)

(Study No. 192)

December 19, 1986

Quality Assurance Unit

San-Ei Gen F.F.I., Inc.

Summary

In order to evaluate mutagenicity of enzymatically modified isoquercitlin (SANMELIN®), the present bacterial reverse mutation study was performed with pre-incubation method using 6 strains including *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538, and *Escherichia coli* WP-2 *uvr A*.

Eight concentrations of the test material (20000, 10000, 5000, 2000, 1000, 500, 200, and 100 μg/plate) were established for the studies in the presence or absence of metabolic activation.

The test results revealed that enzymatically modified isoquercitlin (SANMELIN®) did not increase the number of reverse-mutated colonies to more than 2 times that of a negative control in the presence or absence of the metabolic activation for the 6 strains used in this study.

Based on these results, it was concluded that enzymatically modified isoquercitlin (SANMELIN®) has no mutagenicity in the presence or absence of metabolic activation under the conditions of this study.

1. Study title

Bacterial Reverse Mutation Study of Enzymatically Modified Isoquercitlin (SANMELIN®) (Study No. 192)

2. Study purpose

To evaluate the mutagenicity of enzymatically modified isoquercitlin (SANMELIN®), a bacterial reverse mutation assay was performed with pre-incubation method using 6 strains including *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538, and *Escherichia coli* WP-2 *uvr A*.

3. Testing facility

Name: Assay Group, Quality Assurance Unit, San-Ei Gen F.F.I., Inc.
Address: 1-1-11, Sanwa-cho, Toyonaka, Osaka, 561-8588

4. Study period

Study period: November 18, 1986 to December 19, 1986

5. Study director

Assay Group, Quality Assurance Unit, San-Ei Gen F.F.I., Inc.
Mikio Nakamura December 19, 1986

6. Study performer

Assay Group, Quality Assurance Unit, San-Ei Gen F.F.I., Inc.
Youichi Ogawa December 19, 1986

7. Material and method

1) Test material

Name:	Enzymatically modified isoquercitlin (SANMELIN®)
Lot. No.:	610701
Description and solubility:	Yellow or yellowish brown powder. Having high solubility in water and hydrous ethanol and extremely low solubility in oil.

2) Preparation method of the test material mixed solution

Distilled water was used as a vehicle. The test material was dissolved with water to prepare 10000 μg/plate (concentration of the test material: 10%) and 20000 μg/plate (concentration of the test material: 20%) solutions, and solutions with lower concentrations were prepared by sequentially diluting the 10000 μg/plate solution with distilled water.

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3) Type of strains tested

A total of 6 strains were used in this study: for detection of base substitution mutation, *Salmonella typhimurium* TA100 and TA1535 and *Escherichia coli* WP-2 *uvr A* were used; for detection of frameshift mutation, *Salmonella typhimurium* TA98, TA1537 and TA1538 were used. These strains were purchased from Osaka Prefectural Institute of Public Health on December 18, 1979. Each of the bacterial suspensions and DMSO were put into a test tube at a volumetric ratio of 0.8 mL vs. 0.07 mL, and stored at −80°C in a freezer (Storage apparatus: Ultra-deep freezer, MDF-290AT).

4) Medium used in this study

Nutrient broth (BACTO NUTRIENT BROTH, DIFCO) was used as a medium for pre-culture. Minimum glucose agar plate medium (powder agar for bacteria; Nissui Pharmaceutical, Co., Ltd.; Lot. No.: 660606) was used for the study.

5) Preparation of S-9 Mix

S-9 Mix was prepared as needed using routine methods, with the composition described bellow. Marketed S-9 (Oriental Yeast, Co., Ltd.; purchased on December 3 1986; Lot. No.: 86103110), which is prepared using livers from male rats (Sprague-Dawley, 7 weeks of age, 218 ± 9.1 g) receiving 4-day intraperitoneal administration of 30 mg/kg (Day 1) and 60 mg/kg (Days 2 to 4) of Phenobarbital and 80 mg/kg of 5,6-benzoflavone (Day 3) to induce the drug metabolizing enzyme system.

[Composition of 1 mL of S-9 Mix]	
S-9	0.1 mL
NADH	4 μmol
NADPH	4 μmol
$MgCl_2$	8 μmol
KCl	33 μmol
G.6.P	5 μmol
Sodium-phosphate buffer solution	100 μmol

6) Study methods

(i) Pre-culture of strains tested

Bacterial suspensions were thawed and put into a flask containing nutrient broth and incubated at 37°C for 16 hrs with shaking.

(ii) Assay procedures

In this study pre-incubation method was used in the presence or absence of metabolic activation. Study conditions are shown in the following table.

Composition	
Bacterial suspensions to be used in the study	0.1 mL
Test material solution	0.1 mL
Sodium-phosphate buffer solution	0.5 mL
S-9 Mix (for metabolic activation)	0.5 mL
Soft agar solution	2.0 mL

Conditions for incubation		
Pre-incubation:	Temperature / duration:	37°C / 20 min
Incubation:	Temperature / duration:	37°C / 48 hrs

Non-bacterial reverse mutation study was conducted in the absence of S-9 Mix for the test material solutions, and the results revealed no colonies.

7) Evaluation criteria for results

Mutagenicity was judged to be negative except when the number of reverse-mutated colonies per plate was 2 times higher in the test material groups than in the negative control group and the number increased dose-dependently.

8. Study results

Average values (n = 4) obtained from the studies in the absence or presence of metabolic activation are shown in Tables 1 and 2, respectively.

1) Studies in the absence of metabolic activation

This study was conducted at 8 concentration of the test material; 100, 200, 500, 1000, 2000, 5000, 10000 and 20000 μg/plate. The results showed that the number of reverse-mutated colonies did not increase 2 times more than that in the negative control group for each of the 6 strains (*Salmonella typhimurium* TA98, TA100,. TA1535, TA1537 and TA1538 and *Escherichia coli* WP-2 *uvr A*) used in this study.

2) Studies in the presence of metabolic activation

This study was conducted at 8 concentration of the test material; 100, 200, 500, 1000, 2000, 5000, 10000 and 20000 μg/plate. The results showed that the number of reverse-mutated colonies did not increase by more than 2 times that in the negative control group for each of the 6

strains (*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538 and *Escherichia coli* WP-2 *uvr A*) used in this study.

9. Conclusion

Mutagenicity of enzymatically modified isoquercitlin (SANMELIN®) was evaluated with pre-incubation method using 6 strains including *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538 and *Escherichia coli* WP-2 *uvr A*. Eight doses of the test material (100, 200, 500, 1000, 2000, 5000, 10000 and 20000 μg/plate) were established for the studies in the presence or absence of metabolic activation.

Enzymatically modified isoquercitlin (SANMELIN®) did not increase the number of reverse-mutated colonies to more than 2 times that of the negative control in the presence or absence of metabolic activation for the 6 strains used in this study. Therefore, the test material was concluded to have no mutagenicity.

Besides, the numbers of reverse-mutated colonies of both the negative and positive controls were within the background data of our institute, and thus the study was considered to have been conducted under adequate conditions.

Based on these results, it was concluded that enzymatically modified isoquercitlin (SANMELIN®) has no mutagenicity in the presence or absence of metabolic activation under the conditions of this study.

<Study Results>

Table 1 Results of the study in the absence of metabolic activation[a] (without S-9 Mix)

	Concentration µg / plate	Number of reverse-mutated colonies / plate					
		Base substitution mutation			Frameshift mutation		
		TA100	TA1535	WP-2	TA98	TA1537	TA1538
Test material	Vehicle control	105	5	14	27	11	17
	100	101	3	13	24	9	17
	200	105	4	11	27	10	11
	500	105	5	13	31	8	17
	1000	96	3	10	26	8	16
	2000	97	5	9	29	10	14
	5000	101	3	9	26	9	15
	10000	102	3	10	24	9	12
	20000	100	4	11	29	10	11
Positive control	Compound	ENNG*	ENNG	ENNG	2NF**	9AA***	2NF
	µg / plate	3	5	2	1	80	2
	No. of mutated colonies	901	3368	1382	979	712	1309

[a] Average value (n = 4)

* ENNG: *N*-Ethyl-*N'*-nitro-*N*-nitrosoanidine

** 2-NF: 2-Nitro-fluorene

*** 9AA: 9-Aminoacridine hydrochloride monohydrate

Table 2 Results of the study in the presence of metabolic activation[a] (with S-9 Mix)

	Concentration µg / plate	Number of reverse-mutated colonies / plate					
		Base substitution mutation			Frameshift mutation		
		TA100	TA1535	WP-2	TA98	TA1537	TA1538
Test material	Vehicle control	126	9	14	40	12	25
	100	133	7	12	34	11	20
	200	122	7	10	29	8	25
	500	132	6	10	36	8	24
	1000	133	8	12	35	10	23
	2000	143	6	10	32	8	23
	5000	137	7	11	32	7	21
	10000	121	8	10	32	8	23
	20000	117	10	11	38	10	24
Positive control	Compound	B(a)P*	2AA**	2AA	B(a)P	B(a)P	B(a)P
	µg / plate	5	2	5	5	5	5
	No. of mutated colonies	1304	119	899	387	123	129

[a] Average value (n = 4)
* B(a)P: Benzo[a]pyrene
** 2AA: 2-Aminoanthracene

Appendix 11

Appendix 11

Acute oral toxicity study of SANMELIN® 11130 in rats

January 10, 1987

Yukio Yanagimoto, MD, Ph.D.

Director
Life Science Laboratory
New Okazakibashi building 4F, 2-5-19, Nishi-Honmachi Nishi-ku, Osaka
Tel: 06-531-1881

Summary

An acute oral toxicity study of SANMELIN® 11130 was conducted in rats. SANMELIN® 11130 at the maximum physically-administrable dose of 25 g/kg caused no deaths in both sexes. Therefore, the LD_{50} of the test material was estimated at 25 g/kg or more for both sexes.

Objective:
Single oral administration of SANMELIN® 11130 was conducted in male and female rats to observe mortality and the number of surviving animals and general conditions, and calculate its LD_{50}.

Sponsor:

Name:	San-Ei Chemical Industries Co., Ltd.
Address:	1-1-11, Sanwa-cho, Toyonaka, Osaka

Contractor:

Name:	Life Science Laboratory
Address:	New Okazakibashi building 4F 2-5-19, Nishi-Honmachi, Nishi-ku, Osaka
Testing facility:	Hirano Laboratory, Life Science Laboratory 6-9-8, Uuriwari, Hirano-ku, Osaka

Study period:

Start of the study:	December 17, 1986
End of the study:	January 10, 1987

Test material:

Name:	SANMELIN® 11130
Composition:	Drying loss, 2.6%; crude protein, 0.06%; crude fat, 0.05%; ash content, 0.57%; carbohydrate, 96.92%; arsenic, less than 1 ppm; heavy metal, less than 1 ppm; no. of live bacteria, 0; no. of funguses, 0; no. of yeasts, 0; *Escherchia coli*; (-); *Staphylococci*, (-); having no mutagenicity
Lot No.:	611130
Description:	Hygroscopic yellowish brown powder
Solubility:	Very soluble in water

Stability:	Stable at room temperature
Storage conditions:	Stored in an airtight container
Supplier:	Name: San-Ei Chemical Industries Co., Ltd.
	Address: 1-1-11, Sanwa-cho, Toyonaka, Osaka
Receipt date:	The test material was supplied by the sponsor on December 20, 1986.

Control compound:
Control compounds were not used.

Preparation of the test material:
Fifty grams of the test material was placed in a mortar, mixed with 5 % Arabic gum water solution and made up to 100 mL to prepare 50 %(w/v) suspension.

Test system

Animals used:
Four-week-old Sprague-Dawley rats (SPF; body weight at the time of receipt: 63.5 – 72.2 g [males] and 57.9 – 68.3 g [females]) were purchased from Charles River Japan, Inc, on December 19, 1986. These animals were acclimated for 7 days, after which animals in good general conditions at 5 weeks of age were used for the study. The number of animals used for the study was 10 for each sex per group, and body weight ranged from 128.2 – 143.8 g for males and 117.2 – 131.4 g for females. Remaining animals were sacrificed.

Animal identification method:
For identification, animal number was written on the tail, and also on each of the cages and cage racks.

Group assignment:
All animals were weighed before administration and randomly assigned to each study group so as to minimize the difference in mean body weight between each group.

Animal husbandry:
Animals were housed in stainless steel wire mesh cages (W185 x D260 x H175 mm) and reared in a barrier-system animal room at a controlled room temperature of 23 ± 3°C, relative humidity of 55 ± 10%, with ventilation of 12 times or more per hour, and in the artificial lightening duration of 12 h per day (7:00 – 19:00). The cage racks and the animal room were cleaned and the floor was washed daily, and then they were sterilized with Tego-51.

Animals were provided with pellet NMF (Oriental Yeast, Co., Ltd.) and with Osaka City tap water via a polycarbonate bottle *ad libitum*.

Study method

1. Administration route: Oral gavage administration
   Reason for selection: Followed instruction by the sponsor

2. Administration method

Pellets were removed from cages before administration, and animals were fasted for more than 10 hrs. After that, gavage administration of the test material was conducted using an intragastric tube for rats.

3. Establishment of study groups and doses

Based on results of a preliminary study, the study groups and dosages described below were selected for the present study. Since no cases of death were observed at a dose of 25 g/kg in a preliminary study, this dose was selected as the highest dose, and 2 other doses of 20 g/kg, the dose into which 25 g/kg was divided by common ratio of 1.25, and 16 g/kg were established by sequential dilution.

Sex	Dose (g/kg)	Volume administered (mL/100g)	No. of animals	Animal No.
Male	25	5.0	10	1 – 10
	20	4.0	10	11 – 20
	16	3.2	10	21 – 30
Female	25	5.0	10	1 – 10
	20	4.0	10	11 – 20
	16	3.2	10	21 – 30

4. Frequency of administration: Single administration
   Reason for selection: Followed instruction by the sponsor (acute toxicity study)

5. Observation period

For 14 days after administration

Reason for selection: Followed SOP for acute toxicity study used by Life Science Laboratory

6. Observation of clinical conditions

Animals were observed for clinical conditions including mortality and the number of surviving animals, external appearance and behavior frequently on dosing day and thereafter once daily for 14 days.

7. Measurement of body weight

Day of dosing was set as Day 0, and body weight was measured before administration and on Days 1, 3, 7 and 14.

8. Necropsy

All animals surviving at the end of the observation period (Day 14) were sacrificed under ether anesthesia on Day 14, followed by necropsy and gross pathological observation.

9. Factors that might have affect reliability of the study results

Humidity range increased by 5% (55 ± 15%) during the study period, which deviated from the study protocol. However, it was caused by running a dehumidifier to adjust humidity and persisted transiently. Additionally, humidity was not beyond the range of 40 to 70%, and thus it was not considered to have affected the reliability of the study results.

10. Study personnel

Responsible personnel for management of breeding:	Yoshihisa Fujii
Responsible personnel for administration and observation:	Atsunobu Matsuo
Responsible personnel for pathological necropsy:	Yukio Yanagimoto

11. Statistical analysis

Administration of SANMELIN® 11130 at the maximum physically-administrable dose of 25 g/kg caused no deaths. Therefore, Bliss's Probit analysis was not used for calculation of LD_{50} value.

Study Results

Mean body weight of each group measured before dosing and on Days 1, 3, 7 and 14 of administration are shown in Tables 1 and 2, body weight curve during the observation period are presented in Figures 1 and 2, and mortality and surviving animals for 14 days are shown in Tables 3 and 4.

1. Mortality and the number of surviving animals

There were no cases of death during the observation period (14 days) in both sexes of the 16, 20 and 25 g/kg groups.

2. Observation of clinical conditions

   1) Spontaneous activities

      Although spontaneous activities slightly decreased immediately after administration in both sexes of the 25 mg/kg group, they recovered within a few hours.

   2) Other abnormal findings

Other remarkable abnormal findings were not observed.

3. Changes in body weight

Steady body weight gain was observed in males and females of each group. There was no significant difference in mean body weight between each group.

4. Necropsy findings

All the males and females of each group were sacrificed under ether anesthesia at the end of the observation period (14 days after administration), and were conducted on necropsy. No remarkable abnormalities were noted in gross observation of the viscera.

Discussion

In the present acute oral toxicity study of SANMELIN® 11130, there were no cases of deaths in both sexes at the maximum physically-administrable dose of 25 g/kg. Also, no abnormalities were noted in clinical conditions and in gross observation of the viscera during the necropsy at the end of the observation period.

Therefore, the test material was considered to have an excellent safety profile.

Conclusion

Based on the study results, LD_{50} values of the test material for SD rats are as follows;

Name of test material: SANMELIN® 11130

LD_{50} for SD rats (in terms of weight)

Male rats: 25 g/kg or more

Female rats: 25 g/kg or more

(There is a physical limit with administration at doses higher than 25 g/kg.)

Study personnel

Responsible personnel for animal husbandry:

_____ January 9, 1987

Yoshihisa Fujii

Responsible personnel for administration and observation:

_____ January 9, 1987

Atsunobu Matsuo

Responsible personnel for pathological necropsy:

_____ January 9, 1987

Yukio Yanagimoto

Study Director:

_____ January 10, 1987

Atsunobu Matsuo

Management:

_____ January 10, 1987

Yukio Yanagimoto

Table 1: Changes in body weight during the observation period (14 days) in the acute toxicity study

(Animal species, SD rats; administration route, oral; sex, male)

Name of test material: SANMELIN® 11130 (50 w/v% - 5% Arabic gum)

(Unit: g)

Sex	Dose g/kg (mL/kg)	No. of animals used	Day of dosing (Day 0)	Day 3	Day 7	Day 14
Male	25 (50.0)	10	135.25 ±1.23	156.49 ±1.49	187.29 ±1.61	249.01 ±2.48
	20 (40.0)	10	136.13 ±1.47	157.71 ±2.79	189.50 ±3.38	249.31 ±4.75
	16 (32.0)	10	136.99 ±1.50	159.28 ±1.83	190.37 ±2.00	248.26 ±3.17

Mean ± S.E.

Table 2: Changes in body weight during the observation period (14 days) in the acute toxicity study

(Animal species, SD rats; administration route, oral; sex, female)

Name of test material: SANMELIN® 11130 (50 w/v% - 5% Arabic gum)

(Unit: g)

Sex	Dose g/kg (mL/kg)	No. of animals used	Day of dosing (Day 0)	Day 3	Day 7	Day 14
Female	25 (50.0)	10	123.94 ±1.25	136.74 ±1.69	156.59 ±1.92	181.05 ±2.81
	20 (40.0)	10	122.94 ±1.13	138.35 ±1.20	158.39 ±2.22	185.14 ±2.68
	16 (32.0)	10	123.61 ±1.30	139.58 ±1.92	158.42 ±3.01	183.53 ±3.23

Mean ± S.E.

Figure 1: Body weight curve during the observation period (14 days) in the acute toxicity study

(Animal species, SD rats; administration route, oral; sex, male)

Name of test material: SANMELIN® 11130 (50 w/v% - 5% Arabic gum)

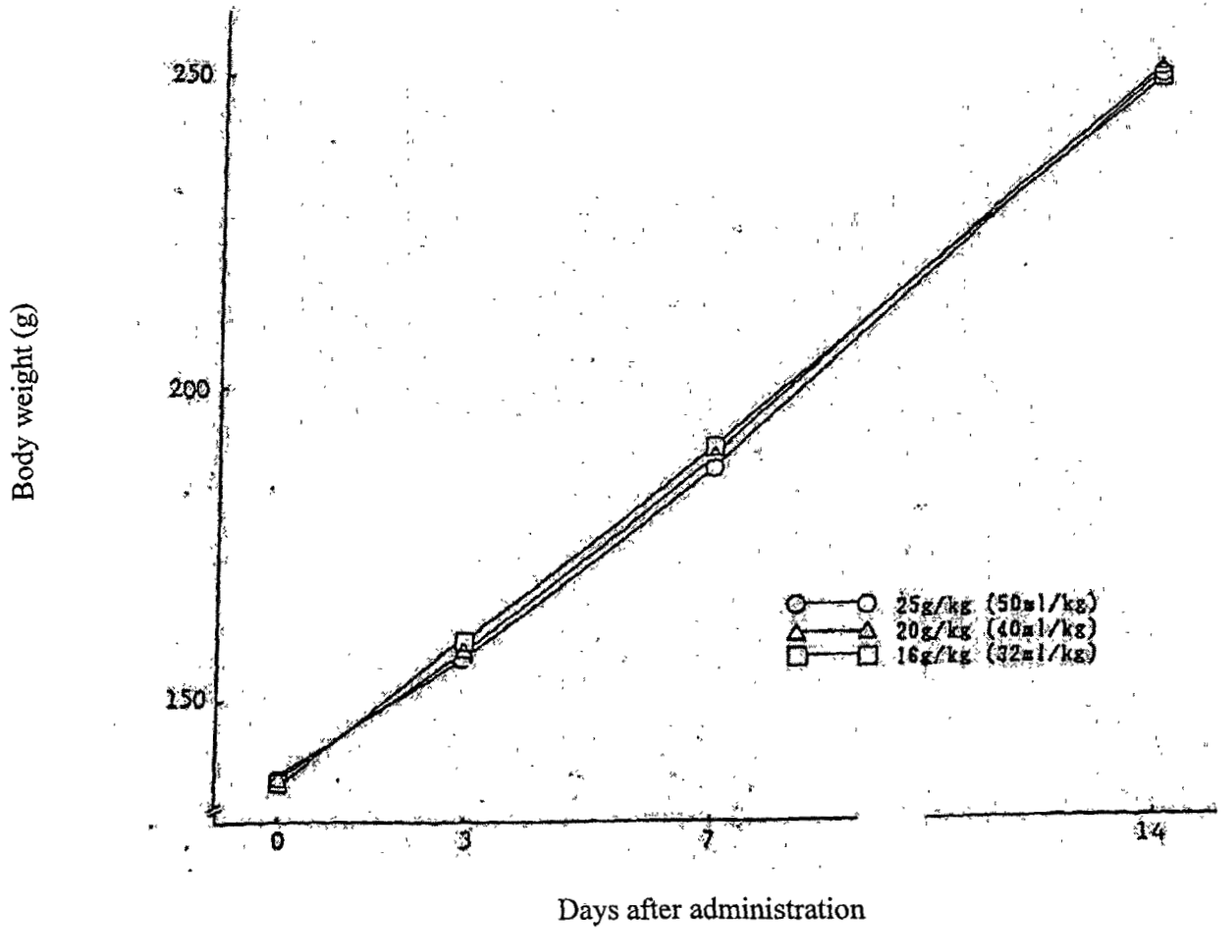

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Figure 2: Body weight curve during the observation period (14 days) in the acute toxicity study

(Animal species, SD rats; administration route, oral; sex, female)

Name of test material: SANMELIN® 11130 (50 w/v% - 5% Arabic gum)

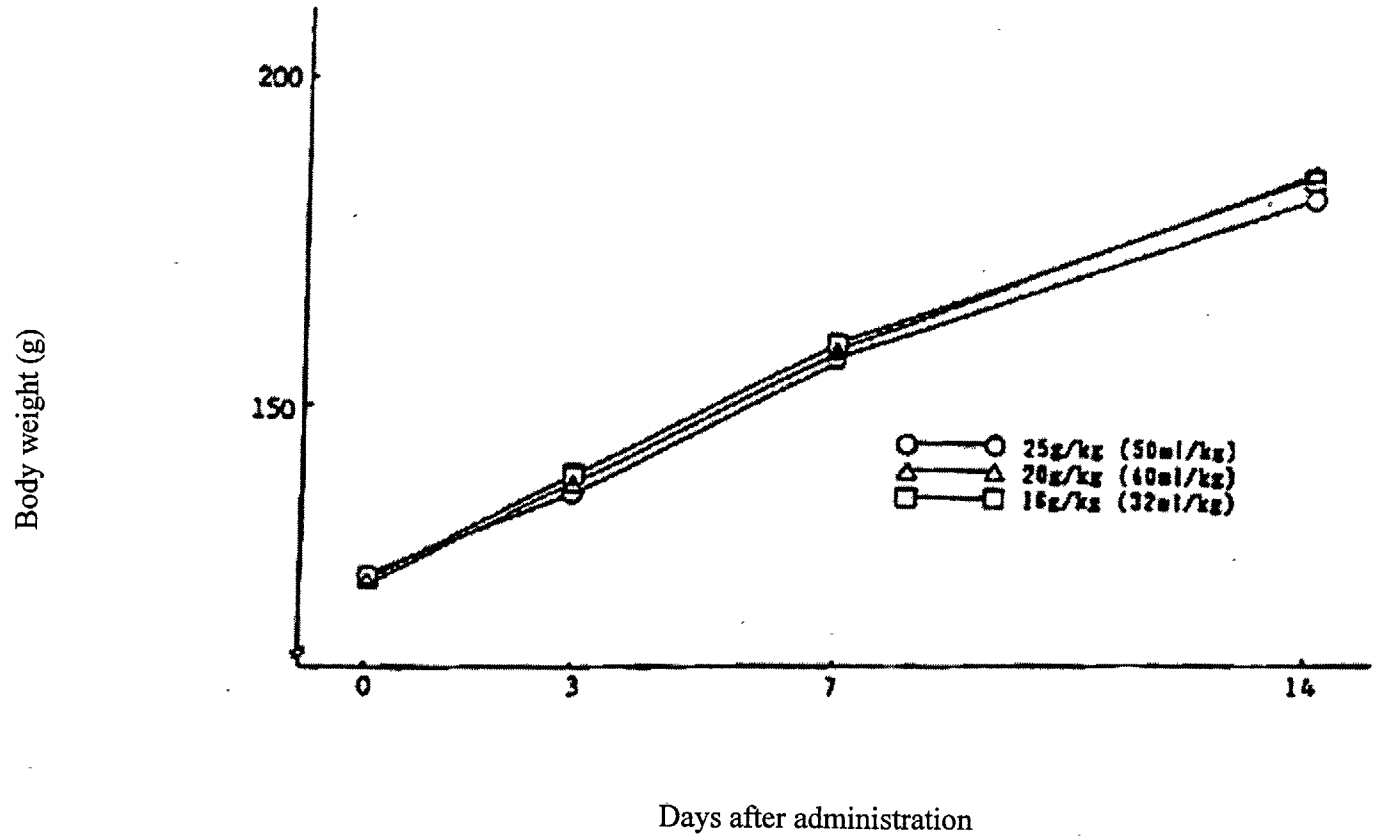

Table 3: Mortality and the number of surviving animals

(Animal species, SD rats; Administration route, oral; Sex, male)

Name of test material: SANMELIN® 11130 (50 w/v % - 5% Arabic gum)

Sex	Dose g/kg (mL/kg)	No. of animals	Days after administration, mortality and the number of surviving animals													
			Day 1	Day 2	Day 3	Day 4	Day 5	Day6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Male	25 (50.0)	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	20 (40.0)	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	16 (32.0)	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4: Mortality and the number of surviving animals

(Animal species, SD rats; administration route, oral; sex, female)

Name of test material: SANMELIN® 11130 (50 w/v% - 5% Arabic gum)

Sex	Dose g/kg (mL/kg)	No. of animals	Days after administration, mortality and the number of surviving animals													
			Day 1	Day 2	Day 3	Day 4	Day 5	Day6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Female	25 (50.0)	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	20 (40.0)	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	16 (32.0)	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 12

Preliminary Study for 90-Day Oral Toxicity of Enzymatically Modified Isoquercitrin in F344/DuCrj Rats

Project No. 9701

FINAL REPORT

-English version translated from the Japanese original-

Submitted to

San-Ei Gen F.F.I., Inc., Osaka, Japan

January 28, 2003

Daiyu-Kai Institute of Medical Science

Ichinomiya, Japan

000186

CERTIFICATE OF TRANSLATION

Study Title: Preliminary Study for 90-Day Oral Toxicity of Enzymatically Modified Isoquercitrin in F344/DuCrj Rats

Project No.: 9701

This is to certify that I have reviewed the attached translation and that the English is faithful to the original Japanese text. Occasionally certain words may have been changed from dictionary alternatives in order to retain the original meaning and intent.

Date:

Seiko Tamano, Ph.D., Dip.J.S.T.P. and J.S.T.
Management Director
Daiyu-Kai Institute of Medical Science

000187

Preliminary Study for 90-Day Oral Toxicity of Enzymatically Modified Isoquercitrin in F344/DuCrj Rats

Project No. 9701

FINAL REPORT

(English version translated from the Japanese Original)

Study completed: August 7, 1997

Daiyu-Kai Institute of Medical Science

Ichinomiya, Japan

GLP COMPLIANCE STATEMENT

Study Title: Preliminary Study for 90-Day Oral Toxicity of Enzymatically Modified Isoquercitrin in F344/DuCrj Rats

Project No.: 9701

The investigation described in this report was carried out under my supervision in accordance with the Japanese Good Laboratory Practice Standard Pharmaceutical Affairs Bureau Ministry of Health and Welfare (Ministerial Ordinance No. 21, March 26, 1997).

Tadaomi Kadota Date: August 8, 1997

Management Director
Daiyu-Kai Institute of Medical Science

QUALITY ASSURANCE STATEMENT

Study Title: Preliminary Study for 90-Day Oral Toxicity of Enzymatically Modified Isoquercitrin in F344/DuCrj Rats

Project No.: 9701

Quality assurance inspections of the study and the review of this final report were conducted according to the standard operating procedures of the Quality Assurance Unit on the following dates, and we herewith certify that the study was properly performed and the methods and procedures used were correctly recorded in the final report and this presents accurately all data generated (see next page).

OFFICE OF QUALITY ASSURANCE (Continued)

Items	Dates	
	Inspection or audit	Report to management and study director
Protocol	4/22/1997	4/22/1997
Amendment 1	4/24/1997	4/24/1997
Procedures	4/28/1997	4/28/1997
	5/6/1997	5/6/1997
	5/8, 13, 14/1997	5/19/1997
	5/28/1997	5/28/1997
	6/12/1997	6/12/1997
Raw data and notes	6/19, 20/1997	6/23/1997
	7/22, 23/1997	7/23/1997
Draft report	6/27/1997	6/27/1997
Final report	8/8/1997	8/8/1997

Quality Assurance

Akihiro Hagiwara Date: August 8, 1997

Director of the Quality Assurance Unit

STUDY INFORMATION

Study Title: Preliminary Study for 90-Day Oral Toxicity of Enzymatically Modified Isoquercitrin in F344/DuCrj Rats

Sponsor: San-Ei Gen F.F.I., Inc.
1-1-11 Sanwa-cho Toyonaka-city, 561-8588 Japan

Testing Facility: Daiyu-Kai Institute of Medical Science
25 Nikenya, Nishi-azai Azai-cho
Ichinomiya-city, 491-0113 Japan

Commencement of the study: April 22, 1997

Schedule:

Animal receipt:	May 8, 1997
Commencement of the dosing of test chemical:	May 14, 1997
End of the dosing of test chemical:	June 11, 1997
Sacrifice:	June 12, 1997

Completion of the study: August 7, 1997

Unforeseeable circumstances that may have ill effects on the reliability of the study and deviation from the protocols: No particulars to mention.

Archives: The records, raw data, specimens generated in the course of the study and a copy of final report are to be stored in the archives of Daiyu-Kai Institute of Medical Science for three years after submission of the final report, the original of which is to be stored by the sponsor. After expiration of the contracted retention period, the institute and the sponsor will decide on future steps.

STUDY PERSONNEL

Study Title: Preliminary Study for 90-Day Oral Toxicity of Enzymatically Modified Isoquercitrin in F344/DuCrj Rats

Project No.: 9701

Study Director :

Date: August 7, 1997

Assistant Study Director :

Date: August 7, 1997

Principal Responsibility:
Animal Care :

Date: August 7, 1997

Pathology :

Date: August 7, 1997

Data Processing :

Date: August 7, 1997

STUDY ATTENDANTS

Animal Care :

Diet Preparation :

Clinical Observation, Measurement of Body Weights and Food and Water Consumption:

Autopsy and Measurement of Organ Weights:

Data processing and statistical analysis:

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I. SUMMARY

The potential repeated oral toxicity of enzymatically modified isoquercitrin (EMI) was investigated in F344/DuCrj (Fischer) rats of both sex given powdered diet containing the compound at 0, 0.625, 1.25, 2.5 or 5% for 4 weeks.

No clinical signs or mortality related to the treatment were found throughout the experiment.

Regarding body weight change, a tendency for lowering mean body weights was observed in both sexes of the 2.5 and 5% groups.

Food and water consumption in males of 2.5 and 5% groups also tended to be lowered.

On macroscopic examination, yellow coloration of bones (cranium and fore- and hind-limb bone) was found in both sexes receiving 1.25% or more.

No alterations due to the EMI treatment were found for liver, kidney or spleen weights.

The present study demonstrated a tendency for lowering body weights in both sexes given 2.5 and 5% and food and water consumption in males of the 2.5 and 5% groups, as well as yellow coloration of bones in both sexes of the 1.25% or more group. Thus, it was concluded that 2.5% should be selected as the highest dietary level for the 90-day repeated oral toxicity study with apparent adverse effects. The lower levels were set at 1.25%, 0.625% and 0.3% with a proportional factor of 2.

Ⅱ. INTRODUCTION

The present 28-day repeated dose toxicity study was conducted on F344 rats of both sexes to determine the dosage level for a 90-day repeated oral toxicity study of enzymatically modified isoquercitrin (EMI).

Ⅲ. MATERIALS AND METHODS

1. Test chemical

The test chemical used in this study was supplied by San-Ei Gen F.F.I., Inc. (Osaka, Japan). Its name, lot No., Purity, properties, storage condition, manufacturing date, expiration date and receipt date are given below.

Name: Enzymatically modified isoquercitrin (EMI)
Lot No.: 970407
Purity: 71.30% (as rutin content)
Properties: Fine yellow, specific mild flavor
Storage conditions: In a refrigerator. After opening, the package was sealed up as instructed by the supplier.
Manufacturing date: April 7, 1997
Expiration date: April 7, 1999
Supply date: April 12, 1997

2. Diet preparation and dietary analysis

(1) Diet preparation

The test chemical was added to powdered MF (Oriental Yeast Co., Ltd., Tokyo, Japan) to the required levels and mixed in a mechanical mixer (type KS101; Shin-Kanto Co., Osaka, Japan) for 30 minutes. The diet preparation was carried out one time and the prepared diets were stored in a refrigerator (4~10℃).

(2) Dietary analysis for stability, homogeneity and content

In order to investigate the stability, homogeneity and content of the test compound in the prepared diet, samples were shipped to the following institute:

Japan Food Research Laboratories, Tama Laboratories
6-11-10, Nagayama,
Tama, Tokyo 206-0025 Japan

The results of the analysis summarized from the final report, Japanese version (Ⅷ. Appendices 1), are shown below.

(a) Stability

Stability of test chemical in diet containing 5 and 0.1% was analyzed 0, 1, 2, 4, 8 weeks after preparation with storage under conditions of refrigeration or at room temperature. The results of the analysis summarized from the final report, Japanese version are shown below. The contents of EMI at each dietary level remained within the acceptable range (±16%) for up to 8 weeks under storage conditions.

(Refrigeration)

Nominal Concentration (%)	Results of the analysis (%) Weeks after the preparation				
	0	1	2	4	8
0.1	-	0.0974	0.101	0.100	0.101
5	-	5.07	5.24	5.10	5.27

(Room temperature)

Nominal Concentration (%)	Results of the analysis (%) Weeks after the preparation				
	0	1	2	4	8
0.1	0.101	0.0986	0.101	0.103	0.103
5	5.01	5.06	4.95	4.94	5.28

Preparation Date: April, 28, 1997

(b) Homogeneity

One hundred grams of each diet sampled from the top, middle and bottom of the mixer in the first preparation were subjected to analyses.

The results of the analysis were summarized below. The data were judged to demonstrate homogeneity.

Nominal Concentration (%)	Results of the analysis (%) Layer		
	Top	Middle	Bottom
0.625	0.615	0.626	0.624
1.25	1.27	1.23	1.28
2.5	2.50	2.53	2.49
5	4.95	5.13	5.01

Preparation date: May, 6, 1997

(c) Concentration

Averages of three values for homogeneity were applied for the concentration.

The contents for dietary levels were within the acceptable range (±16%).

Nominal Concentration(%)	Results of the analysis(%)
0.625	0.622
1.25	1.26
2.5	2.51
5	5.03

3. Animals

Thirty one male and female F344/DuCrj (Fischer) rats (actual ordered numbers:30 each) were purchased from Charles River Japan (Atsugi, Japan) at the 4 weeks of age on May 8, 1997 and allowed a 1-week acclimatization period during which body weights and health conditions were monitored. Only after confirmation of normal status were they allocated for the study at the age of 5 weeks. This strain of rats was selected for use in the present study since it is frequently used in toxicity studies and these are abundant compiled data available.

4. Animal husbandry

The animals were housed 5 to a plastic cage (L 412 x W 260 x H 195mm), on hardwood chip bedding (Beta Chip : Northeastern Products Co., NY, USA). The cages with bedding were changed twice a week. The environmental conditions in the animal room were controlled to maintain the temperature at 22±2℃ and the humidity at 55±10%, ventilation 15 times/hr or more (all fresh system) and illumination for 12h each day (lights on at 7:00 am and off at 7:00 pm). The rats were allowed free access to the basal or test chemical-containing diet from feeders (CL-0921: Clea Japan, Inc., Tokyo, Japan). Drinking water (Ichinomiya City Water Supply) was also supplied *ad libitum* via bottles. Food was refilled every 2 or 3 days, the feeders were replaced once a week, and the water bottles every 2 or 3 days. The values from contaminant analyses of food, water and bedding are given in the appendices 2, 3 and 4 (Japanese version). The results for food and bedding were within the acceptable ranges. The drinking water was confirmed to be

within the regulations concerning the quality of water.

Individual rats were identified by a combination of Cage No. and In-Cage animal No. (ear punching and marking with dry ink). Cage cards indicated the project No., cage No., dosage level, group No., animal No., duration, commencement and termination dates of the experiment and the names of the study director and the assistant study director, and different color tapes for each dietary group were attached to each cage. During the acclimatization period, individual animals were identified by a combination of Cage No. and In-Cage No. (marking with dry ink).

5. Testing methods

(1) Administration duration

A four-week period of administration was selected.

(2) Groups

Twenty five out of 31 rats in each sex were allocated to 5 groups (5 rat/group each sex) using a computerized stratified body weight technique, as indicated in the following table. The remaining animals were excluded from the present study. The body weight ranges at the commencement of study were 72~77 grams for females and 85~94 grams for males.

Dietary level (%)	Females		Males	
	No. of rats	Animal Number	No. of rats	Animal Number
0	5	9701001~005	5	9701026~030
0.625	5	9701006~010	5	9701031~035
1.25	5	9701011~015	5	9701036~040
2.5	5	9701016~020	5	9701041~045
5	5	9701021~025	5	9701046~050

(3) The rationale for selection of the dietary levels and the administration route

The highest dietary level, 5% was selected in compliance with the Guidelines for Designation of Food Additives and for Revision of Standards for Use of Food Additives of the Japanese Ministry of Health and Welfare. The lower levels were set at 2.5%, 1.25% and 0.625% with a proportional factor of 2. The test compound was given in the diet, since the oral route is the most likely anticipated for human exposure.

(4) Observation and measurement during the experiment

1) Clinical signs

All animals were checked for general behavior, signs of toxicity and mortality twice daily (in the morning and afternoon), and findings were individually recorded.

2) Palpation

Palpation was performed on all animals when body weights were measured once a week.

3) Body weights

Body weights were measured using an electric balance (Sartorius LC2200) for all animals at the commencement of treatment, and then at weekly intervals. At the final sacrifice, body weights were also determined after overnight (ca. 16 hours) fasting.

4) Food consumption, test material intake, and food efficiency

Two-day food consumption per cage was measured at weekly intervals, and mean daily food consumption values per rat were calculated from the number of animals housed in one cage and the number of days. Test material intake was calculated from the nominal dietary levels, group mean food consumption and body weight data. Food efficiency was calculated by dividing 1-week body weight gain values by the total amount of food consumed during the same period.

5) Water consumption

Two-day water consumption per cage was measured at weekly intervals, and mean daily water consumption values per rat were calculated from the number of animals housed in one cage and the number of days.

(5) Pathology

At the termination, all surviving animals were fasted from the evening (16:00~17:00) of the day before. They were killed

by exsanguination from the abdominal aorta under ether anesthesia and subjected to the following pathological examinations.

1) Gross pathology

Systemic organs/tissues were grossly examined for all animals. The liver, spleen, kidney and any grossly visible lesions were removed and preserved in 10% buffered formalin.

2) Organ weights

The liver, spleen and kidney were weighed. Organ to body weight ratios were calculated using the final body weights (after over-night fasting) before sacrifice.

6. Statistical Analysis

The significance of differences between control and treated groups for each parameter, excluding food consumption, test material intake, food efficiency and water consumption, were analyzed, and evaluated at $P<0.05$ or $P<0.01$. Dunnett's multiple comparative test was employed for analysis of variance of mean values for each group. The significances of inter group differences in incidences were analyzed with the Fisher's exact probability test.

IV. RESULTS

1. Survival rate and clinical observations (Tables 1 and 2, Appendix A)

No deaths or clinical signs related to treatment were observed in any rats of either sex of control and treated groups during the course of the study.

2. Body weights (Table 3, Figures 1 and 2, Appendix B)

Body weights in both sexes of all EMI-treated groups did not significantly differ from control values. Mean body weights in females of the 5% group at week 4, males of the 2.5% group at weeks 3 and 4, and males of the 5% group from weeks 1 to 4 tended to be lowered. Mean body weights for the 2.5 and 5% groups at week 4 were decreased by 2% in females and 6% in males, and 4% in females and 6% in males, respectively compared to the controls.

3. Food consumption, test material intake and food efficiency (Tables 4-6, Appendix C)

Food consumption by males of the 2.5 and 5% groups tended to be lowered at week 4. Food consumption in females of the 0.625% group tended to be elevated at week 4, but this was considered unrelated to the treatment, since it was incidental and no dose relationship was observed.

The average EMI intakes for females and males are given below. Dose-dependent proportional increase was apparent.

Nominal Concentration (%)	Average EMI intake (mg/kg/day)	
	Female	Male
0	0	0
0.625	555	544
1.25	1098	1075
2.5	2116	2064
5	4234	4330

The food efficiencies in both sexes of all EMI-treated groups were comparable to the control groups.

4. Water consumption (Table 7, Appendix D)

Water consumption by males of the 2.5 and 5% groups tended to be lowered at week 4. Consumption by females of the 0.625% group at week 4 tended to be elevated, but this was considered unrelated to the treatment, since no dose relationship was observed.

5. Pathology

(1) Gross pathology (Table 8, Appendix E)

Yellow coloration of bones (cranium and fore- and hind-limbs) was observed in both sexes given 1.25% or more.

(2) Final body weight and organ weights (Tables 9 and 10, Appendices F and G)

Final body weights for both sexes of all EMI-treated groups did not significantly differ from control values. Similarly, the absolute and relative weights for liver, kidney and spleen were comparable in all groups.

V. DISCUSSION AND CONCLUSION

The present study was conducted on F344 rats of both sexes to determine appropriate dose levels for a 90-day repeated oral toxicity study of enzymatically modified isoquercitrin (EMI).

No clinical signs or mortality related to the treatment were found throughout the experiment.

A tendency for lowering mean body weights, considered to be related to the treatment, was observed in both sexes of the 2.5 and 5% groups. The proportional rates for decrease as compared to the control values at week 4 were 2~4% in females and 6% in males.

Food and water consumption in males of 2.5 and 5% groups also tended to be lowered at week 4. This was considered to be related to the treatment.

On macroscopic examination, yellow coloration of bones (cranium and fore- and hind-limb bone), evidently related to the treatment, was found in both sexes receiving 1.25% or more, the test chemical being yellow.

No adverse effects on the organ weights were found with no statistically significant differences from the control values.

The present study demonstrated a tendency for lowered body weights in both sexes of the 2.5 and 5% groups, and food and water consumption in males of the 2.5 and 5% groups, as well as yellow coloration of bones in both sexes given 1.25% or more. Thus, it was concluded that the highest dietary level for a 90-day repeated oral toxicity study with apparent adverse effects, should be set at 2.5% with lower levels at 1.25%, 0.625% and 0.3% with a proportional factor of 2.

VI FIGURES AND SUMMARY TABLES

FIGURES AND UMMARY TABLES

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FIGURE 1
PRELIMINARY STUDY FOR A 90-DAY REPEATED DOSE TOXICITY STUDY OF POWDERED ENZYMATICALLY MODIFIED ISOQUERCITRIN EXTRACT IN F344 RATS
BODY WEIGHT CURVE -FEMALE
PROJ. NO. 9701

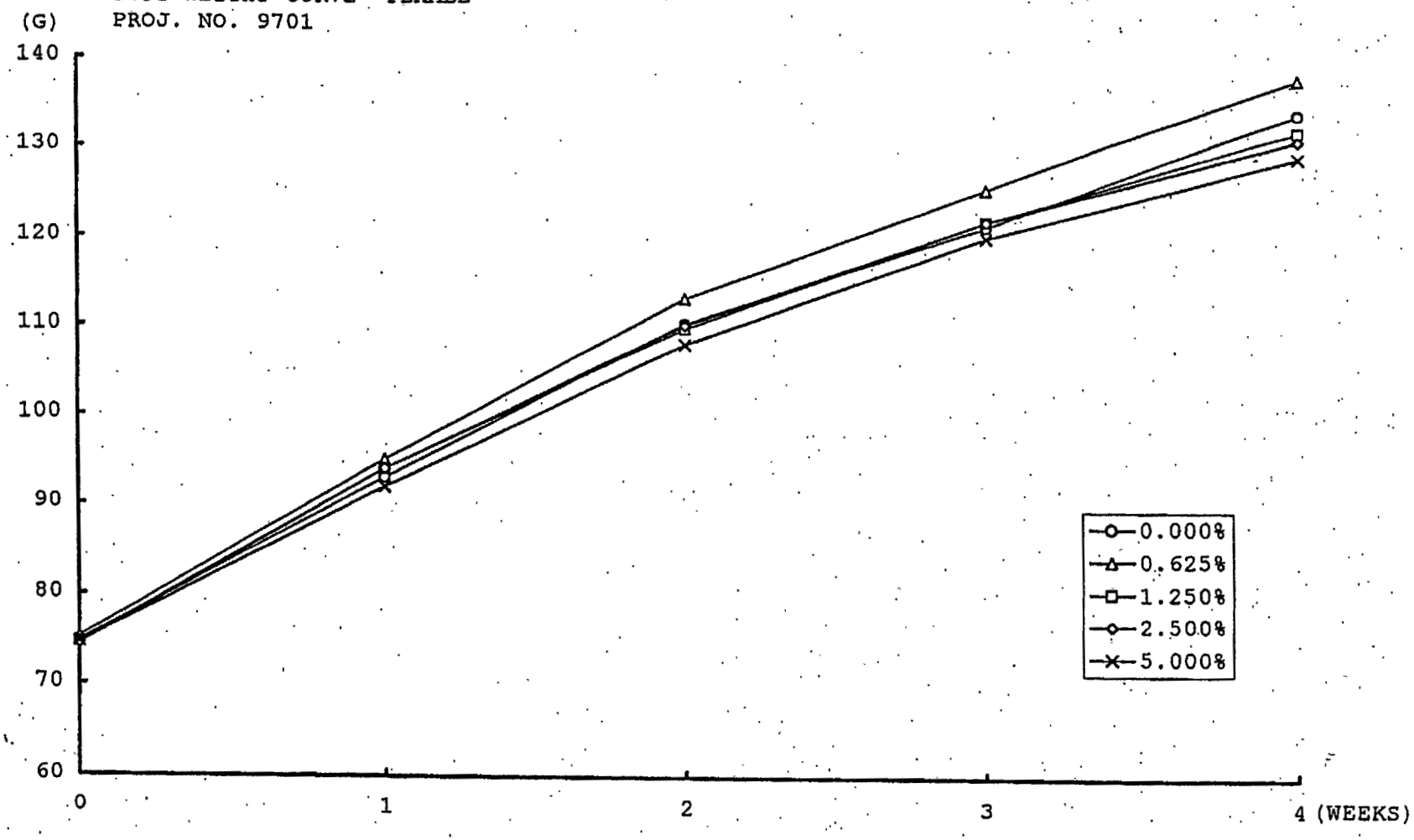

FIGURE 2
PRELIMINARY STUDY FOR A 90-DAY REPEATED DOSE TOXICITY STUDY OF POWDERED ENZYMATICALLY MODIFIED ISOQUERCITRIN EXTRACT IN F344 RATS
BODY WEIGHT CURVE -MALE
PROJ. NO. 9701

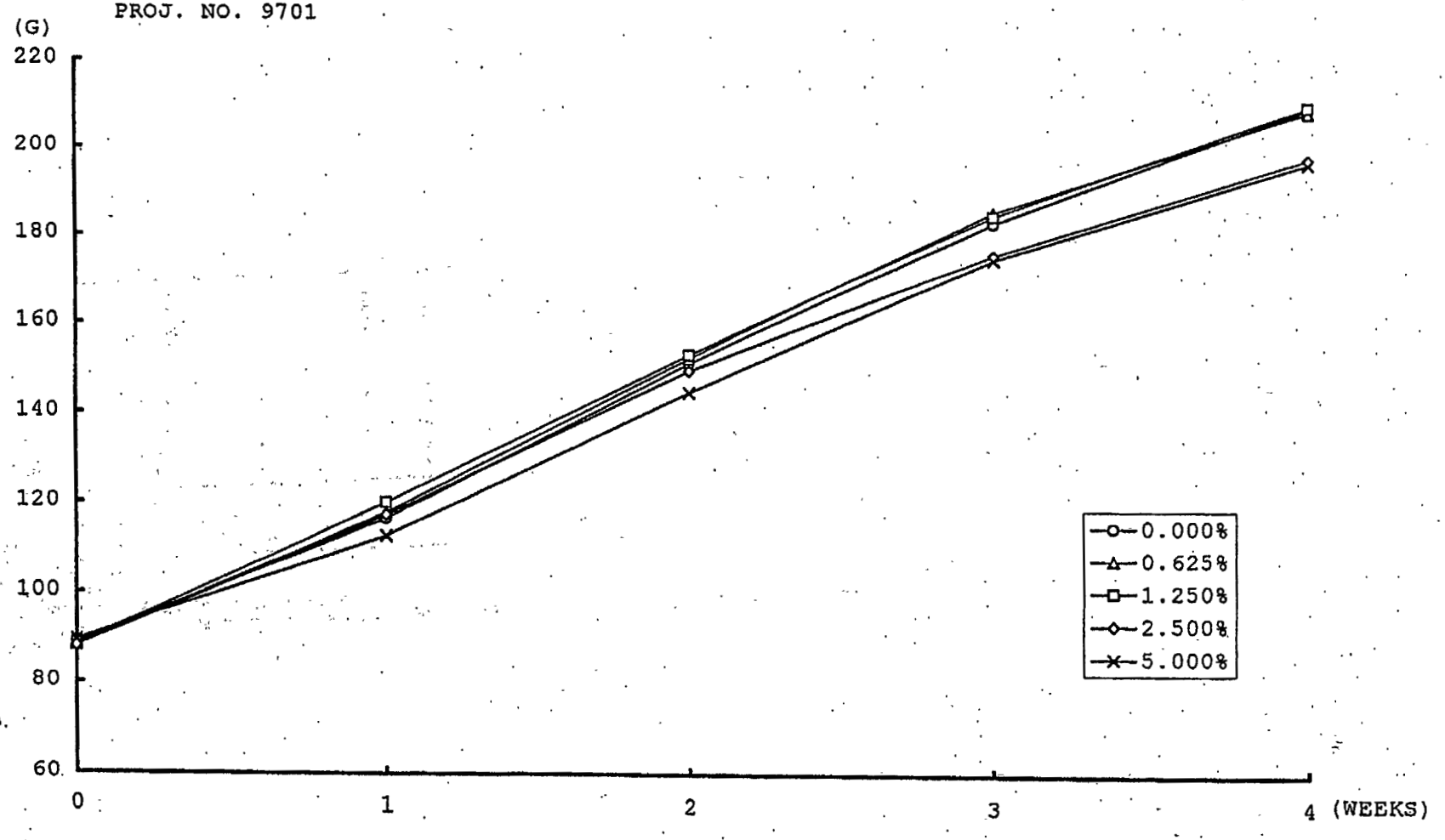

TABLE 1
PRELIMINARY STUDY FOR A 90-DAY REPEATED DOSE TOXICITY STUDY OF POWDERED ENZYMATICALLY MODIFIED ISOQUERCITRIN EXTRACT IN F344 RATS
SURVIVAL DATA
PROJ. NO. 9701

SEX	LEVEL (%)	NO. OF ANIMALS	NO. OF SURVIVALS [WEEKS] 0	1	2	3	4	TOTAL NO. OF SURVIVALS(%)
FEMALE	0.000	5	5	5	5	5	5	5(100)
	0.625	5	5	5	5	5	5	5(100)
	1.250	5	5	5	5	5	5	5(100)
	2.500	5	5	5	5	5	5	5(100)
	5.000	5	5	5	5	5	5	5(100)
MALE	0.000	5	5	5	5	5	5	5(100)
	0.625	5	5	5	5	5	5	5(100)
	1.250	5	5	5	5	5	5	5(100)
	2.500	5	5	5	5	5	5	5(100)
	5.000	5	5	5	5	5	5	5(100)

TABLE 2
PRELIMINARY STUDY FOR A 90-DAY REPEATED DOSE TOXICITY STUDY OF POWDERED ENZYMATICALLY MODIFIED ISOQUERCITRIN EXTRACT IN F344 RATS
CLINICAL OBSERVATION DATA
PROJ. NO. 9701

SITE AND FINDING	SEX ----------	FEMALE				
	GROUP NO. --------	1	2	3	4	5
	LEVEL (%) --------	0.000	0.625	1.250	2.500	5.000
Initial No. of animals		(5)	(5)	(5)	(5)	(5)

No clinical signs were observed in any animals.

TABLE 2
PRELIMINARY STUDY FOR A 90-DAY REPEATED DOSE TOXICITY STUDY OF POWDERED ENZYMATICALLY MODIFIED ISOQUERCITRIN EXTRACT IN F344 RATS
CLINICAL OBSERVATION DATA
PROJ. NO. 9701

SITE AND FINDING	SEX	MALE				
	GROUP NO.	6	7	8	9	10
	LEVEL (%)	0.000	0.625	1.250	2.500	5.000
Initial No. of animals		(5)	(5)	(5)	(5)	(5)

No clinical signs were observed in any animals.

TABLE 3
PRELIMINARY STUDY FOR A 90-DAY REPEATED DOSE TOXICITY STUDY OF POWDERED ENZYMATICALLY MODIFIED ISOQUERCITRIN EXTRACT IN F344 RATS
BODY WEIGHT DATA (G, MEAN ± S.D.)
PROJ. NO. 9701

SEX	GROUP	LEVEL (%)	WEEKS 0	1	2	3	4
FEMALE	1	0.000	74.8± 0.8	93.0± 3.9	110.4± 5.0	121.4± 4.7	134.0± 5.3
	2	0.625	75.2± 1.5	95.0± 1.6	113.2± 1.9	125.6± 1.5	138.0± 2.2
	3	1.250	74.6± 1.7	94.0± 2.1	109.8± 3.0	122.0± 3.6	132.0± 5.8
	4	2.500	74.4± 0.9	94.0± 2.3	110.2± 3.3	122.0± 2.2	131.0± 2.1
	5	5.000	74.6± 1.8	92.0± 1.9	108.0± 2.9	120.2± 0.8	129.2± 2.3
MALE	6	0.000	89.0± 3.4	116.6± 5.5	151.2± 10.5	182.8± 10.6	209.0± 10.1
	7	0.625	88.2± 2.2	118.0± 6.0	152.4± 9.8	185.4± 10.4	208.2± 9.5
	8	1.250	88.4± 2.2	120.2± 5.4	153.2± 4.3	184.4± 7.1	209.4± 8.0
	9	2.500	88.0± 2.4	117.4± 3.4	149.6± 4.8	175.6± 7.8	197.4± 8.7
	10	5.000	89.4± 2.8	112.8± 6.9	144.8± 7.8	174.6± 7.6	196.4± 9.9

TABLE 4
PRELIMINARY STUDY FOR A 90-DAY REPEATED DOSE TOXICITY STUDY OF POWDERED ENZYMATICALLY MODIFIED ISOQUERCITRIN EXTRACT IN F344 RATS
FOOD CONSUMPTION DATA (G/ANIMAL/DAY, MEAN)
PROJ. NO. 9701

SEX	GROUP	LEVEL (%)	WEEKS 1	2	3	4
FEMALE	1	0.000	8.40	9.20	9.30	9.50
	2	0.625	9.10	10.20	10.10	12.30
	3	1.250	9.30	9.70	10.30	10.50
	4	2.500	9.40	9.80	9.40	9.50
	5	5.000	9.10	9.40	9.80	9.20
MALE	6	0.000	11.50	13.10	14.30	18.10
	7	0.625	12.40	12.70	14.40	17.10
	8	1.250	11.70	13.20	14.70	16.90
	9	2.500	11.50	12.30	13.90	14.00
	10	5.000	10.90	12.80	14.40	15.50

000217

TABLE 5
PRELIMINARY STUDY FOR A 90-DAY REPEATED DOSE TOXICITY STUDY OF POWDERED ENZYMATICALLY MODIFIED ISOQUERCITRIN EXTRACT IN F344 RATS
TEST MATERIAL INTAKE DATA (MG/KG/DAY, MEAN)
PROJ. NO. 9701

SEX	GROUP	LEVEL (%)	WEEKS 1	2	3	4
FEMALE	1	0.000	0.00	0.00	0.00	0.00
	2	0.625	598.68	563.16	502.59	557.07
	3	1.250	1236.70	1104.28	1055.33	994.32
	4	2.500	2500.00	2223.23	1926.23	1812.98
	5	5.000	4945.65	4351.85	4076.54	3560.37
MALE	6	0.000	0.00	0.00	0.00	0.00
	7	0.625	656.78	520.83	485.44	513.33
	8	1.250	1216.72	1077.02	996.48	1008.83
	9	2.500	2448.89	2055.48	1978.93	1773.05
	10	5.000	4831.56	4419.89	4123.71	3946.03

TABLE 5
PRELIMINARY STUDY FOR A 90-DAY REPEATED DOSE TOXICITY STUDY OF POWDERED ENZYMATICALLY MODIFIED ISOQUERCITRIN EXTRACT IN F344 RATS
TEST MATERIAL INTAKE DATA
PROJ. NO. 9701

SEX	GROUP NO.	LEVEL (%)	TOTAL (MG/KG)	AVERAGE (MG/KG/DAY)
FEMALE	1	0.000	0.00	0.00
	2	0.625	15550.50	555.38
	3	1.250	30734.41	1097.66
	4	2.500	59237.08	2115.61
	5	5.000	118540.87	4233.60
MALE	6	0.000	0.00	0.00
	7	0.625	15234.66	544.10
	8	1.250	30093.35	1074.76
	9	2.500	57794.45	2064.09
	10	5.000	121248.33	4330.30

000219

TABLE 6
PRELIMINARY STUDY FOR A 90-DAY REPEATED DOSE TOXICITY STUDY OF POWDERED ENZYMATICALLY MODIFIED ISOQUERCITRIN EXTRACT IN F344 RATS
FOOD EFFICIENCY DATA (%, MEAN)
PROJ. NO. 9701

SEX	GROUP	LEVEL (%)	WEEKS 1	2	3	4
FEMALE	1	0.000	30.95	27.02	16.90	18.95
	2	0.625	31.08	25.49	17.54	14.40
	3	1.250	29.80	23.27	16.92	13.61
	4	2.500	29.79	23.62	17.93	13.53
	5	5.000	27.32	24.32	17.78	13.98
MALE	6	0.000	34.29	37.73	31.57	20.68
	7	0.625	34.33	38.70	32.74	19.05
	8	1.250	38.83	35.71	30.32	21.13
	9	2.500	36.52	37.40	26.72	22.24
	10	5.000	30.67	35.71	29.56	20.09

000220

TABLE 7
PRELIMINARY STUDY FOR A 90-DAY REPEATED DOSE TOXICITY STUDY OF POWDERED ENZYMATICALLY MODIFIED ISOQUERCITRIN EXTRACT IN F344 RATS
WATER CONSUMPTION DATA (G/ANIMAL/DAY, MEAN)
PROJ. NO. 9701

SEX	GROUP	LEVEL (%)	WEEKS 1	2	3	4
FEMALE	1	0.000	12.80	14.30	15.40	15.20
	2	0.625	13.20	15.20	15.00	17.80
	3	1.250	13.70	14.00	15.60	15.20
	4	2.500	14.20	15.00	15.00	14.60
	5	5.000	13.00	13.50	14.40	13.20
MALE	6	0.000	16.00	18.40	20.40	23.30
	7	0.625	15.80	17.10	20.50	23.50
	8	1.250	15.70	17.00	22.10	23.50
	9	2.500	14.30	16.10	18.30	19.00
	10	5.000	14.90	16.20	18.90	20.00

000221

TABLE 8
PRELIMINARY STUDY FOR A 90-DAY REPEATED DOSE TOXICITY STUDY OF
POWDERED ENZYMATICALLY MODIFIED ISOQUERCITRIN EXTRACT IN F344 RATS
GROSS PATHOLOGY DATA
PROJ. NO. 9701

ORGAN AND FINDING	SEX	FEMALE				
	GROUP NO.	1	2	3	4	5
	LEVEL (%)	0.000	0.625	1.250	2.500	5.000
	No. of rats examined	(5)	(5)	(5)	(5)	(5)
Not remarkable		5	5	0	0	0
Bone (cranium, fore- and hind-limb) : Yellow coloration		0	0	5**	5**	5**

** : SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT P<0.01.

000222

TABLE 8
PRELIMINARY STUDY FOR A 90-DAY REPEATED DOSE TOXICITY STUDY OF
POWDERED ENZYMATICALLY MODIFIED ISOQUERCITRIN EXTRACT IN F344 RATS
GROSS PATHOLOGY DATA
PROJ. NO. 9701

ORGAN AND FINDING	MALE				
	6 0.000	7 0.625	8 1.250	9 2.500	10 5.000
No. of rats examined ------	(5)	(5)	(5)	(5)	(5)
Not remarkable	5	5	0	0	0
Bone (cranium, fore- and hind-limb) : Yellow coloration	0	0	5**	5**	5**

** : SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT P<0.01.

000223

TABLE 9
PRELIMINARY STUDY FOR A 90-DAY REPEATED DOSE TOXICITY STUDY OF POWDERED ENZYMATICALLY MODIFIED ISOQUERCITRIN EXTRACT IN F344 RATS
FINAL BODY AND ORGAN WEIGHT DATA (G, MEAN ± S.D.)
PROJ. NO. 9701

SEX	GROUP	LEVEL (%)	NO.OF EXAMINED	FINAL BODY WEIGHT	LIVER	KIDNEYS	SPLEEN
FEMALE	1	0.000	5	125.0 ± 5.7	3.3372 ± 0.0949	0.9794 ± 0.0280	0.3410 ± 0.0256
	2	0.625	5	128.2 ± 1.6	3.5886 ± 0.4058	1.0186 ± 0.0481	0.3552 ± 0.0180
	3	1.250	5	124.2 ± 6.2	3.3182 ± 0.1302	0.9728 ± 0.0189	0.3328 ± 0.0284
	4	2.500	5	123.2 ± 1.5	3.4766 ± 0.0817	0.9686 ± 0.0222	0.3566 ± 0.0214
	5	5.000	5	120.4 ± 2.5	3.4022 ± 0.1282	0.9640 ± 0.0289	0.3452 ± 0.0230
MALE	6	0.000	5	193.8 ± 9.6	5.6600 ± 0.2812	1.4466 ± 0.1401	0.4622 ± 0.0331
	7	0.625	5	195.0 ± 9.8	5.6560 ± 0.3272	1.4744 ± 0.0947	0.4692 ± 0.0266
	8	1.250	5	196.2 ± 7.7	5.7582 ± 0.2967	1.5006 ± 0.0612	0.4712 ± 0.0285
	9	2.500	5	185.2 ± 7.7	5.5406 ± 0.3640	1.4472 ± 0.0982	0.4534 ± 0.0209
	10	5.000	5	185.2 ± 6.1	5.5686 ± 0.3013	1.4500 ± 0.0491	0.4536 ± 0.0319

TABLE 10
PRELIMINARY STUDY FOR A 90-DAY REPEATED DOSE TOXICITY STUDY OF POWDERED ENZYMATICALLY MODIFIED ISOQUERCITRIN EXTRACT IN F344 RATS
ORGAN TO BODY WEIGHT RATIO DATA (G/100G BW, MEAN ± S.D.)
PROJ. NO. 9701

SEX	GROUP	LEVEL (%)	NO.OF EXAMINED	LIVER		KIDNEYS		SPLEEN	
FEMALE	1	0.000	5	2.6734 ±	0.1262	0.7848 ±	0.0391	0.2728 ±	0.0160
	2	0.625	5	2.7990 ±	0.3100	0.7946 ±	0.0365	0.2772 ±	0.0125
	3	1.250	5	2.6744 ±	0.1071	0.7844 ±	0.0274	0.2678 ±	0.0192
	4	2.500	5	2.8226 ±	0.0990	0.7860 ±	0.0115	0.2898 ±	0.0207
	5	5.000	5	2.8252 ±	0.0517	0.8006 ±	0.0105	0.2866 ±	0.0185
MALE	6	0.000	5	2.9206 ±	0.0271	0.7454 ±	0.0366	0.2384 ±	0.0117
	7	0.625	5	2.9010 ±	0.0943	0.7558 ±	0.0141	0.2406 ±	0.0069
	8	1.250	5	2.9346 ±	0.1002	0.7648 ±	0.0148	0.2400 ±	0.0064
	9	2.500	5	2.9894 ±	0.0762	0.7808 ±	0.0252	0.2448 ±	0.0083
	10	5.000	5	3.0062 ±	0.1100	0.7832 ±	0.0242	0.2450 ±	0.0120

Appendix 13a

Pages 000227 - 000233 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Appendix 13b

Results of Peer Review on the Final Report of "Ninety-Day Repeated Oral Toxicity Study and 28-Day Recovery Study of Enzymatically Modified Isoquercitrin in Rats", Conducted in the Daiyu-kai Institute of Medical Science, Inc.

October 8th, 2002

Michihito. Takahashi, MD, PhD
Pathology Peer Review Center

Certified Toxicologist. Japanese Society of Toxicology
Diplomate, Japanese Society of Toxicologic Pathology
Certified Medical Pathology, Japanese Society of Pathology

1) Mortality and Clinical Signs: No cases of mortality occurred during the study period and no abnormal clinical signs were noted.
2) Changes in Body Weight: No differences were seen between any of the enzymatically modified isoquercitrin(EMI)-treated groups and the control group for both males and females, but in the groups set as a recovery groups, low values were measured in the males and females of the 2.5% group. This finding was deemed by the study director suggestive of an effect of the test substance administration. Indisputably, significantly low values can be seen in the males starting from the 2nd week of administration and in the females starting from the 5th week of administration. Essentially, recovery groups should be included in this study, and the division of groups as independent study groups is disadvantageous and inappropriate for statistical analysis.
3) Food and Water Consumption: No differences were seen between any of the administration groups and the control group for both males and females, and the test substance intake correlated with the dose.
4) Urinalysis: Except for a significantly high level of ketones in the male 2.5% group, a yellowish or yellowish-brown discoloration of the urine was noted, and the greater was the urinary volume the stronger was the brown discoloration.
5) Hematological Tests: In differential counts of the white blood cells, a low band cell ratio was found in the male 1.25% group. The study director interpreted it as an accidental change and the reviewer agreed with this decision. In addition, high values of reticulocyte counts were seen in the female 0.625% and 1.25% groups and in the male 0.625% and 2.5% groups, but because the correlation with the dose was unclear and

because no statistically significant differences were found in other parameters, this finding seems to be toxicologically insignificant.

6) Blood Biochemistry: γ-glutamyl transpeptidase (γ-GTP) levels were significantly high in the male 1.25% and 2.5% groups, and BUN was also significantly high in the male 1.25% and 2.5% groups. While these findings were statistically significant, they were not deemed abnormal and they were considered toxicologically insignificant. Though high levels of alkaline phosphatase (ALP) were measured in the male 0.625% group, this finding was deemed accidental since no significant changes were found in the other EMI-treated groups. A low albumin / globulin (A/G) ratio was found in the female 1.25% group, but because no changes were found in the other groups or in the males, the study director deemed it an accidental change and the reviewer agreed with this decision. Low levels of sodium (Na) were measured in the male 0.3% group, but because no changes were found in the other groups or in the females, the study director deemed it an accidental change and the reviewer agreed with this decision. No statistically significant changes were found in any of the other parameters.

7) Final Body Weight and Absolute and Relative Organ Weight at Autopsy: A high relative weight of the kidneys was measured in the female 2.5% group. A low relative weight of the spleen was measured in the male 2.5% group. A high relative weight of the thyroid gland was measured in the male 1.25% and 2.5% groups, but no histopathological changes were detected, the toxicological significance of the change is unclear and it does not seem to be related to the administration. A high absolute weight of the pituitary gland was measured in the male 1.25% group, but no such change was seen in the other groups and it seemed to be an accidental change.

8) Gross Findings: Yellow coloration of the cranium and femoral bone was seen in the female groups administered with 1.25% or higher and the male groups administered with 0.625% or higher. Diaphragmatic hernia of the liver was noted in 2 cases in the male 0.625% group and uterine dilation was seen in 1 to 3 cases in each of the female groups except for the 0.625% group, but none of these changes were seemed to be related to administration and they seemed to be a spontaneous occurrence.

9) Histopathological Examination: In histopathological examination, the incidences of kidney calcification in the female groups were 9 of 10 in the control group, 9 of 10 in the 0.3% group, 4 of 10 in the 0.625% group, 7 of 10 in the 1.25% group and 0 of 10 in the 2.5% group, showing a significantly lower incidence in the 0.625% and 2.5% groups. The dose correlation of this finding was, however, unclear, and because such calcification is a natural occurrence whose incidence is known to greatly fluctuate, this finding did not appear to be toxicologically significant. No histopathological abnormalities were found,

on the other hand, in examination of the yellow coloration of the cranium and femoral bone, seen in gross observation in the female groups administered with 1.25% or higher and the male groups administered with 0.625% or higher, and the coloration appeared to be related to the administration of the test substance but with no toxicological significance.

10) Based on the above results, the toxicological significance of none of the changes observed, after 90-day administration to rats of EMI mixed with the diet, was clear. The decrease in body weight, observed in the 2.5% group set as a recovery group, was not observed during the study itself, and is therefore unlikely to be significant. It is necessary to repeat the statistical analysis in conjunction with this study. If the results of such analysis reveal a significant difference, such a change would have to be regarded as related to the administration, and if no significant difference is found, the finding would be deemed an accidental change. Each of the other statistically significant changes, which included a mild increase in the reticulocyte counts in the male 2.5% group, and mildly high values of γ-GTP and BUN in the male 2.5% group, is barely an indicator of toxicity, and they suggest, therefore, no toxicological significance. Moreover, the toxicological significance of the high level of ketones, found in the male 2.5% group, is unclear. Therefore, whatever effect of the administration was seen in the male 2.5% group, it did not seem to produce toxic findings, and the no observed adverse effect level (NOAEL) seems, therefore, to be the 2.5% group. The decision on 0.3% for both males and females, cited in the report, seems therefore inappropriate. If the combination of recovery groups and the groups of the study itself yields changes in body weight, which would appear to be statistically toxic findings, I recommend to determine the 1.25% group as NOAEL.

– End –

I hereby confirm that I wrote the above peer review results.

Michihito Takahashi, MD, PhD.
October 8th, 2002
Pathology Peer Review Center
#104, Uehara 3-45-11, Shibuya-ku, Tokyo 151-0064 Japan.

Appendix 13c

ANSWER FOR THE PEER REVIEW

Objective final report: the 90-day oral toxicity and 28-day recovery study of enzymatically modified isoquercitrin in F344/DuCrj rats (Project No. 9702 and 9703), performed by Daiyu-Kai Institute of Medical Science

I have responded below to the peer review reported by Dr Michihito Takahashi on October 8, 2002 concerning the 90-day oral toxicity and 28-day recovery study of enzymatically modified isoquercitrin (EMI) in F344/DuCrj rats performed by Daiyu-Kai Institute of Medical Science.

The study director estimated 0.3% as the no observed adverse effect level (NOAEL) on the basis of the items given in the Table below.

Items of investigation	Sex・group	Alteration
Body weight change	Both sexes of the 2.5% group	Suppressed body weight gain during the treatment period. Recovery to the control level in males.
Urinalysis (Ketones)	Males of the 2.5% group	Elevation at the end of the treatment period. Recovery to the control level.

continued

(Appearance)	Both sexes of all EMI-treated groups	Yellow or brown yellow at the end of the treatment period. Recovery to the normal color.
Hematology (Reticulocyte count)	Females of the 0.625 and 1.25% groups, and males of the 0.625 and 2.5% groups	Elevation at the end of the treatment period. Recovery to control level.
Blood chemistry (BUN)	Males of 1.25 and 2.5% groups.	Elevation at the end of the treatment period. Recovery to the control level.
(γ-GTP)	Males of 1.25 and 2.5% groups	Elevation at the end of the treatment.
Gross pathology	Females of the 1.25 and 2.5%groups, and males of the 0.625% or more groups.	Yellow coloration of bone (cranium and/or femur) at the end of the treatment period.
Histopathology	Females of the 0.625% or more groups.	Lowered incidences of calcification in the kidney at the end of the treatment period.

The alterations detailed above are concluded to be related to EMI treatment. However, the changes were very slight or had negligible toxicological significance, since no the other associated alterations were found as mentioned in the discussion and evaluation of the final report. However, it could not be judged whether the yellow coloration observed in the cranium and/or femur in males receiving 0.625% or more and females given 1.25% or more

was adverse or not, in spite of no histopathological changes, because no recovery was noted. Therefore, the NOAEL was evaluated as 0.3% with significant effects at 0.625% or more.

Recently, it was reported that simple coloration induced by test chemical treatment is not toxicologically significant[1]. Therefore, it might be appropriate to judge that the yellow coloration with EMI treatment is not an adverse effect.

The peer reviewer indicated that body weight changes should be evaluated based on data for both 90-day repeated dose and 28-day recovery groups. The combined results are now given in Table 16 and Appendix M. Body weights of females of the 0.625 and 2.5% groups were statistically significantly lowered at weeks 7, 8, 9, 10, 12 and 13. The lowered body weight in females of the 0.625% group was not considered to be related to EMI treatment, since no dose-relation was apparent. Additionally, body weights in male rats of all EMI-treated groups did not significantly differ from control values. Thus, the NOAEL is estimated to be 1.25%.

REFERENCE

1. Teo, S.K., Evans, M.G., Brockman, M.J., Ehrhart, J., Morgan, J.M., Stirling, D.I. and Thomas, S.D.: Safety profile of thalidomide after 53 weeks of oral administration in beagle dogs. Toxicol. Sci. 59, 160-168, 2001.

<signed> November 14, 2002

Seiko Tamano, Ph.D.,
Dipls. J.S.T.P. and J.S.T.
Management Director
Daiyu-kai Institute of Medical Science

Appendix 14a

Pages 000243 - 000263 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Appendix 14b

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January 5, 2006

Joseph F. Borzelleca, Ph.D.
Professor
Department of Pharmacology and Toxicology
of the Medical College of Virginia,
Virginia Commonwealth University

Dear Prof. Borzelleca,

I am the principal investigator of our published article entitled "Lack of carcinogenicity of enzymatically modified isoquercitrin in F344/DuCrj rats" in Food and Chemical Toxicology (42), pp1949-1969, 2004. To clarify the conclusions of our publication, I reviewed the H&E stained spleen sections from all dosage groups together with the controls and confirmed that the decrease in spleen weights was not toxicologically significant due to the absence of accompanying microscopic lesions.

Based on the above results, the NOAEL for enzymatically modified isoquercitrin is at least 1.5% in the diet, corresponding to 598 mg/kg/day for males and 489 mg/kg/day for females, respectively.

Please let me know if you have any questions.

Best Regards,

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Date

Appendix 15

Pages 000267 - 000275 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

EXPERT REPORT ON RE-EVALUATION OF RENAL HISTOPATHOLOGY IN THE NTP 2-YEAR CARCINOGENICITY BIOASSAY OF QUERCETIN

PREPARED BY:

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000276

AUTHOR STATEMENT REGARDING AUTHENTICATION

I, the undersigned, hereby declare that the findings of this point-by-point evaluation, conducted by me, of glass histology slides from toxicity and carcinogenicity studies with F344 rats ingesting quercetin in the diet are accurately reflected in the body of this report, which was compiled by me, and which comprises 20 pages, including six (6) tables and one (1) appendix.

Gordon C Hard
BVSc, PhD, DSc
FRCPath, FRCVS, FAToxSci, DACVP

Date

INTRODUCTION

Quercetin, a naturally occurring plant flavonoid widely distributed in fruits and vegetables, has been tested by the National Toxicology Program (NTP) in a 2-year carcinogenicity bioassay in Fischer 344 rats (F344) (Dunnick and Hailey, 1992; NTP 1992). From this study, the NTP concluded that there was *some evidence of carcinogenic activity* for quercetin in male F344/N rats based on a modest increased incidence of renal tubule adenomas. Enzymatically modified isoquercitrin (EMIQ) is a related compound manufactured by San-Ei Gen F.F.I., Inc., Japan, intended for use as an antioxidant, stabilizer, and ultraviolet ray absorbent in a wide variety of applications, including flavored beverages, other food products, and cosmetics. Dr Shim-mo Hayashi of San-Ei Gen F.F.I., Inc. requested that I re-evaluate the renal histopathology associated with quercetin in rats in the NTP 2-year carcinogenicity study. The issue of quercetin carcinogenicity is relevant because it is the aglycone of EMIQ and it is possible that EMIQ is metabolized to quercetin. Quercetin may also be an impurity in EMIQ. In this re-evaluation of the histopathology of the rat kidney from the NTP 2-year study, the emphasis was on attempting to determine a mode of action for the quercetin-associated renal tumors.

MATERIALS AND METHODS

This review of histology slides and pathology data was conducted at the NTP Archives on the premises of Experimental Pathology Laboratories Inc. (EPL) at 615 Davis Drive, Suite 400, Durham, NC 27713, from March 20 to 30, 2006. The NTP 2-year carcinogenicity study of quercetin (TDMS no. 05033-01; TSI Mason Laboratories Inc. study no. C60106) was conducted in male and female F344/N rats with 50 animals per group scheduled for the 2-year sacrifice. The test compound was administered orally in the diet at doses of 0, 1000, 10000, and 40000 ppm, corresponding respectively to 0, 0.1, 1.0, and 4.0% (the units used in this report). An additional 10 rats per group were included for interim examinations at 6 and 15 months during the study. Kidney sections in the NTP studies had been stained with hematoxylin and eosin (H&E). Based on the finding in the standard (single) sectioning of each kidney of 4 renal tumors (3 adenomas and 1 carcinoma) in the high-dose males, the NTP performed step sectioning of the rat kidneys at approximately 1-mm intervals to produce 6-8 additional kidney sections per animal for further histopathological evaluation.

The kidneys were examined with an Olympus BX 41 microscope using magnifications ranging from 4 to 40x. In the carcinogenicity bioassay, all male rats in the control (0 %), low-dose (0.1%), mid-dose (1.0%), and high-dose (4.0%) groups were evaluated, as well as all control and high-dose male rats at the interim sacrifice of 6 and 15 months. In addition, the kidneys of the female rats recorded by NTP as having renal tubule hyperplasia or renal tumor were examined, and all control and high-dose females from the 6 and 15 month, and 2 year time-points were graded for spontaneously occurring nephropathy. The groups and the animal numbers included in this re-evaluation are listed in Appendix 1.

Criteria used for diagnosing tubule hyperplasia and chronic progressive nephropathy (CPN) were those described by the Society of Toxicologic Pathologists and the International Agency for Research on Cancer (Alden et al 1992; Hard et al, 1995; Hard et al, 1999). Simple tubule hyperplasia is an increase in the number of epithelial cells without altering the single-cell-layer aspect of the tubule lining. Atypical tubule hyperplasia (ATH) represents complex internal proliferation of the epithelial lining of a tubule beyond the normal single cell layer into, and usually obliterating, the lumen, but essentially retaining the integrity of the tubule outline. Tubule proliferation transcends from atypical hyperplasia to adenoma when it exceeds the integrity of a single tubule, and/or vascular ingrowth into the proliferative focus can be discerned. The distinction between adenoma and carcinoma is the presence of multiple areas of necrosis/hemorrhage in the latter, usually accompanied by a trend to cellular pleomorphism. It is widely accepted that ATH in the rat kidney is a preneoplastic lesion, that is, ATH, adenoma and carcinoma represent sequential stages in the continuum from preneoplastic to malignant proliferation (Hard, 1990; Dietrich and Swenberg, 1991; Nogueira et al, 1993).

CPN is an age-related renal disease of laboratory rodents that occurs spontaneously in high incidence. It is characterized in the early stages by single tubule profiles or focal lesions of cortical tubule basophilia associated with thickened basement membrane, often with hyaline cast formation involving the inner medullary segments of the same tubule. CPN is a degenerative/regenerative disease process, and the basophilic tubules represent simple hyperplasia accompanied by single cell death and mitotic activity. With progression of the disease, the foci of tubule alteration enlarge and coalesce into areas of affected tubules, ultimately involving

the entire kidney bilaterally. Transitional cell hyperplasia of the renal pelvis lining also occurs as part of the lesion spectrum in the advanced stages of CPN. (Hard and Khan, 2004). CPN can be graded according to a semiquantitative system devised by the Reviewer based on lesion pathogenesis on a scale of 1 to 8 (Hard and Khan, 2004), where 1 represents *minimal* involvement (1-5 focal lesions in right and left kidney sections together); 2, *mild* (6-15 foci); 3, *low-moderate* (16-30 foci and with occasional medullary hyaline casts); 4, *mid-moderate* (30-50 foci and with scattered medullary casts); 5 *high-moderate* (lesions still focal but too numerous to count, and with many medullary casts), 6, *low-severe* (lesions beginning to coalesce, and with many medullary casts); 7, *high-severe* (only small islands of normal parenchyma remaining, with numerous medullary casts); and 8 an *end-stage* kidney (no or almost no normal parenchyma remaining) signaling imminent death from chronic renal failure. Advanced grades of CPN (grades 6-8), especially where CPN is exacerbated by chemical exposure, can display sporadic dilated or solid tubule profiles that may be confused with preneoplastic tubule foci. However, these appear to be regenerative lesions associated with atrophic tubules, and different from ATH (Hard and Seely, 2005).

In particular, this re-evaluation took account of recently published criteria (Hard and Seely, 2005) for distinguishing preneoplastic tubule hyperplasia from the simple tubule hyperplasia and proliferative tubules (referred to here as *CPN tubule profile*) that can be a characteristic of advanced to end-stage CPN.

RESULTS

Evaluation for compound-induced toxic injury

The standard kidney sections from each animal in the male dose groups at 6 months, 15 months, and 2 years, were carefully examined for the presence of tubule alterations, including cytoplasmic vacuolation, basophilic tubules, single cell death, nuclear variability in size and/or karyomegaly, and increase in mitotic figures, that individually would be indicative of some test compound-induced toxic injury. This evaluation focused on renal parenchyma that was not affected by spontaneous, age-related CPN.

None of these individual cellular alterations were observed in renal tubules that were uninvolved in the CPN process, and the general

appearance of these areas was considered to be compatible with normal kidney.

Evaluation for chronic progressive nephropathy (CPN)

The results concerning the group distribution and severity grading of CPN in male rats for the 6-month, 15-month, and 2-year time-points are summarized in Table 1. The mean grades of severity for each male group are shown in Table 2. Apart from one control male rat dying at day 226, the incidence of CPN in all male groups was 100%.

At the 6-month time point, the mode for CPN severity in the control males was grade 1 (*minimal*), compared to grade 2 (*mild*) in the high-dose males. The distribution was restricted to grades 1 and 2 for the control males, but ranged from grade 1 to grade 4 in the high-dose group. The mean grade of CPN severity on the scale of 0-8, was 1.4 in the controls, and 2.4 in the high-dose males.

At the 15-month time point, the mode for CPN severity was grade 5 (*high moderate*) for both control and high-dose male groups, but the distribution of CPN grades ranged from 3 to 5 in the controls, and 5 to 6 for the high-dose males. The mean grade of severity (range 0-8) was 4.4 in control males, and 5.3 in high-dose males.

In the 2-year study, the mode for the control, low-dose, and mid-dose males was grade 6 (*low severe*), and for the high-dose it was grade 7 (*high severe*). The number of rats with end-stage (grade 8) CPN was 10 (20% of the group) at the high-dose, and 6 (12%) at the mid-dose. These numbers contrasted with one rat only (2%) with end-stage disease in the control and low-dose males. The mean grade (range 0-8) across the groups was 5.7 for controls, 5.9 for the low-dose, 6.2 for the mid-dose, and 6.5 for the high-dose.

The results for CPN grading in the females are shown in Table 3. The mode for both control and high-dose females at 6 months was grade 1 (*minimal*), at 15 months grade 4 (*mid moderate*), and at 2 years grade 5 (*high moderate*). There were no female rats with end-stage kidney.

Incidence and phenotype of renal tubule tumors in male rats

The combined incidence (standard single plus step sections) of renal tubule tumors diagnosed in male rats in this re-evaluation of both the standard and step sections of the 2-year study is summarized in Table 4.

The distribution of these tumors according to severity grade of CPN is presented in Table 5.

In the high-dose males there were 5 adenomas and 1 carcinoma, and in the mid-dose males, 5 adenomas. This incidence contrasted with no renal tubule tumors in the control and low-dose males. These numbers were less than those recorded in the NTP report (NTP, 1992) (see Table 4).

All of the 10 adenomas, except one in a mid-dose male (animal no. 204) were small basophilic lesions occurring within CPN affected tissue, and in rats with advanced CPN graded as 6, 7, or 8. Three of the 5 high-dose adenomas occurred in rats with end-stage (grade 8) CPN. Most of the adenomas were solid lesions, but 2 (animal no. 187 in the mid-dose group, and animal no. 216 in high-dose group) were cystic. Six of the 9 basophilic adenomas occurring in treated male rats (2 in the mid-dose and 4 in the high-dose groups) were incipient tumors, borderline with ATH. These animal nos. were: 170 and 178 in the mid-dose males, and 240, 241, 274, and 276 in the high-dose males).

The exception noted above, animal no. 204 in the mid-dose males, and the carcinoma in the high-dose male (animal no. 238), had a distinctive phenotype with eosinophilic staining (as opposed to basophilic), large cells with ample cytoplasm, frequent large vacuoles or circular spaces suggestive of minilumens, and prominent, often enlarged nucleoli. The carcinoma also had multiple areas of necrosis.

Incidence of atypical tubule hyperplasia (ATH) in male rats

The combined incidence (standard single plus step sections) of foci of ATH diagnosed in male rats in this re-evaluation of both the standard and step sections of the 2-year study is summarized in Table 4. The distribution of these preneoplastic lesions according to severity grade of CPN is presented in Table 5.

In the control males, there were 2 rats diagnosed with ATH, 4 rats in the low-dose group, 4 rats in the mid-dose group, and 6 rats in the high-dose males. One rat in the high-dose male group (with *end-stage* CPN) had 2 foci of ATH (animal no. 220), and 2 high-dose rats (animal nos. 240 and 274) had an early, borderline adenoma as well as a focus of ATH.

All of the foci of ATH in male rats were of the solid basophilic type (except one cystic lesion in high-dose male no. 249), compatible with the basophilic phenotype of the adenomas (but not the eosinophilic adenoma in animal no. 204 described above). The foci of ATH were located within, and/or intimately associated with, CPN-affected tissue.

Incidence and phenotype of renal tubule ATH and tumors in female rats

The incidence, and distribution according to severity grade of CPN, of renal tubule neoplastic and preneoplastic lesions diagnosed in this re-evaluation is summarized in Table 6.

Only 2 adenomas were diagnosed in the female rats, one each in the control (animal no. 053) and mid-dose (animal no. 168) groups. Both were of the eosinophilic, vacuolar phenotype identical to that of the male rat carcinoma at the high-dose, and an adenoma in the mid-dose males.

There was one rat with a focus of ATH in each of the control and low-dose female groups, and 2 in each of the mid- and high-dose groups. One of these foci of ATH in a mid-dose female (animal no. 149) was of the eosinophilic, vacuolar phenotype. The remaining cases were basophilic lesions similar to the ATH foci in the male rats.

Several of the basophilic ATH were associated with and located in CPN-affected tissue, but the eosinophilic adenomas and ATH were not associated with CPN (Table 6).

DISCUSSION

Because of its wide distribution in many food plants, quercetin has been tested for carcinogenicity in rodents a number of times previously (Pamukcu et al, 1980; Saito et al, 1980; Hirono et al; 1981; Takanashi et al, 1983; Ito et al, 1989), all with negative kidney results. The NTP study has been the only carcinogenicity bioassay of quercetin identifying the kidney as a target organ (Dunnick and Hailey, 1992; NTP, 1992). Quercetin has tested positively in most *in vitro* assays for mutagenicity/genotoxicity, but in contrast, most of the *in vivo* tests have been negative (IARC, 1983, 1999). Accordingly, the International Agency for Research on Cancer (IARC) has concluded that there is *limited* evidence in experimental animals for the carcinogenicity of quercetin (IARC, 1999).

Based on recently published recommendations for discriminating proliferative tubules of unusual appearance in advanced stages of CPN from preneoplastic tubule hyperplasia (ATH), along with criteria for distinguishing ATH and adenomas (Hard and Seely, 2005), the tally of foci of ATH and adenomas in this re-evaluation of the quercetin 2-year carcinogenicity study was less than recorded by the NTP (Dunnick and Hailey, 1992; NTP, 1992), due to downgrading of some adenomas to ATH and/or of ATH to CPN tubule profile. The latter are not considered to be preneoplastic lesions (Hard and Seely, 2005). However, this difference from the NTP report numbers (summarized in Table 5) did not alter the NTP conclusion that there was an increase in renal tubule tumors in male rats, at least at the high dose of 4.0% of quercetin in the diet. There was agreement with the NTP report that one of the tumors (animal no. 238 in a high-dose male) was a carcinoma. This review also confirmed the NTP findings that there was an exacerbation by the chemical of age-related, spontaneous CPN in the male rats, but not in the females.

It is significant that the one carcinoma occurring in the study (animal no. 238 in the high-dose males), along with one adenoma in the mid-dose males (animal no. 204), and in the females, 2 adenomas (animal no. 053 in the controls, and 168 in the mid-dose group) and one ATH focus (animal no. 149 in the mid-dose group), that is, 5 lesions in all, possessed a phenotype that was different from the basophilic character of the rest of the neoplastic and preneoplastic lesions. This was an eosinophilic, vacuolar morphology that has been observed as occurring spontaneously in rats in 90-day studies (Crissman et al, 1992; Hard et al, 1994), spontaneously in F344 littermates (Thurman et al, 1995), and in the Eker rat familial renal tubule cancer syndrome (Everitt et al, 1992). Collectively, the evidence suggests that this morphology represents a familial type of tumor of spontaneous origin. The Reviewer has observed this phenotype across gender and groups, including controls, in a number of NTP 2-year studies using the F344 rat, and he has also seen the same phenotype in control Sprague-Dawley and Wistar rats. In the studies where the Reviewer has encountered this phenotype, there have always been at least 2, and up to 5 examples (as seen in the quercetin study) of the lesion in each study, suggesting that this phenotype is occurring in a single litter in each of the affected studies as has been observed on a previous occasion (Thurman et al, 1995). If these particular tumors are excluded from the male rat groups as most likely being of spontaneous origin, the resulting tumor count associated with quercetin becomes 4 adenomas in the mid-dose males, and 5 adenomas in the high-dose males,

the majority of which were incipient tumors borderline with ATH, and with no carcinoma.

CPN was exacerbated in the high-dose males at each of the time points examined, i.e. at 6 months, 15 months, and 2 years. Dunnick and Hailey (1992) noted no increase in the severity of CPN at the 6 and 15 month interim sacrifice times, but the 8-grade assessment system employed by the Reviewer is more sensitive, particularly at the early stages of the CPN process, than the 4-grade system used by the NTP. The enhancement of CPN was observed as an increase in the mode and mean values of CPN severity, as well as an increase in the number of rats reaching end-stage CPN. There was also a modest exacerbation of this spontaneous disease in the mid-dose males at 2 years. Apart from the familial-like, eosinophilic examples discussed above, the adenomas and foci of ATH, which were basophilic, occurred in association with CPN, supporting the view that these proliferative tubule lesions were a consequence of the exacerbation of CPN. CPN is both a degenerative and regenerative disease, showing a high rate of cell turnover (Konishi and Ward, 1989; Hard and Khan, 2004) at all stages of progression. Because of this proliferative nature of CPN, unusual but non-neoplastic tubule profiles can be encountered in advanced CPN (Hard and Seely, 2005), and there can be a small increase in the incidence of ATH and renal tubule adenomas (Hard and Khan, 2004). This association between advanced CPN and the exacerbating effect of a test chemical, resulting in a modest increase in renal tubule tumors, has been demonstrated for hydroquinone (Hard et al, 1997) and ethyl benzene (Hard, 2002).

The available database on renal chemical carcinogenesis in rats demonstrates that renal tumor induction can be classified into a number of categories based on mechanism or mode of action (Hard, 1998; Hard, 1999; Lock and Hard, 2004). Thus, renal tubule tumors can be induced by chemicals through mechanistic processes involving either:

1. direct DNA reactivity (eg several nitrosocompounds),
2. indirect DNA reactivity mediated by oxidative stress (eg potassium bromate),
3. sustained cytotoxicity/regeneration mediated directly by the chemical but unassociated with DNA reactivity (eg chloroform),
4. sustained cytotoxicity/regeneration associated with alpha-2u-globulin nephropathy (eg d-limonene), or
5. interaction with spontaneous CPN (eg ethyl benzene).

If quercetin was inducing renal tubule tumors through a mode of action involving either direct or indirect DNA reactivity, or via sustained cytotoxicity and regeneration, this histologic evaluation would have revealed evidence of toxic injury in those renal tubules that were not affected by CPN. Renal tubule injury would have been manifest by one or several of the following: cytoplasmic vacuolation, basophilic tubules (unrelated to CPN), single cell death (as indicated by pyknosis, karyorrhexis, or apoptosis), variability in nuclear size and/or sporadic karyomegaly (representing polyploidy), or increase in mitotic figures. Careful examination of the renal parenchyma showed no evidence of any of these alterations in treated male rats at 6 months, 12 months or 2 years of exposure to quercetin. In addition, hyaline droplet accumulation, and granular casts at the junction of the outer and inner stripes of outer medulla were absent at the 6-month interim time point, and linear papillary mineralization was absent at 2-years, thus ruling out a mode of action involving alpha-2u-globulin nephropathy (Hard et al, 1993). Thus, there were no alterations found in this histopathological re-evaluation indicative of toxicity observed in the renal tubules of male rats from the 6-month, 15-month or 2-year time-points that would support a mode of action directly related to the chemical. Furthermore, Dunnick and Hailey (1992) stated that there was no evidence of kidney toxicity in 13-week studies with quercetin.

Instead, the evidence regarding quercetin fulfils the criteria for interaction with CPN (Hard and Khan, 2004). These criteria include a slight to modest increase in renal tubule tumors, exacerbation of the disease to advanced grades of severity at doses associated with tumor increase, preponderance of adenomas which are either borderline with ATH or of small size, occurrence of the tumors and foci of ATH predominantly in rats with advanced CPN and in tissue affected by CPN, and an absence of any cellular alterations indicative of chemical toxicity in parenchyma that is not involved in the CPN process. Importantly, the modest renal tumor increase associated with quercetin administration occurred only at dose levels in males at which an exacerbation of CPN was evident, and there was no increase in renal tumors in treated female rats correlating with no exacerbation of CPN in female groups. The interaction of quercetin with CPN provides a mode of action explaining the modest renal tubule tumor increase, and refutes the conclusion of Dunnick and Hailey (1992), who speculated that quercetin could be exerting a toxic and carcinogenic effect in male rat kidney because older animals may have impaired metabolism and detoxifying mechanisms. Interaction of the test chemical with CPN

represents a secondary mechanism for renal tumor development, which is believed to be not relevant for interspecies extrapolation because the syndrome of rat CPN appears to have no strict counterpart in humans (Hard and Khan, 2004).

CONCLUSION

Re-evaluation of the rat kidneys from the NTP 2-year carcinogenicity study of quercetin administered in the diet provided evidence that the small increase in renal tubule adenomas occurring in the high- and mid-dose males was associated with an exacerbation of CPN to advanced stages. This mode of action is considered to have no relevance for extrapolation to humans. In addition, the carcinoma present in the high-dose males, along with 4 other lesions ranging from ATH to adenoma in male and female groups, was considered to have a unique phenotype indicative of spontaneous origin.

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Table 1. **Group Distribution of CPN Severity in Male Rats at 6 and 15 Months, and 2 Years**

Dose group (%)	Rats in group	Rats scored for CPN*	Rats with severity grade**:								
			0	1	2	3	4	5	6	7	8
6-mth											
0	10	10	0	**6**	4	0	0	0	0	0	0
4.0	10	10	0	2	**4**	2	2	0	0	0	0
15-mth											
0	10	10	0	0	0	1	4	**5**	0	0	0
4.0	10	10	0	0	0	0	0	**7**	3	0	0
2-year											
0	50	49	1	0	0	0	3	13	**22**	9	1
0.1	50	50	0	0	0	0	4	9	**25**	11	1
1.0	50	50	0	0	0	0	1	10	**23**	10	6
4.0	50	49	0	0	0	0	0	10	13	**16**	10

* excluding rats that could not be graded because of autolysis or leukemia
** the mode for each group is in bold

Table 2. **Mean CPN Grades in Male Rats at 6 and 15 Months, and 2 Years**

Dose group (%)	6 Months	15 Months	2 Years
0	1.4	4.4	5.7
0.1	na	na	5.9
1.0	na	na	6.2
4.0	2.4	5.3	6.5

na - not available

Table 3. Group Distribution of CPN in Control and High-dose Female Rats

Dose group (%)	Rats in group	Rats with severity grade:								
		0	1	2	3	4	5	6	7	8
6-mth										
0	9	1	**8**	0	0	0	0	0	0	0
4.0	10	3	**7**	0	0	0	0	0	0	0
15-mth										
0	10	0	0	1	2	**5**	2	0	0	0
4.0	10	0	0	0	1	**6**	3	0	0	0
2-year										
0	50*	0	1	0	3	6	**22**	11	5	0
4.0	50*	0	0	0	2	11	**23**	9	3	0

* 2 rats not graded because of autolysis or leukemia

Table 4. Incidences of Renal Tubule Proliferative Lesions Recorded by NTP and in the Re-evaluation

Dose group (0%)	ATH		Adenoma		Carcinoma	
	NTP	GCH	NTP	GCH	NTP	GCH
Males						
0	3	2	1	0	0	0
0.1	3	4	2	0	0	0
1.0	8	4	7	5*	0	0
4.0	11	6	8	5	1	1*
Females						
0	2	1	1	1*	0	0
0.1	1	1	0	0	0	0
1.0	3	2*	1	1*	0	0
4.0	4	2	0	0	0	0

* includes one rat with a lesion of a familial-like phenotype suggestive of spontaneous origin

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Table 5. **Distribution of Proliferative Lesions in Male Rats According to CPN Severity Grade**

Dose group (%)	Rats with CPN severity grade:								
	0	1	2	3	4	5	6	7	8
ATH									
0							1	1	
0.1							2		2
1.0							2		2
4.0							2	1	3
Adenoma									
0									
0.1									
1.0					1*		3	1	
4.0							1	1	3
Carcinoma									
0									
0.1									
1.0									
4.0							1*		

* familial-like phenotype suggestive of spontaneous origin
Note: 6 of 9 adenomas in treated males were borderline, incipient tumors

Table 6. Distribution of Proliferative Lesions in Female Rats According to CPN Severity Grade

Dose group (%)	Rats with CPN severity grade:								
	0	1	2	3	4	5	6	7	8
ATH									
0							1		
0.1						1			
1.0				1*	1				
4.0							1	1	
Adenoma									
0						1*			
0.1									
1.0				1*					
4.0									
Carcinoma									
0									
0.1									
1.0									
4.0									

* Familial-like phenotype suggestive of spontaneous origin

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APPENDIX 1

Groups and Animals Examined Microscopically

6 Month interim sacrifice

Dose 0% Males 001, 006, 007, 020, 031, 032, 039, 047, 066, 067.
Females 411-419.
4.0% Males 212, 217, 218, 231, 242, 243, 250, 258, 277, 278.
Females 421-430.

15 Month interim sacrifice

Dose 0% Males 008, 024, 026, 033, 040, 036, 048, 052, 059, 070.
Females 001, 018, 019, 023, 026, 033, 040, 048, 052, 067.
4.0% Males 224, 227, 233, 245, 247, 248, 251, 253, 259, 280.
Females 211, 234, 235, 237, 242, 252, 258, 261, 277, 278.

2 Year scheduled study

Dose 0% Males 002-005, 009-019, 021-023, 025, 027-030, 034, 035, 037, 038, 041-046, 049-051, 053-058, 060- 065, 068, 069.
Females 002-004, 006, 007, 009-017, 020-022, 024, 025, 028, 029, 031, 032, 035-037, 039, 041-044, 046, 047, 049 -051, 053, 055-058, 061-066, 068-070.
0.1% Males 073, 076-079, 081, 082, 084-088, 092-094, 096, 098, 099, 101-112, 114, 117, 119-122, 124-126, 128-133, 135-137, 139, 140.
Females 107.
1.0% Males 142, 143, 145, 146, 151, 152, 155, 157-160, 162, 164, 165, 168-171, 173, 175-188, 190, 191, 193-202, 204-207, 209.
Females 145, 149, 156, 168.
4.0% Males 211, 213-216, 219-223, 225, 226, 228-230, 232, 234-241, 244, 246, 249, 252, 254-257, 260-276, 279.
Females 212-215, 217, 218, 220-233, 236, 239, 240, 243, 244, 246-248, 250, 251, 253-255, 257, 259, 260, 262-264, 266-269, 272-276, 279, 280.

SUBMISSION END