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GRAS NOTICE FOR HIGH-PURITY QUERCETIN

Submitted to:

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD, U.S.A.
20740-3835

Submitted by:

Quercegen Pharma LLC
275 Grove Street, Suite 2-400
Newton, MA, U.S.A.
02466

May 20, 2010

GRAS NOTICE FOR HIGH-PURITY QUERCETIN

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GRAS NOTICE FOR HIGH-PURITY QUERCETIN

I GRAS EXEMPTION CLAIM

I.A Claim of Exemption from the Requirement for Premarket Approval

High-purity quercetin (≥99.5% quercetin) has been determined by Quercegen Pharma LLC (hereafter Quercegen) to be Generally Recognized as Safe (GRAS), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use in food, and on the consensus opinion of an independent panel of experts qualified by scientific training and expertise. Therefore, pursuant to proposed 21 CFR §170.36(c)(1) [62 FR 18938 (U.S. FDA, 1997)], the use of high-purity quercetin in food as described below is exempt from the requirement of premarket approval.

Signed,

(b) (6)

20 MAY 2010
Date

Tom Lines
Quercegen Pharma LLC
275 Grove Street, Suite 2-400
Newton, MA, U.S.A.
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I.B Name and Address of Notifier

Thomas C. Lines
Chief Executive Officer
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275 Grove Street, Suite 2-400
Newton, MA, U.S.A.
02466

Telephone: (b) (6)
Facsimile:
E-mail: Bossline3@aol.com

I.C Common Name of the Notified Substance

QU 995 (common or usual name quercetin) is the subject of this Notification.

Quercetin may be produced by multiple purification steps to achieve multiple purity levels with Trade Names QU 985, QU 985A, QU 998, and QU 1000.

I.D Conditions of Intended Use in Food

I.D.1 Background Consumption

Quercetin is one of several naturally-occurring flavonol compounds belonging to the broader group of polyphenolic flavonoid substances, which are characterized by a phenyl benzo(γ)pyrone-derived structure consisting of 2 benzene rings linked by a heterocyclic pyran or pyrone ring (Kühnau, 1976; Morand *et al.*, 1998). Among other biological properties, these compounds have been reported to exhibit strong antioxidant properties in the plant (Wiczkowski *et al.*, 2003). Fruits and vegetables, particularly onions and apples, were identified as the primary sources of naturally-occurring dietary quercetin of the typical Western diet (Hertog *et al.*, 1992; Day and Williamson, 1999; Harnly *et al.*, 2006). Black tea also was identified as a rich dietary source of quercetin (Hertog *et al.*, 1993; Sampson *et al.*, 2002). In the United States, the average daily intake of all flavonoids from a normal mixed diet was estimated to be approximately 1 g/day (expressed as quercetin equivalents) (Kühnau, 1976; Brown, 1980), with flavonol glycosides, expressed as quercetin equivalents, consumed at levels of up to 100 mg/day (Brown, 1980; Jones and Hughes, 1982; NTP, 1992; Rimm *et al.*, 1996; USDA, 2000; Sampson *et al.*, 2002), whereas daily intake levels of quercetin as high as 200 to 500 mg have been reported in high-end consumers of fruits and vegetables (Jones and Hughes, 1982).

National (*e.g.*, Australia, the Netherlands, Finland, Italy, Croatia, Japan, United States) dietary record-based cohort assessments of the intake of quercetin from the habitual diet indicated consumption levels of up to approximately 40 mg quercetin/day (Hertog *et al.*, 1995; Rimm *et al.*, 1996; Knekt *et al.*, 1997; Kimira *et al.*, 1998; Johannot and Somerset, 2006; Lin *et al.*, 2006). Values for the estimated intake of quercetin by the U.S. population from naturally-occurring sources can also be generated using the available data on the quercetin content of foods from the flavonoid database combined with food intake data from the National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) for the years 2003-2004 (NHANES 2003-2004) (CDC, 2006; USDA, 2009a). Individuals that did not report consuming a naturally-occurring food source for quercetin were excluded from these analyses. Total quercetin intakes from background sources are presented in Table 1. Consumption of these types of foods by the total U.S. population resulted in estimated mean and 90th percentile all-

user background intakes of quercetin of 5.9 and 14.7 mg/person/day, respectively. A maximum quercetin all-user intake value of 258.2 mg/day also was generated. This value represents the highest individual reported quercetin intake from all naturally-occurring quercetin sources and occurred in the male adult population group. Given that the quercetin content is only available for selected foods, it is likely that actual background intakes are higher than these estimates.

Table 1 Summary of the Estimated Daily Background Intake of Quercetin from Naturally Occurring Sources in the United States by Population Group (2003-2004 NHANES Data and USDA Database for the Flavonoid Content of Selected Foods)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	Background Quercetin Intake (Users Only)		
				Mean (mg)	90th Percentile (mg)	Maximum (mg)
Infants	0 to 2	54.2	504	1.9	3.7	60.2
Children	3 to 11	85.5	1101	2.6	6.3	96.4
Female Teenagers	12 to 19	79.6	790	4.9	10.7	176.8
Male Teenagers	12 to 19	83.9	838	6.1	15.1	118.2
Female Adults	20 and Up	82.4	1754	5.7	15.6	117.5
Male Adults	20 and Up	84.0	1620	7.6	17.8	258.2
Total Population	All Ages	79.9	6607	5.9	14.7	258.2

Adapted from CDC (2006) and USDA (2007; 2009a)

I.D.2 Intended Use of High-Purity Quercetin and Levels of Use in Foods

Quercegen intends to market high-purity quercetin (≥99.5%) as a food ingredient in a variety of food products including beverages and beverage bases, grain products and pastas, processed fruits and fruit juices, and soft candies. Depending on the particular food category, the ingredient is intended for use at levels up to 500 mg of quercetin/serving. The individual intended food-uses and use-levels for Quercegen's high-purity quercetin ingredient are summarized in Table 2. Quercegen's high-purity quercetin ingredient is not intended to be used in meat or poultry products.

Table 2	Summary of the Individual Proposed Food-Uses and Use-Levels for Quercetin in the U.S.			
Food Category	**Proposed Food-Use**	**Quercetin -Level (mg/serving)***	**Serving Size (g or mL)**	**Use-Level (%)**
Beverages and Beverage Bases	Sports and Energy Drinks	250	240	0.10
Grain Products and Pastas	Cereal and Energy Bars	50	40	0.125
	Grain-Based Beverages	10	240	0.004
Processed Fruits and Fruit Juices	Fruit-Flavored Drinks (non-carbonated and carbonated)	125	240	0.052
	Fruit Smoothie Drinks	125	240	0.052
Soft Candies	Dietetic or Low-Calorie Soft Candies	250 to 500	6	4.2 to 8.3

* Serving Sizes are from Reference Amounts Customarily Consumed per Eating Occasion (21 CFR §101.12) (U.S. FDA, 2009).

High-purity quercetin is proposed to be added to selected foods and beverages for specific technical/functional uses for exercising adults (i.e. Targeted Population) and will be marketed initially through specialty channels (e.g. GNC, sporting goods stores). Foods and beverages to which this ingredient would be added will be labeled to disclose to consumers the presence of high-purity quercetin and to provide directions and/or other relevant information about the technical/functional uses. The technical/functional uses will be a primary reason why consumers will purchase and consume the foods and beverages. In addition, such foods and beverages would be priced at a premium compared to commodity foods that do not contain high-purity quercetin because of the high cost of this ingredient. Thus, foods and beverages to which high-purity quercetin is added will be specially labeled, marketed, and priced to reflect a specific technical/functional use, and such foods and beverages would not be labeled, marketed, or priced in the same manner as commodity foods.

I.D.3 Estimated Dietary Consumption of High-Purity Quercetin Based upon Intended Food Uses

The consumption of quercetin from all intended food-uses of Quercegen's ingredient was estimated using the National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2009a,b), which is a 24-hour dietary recall survey administered on 2 non-consecutive days throughout all 4 seasons of a year. The data from the 2003-2004 and 2005-2006 cycles of the NHANES

survey were combined to provide a larger population from which to estimate quercetin consumption. Approximately 37.4% of the total U.S. population was identified as potential consumers of Quercegen's high-purity quercetin from the intended food-uses (6,252 actual users identified). This is the traditional approach to estimating intake of substances added to food and is based on historical consumption of the foods and beverages to which high-purity quercetin is proposed to be added.

Using a traditional consumption analysis (see Tables 3 and 4), the Total Population All-Users estimated mean intake of high-purity quercetin is 205 mg/person/day (4 mg/kg bw/day); whereas, the 90th percentile All-Users intake is 461 mg/person/day (9 mg/kg body weight/day). On a gender/age group basis, the estimated highest All-Users intake of high-purity quercetin is in male teenagers, with a mean value of 298 mg/person/day and a 90th percentile value of 668 mg/person/day. Male teenagers is a group that plausibly could be consumers of foods and beverages containing high-purity quercetin for their specific technical/functional uses, although to a lesser degree than exercising adults. On the other hand, infants have an All-Users mean intake of high-purity quercetin of 269 mg/person/day and a 90th percentile value of 625 mg/person/day. Clearly, these intake estimates for infants greatly exaggerate the potential for exposure of this age group since they are a Non-targeted Population and would not be consumers of sports and energy drinks or "sports chews" for technical/functional uses. It should be noted that the food category and respective food codes for "Soft Candies/ Dietetic or Low-Calorie Soft Candies" is being used as a surrogate for "Sports Chews", since NHANES food codes do not exist for this specific food-use. The assumption by the traditional approach to estimating intake that Sports and energy drinks are consumed by infants unrealistically skews upward the estimate of their intake of all food categories since the mean intake of high-purity quercetin is 274 mg/person/day and 90th percentile intake is 635 mg/person/day for this food-use alone. The same rationale applies to children also as a Non-targeted Population.

Table 3 Summary of the Estimated Daily Intakes of Quercetin Based on all Proposed Food-Uses and Use-Levels in the United States (NHANES 2003-2004, 2005-2006)

Population Group	Age (Years)	% Users	Actual # of Total Users	All-Person Consumption (mg/day)		All-User Consumption (mg/day)	
				Mean	90th Percentile	Mean	90th Percentile
Infants	0 to 2	28.9	552	85	153	269	625
Children	3 to 11	55.9	1,528	97	250	176	375
Female Teenagers	12 to 19	47.8	949	70	225	156	338
Male Teenagers	12 to 19	49.7	964	144	450	298	668
Female Adults	> 20	27.3	1,167	39	105	152	288
Male Adults	> 20	28.4	1,092	70	214	256	572
Total Population	All ages	37.4	6,252	67	182	205	461

Derived using data and individual food codes from the National Health and Nutrition Examination Surveys (NHANES) 2003-2004, 2005-2006 (CDC, 2006; USDA, 2009a,b).

Table 4 Summary of the Estimated Daily Per Kilogram Body Weight Intakes of Quercetin Based on all Proposed Food-Uses and Use-Levels in the United States (NHANES 2003-2004, 2005-2006)

Population Group	Age (Years)	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw/day)		All-User Consumption (mg/kg bw/day)	
				Mean	90th Percentile	Mean	90th Percentile
Infants	0 to 2	28.9	552	7	12	22	53
Children	3 to 11	55.9	1,528	4	9	7	13
Female Teenagers	12 to 19	47.8	949	1	4	3	6
Male Teenagers	12 to 19	49.7	964	2	7	5	11
Female Adults	> 20	27.3	1,167	1	1	2	4
Male Adults	> 20	28.4	1,092	1	2	3	7
Total Population	All ages	37.4	6,252	1	4	4	9

Derived using data and individual food codes from the National Health and Nutrition Examination Surveys (NHANES) 2003-2004, 2005-2006 (CDC, 2006; USDA, 2009a,b).

Because the addition of high-purity quercetin will make a food or beverage materially different from other commodity foods or beverages of a food category, consumption of high-purity quercetin cannot be accurately estimated for all gender/age groups using only traditional consumption analysis based on historical consumption of the respective commodity foods. Instead, the intake estimate provided by the traditional approach must be further evaluated by considering how high-purity quercetin-containing foods and beverages will be consumed by an exercising adult population for technical/functional

uses as opposed to Non-targeted Populations (i.e. infants, children, teenagers and nonexercising adults).

I.D.4 Discussion of Targeted and Non-targeted Populations

In general, consumption estimates by the traditional approach are highly conservative for a number of reasons. In particular, the estimates assume that all target foods and beverages that could contain high-purity quercetin would in fact be formulated with it at the maximum level of use. Because high-purity quercetin is intended for Targeted Populations for technical/functional uses that will modify the normal intended use and price of the foods and beverages to which it will be added, it is likely that only a small number of the target foods will be formulated with high-purity quercetin.

Targeted Population consumers who select high-purity quercetin containing products will be able to select these foods and beverages at nutrition and sports beverage specialty stores based on their labeling as products with specific technical/functional uses. At this time, there is not a daily recommended intake level for high-purity quercetin, but the highest proposed use-levels are 250 mg/serving for sports and energy drinks and 250 to 500 mg/serving for sports chews so that consumers may choose to conveniently obtain 1000 mg high-purity quercetin/day by consuming two to four servings of the target foods or beverages. Several publications suggest that technical/functional effects related to exercise performance parameters may be achieved at this intake level. It is estimated that two to four servings of the target foods or beverages would be the most likely intake level for consumers who specifically seek high-purity quercetin, and that these Targeted Population consumers would be unlikely to exceed this level over a long term, for the following reasons:

- Targeted Population consumers may seek to supplement their diet with high-purity quercetin and may increase intake for a short period of time; however, it is unlikely that they would significantly change their habitual intake levels of the target foods and beverages over a long period of time.

- Since there is not a wide variety of target foods that could contain high-purity quercetin, and because only a few food codes from the target food and beverage categories would in fact be formulated with high-purity quercetin, it may be difficult for many Targeted Population consumers to consume more than two to four servings a day of such foods.

- The foods and beverages containing high-purity quercetin will be priced at a premium as compared to commodity foods that do not contain high-purity quercetin.

For adult exercising Targeted Population consumers who would intentionally seek high-purity quercetin-containing foods and beverages, consumption is estimated at 1000 mg quercetin/day. It is possible that these adult exercising consumers who were to select high-purity quercetin-containing foods and beverages would consume more than two to four servings/day for short periods of time that would provide 1250 or 1500 mg quercetin/day. However, long-term consumption at this level is considered unlikely based on the rationale presented above. The Non-targeted Population (particularly infants and children) are expected to consume high-purity quercetin at much lower levels and, in fact, their consumption is expected to be much lower than estimated by the traditional approach for consumption analysis. The safety of the anticipated maximum daily intake foreseen by the Targeted Population adult exercising consumers is supported by the following section, IV. Review of Toxicological and Other Safety Related Data.

The Total Population maximum estimated daily background intake of quercetin from naturally occurring sources was 258.2 mg/day, which is within the published range of 200-500 mg/day for high-end consumers of fruits and vegetables (Jones and Hughes, 1982). Therefore, a Targeted Population consumer with a daily intake of 1000 mg high-purity quercetin, who also was high-end consumer of fruits and vegetables, may consume approximately 1250 mg quercetin/day. This daily intake is also considered safe based on the totality-of-the-evidence.

I.E Basis for the GRAS Determination

Pursuant to 21 CFR §170.35, high-purity quercetin has been determined by Quercegen to be GRAS on the basis of scientific procedures (U.S. FDA, 2009). This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of quercetin as a component of food (see Exhibit A).

I.F Availability of Information

The data and information that serve as the basis for this GRAS Notice will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times as arranged by:

Quercegen Pharma LLC
275 Grove Street, Suite 2-400
Newton, MA, USA
02466
(contact: Thomas C. Lines, telephone: (b) (6)

Should the FDA have any questions or additional information requests regarding this notification, Quercegen will supply these data and information.

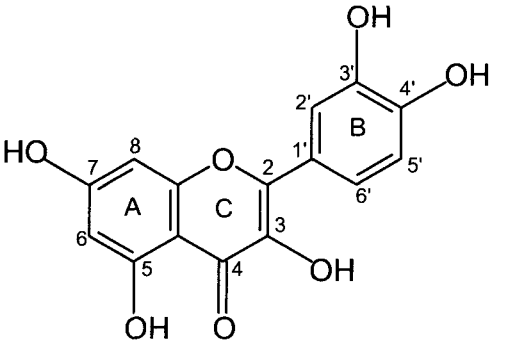
II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE

II.A Identity

II.A.1 Chemical Description

Quercetin is a flavonol, characterized by a phenyl benzo(γ)pyrone-derived structure (Kühnau, 1976; Morand *et al.*, 1998). Flavonols comprise one of several classes of polyphenolic flavonoid compounds. Commercially, quercetin is produced by extraction of the quercetin glycoside, rutin, from plants. Rutin is subsequently subjected to hydrolysis to yield the aglycone. The structural formula and other chemical and physical characteristics of quercetin are presented below.

Structure:



Synonyms:	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one; meletin; cyanidenolon 1522; 3,3',4',5,7-pentahydroxyflavone; sophoretin; C.I. Natural Yellow 10; C.I. 75670; quercetin; quercetol; quertin; quertine; sophoretin; xanthaurine.
CAS Number:	117-39-5
Empirical Formula:	$C_{15}H_{10}O_7$
Molecular Weight:	302.23
Appearance:	A greenish-yellow, crystalline solid
Solubility:	Soluble in glacial acetic acid and aqueous alkaline solutions; slightly soluble in alcohol; insoluble in water

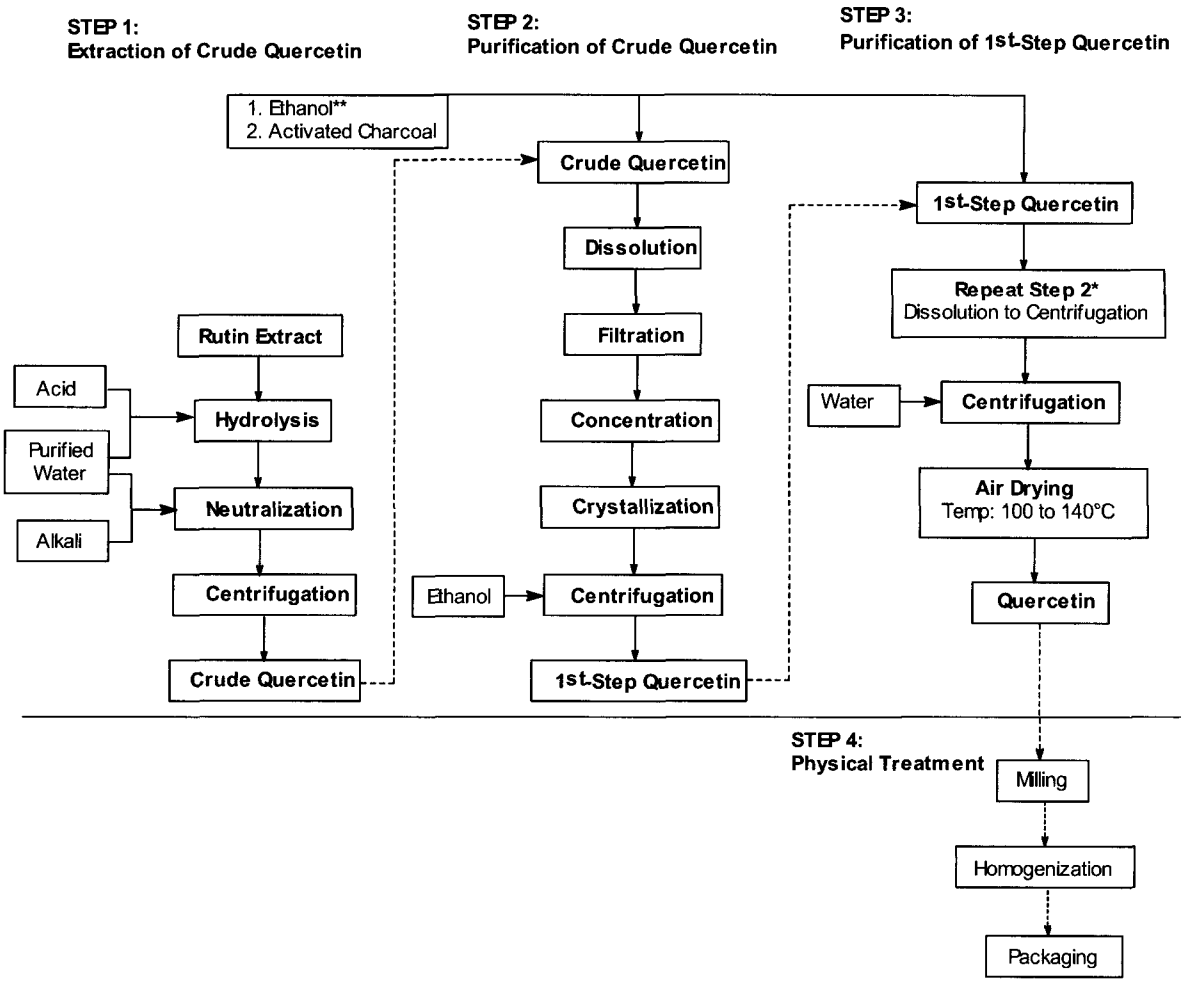
II.B Method of Manufacture

The quercetin product intended for use as an ingredient in food is manufactured by the Natural Products division of Quercegen Agronegócios 1 Ltda. (formerly Merck S/A, Brazil) in

accordance with Good Manufacturing Practices (GMPs). Immature, sun-dried Fava d'Anta beans (*Dimorphandra mollis* or *Dimorphandra gardeneriana*), which are native to several regions of Brazil, or alternatively Uncaria leaves (*Uncaria elliptica*), also domestically grown in Brazil (Unidade Agroindustrial Merck, Barro do Corda, Brazil), are the principal starting materials for the manufacture of high-purity quercetin. Additionally, onions and/or apples may be utilized as alternative starting materials for quercetin. The manufacturing process for quercetin essentially consists of the aqueous extraction of the quercetin glycoside, rutin, from the above-identified plant source(s) and release of the aglycone *via* hydrolysis by the addition of an acidic aqueous solution common to food ingredient manufacturing processes[1] (*i.e.,* CASRN 7697-37-2) and subsequent neutralization (21 CFR § 184.1205) (U.S. FDA, 2009) to produce a crude crystalline quercetin product. Next, the resultant quercetin product is subjected to several purification processes to yield purified quercetin crystals (2 steps to obtain QU 985 and 3 steps to obtain QU 995, QU 998, and QU 1000). All materials involved in the manufacturing process are appropriate for food use. A schematic overview of the manufacturing process of quercetin is provided in Figure 1.

[1] As per the manufacture processes of the direct food substances affirmed as Generally Recognized as Safe provided for by 21 CFR § 184.1009, 184.1021, and 184.1878 (U.S. FDA 2009)

Figure 1 Schematic Overview of the Manufacturing Process for Quercegen's High-Purity Quercetin



* 'Step 2' is repeated at least once, but may be repeated more than one time, depending on the desired level of purity for the final product.
** 21 CFR § 184.1293 (U.S. FDA, 2009).

II.C Specifications for Food-Grade Material

II.C.1 Chemical and Microbiological Specifications

The physical, chemical, and microbiological specifications for high-purity quercetin are presented in Table 5. Analysis of non-consecutive representative batches (Batch MM2-6050, Batch MM2-6051, Batch MM2-6052, and Batch MM2-6053) demonstrated compliance with final product physical, chemical, and microbiological specifications (see Exhibit B-1).

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Table 5	Physical, Chemical, and Microbiological Specifications for Quercegen's High-Purity Quercetin	
Parameter	**Limit**	**Method of Analysis[a]**
Description	Yellow or greenish-yellow, crystalline powder, odorless or with a slight characteristic odor	Visual and olfactory inspection
Solubility, in 2% ethanol (NTU)	Not more than 15	Merck S/A
Solubility, 1% DMF	Clear solution	Visual inspection
Solubility, Water	Insoluble	Visual inspection
Identity	Comparable to quercetin standard	HPLC analysis
Assay (Quercetin) (dry weight basis)	Not less than 99.5%[b]	HPLC analysis
Density (g/mL)	Not less than 0.25	Merck S/A SOP 41/g/1213
Moisture (%)	Not more than 4.0	Karl-Fisher
Sulfated ash (%)	Not more than 0.15	Merck S/A
Chloride (Cl^-) (ppm)	Not more than 1,000	Merck S/A
Sulfate (SO_4^{2-}) (ppm)	Not more than 1,000	Merck S/A
Heavy Metals		
Arsenic (As) (ppm)	Not more than 1	AAS
Cadmium (Cd) (ppm)	Not more than 1	AAS
Cobalt (Co) (ppm)	Not more than 1	AAS
Lead (Pb) (ppm)	Not more than 1	AAS
Mercury (Hg) (ppm)	Not more than 1	AAS
Nickel (Ni) (ppm)	Not more than 1	AAS
Microbiological Specifications		
Aerobic bacteria (CFU/g)	Not more than 5,000	Merck General Method MG 029[c]
Yeasts and molds (CFU/g)	Not more than 500	Merck General Method MG 029
Salmonella species (per 10 g)	Absent	Merck General Method MG 029
Escherichia coli (per 10 g)	Absent	Merck General Method MG 029
Staphylococcus aureus (per 10 g)	Absent	Merck General Method MG 029
Pseudomonas aeruginosa (per 10 g)	Absent	Merck General Method MG 029

AAS = Atomic Absorption Spectroscopy; CFU = Colony-forming units; DMF = N,N-Dimethylformamide; HPLC = high-performance liquid chromatography; NTU = Nephelometric Turbidity Unit
[a] See Exhibit B-2 for details of the methods of analysis.
[b] To obtain quercetin of not less than 99.5% purity, a 3-step purification process is applied.
[c] Merck General Method 029 complies with U.S. Pharmacopeia Monograph for Microbiology Tests (USP, 2008 or a more recent edition) (see Exhibit B-2).

II.C.2 Additional Analyses

Rutin (CAS RN 153-18-4) and isoquercetin (CAS RN 482-35-9), which are glycosides of the quercetin aglycone, may occur in the final quercetin product. The levels of these compounds in the final product are limited to <1%, and batch samples are routinely tested using thin-layer chromatography to ensure compliance with this limit. Glycosides including rutin and

isoquercetin are the primary form of quercetin that occurs naturally in foods, and therefore, their minimal presence in the final product is not considered to be of concern.

A possible impurity occurring in the final quercetin product as a result of the manufacturing process is residual ethanol. Appropriate food-grade criteria have been established to limit the levels of residual ethanol in the final product (*i.e.*, <3,000 ppm) and batch samples are routinely tested to ensure purity of the final ingredient (see Exhibit B-1). More specifically, residual levels of ethanol are verified by gas chromatographic analysis and comply with the limits set for residual solvents by the ICH of Technical Requirements for Registration of Pharmaceuticals for Human Use (*i.e.*, 5,000 ppm). Additionally, analysis of levels of nitrate in the final product demonstrated that it was undetectable at the level of detection used in the assay (0.1 ppm).

II.C.3 Stability of Quercegen's High-Purity Quercetin

II.C.3.1 Bulk Stability (Dry Powder)

In a 60-month long-term stability study, quercetin samples were stored in plastic bags at 30±2°C and 70±5% humidity. The batch sample used for samples for this study was manufactured in 2003 (Batch MM 2-3028). At that time, the specification for quercetin purity was not less than 98.0%, whereas the current specification is not less than 99.5%. Stability data for the 98.0% product are considered to be applicable to the 99.5% product. Results of the analyses of the long-term stability indicated that the level of quercetin in the ingredient was very stable throughout the duration of the study.

In addition to the long-term study conducted using the 98.0% quercetin product, a sample containing not less than 99.5% quercetin (Batch MM 2-6023) was evaluated in a 24-month stability study in which the product was stored in plastic bags at 30±2°C and 75±5% humidity. The level of quercetin in the product was shown to be stable under these conditions for 24 months.

The stability of quercetin (>99.5%, Batch Nos. MM 02-9046, MM 02-9047 and MM 02-9048) was also assessed in 6-month stability studies in which samples were stored in plastic bags at 70±5% relative humidity and 30±2°C (long-term stability samples) or 75±5% relative humidity and 40±2°C (accelerated stability samples). The results of the analysis of quercetin at time 0 and the quantitative and qualitative stability parameters measured at study initiation and at 1, 2, 3 and 6 months thereafter indicated conformity to product specifications, confirming that the quercetin product is stable for at least 6 months even under accelerated conditions of elevated temperature and humidity.

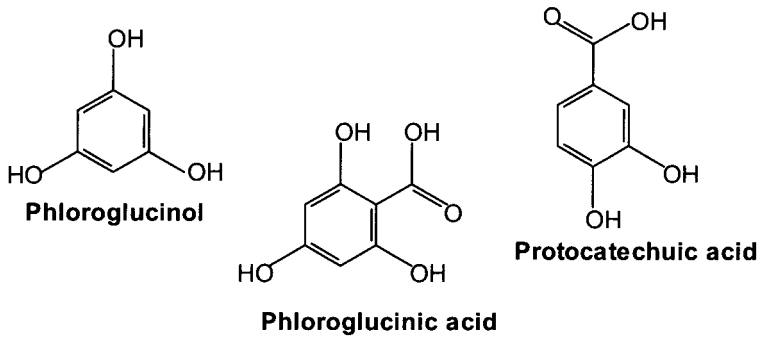
Details of the bulk stability of Quercegen's quercetin ingredient are provided in Exhibit B-3.

II.C.3.2 Stability in Aqueous Solutions

As quercetin will be added to a variety of beverage products, including sports and energy drinks, grain-based beverages, fruit-flavored drinks, and smoothie drinks, the stability of quercetin under varying pH and temperatures conditions is relevant to the GRAS status of the ingredient.

Schmalhausen *et al.* (2007) reported that quercetin auto-oxidized in aqueous solutions of neutral pH (7.5). The compounds that have been identified as the principal oxidative degradation products of quercetin include protocatechuic acid (3,4-dihydroxybenzoic acid; CAS No. 99-50-3) and phloroglucinic acid (2,4,6-trihydroxybenzoic acid; CAS No. 83-30-7), as well as phloroglucinol (1,3,5-trihydroxybenzene; CAS No. 108-73-6), a decarboxylation product of the latter (see Figure II.C.3.2-1) (Zenkevich *et al.*, 2007). The intended conditions of use of high-purity quercetin do not include any beverage categories with a pH as high as 7.5, and therefore, the auto-oxidation of querecetin as a result of neutral pH conditions is not likely. Moreover, should high-purity quercetin undergo a minimal degree of oxidation, its principal oxidative degradation products, protocatechuic acid, phloroglucinic acid, and phloroglucinol, are metabolic products resulting from the colonic microbial degradation of unabsorbed quercetin and therefore, would not be anticipated to result in any adverse effects on human health.

Figure 2 Structural Formulae of Quercetin Degradation Products, Phloroglucinol, Phloroglucinic Acid, and Protocatechuic Acid



Moon *et al.* (2008) subsequently investigated the stability of quercetin at different pH values (2.7, 7, and 10) and different temperatures (room temperature, 4°C, and -20°C). At pH 10, quercetin was fully degraded at all temperatures within 6 to 12 hours. At pH 2.7 and temperatures of 4 or -20°C quercetin remained stable for the duration of the storage period (96 hours). Likewise, greater than 80 and 90% of quercetin was recovered in the pH 7 system stored at 4 and -20°C. At room temperature, the stability of quercetin was reduced, such that 50 and 20% of quercetin degraded in the first 25 hours at pH 7 and 2.7, respectively. Generally,

the study results demonstrated that the stability of quercetin is both pH and temperature dependent, with acidic conditions and low temperatures identified as more conducive to quercetin stability. The intended conditions of use of high-purity quercetin include beverage products with acidic pH and that, in general, will have recommended conditions of storage at low temperatures; therefore, the potential pH and high temperature effects of beverage matrices and/or their storage or processing conditions are not anticpated to affect the stability of high-purity quercetin under the intended conditions of use.

Since the intended uses for quercetin include beverages of various pH, the stability of Quercegen's high-purity quercetin was assessed under different pH conditions (ranging from pH 3 to 7.5), alone and in the presence of other antioxidants [*e.g.*, ascorbic acid, epigallocatechin gallate (EGCG), propyl gallate]. Results of the stability studies demonstrate that depending on the test conditions (*i.e.*, pH of solution, quercetin concentration, and the ratio of quercetin to added antioxidants), quercetin is either fully protected from degradation or the degradation of quercetin is significantly attenuated (see Exhibit B-3).

The stability of a prototype beverage formulation was also tested after storage of up to 32 weeks at 40, 73 or 100°F. Results of this study demonstrated that the concentration of quercetin in the prototype beverage formulation containing a target level of 280 mg quercetin/240 g beverage formulation, niacinamide (0.00483%), ascorbic acid (0.105%), and at a pH range of 2.7-3.3 is stable at 40 or 73°F for at least 32 weeks and at 100°F for at least 16 weeks (see Exhibit B-3).

II.C.3.3 Photostability

One existing study in the published literature pertaining to the stability of quercetin under exposure to UV irradiation was identified, which showed that quercetin was stable following UVB radiation exposure (Vicentini *et al.*, 2007). However, in another study, exposure of aqueous solutions of quercetin at pH 5, 7.5, or 10 at 25°C to UV irradiation resulted in initiation of oxidation product formation at the 2 lower pH levels (5 and 7.5) and acceleration of the quercetin degradation rate at pH 10 (Momić *et al.*, 2007). Appropriate packaging will be utilized to eliminate any potential oxidation product formation or degradation of quercetin under the intended conditions of use in beverage products.

II.C.3.4 Summary

Data from long-term (60 month) and accelerated stability studies indicate that quercetin is stable under bulk storage conditions in dry powder form for the duration of its shelf life (5 years), as demonstrated by analysis of quercetin levels present in the material at the end of the study. The stability of quercetin in a prototype beverage formulation has also been confirmed.

While quercetin has been shown to degrade under certain pH conditions, provisions exist to prevent degradation of high-purity quercetin under the intended conditions of use in foods. These include the inherent acidic pH and low-temperature storage conditions of the intended beverage matrices. Moreover, in order to prevent degradation of quercetin in aqueous solutions, quercetin will be used with an appropriate antioxidant, such as ascorbic acid, that will be added at levels sufficient to preserve the stability of quercetin and prevent its degradation under intended conditions of use. Regardless, the products resulting from possible quercetin degradation (*i.e.*, phloroglucinol, phloroglucinic acid, and protocatechuic acid) are known to occur ubiquitously in nature and have been identified as normal degradation products of anaerobic quercetin fermentation. Based on the intake estimates for quercetin (see Section I.D.3) under the intended conditions of use and assuming a maximum 15% degradation of quercetin to phloroglucinol, phloroglucinic acid, and protocatechuic acid, mean and 90th percentile exposure to these degradation products from the consumption of foods with added quercetin would be less than approximately 31 and 69 mg/person/day, respectively (0.5 and 1.2 mg/kg body weight/day, respectively). There are no data to indicate that ingestion of phloroglucinol, phloroglucinic acid, or protocatechuic acid would be associated with any adverse effects. Data related to the metabolic fate of phloroglucinol and protocatechuic acid indicate that both compounds, much like quercetin, are subjected to extensive phase I and II metabolic reactions following absorption and are subsequently rapidly excreted (Dollo *et al.*, 1999; Xu *et al.*, 2007). Therefore, any unmitigated degradation of quercetin to these compounds is not expected to result in any adverse effects in human health. Additional safety evaluation of potential quercetin degradation products is provided in Section IV.D.6, below.

III. INTENDED TECHNICAL EFFECT AND SELF-LIMITING LEVELS OF USE

Quercegen's high-purity quercetin is intended to be an effective source of flavonols in products proposed for the excercising adult population. An exposure level of 1000 mg quercetin/day may enhance some exercise performance parameters as demonstrated in several published human clinical studies (see attached technical Dossier, Exhibit D) in which safety was also confirmed by a number of endpoints.

The levels of use of quercetin in food products are limited by the aglycone's characteristic bitter taste and its limited solubility. The concentration of quercetin in beverages for consumers proposed in this Notice is 250 mg/8 oz (240 g) or 100 mg/100g and the concentration used in human pharmacology studies was 600 mg/8 oz or 250mg/100g. The sensory effects observed at use levels higher than those intended for human consumption include astringency (mouth drying), bitterness (similar to other flavonoids), and throat irritation. Additional limitations are exhibited by the opacity and bright yellow hue of quercetin beverage formulations.

IV. REVIEW OF TOXICOLOGICAL AND OTHER SAFETY RELATED DATA

IV.A Introduction

The safety evaluation of quercetin is based primarily on the large number of reliable peer-reviewed publications concerning human and animal metabolism, *in vitro* and *in vivo* mutagenicity/ genotoxicity studies, subchronic and chronic toxicity, carcinogenicity, two-stage carcinogenicity, and developmental and reproduction toxicity studies. Furthermore, several published epidemiology studies and human clinical trials were reviewed, and two *in vivo* drug interaction studies with healthy subjects and an oral glucose tolerance test in type 2 diabetics were conducted recently with quercetin, in order to assess the safety of quercetin and corroborate data from the literature. Studies investigating the fate of quercetin following its consumption indicate that it is extensively metabolized, beginning with microbial degradation in the gastrointestinal tract with excretion of the resultant metabolites in the feces and urine. Whereas *in vitro* assays indicate quercetin is a well-established mutagen, mutagenic properties for quercetin have not been confirmed *in vivo* as demonstrated in numerous genotoxicity, carcinogenicity, and anti-carcinogenicity studies. The various metabolic pathways involved for quercetin, as discussed below, act in concert to limit the bioavailability of this flavonol for consumers and as such prevent the occurrence for potential adverse effects associated with its consumption. Additionally, it should be noted that quercetin, one of many flavonoid compounds, is widely distributed mostly in fruits and vegetables of the common human diet. Therefore, adaptive mechanisms such as extensive metabolism contributing to the absence of adverse effects in animals and humans are considered.

The totality of the available published and corroborative data evidence supports the GRAS status of Quercegen's high-purity quercetin. A summary of these data in relation to the GRAS status of high-purity quercetin under the intended conditions of use is presented below. [2]

IV.B Metabolic Fate of Quercetin

Ingestion of free quercetin aglycone may be followed immediately by its extensive microbial degradation in the gastrointestinal tract prior to absorption. Thereafter, quercetin or its derivatives are absorbed along the gastrointestinal tract and subsequently undergo rapid and extensive systemic metabolism. The different pathways involved in the metabolism of quercetin, outlined below (Figure 3), act in concert to limit the bioavailability of quercetin *in vivo*

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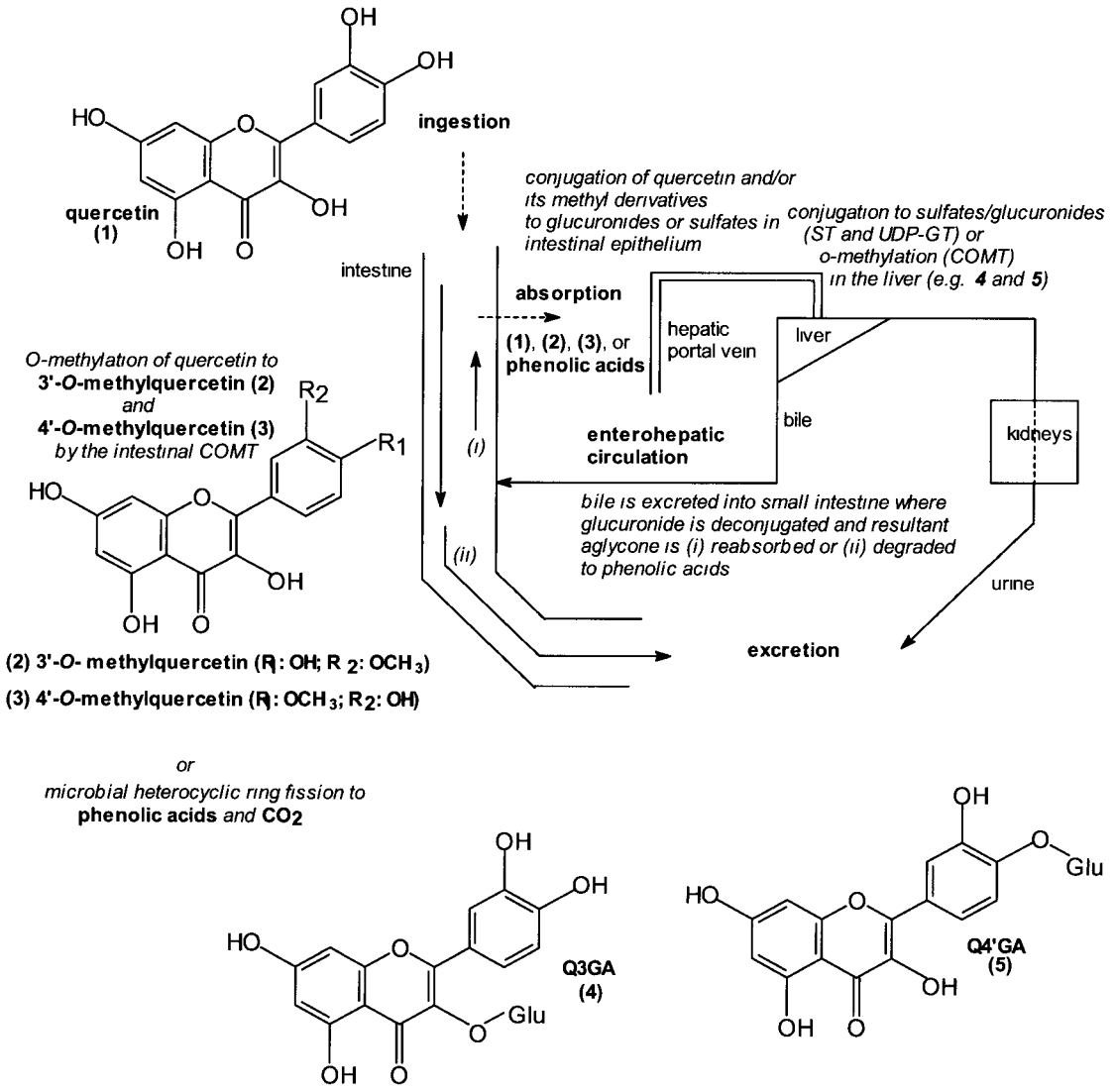
[2] Unless otherwise indicated, the quercetin test material used in the studies described below was the flavonol aglycone.

and prevent potential adverse effects associated with its consumption. Ultimately, quercetin conjugates are eliminated in the urine following metabolism, unmetabolized quercetin or bacterially-degraded quercetin derivatives are excreted in the feces, quercetin metabolites are secreted *via* the bile, or are expired as exhaled CO_2. It should be noted that quercetin, one of many naturally-occuring flavonoid compounds, is widely distributed in the common human diet. Therefore, adaptive mechanisms such as extensive metabolism contributing to the absence of adverse effects in animals and humans cannot be discounted.

Figure 3 **Schematic Representation of the Absorption, Metabolism, and Excretion of Quercetin in Mammals**



Adapted from Day and Williamson (1999)

IV.C Effects on Enzymes and Other Biochemical Parameters

It may be concluded that orally administered quercetin does not adversely impact the following biochemical events based on the totality-of-evidence.

In vitro evidence that quercetin inhibits monosaccharide absorption is equivocal (Kwon *et al.*, 2007; Cermak *et al.*, 2004; Strobel *et al.*, 2005; Barrenetxe *et al.*, 2006); whereas the available human study demonstrates that daily oral intake of a 1500 mg quercetin liquid formulation for five days does not impact blood glucose levels as compared to placebo in subjects with type 2 diabetes (Prism Research, 2010a). Studies to assess the effects of quercetin on plasma glucose levels in normoglycemic and diabetic rats are summarized in Table C-1 (see Exhibit C).

As an antioxidant, GSH binds reactive quercetin and as such a potential exists for GSH depletion following quercetin exposure; however, in the human study that monitored GSH levels during an extended period of quercetin exposure (1000 mg quercetin/day for 12 weeks), there was no impact on oxidized or reduced GSH levels (Nieman, 2008; Shanely *et al.*, 2010). Studies to assess the effects of quercetin on plasma glucose levels in normoglycemic and diabetic rats are also summarized in Table C-2 (see Exhibit C).

In vitro data indicate that quercetin may induce or inhibit the activity of metabolizing enzymes belonging to the CYP450 family and/or are substrates of P-glycoprotein (P-gp). In most cases, the animal data support the *in vitro* findings when quercetin is administered orally and prior to the drug (Hsiu *et al.*, 2002; Choi *et al.*, 2004a; Choi and Han, 2004; Choi and Li, 2005; Umathe *et al.*, 2008; Bansal *et al.*, 2008; Wang *et al.*, 2004; Shin *et al.*, 2006; Li and Choi, 2009). However, the preponderance of human data do not support a conclusion that oral intake of quercetin interferes with drug bioavailability, since 5 of 7 clinical studies show no changes in drug bioavailability following repeated exposure of up to 1500 mg of quercetin/day (Rashid *et al.*, 1993; DiCenzo *et al.*, 2006; Choi *et al.*, 2004b; Kim *et al.*, 2005, 2009; Prism Research, 2010b,c). Studies to assess the potential effect of quercetin on the pharmacokinetics of CYP3A or P-gp substrate drugs in different species are also summarized in Table C-3 (see Exhibit C).

Although quercetin possesses antioxidant properties, under certain *in vitro* conditions quercetin acts as a prooxidant (i.e. "the quercetin paradox") (Boots *et al.*, 2007). *In vivo*, however, there are a number of mechanisms in place to protect against the possible prooxidant effects of quercetin (*e.g.* low bioavailability of quercetin following oral intake, methylation, physiologic conditions, albumin binding, etc.) (Boots *et al.*, 2007; Harwood *et al.*, 2007).

IV.D Toxicological Studies

IV.D.1 Acute Toxicity Studies

Regardless of the source of quercetin (*i.e.*, Podophyllin or quercetron), in adult albino farm mice (sex and strain not specified), oral LD_{50} values of approximately 160 mg quercetin/kg body weight were reported (Sullivan *et al.*, 1951). When quercetin (dissolved in absolute alcohol or suspended in acacia or methocel) was administered subcutaneously to mice, LD_{50} values of approximately 100 mg/kg body weight were established. It should be noted that the purity and source of the quercetin-test substances were not specified. Subsequently conducted animal toxicity studies, including longer term studies, have demonstrated that quercetin is tolerated at dose levels that are several-fold greater than those reflected by these LD_{50} values.

IV.D.2 Genotoxicity Studies

The results of a considerable number of *in vitro* mutagenicity and genotoxicity assays indicated consistent quercetin-related mutagenic effects; however, similar results have been obtained *in vitro* with other antioxidant compounds (*e.g.*, vitamin C, TBHQ) likely due to their prooxidant activity at unrealistically high *in vitro* concentrations, not attainable *in vivo*. Furthermore, the *in vitro* mutagenic properties of quercetin have not been observed *in vivo* following oral administration in at least 10 negative studies. This discrepancy between positive *in vitro* findings versus negative *in vivo* results may be attributed to limited *in vivo* absorption of quercetin as the aglycone, extensive intestinal microbial degradation, and post-absorption enzymatic metabolism acting in concert to limit the potential for *in vivo* adverse effects following quercetin consumption. Short- and long-term animal studies with orally administered quercetin are more reliable and appropriate models for the assessment of toxicity and carcinogenicity of quercetin added to food.

Results of the *in vitro* assays with quercetin in prokaryotic and eukaryotic test systems are summarized in Tables C-4 and C-5, respectively (see Exhibit C). Results of the *in vitro* assays conducted to assess the potential mutagenicity/genotoxicity of quercetin metabolites are presented in Table C-6, whereas studies assessing the potential mutagenicity/genotoxicity of urine, fecal, or plasma samples following treatment of rats with quercetin are summarized in Table C-7 in Exhibit C. Results of the identified *in vivo* mutagenicity/genotoxicity tests are summarized in Table C-8 in Exhibit C.

IV.D.3 Subchronic, Chronic/Carginogenicity, and Other Relevant Short- and Long-term Toxicity Studies

Quercetin has been studied for its carcinogenic potential in numerous long-term animal studies, the majority of which have indicated no evidence of neoplasm formation related to its oral administration (Ambrose *et al.*, 1951; Saito *et al.*, 1980; Hirono *et al.*, 1981; Hosaka and Hirono, 1981; Morino *et al.*, 1982; Takanashi *et al.*, 1983; Stoewsand *et al.*, 1984; Ito *et al.*, 1989; NTP, 1992). The NTP study reported a significant dose-dependent reduction in body weight gains of males and females and an increased severity of chronic nephropathy, hyperplasia, and neoplasia of the renal tubular epithelium (causing primarily benign tumors of the renal tubular epithelium) in male, but not female rats exposed to 40,000 ppm (mean dose level approximately 2,200 mg/kg body weight/day) quercetin in the diet for 2 years. Table C-9 in Exhibit C summarizes the incidence of renal tubular lesions observed in the NTP study. It was concluded, that under the conditions of the study, there was *some evidence of carcinogenic activity* of quercetin in male F344/N rats based on an increased incidence of renal tubule cell adenomas, but *no evidence of carcinogenic activity* of quercetin in female F344/N rats (NTP, 1992). Following re-evaluation of the kidney lesions, Hard *et al.* (2007) concluded that the alterations observed in the renal tissues of male rats in this study were the result of a secondary mechanism for renal tumor development (*i.e.*, exacerbation of late-stage progressive chronic nephropathy), with no relevance for extrapolation to humans. In the absence of any differences in the survival rates between quercetin-treated and control animals, morphological abnormalities, or organ-based toxicity, the body weight differences observed between high-dose test and control rats were not considered to be adverse effects. Based on the results of the NTP study, no biologically significant adverse effects are expected at dose levels at or below 2,200 mg quercetin/kg body weight/day. This is supported by other long-term toxicity and carcinogenicity studies conducted not only in rats, but also in hamsters and mice, which demonstrated the absence of significant histopathological effects, including those of the kidneys, at comparable and in some cases higher dose levels (up to 12 g/kg body weight/day) (Ambrose *et al.*, 1951; Saito *et al.*, 1980; Pamukcu *et al.*, 1980; Hirono *et al.*, 1981; Hosaka and Hirono, 1981; Morino *et al.*, 1982; Takanashi *et al.*, 1983; Stoewsand *et al.*, 1984; Ito *et al.*, 1989). The results of these studies are summarized in Table C-10 in Exhibit C. It is concluded, based on the totality-of-evidence from multiple long-term animal studies, that quercetin has not been shown to be carcinogenic to the kidneys or any other organs/tissues when administered chronically in the diets of laboratory animals.

Several initiation-promotion studies have been performed to determine specifically the effect, if any, of quercetin on chemical-induced carcinogenesis, most of which also included a quercetin control group, and as such, are relevant to the safety assessment of the flavonoid. The results of these studies are summarized in Table C-11 in Exhibit C. Although, a limited number of studies indicated some enhancing activity of quercetin (Werner *et al.*, 1985; Zhu and Liehr, 1994; Pereira *et al.*, 1996; Barotto *et al.*, 1998; Matsukawa *et al.*, 2002; Valentich *et al.*, 2006),

most others failed to demonstrate quercetin-related promotion of carcinogenesis or demonstrated chemoprotective properties (Morino *et al.*, 1982; Fukushima *et al.*, 1983; Hirose *et al.*, 1983; Kato *et al.*, 1984, 1985; Sato *et al.*, 1987; Ito *et al.*, 1988; Verma *et al.*, 1988; Deschner *et al.*, 1991; Akagi *et al.*, 1995; Exon *et al.*, 1998; Tanaka *et al.*, 1999; Yang *et al.*, 2000; Volate *et al.*, 2005; Choi *et al.*, 2006; Devipriya *et al.*, 2006; Dihal *et al.*, 2006; Jin *et al.*, 2006). These studies further serve to corroborate the absence of quercetin-induced tumor formation in a range of organs, including the kidney, colon, and urinary bladder, as evidenced by the lack of neoplasms reported in the uninitiated quercetin-only treated groups.

In summary, the totality-of-evidence from subchronic and chronic toxicology studies in multiple species with dietary administration of quercetin demonstrates a lack of significant adverse systemic toxicity. Systemic toxicity NOAELs of 2,203 and 2,231 mg/kg body weight/day for males and females (NTP, 1992), and 1,926 and 2,372 mg/kg body weight/day for males and females (Ito *et al.*, 1989), respectively, were established by these long-term studies.

IV.D.4 Reproductive and Developmental Studies

The available data demonstrate that orally administered quercetin is not a reproductive or development toxicant based on the following totality-of-evidence. For *in vitro* cell cultures and administration to rodents by intraperitoneal injection, quercetin is not metabolized by intestinal microflora prior to its contact with germ cells, which may account for abnormal cells and/or reproduction effects observed (Nass-Arden and Breitbart, 1990; Swisher *et al.*, 1994 [abstract]; Trejo *et al.*, 1995; Khanduja *et al.*, 2001). Whereas, for *in vivo* rodent studies utilizing oral administration of quercetin during which metabolism by intestinal microflora and first-pass metabolism do occur; sperm abnormalities, developmental toxicity and effects on reproduction endpoints have not been observed. When quercetin was administered orally to male Swiss albino mice at doses up to 150 mg/kg body weight/day, it did not induce any significant increases in the frequency of abnormal sperm (Nandan and Rao, 1983). No dose-dependent developmental toxicity or teratogenic anomalies were observed in relation to oral quercetin administration (GD 6 to 15) to pregnant female rats of up to 2,000 mg/kg body weight (Willhite, 1982). No differences were observed in reproductive and developmental indices, including parturition index, mean viable litter size, live birth index, 3-day survival, lactation index, birth weight, and 21-day weight, following a single-generation reproduction study design in which exposure of male and female rats to quercetin occurred at up to 0.2% in the diet (Stoewsand *et al.*, 1984).

IV.D.5 Human Studies

Clinical data relating to the safety of quercetin were obtained from randomized, double-blind, clinical trials (Conquer *et al.*, 1988; Shoskes *et al.*, 1999; Kiesewetter *et al.*, 2000; Lozoya *et al.*, 2002; Nieman, 2008; Shanely *et al.*, 2010). Lozoya *et al.* (2002) examined the short-term safety of a phytodrug containing a standardized content of quercetin (1 mg flavonoids expressed as quercetin), whereas Kiesewetter *et al.* (2000) evaluated the safety of red vine leaf extract provided for a period of 12 weeks, reported to contain quercetin-3-*O*-beta-glycoside and isoquercetin (levels not specified). No compound-related adverse effects were reported in either of the studies, including the absence of any variations in clinical tests. Similarly, no adverse effects were reported in an efficacy study in which quercetin was provided to male subjects at a dose level of 16.7 mg/kg body weight/day (Shoskes *et al.*, 1999). In a study undertaken to assess the potential of quercetin to positively affect risk factors for heart disease, treatment with quercetin did not significantly alter serum total, LDL or HDL cholesterol, or triglyceride levels, and there were no significant effects on cardiovascular disease or thrombogenic risk factors (*e.g.*, platelet aggregration, platelet thromboxane B_2 production, blood pressure, or resting heart rate) (Conquer *et al.*, 1998). Recently a randomized, double-blind, placebo-controlled 12-week clinical study was conducted in which 1,002 subjects consumed a placebo, 500 or 1,000 mg of quercetin daily (Nieman, 2008; Shanely *et al.*, 2010). Quercetin was provided twice daily in soft chews. There were no significant group differences over time in clinical chemistry measurements with the exception of decreases in serum creatinine and increases in glomerular filtration rate for the quercetin-treated groups. There were no differences observed in adverse events or levels of reduced or oxidized red blood cell glutathione among the 3 treatment groups. Mean arterial blood pressure decreased in the quercetin-treated groups compared to placebo. It was concluded that supplementation with 500 or 1,000 mg quercetin/day for 12 weeks was safe in this study as there were no adverse symptoms or change in blood diagnostic chemistries and GSH.

Two other 4-week studies were identified in which the potential effects of quercetin (730 mg/day) on blood pressure were assessed in hypertensive and pre-hypertensive subjects (Jalili *et al.*, 2006 [abstract]; Edwards *et al.*, 2007). No adverse effects were reported in either study and reductions in blood pressure were observed only in hypertensive subjects. No adverse effects were reported following 6 weeks of quercetin supplementation (150 mg/day); however, quercetin did reduce systolic blood pressure and plasma oxidized LDL concentrations in subjects with a high-cardiovascular disease phenotype (Egert *et al.*, 2009). In a study in which a daily dose of 60 mg quercetin combination with curcumin was provided to patients with FAP for a period of 6 months, no variations were noted in clinical chemistry paramters compared to baseline (Cruz-Correa *et al.*, 2006).

In several studies conducted to investigate various potential effects of quercetin on immune function, exercise-induced oxidative stress, or performance, the ingestion of quercetin by

healthy subjects at a daily dose of 600 mg to 1000 mg for periods ranging from approximately one to six weeks was not associated with any reports of adverse effects (Cureton *et al.*, 2009; Davis *et al.*, 2010; Dumke *et al.*, 2009; Henson *et al.,* 2008; MacRae and Mefferd, 2006; McAnulty *et al.*, 2008; Nieman *et al.,* 2007a,b,c, 2009, 2010; Quindry *et al.,* 2008).

In addition to the controlled clinical trails, a number of epidemiological reports unequivocally support the absence of any relationship between flavonoid consumption from the diet (up to 68 mg/day) and an increased risk of cancer (Hertog *et al.*, 1994, 1995; Goldbohm *et al.*, 1995 [abstract]; Knekt *et al.*, 1997, 2002; Lam *et al.*, in press; Lin *et al.*, 2006).

Most recently, no safety concerns were reported in three corroborative pharmacokinetic studies; two *in vivo* drug interaction studies (*i.e.,* warfarin and digoxin) were conducted with quercetin in healthy subjects as well as an oral glucose tolerance test (OGTT) in patients with type 2 diabetes (Prism Research, 2010a,b,c). Additionally, there were no serious adverse effects reported during or after any of the three studies. The pharmacokinetic data revealed that co-administration of quercetin did not alter warfarin or digoxin plasma levels nor did it affect blood glucose levels in patients with type 2 diabetes.

IV.D.6 Safety of Potential Quercetin Degradation Products

The products resulting from possible quercetin degradation (*i.e.*, phloroglucinol, phloroglucinic acid, and protocatechuic acid (PCA)) are known to occur ubiquitously in nature and are not expected to result in any adverse effects on human health. No signs of toxicity were reported in rats exposed to PCA at a dietary concentration of 1000 ppm (~50 mg/kg body weight/day) for 32 weeks (Tanaka *et al.*, 1993) and there were no effects in mice given drinking water with 0.01% PCA for 60 days (Nakamura *et al.*, 2001). In a human clinical study to assess the efficacy of phloroglucinol (80 mg/day) for acceleration of labor, there were no side effects reported, and no toxic effects were noted in either the mother or fetus (Tabassum *et al.*, 2005). Additionally, data related to the metabolic fate of phloroglucinol and protocatechuic acid indicate that both compounds, much like quercetin, are subjected to extensive phase I and II metabolic reactions following absorption and are subsequently rapidly excreted (Dollo *et al.*, 1999; Xu *et al.*, 2007). Therefore, any unmitigated degradation of quercetin to these compounds is not expected to result in any adverse effects on human health.

IV.E Overall Conclusions Pertaining to Safety

The scientific totality-of-evidence presented in this dossier indicates that under the conditions of intended use in foods, quercetin would not produce any adverse health effects in consumers. A large body of of animal and human literature has demonstrated limited bioavailability of orally administered quercetin (as the aglycone), with rapid and extensive metabolism of the absorbed

portion and efficient excretion. The safety of quercetin is confirmed by published long-term animal toxicity and carcinogenicity studies, as well as recent corroborative and published human clinical trials and epidemiological investigations, indicating no adverse toxicological effects under testing scenarios relevant to the conditions of intended use in foods. The total body of published animal and human data and information summarized in this report demonstrate that quercetin, meeting appropriate high-purity food-grade specifications, is GRAS, based on the scientific procedures, under the conditions of intended use in foods.

V. Basis for GRAS Determination

V.A Introduction

This section of the GRAS Notice fulfills requirements of proposed 21 CFR 170.36(c)(4)(i)(B) and (C) by providing a discussion of the basis for concluding, in light of all generally available and accepted scientific data and information, methods, or principles relevant to establishing safety of the proposed uses of quercetin, that there is a consensus among qualified experts that there is a reasonable certainty that quercetin is not harmful under its intended conditions of use.

This section also includes a reiteration of the available published and unpublished information being relied upon and summarized above (including an assessment of the metabolism, toxicological data and clinical human data available) in light of the probable intake of the substance.

In addition, this section discusses the issues that the FDA has raised previously and the data by which these concerns may be addressed.

V.B Safety Assessment of Quercetin for Its Intended Uses

Previous Evaluation by Authoritative Bodies

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated quercetin for use as a coloring agent in food (JECFA, 1978). Based on the limited toxicity data available for review at the time of the evaluation, it was not possible to establish an acceptable daily intake (ADI). The International Agency for Research on Cancer (IARC) evaluated quercetin for its potential carcinogenic risk to humans in 1998 (IARC, 1999). Based on the data available to the IARC Panel, quercetin was assigned an overall Group 3 classification (*i.e., not classifiable as to its carcinogenicity to humans*). Although, *limited evidence* of quercetin carcinogenicity was noted in experimental animals, evidence for carcinogenicity to humans was deemed to be *inadequate*.

Acceptable Daily Intake (ADI)

The safety of Quercegen's high-purity quercetin (≥99.5%) ingredient is based on published metabolism (indicating limited bioavailability), short- and long-term preclinical toxicity, developmental and reproduction toxicity, carcinogenicity, *in vitro* and *in vivo* mutagenicity/genotoxicity studies, and several published and four recent corroborative clinical studies performed with quercetin. The pivotal toxicology safety study is a 2-year chronic toxicity and carcinogenicity rat feeding study conducted by the National Toxicology Program (NTP) (Dunnick and Hailey, 1992; NTP, 1992). The results of other relevant high dose toxicity and mutagenicity/genotoxicity studies support the safety of this naturally-occurring flavonoid.

Additional information supporting the safety of quercetin is provided by several human clinical trials and epidemiology studies. In summary, the scientific totality-of-evidence from long-term toxicology and carcinogenicity studies of dietary quercetin demonstrates the lack of systemic toxicity, the non-carcinogenicity, and the lack of tumor promoting activity of quercetin.

The totality-of-the-evidence supports the safety of Quercegen's high-purity quercetin in the proposed uses as a food additive. Table 6 provides a summary of the Key Data from chronic toxicity studies supporting the conclusion that an ADI in the range of 19-22 mg/kg bw/day is scientifically justified and appropriate to ensure safety under the conditions of proposed use. Published and corroborating human clinical studies conducted at dose levels up to 1,500 mg/day (25 mg/kg bw/day based on a 60 kg adult human) for up to 12 weeks demonstrated safety and, therefore support this ADI for high-purity quercetin (Conquer *et al.*, 1998; Cureton *et al.*, 2009; Davis *et al.*, 2010; Dumke *et al.*, 2009; Henson *et al.*, 2008; McAnulty *et al.*, 2008; Nieman *et al.*, 2007a,b,c, 2009, 2010; Quindry *et al.*, 2008; Shanely *et al.*, 2010; Nieman, 2008 [unpublished]; Prism Research, 2010a,b,c [unpublished]).

Table 6. Acceptable Daily Intake from Totality-of-Evidence

Basis of ADI (Safety Factor)	Key Data[1]	ADI mg/kg bw/d
NTP, 1992 Chronic toxicity/ carcinogenicity (100×)	NOAEL =2,200 mg/kg bw/d	22
Ito *et al.*, 1989 Chronic toxicity/ carcinogenicity (100×)	NOAEL =1,900 mg/kg bw/d	19
Conclusion for Quercetin		**19-22**

[1] In each case, the Key Data represent the highest dose level in the respective study.

Based on the results of the two 2-year rat feeding studies (Ito *et al.*, 1989; NTP, 1992), similar NOAELs of approximately 1,900 and 2,200 mg/kg body weight/day were established. Published and corroborating clinical studies support a conclusion of safety for consumer intake of up to 1,500 mg/day. The totality-of-the-evidence summarized above supports the safety in the proposed uses of Quercegen's high-purity quercetin.

Estimated Daily Intake (EDI)

The EDI of quercetin was calculated using the most recent food consumption survey results from the National Health and Nutrition Examination Survey (NHANES). Quercetin intake was determined by multiplying each NHANES respondents' 2-day average food intake by the

maximum proposed use level for each category. The total population EDI from all proposed uses is approximately 4 mg/kg bw/day at the mean and 9 mg/kg bw/day at the 90th percentile of intake among all-users. A user is defined as having consumed a food that would contain quercetin during the 2 days of the NHANES survey. Consideration of only 2 days of food consumption data results in a conservative upper estimate of potential quercetin intake.

In reality, high-purity quercetin is proposed to be added to selected foods and beverages for specific technical/functional uses for the Targeted Population of exercising adults and will be marketed initially through specialty channels (e.g. GNC, sporting goods stores). Therefore, the estimates for infants and children by the traditional approach for consumption analysis are not only unrealistic but not reflective of the intended application. For adult exercising consumers who would intentionally seek high-purity quercetin-containing foods and beverages, consumption is estimated at 1000 mg quercetin/day. It is possible that adult exercising consumers who were to select high-purity quercetin-containing foods and beverages would consume more than two to four servings/day for short periods of time; for example, five three servings/day that would provide 1250 or 1500 mg quercetin/day. However, long-term consumption at this level is considered unlikely based on the rationale presented above. The anticipated maximum daily intake foreseen by the Targeted Population of adult exercising consumers is supported as safe based on a robust preclinical and clinical safety database for quercetin.

Comparision of ADI and EDI

Consideration of the totality-of-evidence related to the safety evaluation of quercetin, including acute/subchronic/chronic toxicity, genotoxicity, reproductive/developmental toxicity, and carcinogenicity, as well as absorption, distribution, metabolism and excretion (ADME) data, leads to the conclusion that an ADI of 19-22 mg quercetin/kg bw/day is scientifically justified and appropriate.

The Total Population maximum estimated daily background intake of quercetin from naturally occurring sources (Table 1) was 258.2 mg/day, which is within the published range of 200-500 mg/day for high-end consumers of fruits and vegetables (Jones and Hughes, 1982). Therefore, a Targeted Population consumer with a daily intake of 1000 mg high-purity quercetin, who also was high-end consumer of fruits and vegetables, may consume approximately 1250 mg quercetin/day. This daily intake is also considered safe based on the totality-of-the-evidence.

In conclustion, even using conservative upper estimates of intake (9 mg/kg bw/day) and the lowest potential ADI (19 mg/kg bw/day), consumer intakes of quercetin from the proposed uses would not exceed the ADI.

V.C Discussion of Information Inconsistent With GRAS Determination

The authors of this notification are not aware of information that would be inconsistent with a finding that the proposed use of quercetin as a food additive for selected foods (beverages and beverage bases, grain products and pastas, processed fruits and fruit juices, and soft candies) is generally recognized as safe.

The regulatory framework for determining whether a substance is generally recognized as safe (GRAS) is in 21 CFR 170.30, which states that GRAS status through scientific procedures shall ordinarily be based upon published studies, which may be corroborated by unpublished studies and other data and information. These criteria have been applied to the existing data for quercetin in this notification.

The issues that have been raised by FDA in the past during meetings with Quercegen have been addressed as discussed below.

During a January 14, 2008 pre-submission meeting on a proposed GRAS Notice for Quercegen's high-purity quercetin between the FDA and Quercegen including CANTOX Health Sciences International, and a May 21, 2009 teleconference between the same parties, FDA expressed a range of concerns about the use of quercetin as a food ingredient. A March 30, 2009 memorandum from Melody Harwood to Dr. Karin Ricker, "Responses to Questions from FDA Pre-Submission Meeting on GRAS Notice for Quercegen's High-Purity Quercetin (January 14, 2008)", a May 21, 2009 PowerPoint presentation, "Pre-Submission Meeting on GRAS Notice for Quercegen's High-Purity Quercetin"; and a May 22, 2009 memorandum from Melody Harwood to Dr. Susan Carlson, "Meeting Minutes from Pre-Submission Discussion of High-Purity Quercetin GRAS Notice, Held May 21, 2009" documented these concerns and preliminarily addressed them. These FDA concerns are further responded to below and in the attached GRAS technical documentation (Exhibit D) in the context of the present-day widely known and accepted information available for quercetin as well as corroborating data from recent clinical studies.

- **NTP (1992) and Ito *et al.* (1989) rat bioassays with quercetin**

Issue: The notification document should include a case for the absence of compound-related toxicity in the kidneys. Hard *et al.*'s (2007) re-evaluation of the kidney lesion in the NTP study to be considered by FDA.

Resolution: Hard *et al.* (2007) re-evaluated the kidney tumors and renal histopathology of the NTP study in order to determine whether interaction with chronic progressive nephropathy was the mechanism underlying the small increase in the number of renal lesions. The re-evaluation

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was based on a semiquantatative method for assessment of rat chronic progressive nephropathy. Although the exact etiology of chronic progressive nephropathy is not clearly defined, it is regarded as an age-related, spontaneously-occurring, rodent-specific disease with no relevance for extrapolation in human risk assessment (Hard and Khan, 2004). Chronic nephropathy leading to hyperplasia in response to increased regenerative cell proliferation has been identified as a risk factor for marginal increases in the background incidence of renal tumors and may be exacerbated by exogenous compounds. The results of the reassessment confirmed that the renal lesions in the NTP study occurred only at dose levels associated with severe chronic nephropathy (Hard *et al.*, 2007). Consistent with the criteria for the development of kidney tumors as a result of an interaction with chronic nephropathy response, the majority of tumors were identified as small-sized adenomas, borderline with atypical tubule hyperplasia. The single case of carcinoma, along with a few adenomas and one hyperplastic focus, exhibited a distinctive phenotype characteristic of spontaneous origin. Furthermore, the re-evaluation did not reveal any cellular alterations that would be indicative of chemical-induced toxicity. Although it was previously suggested that the renal lesions observed only at the terminal evaluation may be a reflection of compromised detoxifying mechanisms in older animals (Dunnick and Hailey, 1992), the absence of any lesions at sites unaffected by chronic nephropathy does not support this hypothesis. Hard *et al.* (2007) concluded that the kidney lesions observed in the NTP bioassay were mediated by the exacerbation of chronic nephropathy (*i.e.*, increased incidences of tumor types known to be associated with late-stage chronic progressive nephropathy in the male rat). This is further substantiated by the absence of nephrotoxicity and carcinogenicity in the female rats.

Issue: Reductions in body weights – provide a rationale explaining why body weight reductions should not be considered an adverse effect in the case of quercetin.

Resolution: There are several reasons that support the lack of toxicological significance of the reduced body weight gains in the high-dose groups of the NTP (1992) and Ito *et al.* (1989) study. There were no differences in body weights between treatment and control rats in the first 13 weeks of the study, which is the period of active growth and during which the animals would be most sensitive to any adverse compound-related effects. As there were no differences in body weight gains during this period, the decreases in body weight gains in the quercetin-treated animals by the end of the study were not of toxicological concern. The decreases in body weight gains may have been related in part to a reduced caloric content of the quercetin-containing diet, especially at the highest dose level, compared to the control diet, as quercetin is a non-caloric substance. Body weight reductions occurred in the absence of any compound-related physiological or morphological abnormalities and these reductions were not replicated in other long-term studies and strains of rats with higher doses of quercetin (*e.g.*, Hirono *et al.*, 1981) and, therefore, the reduced body weight gains observed in the NTP (1992) and Ito *et al.* (1989) study were not considered to be of toxicological significance.

- **Prooxidative properties of quercetin raise concerns for potential toxicity**

Issue: Provide a rationale explaining why quercetin does not produce adverse effects *in vivo* despite its known anti- and prooxidant properties

Resolution: Several antioxidant mechanisms protect against potential prooxidant effects of quercetin *in vivo:* 1) Preferential conjugation of reactive oxidative degradation products of quercetin with glutathione rather than DNA/protein–adduct formation; 2) *In vivo* conditions are not conducive to quercetin autooxidation (i.e. transition metals which catalyze the autooxidation reactions are bound to proteins); 3) Highly selective binding of quercetin to albumin diminishes its cellular uptake and the possibility of oxidative damage; 4) Highly limited bioavailability of quercetin aglycone *in vivo* as a result of the degradation of quercetin to phenolic compounds in the gut and postabsorptive metabolic processes; and 5) Complex network of other antioxidants (e.g. vitamin C) that work in concert to regenerate the parent compound. While the relative importance of each mechanism has not been established, it is likely that the different mechanisms act in unison to protect the body against any possibly harmful effects related to quercetin consumption. Moreover, *in vivo* genotoxicity studies and long-term animal toxicity studies confirm that oral exposure to quercetin is not associated with any adverse effects.

- **Binding of methylated quercetin to GSH/Potential for glutathione (GSH) depletion**

Issue: Since GSH-conjugation of the oxidative degradation products of quercetin and quercetin metabolites has been identified as one of several protective mechanisms that is in place *in vivo* to prevent toxic effects related to quercetin oxidation, a potential exists for GSH depletion, particularly in diseased sub-populations with below normal GSH levels (*e.g.*, Alzheimer's patients).

Resolution: Due to the *in vivo* metabolic processes operating for quercetin, GSH conjugation of quercetin is not considered to be of concern following dietary consumption due to the following reasons:

- Quercetin methylated at the 3'- and 4'- positions of the B-ring retains some of the structural requirements for antioxidants (*i.e.*, a free 3-OH); however, while methylation may not fully eliminate quercetin's oxidative properties, it does significantly reduce the anti-oxidative capacity of the compound. It is therefore not surprising that the methylated compound also shows some affinity for GSH; however, in comparison to the aglycone, binding of methylated quercetin derivatives to GSH is considerably lower (van der Woude *et al.*, 2006).

- The contention that methylated quercetin metabolites are less reactive than the aglycone is supported by the observation that the mutagenic/genotoxic activity of quercetin-

methylated compounds is significantly reduced compared to the aglycone or in some cases completely abolished.

- Methylated quercetin derivatives also are subject to sulfation and/or glucuronidation at the 3-OH position.

With respect to the concerns regarding the potential for GSH depletion following dietary consumption of quercetin, depletion of GSH is not considered to be of concern due to the following rationale:

- Conjugation of reactive oxidative degradation products of quercetin with GSH is only one of several protective mechanisms (see above).
- Binding of oxidized quercetin with GSH is reversible.
- Conjugation with GSH is not the primary route for quercetin metabolism/excretion:
    - Mercapturic acid derivatives of quercetin have not been identified in any of the studies evaluating the metabolic fate of quercetin *in vivo*.
    - Following oral administration of quercetin, only methylated, glucuronidated and/or sulphated metabolites have been identified.
- Quercetin also has been noted to up-regulate GSH production, and therefore, as part of an integrated system of antioxidants, *in vivo*, quercetin is not related with any adverse effects.

Moreover, while the results of animal studies in which GSH levels were monitored following oral quercetin administration are equivocal, in humans, no changes in GSH levels were observed following consumption of quercetin at doses of up to 1,000 mg/day for 3 months (Nieman, 2008; Shanely *et al.*, 2010). Therefore, the potential for GSH depletion under the intended conditions of use is not considered to be of concern.

- **Potential for quercetin autooxidative degradation in end-product food uses of quercetin**

Issue: Quercetin is known to autooxidize under certain pH conditions, resulting in the generation of reactive oxygen species (ROS). Quercetin stability in the different food matrices should be considered.

Resolution: The stability of quercetin at different pH values (2.7, 7, and 10) and different temperatures (room temperature, 4°C, and -20°C) was investigated by Moon *et al.* (2008) and

generally, the study results demonstrated that the stability of quercetin is both pH and temperature dependent, with acidic conditions and low temperatures identified as more conducive to quercetin stability.

Since the intended uses for quercetin include beverages of various pH, the stability of Quercegen's high-purity quercetin was assessed at a range of pH values, alone and in the presence of other antioxidants [*e.g.*, ascorbic acid, epigallocatechin gallate (EGCG), propyl gallate]. Results of the studies demonstrated that at low pH and/or including an appropriate antioxidant, quercetin is either fully protected from degradation or the degradation of quercetin is significantly attenuated in aqueous test formulations.

While quercetin has been shown to degrade under certain pH conditions, provisions exist to prevent degradation of high-purity quercetin under the intended conditions of use in foods. Specifically, in order to prevent degradation of quercetin in aqueous solutions, quercetin will be used with an appropriate antioxidant, such as ascorbic acid, that will be added at levels sufficient to preserve the stability of quercetin and prevent its degradation under intended conditions of use.

Moreover, the products resulting from possible quercetin degradation (*i.e.*, phloroglucinol, phloroglucinic acid, and protocatechuic acid (PCA)) are known to occur ubiquitously in nature and have been identified as normal degradation products of anaerobic quercetin fermentation, and there are no data to indicate that ingestion of these compounds would be associated with any adverse effects. Data related to the metabolic fate of phloroglucinol and protocatechuic acid indicate that both compounds, much like quercetin, are subjected to extensive phase I and II metabolic reactions following absorption and are subsequently rapidly excreted (Dollo *et al.*, 1999; Xu *et al.*, 2007). Therefore, any unmitigated degradation of quercetin to these compounds is not expected to result in any adverse effects in human health. Additionally, no signs of toxicity were reported in rats exposed to PCA at a dietary concentration of 1000 ppm (~50 mg/kg body weight/day) for 32 weeks (Tanaka *et al.*, 1993) and there were no effects in mice given drinking water with 0.01% PCA for 60 days (Nakamura *et al.*, 2001). In a human clinical study to assess the efficacy of phloroglucinol (80 mg/day) for acceleration of labor, there were no side effects reported, and no toxic effects were noted in either the mother or fetus (Tabassum *et al.*, 2005).

- **Exposure estimates resulting from the natural occurrence of quercetin in the diet and intake levels of Quercegen's high-purity quercetin**

Issue: Include a discussion of the naturally occurring levels of quercetin in the diet (*e.g.*, onions). Use the USDA database to calculate exposure estimates for quercetin from natural occurrence and compare to intakes from addition to food (2- to 3-fold higher levels from addition as an ingredient would not be a concern).

Resolution: Values for the estimated intake of quercetin by the U.S. population from naturally-occurring sources were generated using the available data on the quercetin content of foods from the flavonoid database combined with food intake data from the National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) for the years 2003-2004 (NHANES 2003-2004), which is a 24-hour dietary recall survey administered on two non-consecutive days throughout all four seasons of a year (CDC, 2006; USDA, 2009a). Individuals that did not report consuming a naturally-occurring food source for quercetin were excluded from these analyses.

- Consumption of these types of foods by the total U.S. population resulted in estimated mean and 90th percentile all-user background intakes of quercetin of 5.9 and 14.7 mg/person/day, respectively.

- A maximum quercetin all-user intake value of 258.2 mg/day was also generated. This value represents the highest individual reported quercetin intake from all naturally-occurring quercetin sources and occurred in the male adult population group.

- Given that the quercetin content is only available for selected foods, it is likely that actual background intakes are higher than these estimates.

These values are similar to various national dietary record-based cohort assessments of the intake of quercetin from the habitual diet which indicated consumption levels of up to approximately 40 mg quercetin/day in the general population (Hertog *et al.*, 1995; Rimm *et al.*, 1996; Knekt *et al.*, 1997; Kimira *et al.*, 1998; Johannot and Somerset, 2006; Lin *et al.*, 2006), while daily intake levels of quercetin as high as 200 to 500 mg have been reported in high-end consumers of fruits and vegetables (Jones and Hughes, 1982). Additionally, quercetin is marketed as a dietary supplement with recommended daily dosages of supplemental quercetin ranging from 200 to 1,200 mg, supplied as capsules or tablets (PDRNS, 2001).

Estimates for the intake of high-purity quercetin by the traditional approach were based on the proposed food-uses and use-levels in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2009a,b). The data from the 2003-2004 and 2005-2006 cycles of the NHANES survey were combined to provide a larger population from which to estimate high-purity quercetin consumption. On an all-user basis, the mean intake of quercetin by the total U.S. population from all proposed food-uses was estimated to be 205 mg/person/day or 4 mg/kg body weight/day. The heavy consumer (90th percentile) all-user intake of quercetin by the total U.S. population from all proposed food-uses was estimated to be 461 mg/person/day or 9 mg/kg body weight/day. On an absolute basis, the highest estimate for the all-user 90th percentile intake of quercetin within an individual population group occurred in male teenagers at 668 mg/person/day. These values are about 2- to 3- times higher than the estimated background dietary intakes of quercetin in high-end users (*i.e.* vegetarians) from its

natural occurrence in the diet. The type of intake methodology utilized is generally considered to be "worst-case" as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, overestimate the consumption of food products that are normally consumed relatively infrequently. Therefore, it is expected that the actual exposure to Quercegen's high-purity quercetin ingredient will be much less than estimated.

In reality, high-purity quercetin is proposed to be added to selected foods and beverages for specific technical/functional uses for the Targeted Population of exercising adults and will be marketed initially through specialty channels (e.g. GNC, sporting goods stores). Therefore, the estimates for infants and children by the traditional approach for consumption analysis are not only unrealistic but not reflective of the intended application. For adult exercising consumers who would intentionally seek high-purity quercetin-containing foods and beverages, consumption is estimated at 1000 mg quercetin/day. It is possible that adult exercising consumers who were to select high-purity quercetin-containing foods and beverages would consume more than two to four servings/day for short periods of time; for example, five three servings/day that would provide 1250 or 1500 mg quercetin/day. However, long-term consumption at this level is considered unlikely based on the rationale presented above. The anticipated maximum daily intake foreseen by the Targeted Population of adult exercising consumers is supported as safe based on a robust preclinical and clinical safety database for quercetin.

The Total Population maximum estimated daily background intake of quercetin from naturally occurring sources (Table 1) was 258.2 mg/day, which is within the published range of 200-500 mg/day for high-end consumers of fruits and vegetables (Jones and Hughes, 1982). Therefore, a Targeted Population consumer with a daily intake of 1000 mg high-purity quercetin, who also was high-end consumer of fruits and vegetables, may consume approximately 1250 mg quercetin/day. This daily intake is also considered safe based on the totality-of-the-evidence.

- **The effects of quercetin on Cytochrome P450, P-Glycoprotein and/or Glucose Transporter GLUT2**

Issue: With respect to the inhibitory effects of quercetin on intestinal glucose transporter 2 (GLUT2) as reported by Kwon *et al.* (2007), the potential hyperglycemic effects of quercetin needs to be addressed.

Resolution: In Kwon *et al.* (2007) the concentrations at which quercetin decreased glucose or fructose GLUT2-mediated uptake by 50% (IC_{50}) were calculated to be 12.7 and 15.9 μM, respectively; much higher than the plasma levels achieved following quercetin ingestion either from natural occurrence in food or quercetin supplements in human subjects. *In vivo*, quercetin did not affect plasma glucose concentration in normoglycemic animals, but in some studies significantly reduced plasma glucose levels in diabetic animals. Although it is difficult to extrapolate these results to human subjects, especially the diabetic population, a recently conducted clinical study in diabetic patients (Prism Research, 2010a) does provide new corroborative data (see below). Furthermore, in the study conducted by Kobori *et al.* (2009), the quercetin plasma levels achieved following ingestion of quercetin in mice, as well as the quercetin concentration used in Kwon *et al.* (2007), are both much higher than the plasma levels of quercetin following ingestion of quercetin containing foods or supplements in human subjects, *i.e.,* around 2 μM or 650 ng/mL (Hollman *et al.*, 1996; Janssen *et al.*, 1998; McAnlis *et al.*, 1999; Nieman *et al.*, 2007a,b,c; Egert *et al.*, 2008; Utter *et al.*, 2009). Therefore, at the estimated intake levels, quercetin is unlikely to affect GLUT2 transporters.

Issue: Since quercetin has been shown to inhibit cytochrome 3A4 (CYP3A4), and thus increase the bioavailability of pioglitazone, an antidiabetic drug and a CYP3A4 substrate, potential effects of quercetin on glucose levels in diabetes needs to be addressed.

Resolution: Co-administration or pretreatment with quercetin significantly increased the oral bioavailability of several drugs such as etoposide, tamoxifen, paclitaxel, pioglitazone, irinotecan, diltiazem, verapamil and digoxin in rats, pigs, and rabbits. As all of the above mentioned drugs are substrates for CYP3A4 and/or P-gp, these effects have been attributed to the inhibitory effects of quercetin on CYP3A4 and/or P-gp. Conversely, co-administration of quercetin significantly decreased the oral bioavailability of cyclosporine in pigs and rats, suggesting that the effect of quercetin on the pharmacokinetics of cyclosporine cannot be attributed to its modulation of CYP3A4 or P-gp. Furthermore, in a clinical setting studying the effect of quercetin on the plasma or intracellular concentration of saquinavir, quercetin co-administration did not influence plasma saquinavir concentrations (DiCenzo *et al.,* 2006).

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A randomized, placebo-controlled, two-way crossover clinical study was conducted in healthy volunteers to assess whether quercetin co-administration inhibited warfarin metabolism via CYP2C9 and as such could increase bleeding risk (Prism Research, 2010c). There were no biologically-relevant changes in clinical chemistry or hematology values (including prothrombin

time), vital signs, or physical exam findings over the course of the study. With respect to the pharmacokinetic results, quercetin co-administration had no effect on the AUC, C_{max}, half-life or clearance of either (S)- or (R)-warfarin. Observed time to maximal concentration (T_{max}) values occurred at the 2-hour time point in all profiles for both enantiomers. No statistical differences ($p<0.05$) were found between treatments in any of the secondary pharmacokinetic parameters. In conclusion, the co-administration of quercetin did not interfere with the metabolism of either (S)- or (R)-warfarin. There was no evidence that 5 daily doses of quercetin would inhibit or induce cytochrome P450 2C9 enzyme-mediated metabolism. Quercetin in doses of 1500 mg/day was safe and well tolerated.

- **The effects of quercetin on glucose levels in diabetic population (clinical data)**

Issue: Provide scientific evidence regarding the effects of quercetin, or lack thereof, on glucose levels in the diabetic population.

Resolution: An open-label, within-subject, repeated measure study was designed to define the effects and pharmacokinetics of multiple doses of quercetin on plasma glucose levels in male and female patients with type 2 diabetes (Prism Research, 2010a). On Study Day 1, subjects received an OGTT 10-minutes following consumption of a 20-ounce (zero-calorie) placebo formulation, and had blood samples drawn for glucose measurements. Subjects received 5 consecutive days of quercetin (1500 mg) in 20-ounces of formulation (zero-calorie), and 10 minutes following the last formulation administration, an OGTT was performed. No serious adverse events were reported. There were no clinically-significant changes in clinical chemistry or hematology values, vital signs, or physical exam findings over the course of the study. No safety concerns were identified. There was no evidence found to demonstrate that 1500 mg of quercetin would significantly alter blood glucose levels in patients with type 2 diabetes, which corroborates published clinical studies.

- **Basis for Quercegen's high-purity quercetin GRAS**

Issue: History of use due to natural occurrence of quercetin, alone does not substantiate the basis for GRAS. This basis for GRAS needs to be elaborated scientifically.

Resolution: This submission contains a complete review of the literature as well as presentation of the intended uses. It is organized to first describe the chemical identity of quercetin, its technological function in the proposed uses and estimated daily intakes (EDI) from those uses. This is followed by the safety evaluation including ADME, toxicology and an in-depth analysis of research in human volunteers. The acceptable daily intake (ADI) is determined and compared to the EDI. The data and information summarized herein and in the

attached Technical Dossier (Exhibit D) demonstrate that quercetin in aqueous products at an appropriate pH and/or in the presence of a suitable antioxidant and meeting appropriate food-grade specifications, is Generally Recognized as Safe (GRAS), *based on scientific procedures*, under the conditions of intended use in foods.

V.D General Recognition of the Safety of Quercetin

Safe for Intended Use

The intended uses of quercetin have been determined to be safe through scientific procedures, using a wide variety of published toxicology and other available safety-related studies. The absorption, distribution, metabolism, and excretion (ADME) of quercetin is well understood for laboratory animals and humans, and has been described in publicly available studies. Understanding the ADME for quercetin has made it possible to bring a wide array of data and information to bear upon the evaluation of its safety. The safety-related studies on quercetin include acute, subchronic, chronic/carcinogenicity, and reproduction and developmental toxicity studies in laboratory animal models as well as an extensive set of genotoxicity studies. Clinical studies have evaluated the potential for effects of quercetin on CYP450 enzymes systems, P-gp, and on blood glucose levels in diabetics.

Common Knowledge – Generally Available

The totality-of-evidence, both that which is published and that which is unpublished but corroborative, provides a basis upon which to conclude that the uses of quercetin described in this GRAS Notice satisfy the safety standard of Reasonable Certainty of No Harm and also conclude that an ADI of 19-22 mg/kg bw/day (expressed as quercetin) is supported by the data and is appropriate for use in assessing the safety of quercetin for use in food.

Common Knowledge – Generally Accepted by Qualified Experts

An Expert Panel of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine the safety and the Generally Recognized as Safe (GRAS) status of the proposed uses of Quercegen's high-purity quercetin. The available scientific evidence was reviewed by the Panel, who concluded that the intended uses of Quercegen's high-purity quercetin are safe and suitable and GRAS, and that other qualified experts would agree with these conclusions (see Exhibit A).

VI. REFERENCES

Akagi, K.; Hirose, M.; Hoshiya, T.; Mizoguchi, Y.; Ito, N.; Shirai, T. 1995. Modulating effects of ellagic acid, vanillin and quercetin in a rat medium term multi-organ carcinogenesis model. Cancer Lett 94(1):113-121.

Ambrose, A.M.; Robbins, D.J.; DeEDS, F. 1951. Comparative toxicities of quercetin and quercitrin. J Am Pharm Assoc. XLI(3):119-122.

Bansal, T.; Awasthi, A.; Jaggi, M.; Khar, R.K.; Talegaonkar, S. 2008. Pre-clinical evidence for altered absorption and biliary excretion of irinotecan (CPT-11) in combination with quercetin: possible contribution of P-glycoprotein. Life Sci 83(7&8):250-259.

Barotto, N.N.; Lopez, C.B.; Eynard, A.R.; Fernandez, Zapico, M.E.; Valentich, M.A. 1998. Quercetin enhances pretumorous lesions in the NMU model of rat pancreatic carcinogenesis. Cancer Lett 129(1):1-6.

Barrenetxe, J.; Aranguren, P.; Grijalba, A.; Martínez-Penuela, J.M.; Marzo, F.; Urdaneta, E. 2006. Effect of dietary quercetin and sphingomyelin on intestinal nutrient absorption and animal growth. Br J Nutr 95(3):455-461.

Boots, A.W.; Li, H.; Schins, R.P.F.; Duffin, R.; Heemskerk, J.W.M.; Bast, A.; Haenen, G.R.M.M. 2007. The quercetin paradox. Toxicol Appl Pharmacol 222(1):89-96.

Boots, A.W.; Wilms, L.C.; Swennen, E.L.R.; Kleinjans, J.C.S.; Bast, A.; Haenen, R.M.M. 2008. *In vitro* and *in vivo* anti-inflammatory activity of quercetin in healthy volunteers. Nutrition 24:703-710.

Brown, J.T. 1980. A review of the genetic effects of naturally occurring flavonoids, anthraquinones and related compounds. Mutat Res 75:243-277.

CDC. 2006. Analytical and Reporting Guidelines: The National Health and Nutrition Examination Survey (NHANES). Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS); Hyattsville, Maryland. Available from: http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/nhanes_analytic_guidelines_dec_2005.pdf.

Cermak, R.; Landgraf, S.; Wolffram, S. 2004. Quercetin glucosides inhibit glucose uptake into brush-border-membrane vesicles of porcine jejunum. Br J Nutr 91(6):849-855.

Choi, J.-S.; Han, H.-K. 2004. The effect of quercetin on the pharmacokinetics of verapamil and its major metabolite, norverapamil, in rabbits. J Pharm Pharmacol 56(12):1537-1542.

Choi, J.-S.; Li, X. 2005. Enhanced diltiazem bioavailability after oral administration of diltiazem with quercetin to rabbits. Int J Pharm 297(1&2):1-8.

Choi, E.J.; Chee, K.-M.; Lee, B.H. 2003. Anti-and prooxidant effects of chronic quercetin administration in rats. Eur J Pharmacol 482(1-3):281-285.

Choi, J.-S.; Jo, B.-W.; Kim, Y.-C. 2004a. Enhanced paclitaxel bioavailability after oral administration of paclitaxel or prodrug to rats pretreated with quercetin. Eur J Pharm Biopharm 57(2):313-318.

Choi, J.-S., Choi, B.C. and Choi, K.E. 2004b. Effect of quercetin on the pharmacokinetics of oral cycloporine. Am J Health Syst Pharm. 61(22):2406-2409.

Choi, E.J.; Lee, B.H.; Lee, K.; Chee, K.-M. 2005. Long-term combined administration of quercetin and daidzein inhibits quercetin-induced suppression of glutathione antioxidant defenses. Food Chem Toxicol 43(5):793-798.

Choi, S.-Y.; Park, J.H.Y.; Kim, J.-S.; Kim, M.K.; Aruoma, O.I.; Sung, M.-K. 2006. Effects of quercetin and β-carotene supplementation on azoxymethane-induced colon carcinogenesis and inflammatory responses in rats fed with high-fat diet rich in ω-6 fatty acids. Biofactors 27(1-4):137-146.

Conquer, J.A.; Maiani, G.; Azzini, E.; Raguzzini, A.; Holub, B.J. 1998. Supplementation with quercetin markedly increases plasma quercetin concentrations without effect on selected risk factors for heart disease in healthy subjects. J Nutr 128(3):593-597.

Cruz-Correa, M.; Shoskes, D.A.; Sanchez, P.; Zhao, R.; Hylind, L.M.; Wexner, S.D.; Giardiello, F.M. 2006. Combination treatment with curcumin and quercetin of adenomas in familial adenomatous polyposis. Clin Gastroenterol Hepatol 4(8):1035-1038.

Cureton, K.J., Tomporowski, P.D., Singhal, A., Pasley, J.D., Bigelman, K.A., Lambourne, K., Trilk, J.L., McCully, K.K., Arnaud, M.J., and Zhao, Q. (2009) Dietary quercetin supplementation is not ergogenic in untrained men. J Appl. Physiol. 107(4):1095-104.

Day, A.J.; Williamson, G. 1999. Human metabolism of dietary quercetin glycosides. In: Gross, G.G.; Hemingway, R.W.; Yoshida, T. (Eds.). Plant Polyphenols 2: Chemistry, Biology, Pharmacology, Ecology. Kluwer Academic / Plenum Publishers; New York. Basic Life Sciences, Vol. 66, pp. 415-434.

Davis, J.M., Carlstedt, C.J., Chen, S., Carmichael, M.D., and Murphy, E.A. 2010. The dietary flavonoid quercetin increases VO2 max and endurance capacity. Int. J Sport Nutr. Exerc. Metab. 20(1):56-62.

Deschner, E.E.; Ruperto, J.; Wong, G.; Newmark, H.L. 1991. Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. Carcinogenesis 12(7):1193-1196.

Devipriya, S.; Ganapathy; V.; Shyamaladevi, C.S. 2006. Suppression of tumor growth and invasion in 9,10 dimethyl benz(α)anthracene induced mammary carcinoma by the plant bioflavonoid quercetin. Chem Biol Interact 162(2):106-113.

DiCenzo, R., Frerichs, V., Larppanichpoonphol, P., Predko, L., Chen, A., Reichman, R. and Morris, M. 2006. Effect of quercetin on the plasma and intracellular concentrations of saquinavir in healthy adults. Pharmacotherapy. 26(9):1255-1261.

Dihal, A.A.; de Boer, V.C.; van der Woude, H.; Tilburgs, C.; Bruijntjes, J.P.; Alink, G.M.; Rietjens, I.M.; Woutersen, R.A.; Stierum, R.H. 2006. Quercetin, but not its glycosidated conjugate rutin, inhibits azoxymethane-induced colorectal carcinogenesis in F344 rats. J Nutr 136(11):2862-2867.

Dollo, G.; Chevanne, F.; Le Corre, P.; Chemtob, C.; Le Verge, R. 1999. Biodisponibilité du phloroglucinol chez l'homme = [Bioavailability of phloroglucinol in man]. J Pharm Belg 54(3):75-82.

Dumke, C.L.; Nieman, D.C.; Utter, A.C., Rigby, M.D.; Quindry, J.C.; Triplett, N.T.; McAnulty, S.R.; McAnulty, L.S. 2009. Quercetin's effect on cycling efficiency and substrate utilization. Appl Physiol Nutr Metab 34:993-1000.

Dunnick, J.K.; Hailey, J.R. 1992. Toxicity and carcinogenicity studies of quercetin, a natural component of foods. Fundam Appl Toxicol 19(3):423-431.

Edwards, R.I.; Lyon, T.; Litwin, S.E.; Rabovsky, A.; Symons, J.D.; Jalili, T. 2007. Quercetin reduces blood pressure in hypertensive subjects. J. Nutr. 137:2405-2411.

Egert, S.; Wolffram, S.; Bosy-Westphal, A.; Boesch-Saadatmandi, C.; Wagner, A.E.; Frank, J.; Rimbach, G.; Müller, M.J. 2008. Daily quercetin supplementation dose-dependently increases plasma quercetin concentrations in healthy humans. J Nutr 138:1615-1621.

Egert, S.; Bosy-Westphal, A.; Seiberl, J.; Kürbitz, C.; Settler, U.; Plachta-Danielzik, S.; Wagner, A.E.; Frank, J.; Schrezenmeir, J.; Rimbach, G.; Wolffram, S.; Müller, M.J. 2009. Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentration in overweight subjects with a high-cardiovascular disease risk phenotype: a double-blinded, placebo-controlled cross-over study. Br J Nutr 102(7): 1065-1074.

Exon, J.H.; Magnuson, B.A.; South, E.H.; Hendrix, K. 1998. Dietary quercetin, immune functions and colonic carcinogenesis in rats. Immunopharmacol Immunotoxicol 20(1):173-190.

Fukushima, S.; Hagiwara, A.; Ogiso, T.; Shibata, M.; Ito, N. 1983. Promoting effects of various chemicals in rat urinary bladder carcinogenesis initiated by *N*-nitroso-*n*-butyl-(4-hydroxybutyl)amine. Food Chem Toxicol 21(1):59-68.

Goldbohm, R.A.; Brandt, P.A.; Hertog, M.G.L.; Brants, H.A.M.; van Poppel, G. 1995. Flavonoid intake and risk of cancer: A prospective cohort study. Am J Epidemiol 141(11, Suppl.):S61 [Abstract No. 243].

Hard, G.C.; Khan, K.N. 2004. A contemporary overview of chronic progressive nephropathy in the laboratory rat, and its significance for human risk assessment. Toxicol Pathol 32(2):171-180.

Hard, G.C.; Seeley, J.C.; Betz, L.J.; Hayashi, S.-M. 2007. Re-evaluation of the kidney tumors and renal histopathology in a 2-year rat carcinogenicity bioassay of quercetin. Food Chem Toxicol 45(4):600-608.

Harnly, J.M.; Doherty, R.F.; Beecher, G.R.; Holden, J.M.; Haytowitz, D.B.; Bhagwat, S.; Gebhardt, S. 2006. Flavonoid content of U.S. fruits, vegetables, and nuts. J Agric Food Chem 54(26):9966-9977.

Harwood, M.; Danielewska-Nikiel, B.; Borzelleca, J.F.; Flamm, G.W.; Williams, G.M.; Lines, T.C. 2007. A critical review of the data related to the safety of quercetin and lack of evidence of *in vivo* toxicity, including lack of genotoxic/carcinogenic properties. Food Chem Toxicol 45(11):2179-2205.

Henson, D.A.; Nieman, D.C.; Davis, J.M.; Murphy, E. A.; Carmichael, M.D.; Dumke, C.L.; Gross, S.J.; Jenkins, D.P.; Quindry, J.C.; Mcanulty, S.R.; Mcanulty, L.S.; Utter, A.C.; Mayer, E.P. 2008. Post-160-km race illness rates and decreases in granulocyte respiratory burst and salivary IgA output are not countered by quercetin ingestion. Int J Sports Med. 29(10):856-863.

Hertog, M.G.L.; Hollman, P.C.H.; Peter, C.H.; Katan, M.B. 1992. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. J Agric Food Chem 40(12):2379-2383.

Hertog, M.G.L.; Hollman, P.C.H.; van de Putte, B. 1993. Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. J Agric Food Chem 41(8):1242-1246.

Hertog, M.G.L.; Kromhout, D.; Aravanis, C.; Blackburn, H.; Buzina, R.; Fidanza, F.; Giampaoli, S.; Jansen, A.; Menotti, A.; Nedeljkovic, S.; Pekkarinen, M.; Simic, B.S.; Toshima, H.; Feskens, E.J.M.; Hollman, P.C.H.; Katan, M.B. 1995. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med 155(4):381-386.

Hirono, I.; Ueno, I.; Hosaka, S.; Takanashi, H.; Matsushima, T.; Sugimura, T.; Natori, S. 1981. Carcinogenicity examination of quercetin and rutin in ACI rats. Cancer Lett 13(1):15-21.

Hirose, M.; Fukushima, S.; Sakata, T.; Inui, M.; Ito, N. 1983. Effect of quercetin on two-stage carcinogenesis of the rat urinary bladder. Cancer Lett 21(1):23-27.

Hollman, P.C.; Vd, Gaag, M.; Mengelers, M.J.; Van Trijp, J.M.; de Vries, J.H.; Katan, M.B. 1996. Absorption and disposition kinetics of the dietary antioxidant quercetin in man. Free Radic Biol Med 21(5):703-707.

Hosaka, S.; Hirono, I. 1981. Carcinogenicity test of quercetin by pulmonary-adenoma bioassay in strain A mice. GANN 72(2):327-328.

Hsiu, S.L., Hou, Y.C., Wang, Y.H., Tsao, C.W., Su, S.F. and Chao, P.D. 2002. Quercetin significantly decreased cyclosporin oral bioavailability in pigs and rats. Life Sci. 72(3):227-235.

IARC. 1999. Quercetin. In: Some Chemicals that Cause Tumours of the Kidney or Urinary Bladder in Rodents and Some Other Substances. International Agency for Research on Cancer (IARC); Lyon, France. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 73, pp. 497-515.

Ito, N.; Tsuda, H.; Tatematsu, M.; Inoue, T.; Tagawa, Y.; Aoki, T.; Uwagawa, S.; Kagawa, M.; Ogiso, T.; Masui, T.; Imaida, K.; Fukushima, S.; Asamoto, M. 1988. Enhancing effect of various hepatocarcinogens on induction of preneoplastic glutathione S-transferase placental form positive foci in rats - An approach for a new medium-term bioassay system. Carcinogenesis 9(3):387-394.

Ito, N.; Hagiwara, A.; Tamano, S.; Kagawa, M.; Shibata, M.; Kurata, Y.; Fukushima, S. 1989. Lack of carcinogenicity of quercetin in F344/DuCrj rats. Jpn J Cancer Res 80(4):317-325.

Jalili, T.; Edwards, R.; Lyon, T.; Symons, J.D. 2006. Quercetin reduces blood pressure in hypertensive subjects. Circulation 114:(18, Suppl. S):890 [Abstract 4122].

Janssen, K.; Mensink, R.P.; Cox, F.J.; Harryvan, J.L.; Hovenier, R.; Hollman, P.C.; Katan, M.B. 1998. Effects of the flavonoids quercetin and apigenin on hemostasis in healthy volunteers: Results from an *in vitro* and a dietary supplement study. Am J Clin Nutr 67(2):255-262.

JECFA. 1978. Evaluation of Certain Food Additives. 21st JECFA Session, Apr. 18-27, 1977, Geneva, Switz. World Health Organization (WHO), Joint FAO/WHO Expert Committee on Food Additives (JECFA) & Food and Agriculture Organization of the United Nations; Geneva, Switz. WHO Technical Report Series, No. 617.

Jin, N.-Z.; Zhu, Y.-P.; Zhou, J.-W.; Mao, L.; Zhao, R.-C.; Fang, T.-H.; Wang, X.-R. 2006. Preventive effects of quercetin against benzo[α]pyrene-induced DNA damages and pulmonary precancerous pathologic changes in mice. Basic Clin Pharmacol Toxicol 98(6):593-598.

Johannot, L.; Somerset, S.M. 2006. Age-related variations in flavonoid intake and sources in the Australian population. Public Health Nutr 9(8):1045-1054.

Jones, E.; Hughes, R.E. 1982. Quercetin, flavonoids and the life-span of mice. Exp Gerontol 17(3):213-217.

Kato, K.; Mori, H.; Fujii, M.; Bunai, Y.; Nishikawa, A.; Shima, H.; Takahashi, M.; Kawai, T.; Hirono, I. 1984. Lack of promotive effect of quercetin on methylazoxymethanol acetate carcinogenesis in rats. J Toxicol Sci 9(4):319-325.

Kato, K.; Mori, H.; Tanaka, T.; Fujii, M.; Kawai, T.; Nishikawa, A.; Takahashi, M.; Hirono, I. 1985. Absence of initiating activity by quercetin in the rat liver. Ecotoxicol Environ Saf 10(1):63-69.

Khanduja, K.L.; Verma, A.; Bhardwaj, A. 2001. Impairment of human sperm motility and viability by quercetin is independent of lipid peroxidation. Andrologia 33(5):277-281.

Kiesewetter, H.; Koscielny, J.; Kalus, U.; Vix, J.-M.; Peil, H.; Petrini, O.; van Toor, B.S.J.; de Mey, C. 2000. Efficacy of orally administered extract of red vine leaf AS 195 (folia vitis viniferae) in chronic venous insufficiency (Stages I-II). Drug Res 50(I)(2):109-117.

Kim, K.-A.; Park, P.-W.; Kim, H.-K.; Ha, J.-M.; Park, J.-Y. 2005. Effect of quercetin on the pharmacokinetics of rosiglitazone, a CYP2C8 substrate, in healthy subjects. J Clin Pharmacol 45(8):941-946.

Kim, K.-A.; Park, P.-W.; Park, J.-Y. 2009. Short-term effect of quercetin on the pharmacokinetics of fexofenadine, a substrate of P-glycoprotein, in health volunteers. Eur J Clin Pharmacol 65(6):609-614.

Kimira, M.; Arai, Y.; Shimoi, K.; Watanabe, S. 1998. Japanese intake of flavonoids and isoflavonoids from foods. J Epidemiol 8(3):168-175.

Knekt, P.; Järvinen, R.; Seppänen, R.; Heliövaara, M.; Teppo, L.; Pukkala, E.; Aromaa, A. 1997. Dietary intake of flavonoids and the risk of lung cancer and other malignant neoplasms. Am J Epidemiol 146:223-230.

Knekt, P.; Kumpulainen, J.; Järvinen, R.; Rissanen, H.; Heliövaara, M.; Reunanen, A.; Hakulinen, T.; Aromaa, A. 2002. Flavonoid intake and risk of chronic diseases. Am J Clin Nutr 76(3):560-568.

Kobori M, Masumoto S, Akimoto Y, Takahashi Y. 2009. Dietary quercetin alleviates diabetic symptoms and reduces streptozotocin-induced disturbance of hepatic gene expression in mice. Mol Nutr Food Res 53(7):859-868.

Kühnau, J. 1976. The flavonoids: A class of semi-essential food components: Their role in human nutrition. World Rev Nutr Diet 24:117-191.

Kwon, O.; Eck, P.; Chen, S.; Corpe, C.P.; Lee, J.-H.; Kruhlak, M.; Levine, M. 2007. Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. FASEB J 21:366-377.

Lam, T.K.; Rotunno, M.; Lubin, J.H.; Wacholder, S.; Consonni, D.; Pesatori, A.C.; Bertazzi, P.A.; Chanock, S.; Burdette, L.; Goldstein, A.M.; Tucker, M.A.; Caporasp, N.E.; Subar, A.F.; Landi, M.T. in press. Dietary quercetin, quercetin-gene interaction, metabolic gene expression in lung tissue, and lung cancer risk. Carcinogenesis. In press.

Li, X.; Choi, J.-S. 2009. Effects of quercetin on the pharmacokinetics of etoposide after oral or intravenous administration of etoposide in rats. Anticancer Res 29(4):1411-1415.

Lin, J.; Zhang, S.M.; Wu, K.; Willett, W.C.; Fuchs, C.S.; Giovannucci, E. 2006. Flavonoid intake and colorectal cancer risk in men and women. Am J Epidemiol 164(7):644 651.

Lozoya X, Reyes-Morales H, Chavez-Soto M.A., Martinez-Garcia Mdel C., Soto-Gonzalez Y., Doubova S.V. 2002. Intestinal anti-sposmodic effect of a phytodrug of *Psidium guajava folia* in the treatment of acute diarrheic disease. Journal of Ethnopharmacology 83:19-24.

MacRae, H.S. and Mefferd, K.M. (2006) Dietary antioxidant supplementation combined with quercetin improves cycling time trial performance. Int. J Sport Nutr. Exerc. Metab. 6(4):405-419.

Matsukawa, Y.; Nishino, H.; Yoshida, M.; Sugihara, H.; Katsura, K.; Takamatsut.; Okuzumi, J.; Matsumoto, K.; Sato-Nishimori, F.; Sakai, T. 2002. Quercetin enhances tumorigenicity induced by *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine in the duodenum of mice. Environ Health Prevent Med 6(4):235-239.

McAnlis, G.T.; Mceneny, J.; Pearce, J.; Young, I.S. 1999. Absorption and antioxidant effects of quercetin from onions, in man. Eur J Clin Nutr 53(2):92-96.

McAnulty, S.R.; McAnulty, L.S.; Nieman, D.C.; Quindry, J.C.; Hosick, P.A.; Hudson, M.H.; Still, L.; Henson, D.A.; Milne, G.L.; Morrow, J.D.; Dumke, C.L.; Utter, A.C.; Triplett, N.T.; Dibarnardi, A. 2008. Chronic quercetin ingestion and exercise-induced oxidative damage and inflammation. Appl Physiol Nutr Metab 33(2):254-262.

Momić, T.; Savić, J.; Černigoj, U.; Trebše, P.; Vasić, V. 2007. Protolytic equilibria and photodegradation of quercetin in aqueous solution. Collect. Czech. Chem.. Commun. 72(11):1447-1460.

Moon, Y.J.; Wang, L.; DiCenzo, R.; Morris, M.E. 2008. Quercetin pharmacokinetics in humans. Biopharmaceuticals & Drug Disposition 29:205-217.

Morand, C.; Crespy, V.; Manach, C.; Besson, C.; Demigne, C.; Remesy, C. 1998. Plasma metabolites of quercetin and their antioxidant properties. Am J Physiol Regul Integr Comp Physiol 275(1, Part 2):R212-R219.

Morino, K.; Matsukara, N.; Kawachi, T.; Ohgaki, H.; Sugimura, T.; Hirono, I. 1982. Carcinogenicity test of quercetin and rutin in golden hamsters by oral administration. Carcinogenesis 3(1):93-97.

Nakamura, Y.; Torikai, K.; Ohigashi, H. 2001. Toxic dose of a simple phenolic antioxidant, protocatechuic acid, attenuates the glutathione level in ICR mouse liver and kidney. J Agric Food Chem 49(11):5674-5678.

Nandan, S.D.; Rao, M.S. 1983. Lack of mutagenic effects of quercetin in the germ cells of mice. IRCS Med Sci 11(3):210.

Nass-Arden, L.; Breitbart, H. 1990. Modulation of mammalian sperm motility by quercetin. Mol Reprod Dev 25(4):369-373.

Nieman, D.C. 2008 [unpublished]. Quercetin Community Trial on 1,002 Subjects. Quercetin, Upper Respiratory Tract Infection, Disease Risk Factors, and Mental Cognition: A Randomized Community Trial. Appalachian State University, Department of Health Leisure & Exercise Science, Human Performance Laboratory; Boone, North Carolina [unpublished study – preliminary results].

Nieman, D.C.; Henson, D.A.; Davis, J.M.; Murphy, E.A.; Jenkins, D.P.; Gross, S.J.; Carmichael, M.D.; Quindry, J.C.; Dumke, C.L.; Utter, A.C.; McAnulty, S.R.; McAnulty, L.S.; Triplett, N.T.; Mayer, E.P. 2007a. Quercetin's influence on exercise-induced changes in plasma cytokines and muscle and leukocyte cytokine mRNA. J Appl Physiol 103(5):1728-1735.

Nieman, D.C.; Henson, D.A.; Davis, J.M.; Dumke, C.L.; Gross, S.J.; Jenkins, D.P.; Murphy, E.A.; Carmichael, M.D.; Quindry, J.C.; McAnulty, S.R.; McAnulty, L.S.; Utter, A.C.; and Mayer, E.P. 2007b. Quercetin ingestion does not alter cytokine changes in athletes competing in the Western States Endurance Run. J Interferon Cytokine Res 27(12):1003-1011.

Nieman, D.C.; Henson, D.A.; Gross, S.J.; Jenkins, D.P.; Davis, J.M.; Murphy, E.A.; Carmichael, M.D.; Dumke, C.L.; Utter, A.C.; Mcanulty, S.R.; Mcanulty, L.S.; Mayer, E.P. 2007c. Quercetin reduces illness but not immune perturbations after intensive exercise. Med Sci Sports Exerc 39(9):1561-1569.

Nieman, D.C.; Henson, D.A.; Maxwell, K.R.; Williams, A.S.; McAnulty, S.R.; Jin, F.; Shanely, R.A.; Lines, T.C. 2009. Effects of quercetin and EGCG on mitochondrial biogenesis and immunity. Med Sci Sports Exerc 41(7):1467-1475.

Nieman, D.C., Williams, A.S., Shanely, R.A., Jin, F., McAnulty, S.R., Triplett, N.T., Austin, M.D., and Henson, D.A. 2010. Quercetin's influence on exercise performance and muscle mitochondrial biogenesis. Med. Sci. Sports Exerc. 42(2):338-45.

NTP. 1992. Toxicology and Carcinogenesis Studies of Quercetin (CAS No. 117-39-5) in F344/N Rats (Feed Study). National Toxicology Program (NTP); Research Triangle Park, North Carolina. NTP Technical Report Series No. 409.

Pamukcu, A.M.; Yalçiner, Ş.; Hatcher, J.F.; Bryan, G.T. 1980. Quercetin, a rat intestinal and bladder carcinogen present in Bracken Fern (Pteridium aquilinum). Cancer Res 40:3468-3472.

PDRNS. 2001. Quercetin. In: PDR For Nutritional Supplements (1st Ed.). Physicians' Desk Reference (PDR); Demoines, Iowa/Medical Economics Data Production Company; Montvale, New Jersey, pp. 390-392.

Pereira, M.A.; Grubbs, C.J.; Barnes, L.H.; Li, H.; Olson, G.R.; Eto, I.; Juliana, M.; Whitaker, L.M.; Kelloff, G.J.; Steele, V.E.; Lubet, R.A. 1996. Effects of the phytochemicals, curcumin and quercetin, upon azoxynethane-induced colon cancer and 7,12-dimethylbenz[*a*]anthacene-induced mammary cancer in rats. Carcinogenesis 17(6):1305-1311.

Prism Research. 2010a. Effect of Multiple Doses of Compound Q on Plasma Glucose Levels after Oral Glucose Tolerance Test in Patients with Type 2 Diabetes. Unpublished report for Project No. 915 (Protocol 102).

Prism Research. 2010b. Effect of Multiple Doses of Compound Q on Plasma Digoxin Disposition after a Single Oral Dose of Digoxin in Normal Healthy Volunteers Unpublished report for Project No. 916 (Protocol 103).

Prism Research. 2010c. Effect of Multiple Doses of Compound Q on Plasma Warfarin Disposition after a Single Oral Dose of Warfarin in Normal Healthy Volunteers. Unpublished report for Project No. 914 (Protocol 101).

Quindry, J.C.; McAnulty, S.R.; Hudson, M.B.; Hosick, P.; Dumke, C.; McAnulty, L.S.; Henson, D.; Morrow, J.D.; Nieman D. 2008. Oral quercetin supplementation and blood oxidative capacity in response to ultramarathon competition. Int. J Sport Nutr. Exerc. Metab. 18(6):601-616.

Rashid, J., McKinstry, C., Renwick, A.G., Dirnhuber, M., Waller, D.G. and George, C.F. 1993. Quercetin, an in vitro inhibitor of CYP3A, does not contribute to the interaction between nifedipine and grapefruit juice. Br J Clin Pharmacol. 36(5):460-463.

Rimm, E.C.; Katan, M.B.; Ascherio, A.; Stampfer, M.J.; Willett, W.C. 1996. Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. Ann Intern Med 125(5):384-389.

Saito, D.; Shirai, A.; Matsushima, T.; Sugimura, T.; Hirono, I. 1980. Test of carcinogenicity of quercetin, a widely distributed mutagen in food. Teratog Carcinog Mutagen 1(2):213-221.

Sampson, L.; Rimm, E.; Hollman, P.C.; de Vries, J.H.; Katan, M.B. 2002. Flavonol and flavone intakes in US health professionals. J Am Diet Assoc 102(10):1414-1420.

Sato, H.; Takahashi, M.; Furukawa, F.; Miyakawa, Y.; Hasegawa, R.; Toyoda, K.; Hayashi, Y. 1987. Initiating potential of 2-2-furyl)-3- (5-nitro-2-furyl) acrylamide (AF- 2), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and 3,3,4,5,7-pentahydroxyflavone (quercetin) in two-stage mouse skin carcinogenesis. Cancer Lett 38(1&2):49-56.

Schmalhausen, E.V.; Zhlobek, E.B.; Shalova, I.N.; Firuzi, O.; Saso, L.; Muronetz, V.I. 2007. Antioxidant and prooxidant effects of quercetin on glyceraldehyde-3-phosphate dehydrogenase. Food Chem Toxicol 45:1988-1993.

Shanely, R.A., Knab, A.M., Nieman, D.C., Jin, F., McAnulty, S.R. and Landram, M.J. 2010. Quercetin supplementation does not alter antioxidant status in humans. Free Radical Research 44(2):224-231.

Shin, S.-C.; Choi, J.-S.; Li, X. 2006. Enhanced bioavailability of tamoxifen after oral administration of tamoxifen with quercetin in rats. Int J Pharm 313(1&2):144-149.

Shoskes,D.A.; Zeitlin, S.I.; Shahed, A.; Rajfer, J. 1999. Quercetin in men with category III chronic prostatitis: A preliminary prospective, double-blind, placebo-controlled trial. Urology 54(6):960-963.

Stoewsand, G.S.; Anderson, J.L.; Boyd, J.N.; Hrazdina, G.; Babish, J.G.; Walsh, K.M.; Losco, P. 1984. Quercetin: A mutagen, not a carcinogen, in Fischer rats. J Toxicol Environ Health 14(2&3):105-114.

Strobel, P.; Allard, C.; Perez-Acle, T.; Calderon, R.; Aldunate, R.; Leighton, F. 2005. Myricetin, quercetin and catechin-gallate inhibit glucose uptake in isolated rat adipocytes. Biochem J 386(3):471-478.

Sullivan, M.; Follis, R.H. (Jr.); Hilgartner, M. 1951. Toxicology of podophyllin. Proc Soc Exp Biol Med 77:269-272.

Swisher, D.K.; Harris, R.D.; Tague, R.M.; Seyler, D.E. 1994. Effects of quercetin on rabbit sperm. Biol Reprod 50(Suppl. 1):107 [Abstract No. 211].

Tabassum, S.; Afridi, B.; Aman, Z. 2005. Phloroglucinol for acceleration of labor: double blind, randomized controlled trial. J Pak Med Assoc 55:270-273.

Takanashi, H.; Aiso, S.; Hirono, I.; Matsushima, T.; Sugimura, T. 1983. Carcinogenicity test of quercetin and kaempferol in rats by oral administration. J Food Saf 5(2):55-60.

Tanaka, T.; Kojima, T.; Suzui, M.; Mori, H. 1993. Chemoprevention of colon carcinogenesis by the natural product of a simple phenolic compound protocatechuic acid: suppressing effects on tumor development and biomarkers expression of colon tumorigenesis. Cancer Res 53:3908-3913.

Tanaka, T.; Kawabata, K.; Honjo, S.; Kohno, H.; Murakami, M.; Shimada, R.; Matsunaga, K.; Yamada, Y.; Shimizu, M. 1999. Inhibition of azoxymethane-induced aberrant crypt foci in rats by natural compounds, caffeine, quercetin and morin. Oncol Rep 6(6):1333-1340.

Trejo, R.; Valadéz-Salazar, A.; Delhumeau, G. 1995. Effects of quercetin on rat testis aerobic glycolysis. Can J Physiol Pharmacol 73(11):1605-1615.

U.S. FDA. 1997. Substances general regarded as safe. Proposed rule—21 CFR Parts 170, 184, 186, and 570 [Docket No. 97N–0103]. Fed Regist (US) 62(74):18937-18964.

U.S. FDA. 2009. U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs (Food and Drug Administration). U.S. Government Printing Office (GPO); Washington, DC. Available from: http://www.access.gpo.gov/nara/cfr/cfr-table-search.html [See Table for CFR sections].

Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	Section §	Section Title
101—Food labelling	101.12	Reference amounts customarily consumed per eating occasion
170—Food additives	170.35	Affirmation of generally recognized as safe (GRAS) status
184—Direct food substances affirmed as generally recognized as safe	184.1009	Adipic acid
	184.1021	Benzoic acid
	184.1205	Calcium hydroxide
	184.1293	Ethyl alcohol
	184.1878	Thiamine mononitrate

Umathe, S.N.; Dixit, P.V.; Kumar, V.; Bansod, K.U.; Wanjari, M.M. 2008. Quercetin pretreatment increases the bioavailability of pioglitazone in rats: involvement of CYP3A inhibition. Biochem Pharmacol 75(8):1670-1676.

USDA. 2000. 1994-1996, 1998 Continuing Survey of Food Intakes by Individuals (CSFII) and Diet and Health Knowledge Survey (DHKS) (On CD-ROM). U.S. Department of Agriculture (USDA); Riverdale, Maryland. PB2000-500027 Supercedes PB98-500457.

USDA. 2007. What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2003-2004. U.S. Department of Agriculture (USDA); Riverdale, Maryland. Available from: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release.

USDA. 2009a. *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2003-2004*. Riverdale (MD): U.S. Department of Agriculture (USDA). Available from: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release.

USDA. 2009b. *What We Eat In America: National Health and Nutrition Examination Survey (NHANES): 2005-2006.* U.S. Department of Agriculture (USDA); Riverdale, Maryland. Available from: http://www.ars.usda.gov/Services/docs.htm?docid=15044.

Utesch, D.; Feige, K.; Dasenbrock, J.; Broschard, T.H.; Harwood, M.; Danielewska-Nikiel, B.; Lines, T.C. 2008. Evaluation of the potential *in vivo* genotoxicity of quercetin. Mutat Res 654(1):38-44.

Utter, A.C., Nieman, D.C., Kang, J., Dumke, C.L., Quindry, J.C., McAnulty, S.R., McAnulty, L.S. 2009. Quercetin does not affect rating of perceived exertion in athletes during the Western States endurance run. Res Sports Med. 17(2):71-83.

Valentich, M.A.; Eynard, A.R.; Barotto, N.N.; Diaz, M.P.; Bongiovanni, G.A. 2006. Effect of the co-administration of phenobarbital, quercetin and mancozeb on nitrosomethylurea-induced pancreatic tumors in rats. Food Chem Toxicol 44(12):2101-2105.

Verma, A.K.; Johnson, J.A.; Gould, M.N.; Tanner, M.A. 1988. Inhibition of 7,12-dimethylbenz(*a*)anthracene-and *N*-nitrosomethylurea-induced rat mammary cancer by dietary flavonol quercetin. Cancer Res 48(20):5754-5758 [Erratum, 1989, 49(4):1073].

Vicentini, F.T.M.C.; Georgetti, S.R.; Jabor, J.R.; Caris, J.A.; Bentley, M.V.L.B.; Fonseca, M.J.V. 2007. Photostability of quercetin under exposure to UV irradiation. Lat. Am. J. Pharm. 26(1):119-124.

Volate, S.R.; Davenport, D.M.; Muga, S.J.; Wargovich, M. 2005. Modulation of aberrant crypt foci and apoptosis by dietary herbal supplements (quercetin, curcumin, silymarin, ginseng and rutin). Carcinogenesis 26(8):1450-1456.

Wang, Y.-H.; Chao, P.-D.L.; Hsiu, S.L.; Wen, K.-C.; Hou, Y.-C. 2004. Lethal quercetin-digoxin interaction in pigs. Life Sci 74(10):1191-1197

Werner, B.; Sprecher, W.; Mitsohke, H. 1985. Quercetin und dünndarmkrebs. [Quercetin and intestinal tumours an experimental study]. Verdauungskrankheiten 3(3):113-115.

Wiczkowski, W.; Nèmeth, K.; Buciński, A.; Piskula, M.K. 2003. Bioavailability of quercetin from flesh scales and dry skin of onion in rats. Pol J Food Nutr Sci 12(Spec. Iss. 1):95-99.

Willhite, C.C. 1982. Teratogenic potential of quercetin in the rat. Food Chem Toxicol 20(1):75-77.

Xu, M.; Zhang, Z.; Fu, G.; Sun, S.; Sun, J.; Yang, M.; Liu, A.; Han, J.; Guo, D. 2007. Liquid chromatography-tandem mass spectrometry analysis of protocatechuic aldehyde and its phase I and II metabolites in rat. J Chromatogr B 856(1&2):100-107.

Yang, K.; Lamprecht, S.A.; Liu, Y.; Shinozaki, H.; Fan, K.; Leung, D.; Newmark, H.; Steele, V.E.; Kelloff, G.J.; Lipkin, M. 2000. Chemoprevention studies of the flavonoids quercetin and rutin in normal and azoxymethane-treated mouse colon. Carcinogenesis 21(9):1655-1660.

Zenkevich, I.G.; Eshchenko, A.Y.; Makarova, S.V.; Vitenberg, A.G.; Dobryakov, Y.G.; Utsal, V.A. 2007. Identification of the products of oxidation of quercetin by air oxygen at ambient temperature. Molecules 12:654-672.

Zhu, B.T.; Liehr, J.G. 1994. Quercetin increases the severity of estradiol-induced tumorigenesis in hamster kidney. Toxicol Appl Pharmacol 125(1):149-158.

Exhibit A

EXHIBIT A

EXPERT PANEL CONSENSUS STATEMENT: THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF HIGH-PURITY QUERCETIN FOR USE IN FOODS

EXPERT PANEL CONSENSUS STATEMENT: THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF HIGH-PURITY QUERCETIN FOR USE IN FOODS

April 30, 2010

INTRODUCTION

At the request of Quercegen Pharma LLC (hereafter Quercegen), 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, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine the safety and the Generally Recognized as Safe (GRAS) status of the proposed uses of high-purity quercetin (≥99.5%). The Panel consisted of Professors Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), William Waddell, M.D. (School of Medicine, University of Louisville), and Gary Williams, M.D. (New York Medical College). *Curricula vitae* evidencing the Panel members' qualifications for evaluating the safety of food ingredients are provided in Attachment 1.

The Panel, independently and collectively, critically examined a comprehensive package of scientific information and data from the literature and other published sources through February 2010, as compiled by CANTOX Health Sciences International and Toxicology Regulatory Services. In addition, the Panel evaluated other information deemed appropriate or necessary, including data and information provided by Quercegen and Merck S/A (Rio de Janeiro, Brazil). The data evaluated by the Panel included information pertaining to the method of manufacture and product specifications, analytical data, intended use-levels in specified food products, consumption estimates for all intended uses, and comprehensive literature on the safety of quercetin.

Following independent, critical evaluation of such data and information, the Panel convened by teleconference on April 30, 2010, and unanimously concluded that the proposed uses of Quercegen's high-purity quercetin (≥99.5%), manufactured consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food grade specifications, are safe and GRAS based on scientific procedures. A summary of the basis for the Panel's conclusions, excluding confidential data and information, is provided below.

MANUFACTURING AND COMPOSITION

The quercetin product intended for use as an ingredient in food is manufactured by Merck S/A (Brazil) in accordance with local cGMP following the requirements of the Brazilian Health Authority (ANVISA)'s guideline RDC249/05, which is compliant with ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) guideline Q7A (Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients). Quercetin is produced *via* a manufacturing process that involves extraction of the

quercetin glycoside, rutin, from plant source(s) (*i.e.*, Fava d'Anta beans and/or Uncaria leaves), release of the aglycone *via* hydrolysis, and subsequent step-wise purification. The starting material, the Fava d'Anta bean, is native to Brazil, where it is grown and harvested. The alternative starting material is Uncaria leaves of the *Uncaria elliptica* plant. These plants are grown in Brazil, without genetic modification or use of pesticides. Alternative sources of quercetin could include onions and/or apples. The processing chemicals used in the manufacture of quercetin are appropriate for food use.

The chemical and microbiological specifications for quercetin are presented in Table 1. Analyses of representative lots demonstrated compliance with final product chemical and microbiological specifications. Results of a long-term storage stability study conducted with quercetin at 70±5% relative humidity and 30±2°C confirmed that the quercetin product is stable for at least 60 months. Possible impurities occurring in the final quercetin product as a result of the manufacturing process are rutin and isoquercetin, which are glycosides of the quercetin aglycone, as well as ethanol residue. Glycosides including rutin and isoquercetin are the primary form of quercetin that occurs naturally in foods. Appropriate criteria have been established to limit the levels of these impurities in the final product and batch samples are routinely tested to ensure compliance with the specifications.

Table 1 Chemical and Microbiological Specifications for Quercegen's High-Purity Quercetin

Parameter	Limit	Method of Analysis
Description	Yellow or greenish-yellow, crystalline powder, odorless or with a slight characteristic odor	Visual and olfactory inspection
Solubility - 2% ethanol (NTU)	Not more than 15	Merck S/A
Solubility - 1% DMF	Clear solution	Visual inspection
Solubility – Water	Insoluble	Visual inspection
Identity	Comparable to quercetin standard	HPLC analysis
Assay (Quercetin) (%)	Not less than 99.5%	HPLC analysis
Density (g/mL)	Not less than 0.25	Merck S/A SOP 41/g/1213
Moisture (%)	Not more than 4.0	Karl-Fisher
Sulfated ash (%)	Not more than 0.15	Merck S/A
Chloride (Cl^-) (ppm)	Not more than 1,000	Merck S/A
Sulphate (SO_4^{2-}) (ppm)	Not more than 1,000	Merck S/A
Heavy Metals		
Arsenic (As) (ppm)	Not more than 1	AAS
Cadmium (Cd) (ppm)	Not more than 1	AAS
Cobalt (Co) (ppm)	Not more than 1	AAS
Lead (Pb) (ppm)	Not more than 1	AAS
Mercury (Hg) (ppm)	Not more than 1	AAS
Nickel (Ni) (ppm)	Not more than 1	AAS

Table 1 Chemical and Microbiological Specifications for Quercegen's High-Purity Quercetin

Parameter	Limit	Method of Analysis
Microbiological Parameters		
Aerobic bacteria (CFU/g)	Not more than 5,000	Merck General Method MG 029*
Yeasts and molds (CFU/g)	Not more than 500	Merck General Method MG 029
Salmonella species (per 10 g)	Absent	Merck General Method MG 029
Escherichia coli (per 10 g)	Absent	Merck General Method MG 029
Staphylococcus aureus (per 10 g)	Absent	Merck General Method MG 029
Pseudomonas aeruginosa (per 10 g)	Absent	Merck General Method MG 029

AAS = Atomic Absorption Spectroscopy; CFU = Colony-forming units; DMF = N,N-Dimethylformamide; HPLC = High-Performance Liquid Chromatography; ICP-MS = Inductively Coupled Plasma Mass Spectrometry; NTU = Nephelometric Turbidity Unit; SOP = Standard Operating Procedure.
* Merck General Method 029 complies with U.S. Pharmacopeia Monograph for Microbiology Tests (USP, 2007 or a more recent edition)

INTENDED USE AND ESTIMATED EXPOSURE

Quercetin is one of several natural-occurring flavonol compounds belonging to the broader group of polyphenolic flavonoid substances, which are characterized by a phenyl benzo(γ)pyrone-derived structure consisting of 2 benzene rings linked by a heterocyclic pyran or pyrone ring (Kühnau, 1976; Morand *et al.*, 1998). Among other biological properties, these compounds have been reported to exhibit strong antioxidant properties in the plant (Wiczkowski *et al.*, 2003). Fruits and vegetables, particularly onions and apples, were identified as the primary sources of natural-occurring dietary quercetin of the typical Western diet (Hertog *et al.*, 1992; Day and Williamson, 1999; Harnly *et al.*, 2006). Black tea also was identified as rich dietary sources of quercetin (Hertog *et al.*, 1993; Sampson *et al.*, 2002). In the United States, the average daily intake of all flavonoids from a normal mixed diet was estimated at approximately 1 g/day (expressed as quercetin equivalents) (Kühnau, 1976; Brown, 1980), with flavonol glycosides, expressed as quercetin equivalents, consumed at levels of up to 100 mg/day (Brown, 1980; Jones and Hughes, 1982; NTP, 1992; Rimm *et al.*, 1996; USDA, 2000; Sampson *et al.*, 2002). Daily intake levels of quercetin as high as 200 to 500 mg have been reported in high-end consumers of fruits and vegetables (Jones and Hughes, 1982). National (*e.g.*, Australia, the Netherlands, Finland, Italy, Croatia, Japan, United States) dietary record-based cohort assessments of the intake of quercetin from the habitual diet indicated consumption levels of up to approximately 40 mg quercetin/day (Hertog *et al.*, 1995; Rimm *et al.*, 1996; Knekt *et al.*, 1997; Kimira *et al.*, 1998; Johannot and Somerset, 2006; Lin *et al.*, 2006).

Quercegen intends to market high-purity quercetin (≥99.5%) as a food ingredient as a source of the flavonol in a variety of food products including beverages and beverage bases, grain products and pastas, processed fruits and fruit juices, and soft candies.. Depending on the particular food category, the ingredient is proposed for use at levels providing up to 500 mg of

quercetin/serving. The individual proposed food-uses and use-levels for Quercegen's quercetin are summarized in Table 2.

Table 2 Summary of the Individual Proposed Food-Uses and Use-Levels for Quercetin in the U.S

Food Category	Proposed Food-Use	Use-Level (mg/serving)*	Serving Size (g)[a]	Use-Level (%)
Beverages and Beverage Bases	Sports and Energy Drinks	250	240	0.10
Grain Products and Pastas	Cereal and Energy Bars	50	40	0.125
	Grain-Based Beverages	10	240	0.004
Processed Fruits and Fruit Juices	Fruit-Flavored Juice-Based Drinks (non-carbonated and carbonated)	125	240	0.052
	Fruit Smoothie Drinks	125	240	0.052
Soft Candies	Dietetic or Low-Calorie Soft Candies	250 to 500	6	4.2 to 8.3

[a] Unless otherwise indicated the serving sized based is based on the Reference Amounts Customarily Consumed per Eating Occasion (21 CFR §101.12 - CFR, 2008b).

High-purity quercetin is proposed to be added to selected foods and beverages for specific technical/functional uses for exercising adults (i.e. Targeted Population) and will be marketed initially through specialty channels (e.g. GNC, sporting goods stores). Foods and beverages to which this ingredient would be added will be labeled to disclose to consumers the presence of high-purity quercetin and to provide directions and/or other relevant information about the technical/functional uses. The technical/functional uses will be a primary reason why consumers will purchase and consume the foods and beverages. In addition, such foods and beverages would be priced at a premium compared to commodity foods that do not contain high-purity quercetin because of the high cost of this ingredient. Thus, foods and beverages to which high-purity quercetin is added will be specially labeled, marketed, and priced to reflect a specific technical/functional use, and such foods and beverages would not be labeled, marketed, or priced in the same manner as commodity foods.

Estimates of the intake of quercetin were based on the proposed food-uses and use-levels in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2009a,b). The data from the 2003-2004 and 2005-2006 cycles of the NHANES survey were combined to provide a larger population from which to estimate quercetin consumption. Approximately 37.4% of the total U.S. population was identified as potential consumers of Quercegen's quercetin from the proposed food-uses (6,252 actual users identified). This is the traditional approach to estimating intake of substances added to food and is based on historical consumption of the foods and beverages to which high-purity quercetin is proposed to be added.

Table 3 Summary of the Estimated Daily Intakes of Quercetin Based on all Proposed Food-Uses and Use-Levels in the U.S. (NHANES 2003-2004, 2005-2006)

Population Group	Age (Years)	% Users	Actual # of Total Users	All-Person Consumption (mg/day)		All-User Consumption (mg/day)	
				Mean	90th Percentile	Mean	90th Percentile
Infants	0 to 2	28.9	552	85	153	269	625
Children	3 to 11	55.9	1,528	97	250	176	375
Female Teenagers	12 to 19	47.8	949	70	225	156	338
Male Teenagers	12 to 19	49.7	964	144	450	298	668
Female Adults	> 20	27.3	1,167	39	105	152	288
Male Adults	> 20	28.4	1,092	70	214	256	572
Total Population	All ages	37.4	6,252	67	182	205	461

Derived using data and individual food codes from the National Health and Nutrition Examination Surveys (NHANES) 2003-2004, 2005-2006 (CDC, 2006; USDA, 2009a,b).

Table 4 Summary of the Estimated Daily Per Kilogram Body Weight Intakes of Quercetin Based on all Proposed Food-Uses and Use-Levels in the U.S. (NHANES 2003-2004, 2005-2006)

Population Group	Age (Years)	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw/day)		All-User Consumption (mg/kg bw/day)	
				Mean	90th Percentile	Mean	90th Percentile
Infants	0 to 2	28.9	552	7	12	22	53
Children	3 to 11	55.9	1,528	4	9	7	13
Female Teenagers	12 to 19	47.8	949	1	4	3	6
Male Teenagers	12 to 19	49.7	964	2	7	5	11
Female Adults	> 20	27.3	1,167	1	1	2	4
Male Adults	> 20	28.4	1,092	1	2	3	7
Total Population	All ages	37.4	6,252	1	4	4	9

Derived using data and individual food codes from the National Health and Nutrition Examination Surveys (NHANES) 2003-2004, 2005-2006 (CDC, 2006; USDA, 2009a,b).

Using a traditional consumption analysis, the highest 90th percentile all-user intake of quercetin for a population group on an absolute basis was observed to occur in male teenagers at 668

mg/person/day, equivalent to 11 mg/kg body weight/day, respectively. On a body weight basis the highest 90th percentile all-user intake was observed in infants at 53 mg/kg body weight/day (see Tables 3 and 4); however, because infants are not a Targeted Population (as described above) and would not be consumers of sports and energy drinks or "sports chews" for technical/functional uses, the intake estimates for this age group are greatly exaggerated. Additionally, it should be noted that the food category and respective food codes for "Soft Candies/ Dietetic or Low-Calorie Soft Candies" is being used as a surrogate for "Sports Chews", since NHANES food codes do not exist for this specific food-use.

In general, consumption estimates by the traditional approach are highly conservative for a number of reasons. In particular, the estimates assume that all target foods and beverages that could contain high-purity quercetin would in fact be formulated with it at the maximum level of use. Because high-purity quercetin is intended for Targeted Populations for technical/functional uses that will modify the normal intended use and price of the foods and beverages to which it will be added, it is likely that only a small number of the target foods will be formulated with high-purity quercetin.

For adult exercising Targeted Population consumers who would intentionally seek high-purity quercetin-containing foods and beverages, consumption is estimated at 1000 mg quercetin/day. It is possible that these adult exercising consumers who were to select high-purity quercetin-containing foods and beverages would consume more than two to four servings/day for short periods of time that would provide 1250 or 1500 mg quercetin/day. However, long-term consumption at this level is considered unlikely based on premium pricing and the limited number of target foods. The Non-targeted Population (particularly infants and children) are expected to consume high-purity quercetin at much lower levels and, in fact, their consumption is expected to be much lower than estimated by the traditional method. The safety of the anticipated maximum daily intake foreseen by the Targeted Population adult exercising consumers is supported by data summarized in the following section, Safety Assessment.

The Total Population maximum estimated daily background intake of quercetin from naturally occurring sources was 258.2 mg/day, which is within the published range of 200-500 mg/day for high-end consumers of fruits and vegetables (Jones and Hughes, 1982). Therefore, a Targeted Population consumer with a daily intake of 1000 mg high-purity quercetin, who also was high-end consumer of fruits and vegetables, may consume approximately 1250 mg quercetin/day. This daily intake is also considered safe based on the weight of the available evidence.

SAFETY ASSESSMENT

The safety of Quercegen's quercetin ingredient is based on metabolic, short- and long-term preclinical toxicity studies, and *in vitro* and *in vivo* mutagenicity/genotoxicity studies performed

with quercetin. The pivotal study is a 2-year rat feeding study conducted by the National Toxicology Program (NTP) (Dunnick and Hailey, 1992; NTP, 1992). The results of other relevant short- and long-term toxicity studies support the safety of this naturally-occurring flavonoid. Further support of the safety of quercetin is provided by several human clinical trials and epidemiology studies, and two *in vivo* drug interaction studies with healthy subjects and an oral glucose tolerance test in type 2 diabetics. The weight of the available evidence supports the safety of the proposed uses of Quercegen's high-purity quercetin.

Unless otherwise indicated, the quercetin test material used in the studies described below was the flavonol aglycone.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION (ADME)

Absorption and Bioavailability

Several *in vitro* and *in situ* investigations have demonstrated some transport of quercetin across the intestinal epithelium (Noteborn *et al.*, 1997; Walgren *et al.*, 1998; Crespy *et al.*, 1999; Murota *et al.*, 2000; Carbonaro and Grant, 2005); however, the majority of quercetin remained bound to the gut wall (Carbonaro and Grant, 2005). Within the absorptive cells of the intestinal epithelium, quercetin may be *O*-methylated primarily resulting in the formation of 3'-*O*-methylquercetin (isorhamnetin) and to a smaller extent, 4'-*O*-methylquercetin (tamaraxetin), sulfated, or glucuronidated at one of its hydroxyl groups (Crespy *et al.,* 1999; Murota *et al.*, 2000; Rechner *et al.*, 2002; Murota and Terao, 2005; Graf *et al.*, 2005, 2006). Subsequently, the quercetin derivatives, as well as the unmetabolized quercetin fraction were shown to enter the circulation *via* the hepatic portal vein (Manach *et al.*, 1995). Recent data also indicated a potential for systemic quercetin uptake *via* lacteals into the lymph (Murota and Terao, 2005).

In male ACI rats, approximately 20% of radiolabeled material was estimated to be absorbed following administration of an oral dose of radiolabeled quercetin (2.3 mg [4-^{14}C]-quercetin), with a large portion remaining in the digestive tract (Ueno *et al.*, 1983). Chen *et al.* (2005) demonstrated absorption of approximately 60% of total quercetin (*i.e.*, free and conjugated quercetin and its metabolites) and 5% of unchanged quercetin following single oral gavage administration of a 10 mg/kg body weight dose of quercetin to male Sprague-Dawley rats. In male Sprague-Dawley rats administered a single oral dose of 165 µmol (50 mg quercetin/kg body weight) by gavage, approximately 53% of the dose was estimated to be absorbed (Yang *et al.,* 2005). The variability observed in the percent of quercetin that is reported to be absorbed in the animal studies may be due to strain differences or differences in analytical methods. Evaluated in ileostomy patients, 24% of ingested quercetin was absorbed (Hollman *et al.*, 1995, 1997). In healthy subjects provided 100 mg of radiolabeled quercetin, oral absorption was estimated to range from 36 to 53% (based on total radioactivity) (Walle *et al.*, 2001).

Total (free and conjugated) plasma flavonoid concentrations (quercetin and isorhamnetin) between 12.2 and 100 nmol/mL were detected in rats (Wistar; male or Hooded Lister; gender not specified) following single oral administration of 250 to 1,000 mg quercetin (Manach *et al.*, 1997, 1999; Morand *et al.*, 2000a,b; Nakamura *et al.*, 2000; Carbonaro and Grant, 2005). In male Wistar rats administered quercetin (250 mg) by gavage for over several (10-13) days, plasma levels of up to 100 nmol/mL were detected (Manach *et al.*, 1997, 1999). Although, free quercetin was not detected in the plasma with single treatment, 0.56 nmol/mL of unconjugated quercetin was present following multiple administrations over a 10-day period (Nakamura *et al.*, 2000). Free quercetin was identified in the plasma, albeit only at a concentration of 0.9 nmol/mL (0.27 µg/mL), following single intragastric treatment of male Sprague-Dawley rats with 50 mg quercetin/kg body weight (Justino *et al.*, 2004). In weanling F344 rats administered quercetin in the diet at 0.45% for a period of 6 weeks (equivalent to doses of up to 58.5 mg/day during the last week of treatment), plasma samples did not contain any detectable levels of the aglycone (Graf *et al.*, 2006). While low concentrations of free quercetin were identified in liver and kidney (*i.e.*, less than 8% of total quercetin identified in these tissues), the authors suggested that this may have occurred as a result of *ex vivo* hydrolysis of the quercetin metabolites. The same study authors reported that plasma, liver, and kidney samples obtained from a group of 6 F344 rats (gender not specified) fed a diet containing 0.5% quercetin (approximately 250 mg/kg body weight/day) for a period 6 weeks contained only quercetin metabolites (concentrations of total quercetin in plasma or the tissues were not reported) (Graf *et al.,* 2005).

Total plasma quercetin levels (*i.e.*, quercetin, quercetin glycosides, glucuronides, and sulfates) between 29 and 248 µg/L were attained following ingestion of single meals consisting of quercetin-rich foods (~50 to 70 mg quercetin) in a total of 22 healthy humans (12M, 10F) (Hollman *et al.*, 1996; de Vries *et al.*, 1998; McAnlis *et al.*, 1999) and daily ingestion of 114 mg quercetin from onions for 7 continuous days by 18 healthy volunteers (9/sex) resulted in total quercetin plasma levels of 447 µg/L (Janssen *et al.*, 1998). In earlier human trials involving 10 healthy subjects and 4 ileostomy, but otherwise healthy patients, the unconjugated quercetin aglycone was not identified in plasma samples following oral administration of the flavonol (Manach *et al.*, 1998; Walle *et al.*, 2000). Using improved detection methods, Mullen *et al.* (2004) reported trace quantities of the aglycone in the plasma of one of 6 healthy (4 males and 2 females) subjects (between 23 to 45 years of age) ingesting fried onions. Wang and Morris (2005) reported plasma levels of up to 8 ng/mL in another study in which a group of 3 study participants (no additional details specified) were provided 500 mg quercetin three times daily for a period of 5 days (120 to 350 ng/mL of total quercetin). Mean peak plasma aglycone levels ranging from 10.8 to 25 ng/mL were identified 30 minutes following single dose administration of 10 mg quercetin per 70 kg of body weight (0.14 mg/kg body weight) dissolved in different beverages (*i.e.*, vegetable juice, white wine, or grape juice) to 4 healthy subjects, with the highest plasma level (25 ng/mL) observed when quercetin was provided in wine (Goldberg *et al.*, 2003). The variability in plasma concentrations of the quercetin aglycone in the above

studies may be due to different methods of analysis or the matrix in which quercetin was administered.

Evaluated in a group of 9 ileostomy patients, 24% of an oral dose of quercetin (112 mg quercetin dehydrate providing 100 mg aglycone) was absorbed (Hollman *et al.*, 1995, 1997). In healthy subjects provided 100 mg of radiolabeled quercetin, oral absorption was estimated to range from 36 to 53% (based on total radioactivity) (Walle *et al.*, 2001).

Graefe *et al.* (2001) exposed 12 healthy volunteers (9 males and 3 females; mean age 24.3 years) to: 1) onion supplement containing 331 µmol quercetin glucosides; 2) 331 µmol quercetin-4'-*O*-glucoside isolated from onions; 3) buckwheat tea powder providing 662 µmol quercetin rutinoside; or 4) 662 µmol quercetin-3-*O*-rutinoside (*i.e.,* rutin). There was no evidence that the active metabolites of quercetin found in the blood differed based on the administered quercetin glycoside; however, bioavailability was approximately 5 times greater when quercetin was administered bound to a glucose compared to rutinose. In general, the plant matrix seemed to play a much less important role when compared to the role of the sugar moiety.

Egert *et al.* (2008) investigated the effects of oral quercetin supplementation on bioavailability and plasma concentrations in 35 healthy human subjects (18 males and 17 females; mean age 26.2 years). Quercetin supplementation resulted in a significant, dose-dependent increase of total quercetin in plasma, and the rate of absorption did not differ among the quercetin dose groups. Plasma concentrations of total quercetin represented both the unconjugated and conjugated forms of quercetin. The variability in plasma quercetin concentrations was higher at the end of supplementation than at baseline suggesting that individuals absorb quercetin differently. Total plasma quercetin concentrations peaked at 120-360 minutes following oral ingestion, and there were no significant differences between males and females.

Distribution

In Wistar rats (sex not specified) receiving a single 3 mg oral dose of radiolabeled quercetin (approximately 7.5 mg/kg body weight) by gavage, the highest amounts of radioactivity were recovered from the gastrointestinal tract; however, systemically-absorbed quercetin was shown to be distributed relatively uniformly across all major tissues (Abrahamse *et al.*, 2005). Conversely, in male F344 rats fed up to 500 to 800 mg/kg body weight/day of quercetin in the diet for 11 weeks, quercetin was observed to concentrate in a few organs (*i.e.*, lung, testes, kidney, thymus, heart, liver), with the highest concentrations of quercetin and its methylated derivatives, particularly isorhamnetin, detected in pulmonary tissue (de Boer *et al.*, 2005). Quercetin was primarily identified in its conjugated form in the tissue samples; however, free quercetin was extracted from some organs (*i.e.*, lungs, liver, kidney, and testes) at concentrations up to 40% of total extracted quercetin. The authors noted that tissue

β-glucuronidase deconjugation of quercetin may occur *in vivo* and the conversion of conjugated quercetin to the aglycone upon extraction also could not be fully discounted.

In male Wistar rats as well as in humans, quercetin and its metabolites were shown to possess high affinity for serum albumin (Manach *et al.*, 1995; McAnlis *et al.*, 1999; Sengupta and Sengupta, 2003; Kaldas *et al.,* 2005). The elimination of absorbed quercetin has been demonstrated to follow a biphasic profile (Gugler *et al.*, 1975; Hollman *et al.*, 1996; Ader *et al.*, 2000; Skibola and Smith, 2000; Walle *et al.*, 2001; Khaled *et al.*, 2003; Schulz *et al.*, 2005a,b). This may be due to enterohepatic recirculation following elimination of quercetin metabolites in bile. Metabolites of quercetin are eliminated from the circulation in the urine (Gugler *et al.*, 1975; Onishi *et al.*, 1982; Ueno *et al.*, 1983; de Vries *et al.*, 1998; Young *et al.*, 1999; Nakamura *et al.*, 2000; Wang *et al.*, 2003a,b; Abrahamse *et al.,* 2005; Jones *et al.,* 2005; Wang and Morris, 2005; Hong and Mitchell, 2006) or alternatively may be secreted into the bile and may potentially enter the enterohepatic circulation following deconjugation to the aglycone (Ueno *et al.*, 1983). While biliary recirculation could extend the bioavailability of quercetin, degradation of quercetin to phenolic acids in the colon may reduce the amount of quercetin re-entering the circulation (Crespy *et al.*, 1999). Furthermore, no enterohepatic recirculation of quercetin and its conjugates was observed in a linked-rat (male Sprague-Dawley) model (Chen *et al.*, 2005).

Metabolism

While more than 65% (exact number not specified) and more than 90% (93.3%) of orally administered doses of quercetin were shown to be metabolized in the gut of F344 (gender not specified) and male Sprague-Dawley rats, respectively, prior to absorption (Chen *et al.*, 2005; Graf *et al.*, 2005), further conjugation of quercetin and its metabolites to sulfate and/or glucuronide derivatives also was reported to occur in the liver (Shali *et al.*, 1991; Morand *et al.*, 1998; Oliveira and Watson, 2000; Boersma *et al.*, 2002; Chen *et al.*, 2005). Additionally, the catechol-*O*-methyltransferase (COMT) enzymes of the liver and kidneys may participate in the methylation of quercetin or its derivatives following absorption (Onishi *et al.*, 1982; Zhu *et al.*, 1994; De Santi *et al.*, 2002; O'Leary *et al.*, 2003; Graf *et al.,* 2005, 2006). Sulfate and glucuronide conjugates of quercetin and its *O*-methylated derivates were identified in plasma samples collected from hamsters, rats (Wistar, Sprague-Dawley, F344), pigs, and humans (males and females) following quercetin administration or consumption of quercetin-rich foods or herbal extracts (Zhu *et al.*, 1994; Manach *et al.*, 1995, 1997, 1998, 1999; Morand *et al.*, 1998, 2000a,b; Ader *et al.*, 2000; Morrice *et al.*, 2000; Oliveira and Watson, 2000; Day *et al.*, 2001; Moon *et al.*, 2001; Justino *et al.,* 2004; Silberberg *et al.,* 2005; Yang *et al.,* 2005; Graf *et al.,* 2005, 2006; Lesser *et al.,* 2006; Bieger *et al.*, 2008). More than 90% of quercetin recovered from plasma, kidney, and liver samples of orally treated male Sprague-Dawley rats was metabolized (Justino *et al.*, 2004; Graf *et al.*, 2005, 2006). Extensive methylation of quercetin combined with the conjugative metabolic process are important contributing factors to the overall lack of toxicity associated with quercetin *in vivo.*

Excretion

Quercetin that is not absorbed was shown to be degraded to one of several different phenolic acids (*e.g.*, 3,4-dihydroxyphenylacetic acid) and carbon dioxide (CO_2) by the colonic microflora of rabbits, rats, cows, and humans (*i.e.*, heterocyclic ring fission) (Murray *et al.*, 1954; Booth *et al.*, 1956; DeEds, 1968; Krishnamurty *et al.*, 1970; Stelzig and Ribeiro, 1972; Ueno *et al.*, 1983; Gross *et al.*, 1996; Pietta *et al.*, 1997; Justesen and Arrigoni, 2001; Braune *et al.*, 2001; Rechner *et al.*, 2002; Olthof *et al.*, 2003; Weldin *et al.*, 2003; Keppler *et al.*, 2006). Unabsorbed quercetin and the microfloral degradation products of quercetin were eliminated in the feces of rats and humans (Gugler *et al.*, 1975; Ueno *et al.*, 1983; Nakamura *et al.*, 2000).

In male ACI rats , quercetin was excreted as expired CO_2 (35%), or *via* the feces (45%) and urine (10%) as glucuronide or sulfate conjugates 72 hours following gavage administration of radiolabeled quercetin (630 mg/kg body weight) (Ueno *et al.*, 1983). In bile duct-cannulated male ACI rats, 12 and 13% of recovered quercetin appeared in the bile and feces, respectively, with the remainder expired as CO_2 (34%) and in the urine (9%) (Ueno *et al.*, 1983). In ileostomized Wistar rats and Wistar rats fitted with portal vein catheters administered a single oral dose of ^{14}C-quercetin, 6.2 and 9.9% of the administered radioactive label was recovered in the feces and urine, respectively (Abrahamse *et al.,* 2005). Since approximately 75% of the administered radioactive dose was not recovered, the authors speculated that the majority of the administered quercetin was metabolized to form CO_2 and was exhaled. Excretion of quercetin in the urine and feces in 6 healthy human subjects (2 female and 4 male; 23 to 44 years of age) accounted for only 3.3 to 5.7 and 0.21 to 4.6%, respectively, of an orally administered dose (100 mg), with the majority of the radioactivity recovered as CO_2 (*i.e.*, 41.8 to 63.9%) (Walle *et al.*, 2001). Wang and Morris (2005) demonstrated that small quantities of unchanged quercetin (15.5 to 74.9 µg) were excreted in the urine within an 8-hour period by human subjects (n=3; no further details provided) ingesting quercetin supplements (500 mg x thrice daily).

Summary

Following oral administration, the majority of quercetin is metabolized in the gastrointestinal tract prior to systemic uptake in both animals and humans. Studies conducted in rats and humans to quantify the absorption of quercetin and its metabolites from the gastrointestinal tract demonstrated highly variable results (20 to 60% in rats and 24 to 53% in humans). While ingestion of the quercetin aglycone or quercetin-rich foods has been associated with significant increases in plasma total (free and conjugated) quercetin levels in both rats and humans, only trace amounts of the unmetabolized aglycone have been identified (8 to 25 ng/mL in humans following intake of 10 to 500 mg of quercetin). Once absorbed, quercetin is subjected to further metabolism in the liver and kidneys and is eliminated in bile, feces and urine. Unabsorbed quercetin passes into the colon where it is degraded by the microflora to phenolic acids and

CO_2, with the latter exhaled in the expired air. Quercetin that has been secreted in the bile and is not reabsorbed, as well as the phenolic degradation products are excreted in the feces.

EFFECTS ON ENZYMES AND OTHER BIOCHEMICAL PARAMETERS

Studies to Assess Potential Effects of Quercetin on Monosaccharide Absorption

In *Xenopus laevis* oocytes with forced expression of human glucose transporter 2 or 5 (GLUT2 and GLUT5, respectively) or the sodium-dependent glucose transporter 1 (SGLT1), quercetin at concentrations of 10 to 50 µM was shown to inhibit GLUT2-uptake of both ^{14}C-deoxyglucose (10 mM) and ^{14}C-fructose (10 mM) in a dose-dependent manner, but not the transport of either glucose or fructose *via* SGLT1 (glucose-transporter only) and GLUT5 (fructose-transporter only), respectively (Kwon *et al.,* 2007). The concentrations at which quercetin decreased glucose or fructose GLUT2-mediated uptake by 50% (IC_{50}) were calculated to be 12.7 and 15.9 µM, respectively. Lack of an inhibitor effect of quercetin on SGLT1-facilitated glucose uptake was confirmed in another *in vitro* assay in brush-border-membrane vesicles (BBMV) isolated from pig jejunum (Cermak *et al.,* 2004). However, at concentrations of 10 to 100 µM, quercetin also was shown to inhibit insulin-stimulated glucose transport *via* glucose transporter 4 (GLUT4; main glucose transporter in adipose and muscle tissue) in adipocytes isolated from the epididymal fat pads of male Wistar rats (Strobel *et al.,* 2005).

The potential inhibitory effect of quercetin on galactose absorption was evaluated in jejunal BBMV isolated from Swiss mice (20 to 30/group) following 28-day administration of quercetin in the diet at a concentration of 0 (control) or 0.5% (providing an average dose of 950 mg quercetin/kg body weight/day) (Barrenetxe *et al.,* 2006). At the end of the treatment period, all animals were killed, the jejunum was removed, and BBMV were isolated and pooled from 10 animals per group for the absorption assay. Relative to the control group, SGLT1-mediated uptake of ^{14}C-galactose (1 mM) in BBMV obtained from the quercetin-treated group was inhibited only following short incubation periods of 5 or 10 seconds, but not following longer incubation times of 10 or 60 minutes.

In an oral glucose tolerance test (OGTT) conducted in 20 male and female human volunteers with confirmed type 2 diabetes (33-69 years of age), quercetin did not impact blood glucose levels. There were no clinically-significant changes in clinical chemistry or hematology values, vital signs, or physical exam findings over the course of the study. There was no evidence found to demonstrate that quercetin would significantly alter blood glucose levels; therefore, orally administered quercetin did not alter monosaccharide absorption in the gut (Prism Research, 2010a).

Studies to assess the effects of quercetin on plasma glucose levels in normoglycemic and diabetic rats are also summarized in Table A2-1 (see Attachment 2).

Studies to Assess Potential Effects of Quercetin on Cytochrome P450 (CYP450), Mitochondrial, and Intestinal Enzymes

In vitro experimentation has demonstrated that quercetin is able to induce or inhibit the catalytic activity of metabolizing enzymes belonging to the cytochrome P450 (CYP) family of enzymes, including that of human CYP1A1, CYP1A2, CYP1B1, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 (Obach, 2000; Rahden-Staron *et al.*, 2001; Dierks *et al.*, 2001; Zou *et al.*, 2002; Bun *et al.*, 2003; Kim *et al.*, 2003; Schwarz *et al.*, 2003; He and Edeki, 2004; Patel *et al.*, 2004; von Moltke *et al.*, 2004; Walsky *et al.*, 2005, 2006; Chang *et al.*, 2006; Lau and Chang, 2009; Rajaraman *et al.*, 2009; Sergent *et al.*, 2009; Si *et al.*, 2009). In contrast to the *in vitro* findings, an *in vivo* study indicated that quercetin had no effect on CYP2C8 activity (Kim *et al.,* 2005). Specifically, in order to evaluate the implications of the *in vitro* inhibitory activity of quercetin on CYP450 enzymes *in vivo*, 10 male subjects were provided 0 or 500 mg quercetin/day for a period of 3 weeks in a cross-over trial (4-week washout period) and the effect of quercetin on CYP2C8 was subsequently evaluated by assessing blood levels of rosiglitazone, an antidiabetic metabolized by CYP2C8, following oral administration of a single dose (4 mg) (Kim *et al.,* 2005). In comparison to the placebo-control phase, no differences were observed in any of the pharmacokinetic parameters [including peak plasma concentration (C_{max}), time to C_{max}, half-life, area under the plasma concentration-time curve, and oral clearance] for rosiglitazone or its *N*-desmethyl metabolite (*N*-desmethylrosiglitazone) following the quercetin treatment phase.

In a drug interaction study conducted in human volunteers with digoxin, quercetin did not interfere with the P-glycoprotein- (P-gp) mediated efflux. A total of 14 healthy male and female subjects (20-55 years of age) completed the study. There were no clinically-significant changes in clinical chemistry or hematology values, vital sign measurements, or physical examination findings over the course of the study. There was no evidence that 5 daily doses of quercetin affected the P-glycoprotein transporter, as shown by a lack of effect on digoxin AUC, T_{max} or C_{max} (Prism Research, 2010b).

A human volunteer drug interaction study conducted with warfarin and quercetin demonstrated that quercetin did not interfere with the cytochrome P450 system. The potential effect of multiple doses of quercetin on plasma warfarin disposition after a single dose of warfarin was evaluated in 16 healthy males (20-50 years of age). Pharmacokinetics of (S)- and (R)-warfarin were monitored for 10 days following co-administration with quercetin. There were no biologically-relevant changes in clinical chemistry or hematology values (including prothrombin time), vital signs, or physical exam findings over the course of the study. With respect to the pharmacokinetic results, quercetin co-administration had no effect on the AUC, C_{max}, half-life or clearance of either (S)- or (R)-warfarin (Prism Research, 2010c).

Significant increases in the soleus muscle content of mitochondrial enzymes, cytochrome C, and citrate synthase were observed following gavage administration of quercetin to groups of 8 mice (sex and strain not specified) at doses of 12.5 or 25 mg/kg body weight/day for 7 days in comparison to a vehicle-treated control group (Davis *et al.,* 2007). In the brain, only cytochrome C content of quercetin-treated mice was increased significantly.

Enzymatic assays were conducted with jejunal BBMV isolated from Swiss mice administered 5% quercetin in the diet (providing approximately 950 mg/kg body weight/day) for 28 days to determine the potential effect of quercetin on several intestinal enzymes (sucrase, maltase, aminopeptidase N, and alkaline phosphatase) (Barrenetxe *et al.*, 2006). In comparison to BBMV isolated from a control group, activities of the enzymes involved in dipeptide absorption (aminopeptidase and alkaline phosphatase) were significantly increased in quercetin-treated mice, whereas activities of sucrase and maltase, enzymes involved in glucose absorption, were decreased, although only the latter reached statistical significance.

Studies to assess the potential effect of quercetin on the pharmacokinetics of CYP3A or P-gp substrate drugs in different species are also summarized in Table A2-3 (see Attachment 2).

Studies to Assess Potential Prooxidant Properties of Quercetin

Although quercetin possesses antioxidant activities, under certain conditions *in vitro*, it has been shown to elicit prooxidant properties as a result of its oxidation to a reactive metabolite; prooxidation has not been demonstrated *in vivo*. *In vivo* there are a number of mechanisms in place to protect against the possibility of prooxidant effects of quercetin, including: (i) a highly limited bioavailability of the quercetin aglycone resulting from the degradation of quercetin to phenolic compounds in the gut and post-absorptive metabolic processes (*i.e., O*-methylation, glucuronidation, and sulfation); (ii) preferential conjugation of the reactive oxidative degradation products of quercetin with reduced glutathione (GSH), rather than DNA/protein-adduct formation; and (iii) a complex network of other antioxidants that work in concert to regenerate the parent compound (Boots *et al.*, 2007; Harwood *et al.*, 2007). Canada *et al.* (1990) reported that the rate of autooxidation for quercetin was pH-dependent with no autooxidation detected at a physiologic pH *in vitro*; quercetin-produced hydroxyl radicals were not detected below a pH of 9. As such, *in vivo,* extracellular production of reactive oxygen species following quercetin exposure would not be expected.

An *in vivo* study was conducted to specifically assess the potential effects of quercetin on antioxidant status in bone marrow and spleen cells of male brown Norway rats following a 40-day treatment period (Papiez *et al.*, 2008). Levels of malondialdehyde (MDA), an indicator of lipid peroxidation, in the spleen and bone marrow cells of the quercetin-exposed animals were significantly reduced. The reduction in MDA levels was accompanied by a significant decrease

in the ferric ion reducing ability of plasma (FRAP) in both cell types. Levels of heme oxygenase-1 (HO-1) (associated with protection against oxidative stress) mRNA expression increased in a dose-dependent manner. Treatment with quercetin was not associated with DNA damage in either cell type, nor with an increase in the number of apoptotic cells. Similarly, no DNA damage was observed in bone marrow cells of male Wistar rats following gavage administration of quercetin for a period of 3 days (Cierniak *et al.*, 2004).

Studies to Assess Potential Effects of Quercetin on Levels of Glutathione

Glutathione (GSH) is a scavenger of reactive oxygen species, but has several other related functions [*e.g.*, cosubstrate for detoxification of peroxides by glutathione peroxidase (GPx)]. It has been noted that binding of oxidized quercetin with glutathione (GSH) is reversible and is one of several protective mechanisms that exist to prevent potential prooxidant properties of quercetin related to its antioxidant activity (Boots *et al.*, 2007).

Conjugation of quercetin with GSH is not the primary route for quercetin metabolism; one study has identified glutathione conjugates as metabolites of quercetin; a total of 18 metabolites of quercetin were identified, including not only glucuronides and methylated and sulfated conjugates, but also glutathione-related conjugates (glutathionylated quercetin and methylated or glucuronidated forms of glutathionylated quercetin). One of the glutathione conjugates was a quinine (Hong and Mitchell, 2006).

Several *in vivo* animal studies were identified that specifically evaluated the potential effects of quercetin on systemic levels of GSH and several other parameters related to quercetin's potential antioxidative/prooxidative properties. Choi *et al.* (2003, 2005) conducted studies to evaluate the effect of quercetin on the glutathione antioxidant system in the liver of rats, reporting that quercetin protected against lipid peroxidation, and in doing so it also reduced levels of GSH. In another study in which quercetin was provided to normotensive rats at a level decreases were observed in liver GSH levels, with no changes observed in MDA levels (Duarte *et al.*, 2001). In a similar study conducted to assess the effects of quercetin on the GSH-defense systems in both normotensive and hypertensive rats, García-Saura *et al.* (2005) also noted a decrease in GSH levels in the former rat model and no change in GSH levels in rats with elevated blood pressure.

The GSH redox status was also evaluated in mice. An increase in pulmonary and hepatic GSH levels was observed in mice following quercetin exposure (Gandhi and Khanduja, 1993). Meyers *et al.* (2008) reported an increase in hepatic tissue GSH levels, but not plasma or cardiac tissue. The differences in the GSH redox status in various tissues could be due to differences in the exposure of these tissues to quercetin and/or its metabolites. Conversely, evaluated in mitochondria, which have a GSH pool separate of the intracellular GSH pool, an

increase was observed in heart GSSG levels and a reduction in the GSH:GSSG ratio in the heart and liver. The increases in GSH levels were likely the result of quercetin-mediated up-regulation of GSH production, in response to reduced GSH levels. Quercetin is recognized to activate the γ-glutamylcysteine synthetase (GSH-synthesizing enzyme) promoter (Myhrstad *et al.*, 2002).

Although administration of quercetin has generally been shown to be associated with reduced GSH levels in rats, no adverse effects related to treatment with quercetin have been observed in long-term feeding studies up to 2 years in duration (Ito *et al.*, 1989; NTP, 1992). In *vivo*, quercetin exerts its antioxidant functions as part of a complex system that also includes other antioxidants such as β-carotenes, vitamin E, or vitamin C. Although GSH levels may decline initially following exposure to quercetin as a result of its antioxidative role, GSH levels are replenishable. Furthermore, quercetin also has been noted to up-regulate GSH production. Therefore, as part of an integrated system of antioxidants, *in vivo*, quercetin was not related with any adverse effects.

In a study conducted in 1,002 non-institutionalized human subjects, red blood cell total GSH levels (reduced and oxidized state) were unaffected by 12 weeks of oral quercetin supplementation (Nieman, 2008; Shanely *et al.*, 2010). In another study, 7 healthy volunteers (3 males and 4 females) consumed quercetin for a period of 4 weeks. Following the supplementation period, a significant increase was observed in the mean plasma quercetin level as well as in the total plasma antioxidant capacity. No changes were observed in the mean plasma GSH levels and levels of TNF-α, uric acid, or vitamin C compared to pre-treatment values.

A summary of studies conducted to assess GSH status in animals following quercetin administration is also presented in Table A2-2 (see Attachment 2).

In conclusion, the data summarized above demonstrate that orally administered quercetin does not adversely impact monosaccharide absorption or the activity of metabolizing enzymes belonging to the CYP450 family and/or substrates of P-gp. Based on the available evidence, quercetin does not appear to deplete glutathione levels nor act as a prooxidant under *in vivo* conditions.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

No symptoms of toxicity were reported in four rabbits receiving single intravenous administrations of solubilized quercetin at a dose level of 100 mg/kg body weight (Ambrose *et al.*, 1951).

Oral LD_{50} values in albino mice (sex and strain not specified) for quercetin were reported to be approximately 160 mg/kg body weight (Sullivan *et al.*, 1951). Administered subcutaneously to mice, LD_{50} values of approximately 100 mg/kg body weight were reported. Symptoms of toxicity following parenteral administration of quercetin included atypical movements followed by labored respiration, and finally a state of moribundity and death. However, the purity of the quercetin administered in these studies was not reported. Subsequently-conducted longer term animal toxicity studies with relative pure quercetin (≥95% purity) demonstrated that animals tolerated doses several fold higher than the reported LD_{50}.

Genotoxicity Studies

In vitro

Evaluated in the Ames assay in several strains of *Salmonella typhimurium* (TA100, TA102, TA98, TA97, TA1537, and TA1538) with and without metabolic activation (S9), at concentrations of up to 1,000 µg/plate, quercetin consistently tested positive for mutagenic activity (Bjeldanes and Chang, 1977; Hardigree and Epler, 1978; MacGregor and Jurd, 1978; Seino *et al.*, 1978; Brown and Dietrich, 1979; Ochiai *et al.*, 1984; Stoewsand *et al.*, 1984; Ueno *et al.*, 1984; Hatcher and Bryan, 1985; Busch *et al.*, 1986; Rueff *et al.*, 1986, 1992; Crebelli *et al.*, 1987; Schimmer *et al.*, 1988; Nguyen *et al.*, 1989; Vrijsen *et al.*, 1990; NTP, 1992; Czeczot, 1994; Makena *et al.*, 2009; Cross *et al.*, 1996). *S. typhimurium* TA1535 provided the only exception to quercetin-related mutagenicity in the Ames test, in the absence as well as presence of metabolic activation at concentrations of up to 100 µg/plate (Bjeldanes and Chang, 1977; Czeczot *et al.*, 1990). In *Escherichia coli*, quercetin exhibited positive activity in a number of SOS chromotest assays with and without metabolic activation (Rueff *et al.*, 1986; Dayan *et al.*, 1987; Llagostera *et al.*, 1987; Czeczot and Kusztelak, 1993; Czeczot *et al.*, 1993). Positive results also were reported for reverse mutations and DNA single strand breaks in *E. coli* (Hardigree and Epler, 1978; Czeczot *et al.*, 1993; Czeczot, 1994), but not for forward mutations (Hardigree and Epler, 1978). Evaluated in 2 *Bacillus subtilis* strains, quercetin did not exhibit any mutagenic potential in a forward mutation assay with and without metabolic activation (MacGregor, 1979).

Examined at relatively high concentrations (up to 10 mg/2 mL of incubation mix), the mutagenicity observed in bacterial test systems also was found in yeast cells (Hardigree and Epler, 1978). In mammalian cells, chromosomal aberrations were observed in Chinese hamster

ovary, V79 lung, and fibroblast cells (Yoshida *et al.*, 1980; Carver *et al.*, 1983; Kubiak and Rudek, 1990; NTP, 1992; Gaspar *et al.*, 1994), and single strand breaks also were reported in hamster ovary and fibroblast cells (Yoshida *et al.*, 1980; Carver *et al.*, 1983; Kubiak and Rudek, 1990; NTP, 1992), but not in V79 hamster lung cells (van der Hoeven *et al.*, 1984). Additionally, an increase in micronucleus frequency was observed following incubation of Chinese hamster V79 cells with quercetin at up to 22 µg/mL, with and without metabolic activation (Caria *et al.*, 1995). DNA single-strand breaks were induced in mouse L5178Y cells (Meltz and MacGregor, 1981) and rat H4IIE hepatoma cells (Watjen *et al.*, 2005) at cytotoxic cell culture concentrations. Mutations also were detected at the *tk* locus in mouse lymphoma cells (Meltz and MacGregor, 1981; van der Hoeven *et al.*, 1984), but were not identified at the HPRT locus (van der Hoeven *et al.*, 1984). Similarly, in Chinese hamster lung cells, forward mutations were not present at the HPRT locus (van der Hoeven *et al.*, 1984). Carver *et al.* (1983) observed a positive mutagenic response only at the *tk* locus, but not at several others including the *hgprt*, *aprt*, and ATPase loci, in Chinese hamster ovary cells without metabolic activation at up to 15 µg/mL. In Chinese hamster cells, induction of 8-azaguanine- and diphtheria toxin-resistant mutants was observed at quercetin concentrations of up to 200 µg/mL in both the absence and presence of metabolic activation (Maruta *et al.*, 1979; Nakayasu *et al.*, 1986). Suzuki *et al.* (1991), detected recombinational mutations using DNA fingerprinting in cultured mouse cells following exposure to quercetin at concentrations of 16.6 µg/mL. In rat hepatocytes, quercetin did not elicit unscheduled DNA synthesis at a concentration of up to 20 µM (Cross *et al.*, 1996) and was negative in the DNA repair test at a concentration of up to 10 µM (Kato *et al.*, 1985). In human lymphocytes, at concentrations of up to 50 µg/mL, quercetin also induced an increased frequency of micronuclei (Popp and Schimmer, 1991; Caria *et al.*, 1995), chromosomal aberrations (Yoshida *et al.*, 1980), and sister chromosome exchanges (Yoshida *et al.*, 1980; Rueff *et al.*, 1986; Popp and Schimmer, 1991). Incubation of human fibroblast cells in the presence of quercetin at concentrations of up to 5.0 µg/mL also resulted in the formation of significantly elevated incidences of chromosomal aberrations and sister chromosome exchanges (Yoshida *et al.*, 1980). Additionally, positive results were obtained in two cell transformation assays performed in BALB/c 3T3 mouse cells (Meltz and MacGregor, 1981) and hamster embryo cells (Umezawa *et al.*, 1977).

The quercetin-related mutagenicity observed *in vitro* appears to be mediated *via* a pro-oxidant mechanism in the presence of O_2, and mirrors the genotoxic mechanism of action characteristic of other strong antioxidants, such as ascorbic acid (vitamin C) and *tert*-butylhydroquinone (TBHQ), which also demonstrate positive mutagenicity *in vitro*, including clastogenicity (JECFA, 1998, 2006). More specifically, *in vitro*, the oxidative degradation of quercetin has been reported to result in the formation of an *ortho*-semiquinone intermediate, which is subsequently reduced to an *ortho*-quinone, accompanied by the production of reactive oxygen species (ROS), such as superoxide and hydrogen peroxide (Metodiewa *et al.*, 1999; Boots *et al.*, 2003). The *ortho*-quinone is further subject to tautomerization to yield 1 of 3 possible quinone methides. It was demonstrated that the reactive quercetin oxidation products are effectively scavenged by

glutathione (GSH) forming 1 of 2 possible adducts (*i.e.*, 6-GS-quercetin and 8-GS-quercetin) (Metodiewa *et al.*, 1999; Boots *et al.*, 2003); however, as indicated by the metabolism data obtained *in vivo*, the potential for oxidative stress-induced genotoxicity is minimized by the preferential microbial degradation of quercetin prior to its absorption, as well as the *O*-methylation and glucuronidation of the flavonol and its subsequent excretion in the urine and feces.

Consequently, in the Ames assay, in which the mutagenic activity is attributed to the quercetin parent compound, and in particular the presence of hydroxyl (-OH) groups at positions 3' and 4' (Caria *et al.*, 1995), the positive results obtained in the absence of exogenous enzymatic activation were enhanced in the presence of metabolic activation, as oxidative scavengers such as superoxide dismutase (SOD) and catalase present in the S9 system effectively prevented the auto-oxidative degradation of quercetin (Ueno *et al.*, 1984). Conversely, decreased mutagenic activity was demonstrated in a study in which xanthine and xanthine oxidase were added to the test system as generators of superoxide radicals, which enhanced the degradation of quercetin (Ueno *et al.*, 1984). Likewise, evaluated in test systems at 2 different pH levels, SOS activity, which is indicative of DNA damage, was greater at pH 6.0 than at pH 7.4, as the more basic pH supported the oxidative degradation of quercetin and consequently reduced quercetin-related mutagenicity (Rueff *et al.*, 1992). An increase in the mutagenic response was noted at the more basic pH in the presence of the oxidative scavenger, SOD. In contrast, in eukaryotic test systems in which the generation of reactive oxygen species underlies the positive mutagenic response, quercetin-related mutagenicity was increased under basic conditions (pH 8.0) (Gaspar *et al.*, 1994), and either not enhanced (Gaspar *et al.*, 1994; Caria *et al.*, 1995) or reduced following addition of scavenging enzymes added as part of the S9 system (Meltz and MacGregor, 1981; Rueff *et al.*, 1986; NTP, 1992; Caria *et al.*, 1995).

Results of the *in vitro* assays in prokaryotic and eukaryotic test systems are summarized in Tables A2-4 and A2-5, respectively (see Attachment 2).

Additionally, several *in vitro* and *ex vivo* assays were conducted in order to assess the potential mutagenicity/genotoxicity of quercetin metabolites, as well as urinary and fecal extracts and plasma samples collected from animals following quercetin administration. In the Ames assay, methylation of quercetin at the different hydroxyl groups (*e.g.*, 3'-OH, 4'-OH, 3-OH, 4-OH, 5-OH, 7-OH) significantly attenuated or entirely abolished its mutagenic activity (MacGregor and Jurd, 1978; Brown and Dietrich, 1979; Hatcher *et al.*, 1981; Onishi *et al.*, 1982; Czeczot *et al.*, 1990). Urine and fecal samples collected from rats orally administered quercetin demonstrated detectable levels of mutagenicity in some (MacGregor, 1979; Stoewsand *et al.*, 1984; Crebelli *et al.*, 1987), but not all (Hatcher *et al.*, 1981) *Salmonella* assays. Conversely, plasma samples obtained from rats administered quercetin by gavage or *via* the diet exhibited no mutagenic activity in *S. typhimurium* (Crebelli *et al.*, 1987), thus clearly indicating that absorbed quercetin is rapidly and efficiently metabolized to non-mutagenic derivatives.

Results of the *in vitro* assays conducted to assess the potential mutagenicity/genotoxicity of quercetin metabolites are presented in Table A2-6, whereas studies assessing the potential mutagenicity/genotoxicity of urine, fecal, or plasma samples following treatment of rats with quercetin are summarized in Table A2-7 (see Attachment 2).

In vivo

Quercetin was not mutagenic in the recessive sex-linked lethal test (RSLLT) in *Drosophila melanogaster* exposed to 13 mg quercetin/mL for 18 hours; however, at 25 mg/mL and greater, quercetin was reported to induce a positive mutagenic response (Watson, 1982). Female *Drosophila melanogaster* exposed to 5% quercetin in the diet for 7 days in a Meiotic Chromosome Segregation study did not result in a significant increase in levels of non-disjunction (Schramm *et al.*, 1998).

Administration of quercetin in the diet at a concentration of 0.1% [approximately 50 mg/kg body weight/day (U.S. FDA, 1993)] for a period of 1 week to male and female Wistar rats did not induce chromosome aberrations in bone marrow stem cells or micronucleus formation in bone marrow erythrocytes compared to solvent control groups (Taj and Nagarajan, 1996); that is, quercetin was not clastogenic. As determined by the Comet assay, no significant DNA damage was observed in bone marrow cells collected from femurs of male Wistar rats administered 135 mg quercetin/kg body weight/day for a period of 3 days by gavage relative to an untreated control group (Cierniak *et al.*, 2004). In a host-mediated assay (*S. typhimurium* TA98) and a micronucleus assay performed in male mice (strain not specified) orally administered up to 500 and 1,000 mg quercetin/kg body weight/day, respectively, no significant increases in the number of revertants and micronuclei, respectively, were observed (Aeschbacher *et al.*, 1982). In another micronucleus assay, MacGregor (1979) did not observe an increase in micronuclei in bone marrow cells of mice (strain not specified) administered orally up to 1 g quercetin/kg body weight. Ngomuo and Jones (1996) also reported an absence of quercetin-related induction of unscheduled DNA synthesis in gastric mucosal cells of Wistar albino rats orally administered up to 800 mg quercetin/kg body weight. The frequency of sister chromatid exchanges assessed in bone marrow cells of female C57BL/6 mice 24 hours post-administration of 100 mg quercetin by gavage was comparable to that observed in the control group (Ishikawa *et al.*, 1985).

Orally administered quercetin consistently did not induce any significant changes in several endpoints of mutagenicity/genotoxicity (*i.e.*, micronuclei, chromosomal aberrations, sister chromatid exchange, unscheduled DNA synthesis, and alkali-labile DNA damage) in somatic cells in comparison to untreated controls in mice and rats (MacGregor, 1979; Aeschbacher *et al.*, 1982; Ishikawa *et al.*, 1985; Ngomuo and Jones, 1996; Taj and Nagarajan, 1996; Cierniak *et al.*, 2004). In contrast, when administered twice intraperitoneally to groups of male Swiss mice at levels ranging between 200 and 800 mg quercetin/kg body weight, increases in the percent of micronuclei (statistical significance not specified) were reported at 200 and 400 mg/kg body weight, but not at the highest dose level tested (Sahu *et al.*, 1981). In male and female outbred

Swiss Webster mice receiving intraperitoneal quercetin administrations at dose levels of up to 2,500 mg/kg body weight, positive results were reported in a standard micronucleus assay and in the comet assay at the mid-dose level (1,250 mg/kg body weight) and at every dose level, respectively (da Silva *et al.*, 2002). However, the relevance of these results is questionable due to the intraperitoneal route of administration of the test material, since human exposure to Quercegen's quercetin will be oral. Furthermore, no statistically significant variations were observed in micronucleus frequency evaluated in bone marrow cells of mice following intraperitoneal treatment at quercetin dose levels of up to 1 g/kg body weight (MacGregor, 1979; Caria *et al.*, 1995). Frequencies of micronuclei reported in bone marrow and peripheral blood erythrocytes of quercetin-treated mice following administration of 2 intraperitoneal doses (200 and 400 mg quercetin/kg body weight, respectively) also were comparable to those observed in controls (Ngomuo and Jones, 1996).

Results of the identified *in vivo* mutagenicity/genotoxicity tests are summarized in Table A2-8 (see Attachment 2).

In conclusion, quercetin is mutagenic in selected *in vitro* test systems; but the limited absorption of quercetin and almost complete metabolism in the intestinal tract must be considered when extrapolating these results to humans (Gross *et al.*, 1996). Quercetin is extensively metabolized *in vivo* to non-mutagenic compounds such as 3'-*O*-methylquercetin and 4'-*O*-methylquercetin, which may contribute to the absence of quercetin-induced mutagenicity *in vivo* and absence of carcinogenic activity (Zhu and Liehr, 1993). Short- and long-term animal studies with orally administered quercetin are more reliable and appropriate models for the assessment of toxicity and carcinogenicity of quercetin added to food.

Subchronic and Chronic Studies

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated quercetin for use as a coloring agent in food (JECFA, 1977). Based on the limited toxicity data available for review at the time of the evaluation, it was not possible to establish an acceptable daily intake (ADI). The International Agency for Research on Cancer (IARC) evaluated quercetin for its potential carcinogenic risk to humans in 1998 (IARC, 1999). Based on the data available to the IARC Panel, quercetin was assigned an overall Group 3 classification (*i.e.*, *not classifiable as to its carcinogenicity to humans*). Although, *limited evidence* of quercetin carcinogenicity was noted in experimental animals, evidence for carcinogenicity to humans was deemed to be *inadequate*.

In a 21-day feeding toxicity study in male Wistar rats with 2 different doses of quercetin, enhanced proteinuria was observed in the high-dose group (1,133 mg/kg body weight/day), but not in the low-dose group (285 mg/kg body weight/day) (Rangan *et al.*, 2002). With the exception of variations in the levels of fecal bile acids, no significant effects were reported in groups of male Wistar rats administered quercetin in the diet at doses of up to 1,000 mg/kg

body weight/day for 22 consecutive days (Nakamura *et al.*, 2000). No biologically significant effects were observed in either of two 28-day studies in which Swiss mice were administered quercetin in the diet at daily dose levels of up to 950 mg/kg body weight (sex of mice not specified) (Barrenetxe *et al.*, 2006) or 3,000 mg/kg body weight (female mice) (Ruiz *et al.*, 2006). Exon *et al.* (1998) noted increased natural killer cell activity in female Sprague-Dawley rats provided a nominal dose of 100 mg quercetin/kg body weight/day in the diet for a period of 7 weeks; however, all other examined parameters related to immune function, including spleen weight, were unaffected by compound administration. Significantly decreased liver α-tocopherol content and increased hepatic SOD activity, but no changes in hepatic GPx activity or urinary 8-hydroxydeoxyguanosine content (marker of oxidative stress) were observed in female BALB/c mice fed a diet containing 0.1% quercetin (providing approximately 150 mg/kg body weight/day) for a 40-day period relative to a control group (Bando *et al.,* 2007). In comparison to a control group, significant increases in the levels of hepatic and pulmonary glutathione-*S*-transferase (GST) and GSH levels were noted in an 8-week study in which male Swiss NMRI mice received up to 2.6 mg quercetin/kg body weight/day in the drinking water (Gandhi and Khanduja, 1993). In contrast, Duarte *et al.* (2001) reported a decrease in levels of liver GSH in quercetin-treated (10 mg quercetin/kg body weight/day by gavage for a period of 5 weeks) normotensive male Wistar Kyoto rats compared to untreated controls, but no changes in glutathione peroxidase (GPx) activity or glutathione reductase (GR) levels. While a slight reduction in liver GSH levels in male Wistar rats also was reported following daily gavage administrations of 10 mg quercetin/kg body weight in another 5-week study, no variations were noted in liver GST, GPx, and GR activities and plasma levels of markers of oxidative liver stress [thiobarbituric acid (TBARS) and nitrates plus nitrites (NO_x)] (García-Saura *et al.*, 2005). Choi *et al.* (2003, 2005) reported increased liver GPx activity and reductions in hepatic GSH and malondialdeyde (MDA) levels and increased GR activity in Sprague-Dawley rats administered up to 80 mg quercetin/kg body weight/day for 4 to 6 weeks. Although increases in GST and GR activities were observed in segments of the colon mucosa of Sprague-Dawley rats maintained on quercetin-supplemented diets (up to 1% or 500 mg/kg body weight/day) for 6 weeks, no changes in activities of liver enzymes were reported (Fischer *et al.*, 2002). Levels of oxidative scavenger enzymes (SOD and catalase) were not increased in Wistar rats following 3 days of daily quercetin gavage administrations of 135 mg/kg body weight (Cierniak *et al.*, 2004). In a 9-week study wherein a group of 15 male Wistar rats was administered 50 mg quercetin/kg body weight by intraperitoneal injection 5 times a week, no evident signs of nephrotoxicity were observed based upon the absence of changes in renal SOD or GR activities, plasma TBARs, or urinary or plasma markers of renal toxicity (*N*-acetyl-β-d-glucosaminidase, alkaline phosphatase, γ-glutamyl-transpeptidase, and creatinine) in the quercetin-treated group compared to vehicle-treated controls (Morales *et al.,* 2006).

The potential chronic toxicity and carcinogenicity of quercetin were evaluated in several long-term studies. In a 64-week study with F344 rats provided quercetin as a dietary admixture at concentrations of 0.1 or 0.2% (approximately 41 and 76 mg quercetin/kg body weight/day and

29 and 58 mg quercetin/kg body weight/day, in males and females, respectively), there was no increase in the incidence of neoplastic lesions and the lesions that were observed were typical of aging rats and were not attributed to quercetin exposure *per se* (Stoewsand *et al.*, 1984). Statistically significant reductions in levels of glutamic oxaloacetic transaminase (GOT) and blood urea nitrogen (BUN) were observed in male rats at the higher dose (approximately 76 mg quercetin/kg body weight/day). These variations in clinical chemistry values were not accompanied by any observed histopathological changes. Based on the absence of any adverse effects observed in rats following dietary administration of quercetin, the no-observed-effect level (NOAEL) was determined to be 76 mg quercetin/kg body weight/day, the highest dose level tested.

There were no reported treatment-related adverse effects in a 410-day feeding study in which groups of male and female rats (strain not specified) were provided up to 1% of quercetin in the diet (approximately 500 mg quercetin/kg body weight/day) (Ambrose *et al.*, 1951). Based on the absence of any adverse effects observed in rats following dietary administration of quercetin, the no-observed-effect level (NOEL) was determined to be 500 mg quercetin/kg body weight/day, the highest dose level tested.

Quercetin, the aglycone, was nominated by the U.S. Food and Drug Administration (FDA) for toxicity and carcinogenicity studies. In the 2-year NTP study, no statistically significant effects were reported in male or female F344/N rats provided quercetin in the diet at levels of up to 10,000 ppm (calculated using mean body weights and mean food consumption data to provide approximately 517 and 525 mg/kg body weight/day for males and females, respectively) (Dunnick and Hailey, 1992; NTP, 1992). Epithelial cells of the proximal segments of the gastrointestinal tract, including the glandular stomach and to a smaller degree the distal portions (*i.e.*, duodenum and colon), were heavily stained with a yellow-brown, granular pigment, which increased in intensity with increased exposure to quercetin. Both sexes of quercetin-treated animals exhibited reduced body weights and body weight gains in comparison to controls, with the differences reaching statistical significance at the highest dose level tested (40,000 ppm, which was equivalent to approximately 2,203 and 2,231 mg/kg body weight/day for males and females, respectively). The study authors reported no differences in food consumption between test and control animals and concluded that the body weight variations were attributable to quercetin toxicity. Quercetin is a non-caloric ingredient. Thus, while the absolute amount of food consumed by high-dose and control males remained comparable throughout the study period, high-dose males consumed a diet that was lower in caloric content than the diet of the control animals, but did not compensate for the caloric dilution by ingesting more chow. The body weight differences observed between high-dose females and female controls (*i.e.*, 10% at Week 25 and 13 to 15% from Week 65 to study end) were greater than in males (*i.e.*, 5% at Week 25 and 6 to 13% from Week 65 to study end). However, not only did the high-dose diet of females contain 4% more non-nutritive material compared to the control diet, but high-dose females also consumed approximately 1 and 2 grams less food (6 and 13%, respectively) than

controls between Weeks 1 and 13 and 14 and 52, respectively. Furthermore, no significant differences in body weights were observed in either sex of high-dose animals in comparison to controls in the first 13 weeks of the study, a period characterized by active growth that would be expected to be most sensitive to any compound-related toxicity. Recent *in vitro* assays have shown that quercetin inhibits GLUT2 and as such may inhibit glucose absorption from the gastrointestinal tract (Kwon *et al.,* 2007).

The variations in body weights occurred in the absence of any compound-related physiological or morphological abnormalities. Most notably, the lower body weights and body weight gains of treated animals compared to controls were not associated with any clinical symptoms of toxicity and did not adversely affect survival rates or results of hematology, clinical chemistry, and urinalysis. Increases observed in the relative organ weights of quercetin-treated males (kidney and liver) and females (liver, kidney, and brain) in comparison to controls were attributed by the study authors to the lower body weights of treated animals. No significant differences were noted in absolute organ weights. As pointed out by the study authors, the dose-dependent and significant trend observed for lower incidences of mammary gland lesions in females was likely associated with the reduction in body weights. In light of these considerations, the reductions in body weights and weight gains in animals consuming quercetin at the highest dose level were determined to be not adverse.

The renal effects that were observed in high-dose males included an increase in the severity of nephropathy and in the incidence of focal renal tubule and pelvic hyperplasia, accompanied by secondary hyperparathyroidism. Table A2-9 in Attachment 2 summarizes the incidence of renal tubular lesions observed in the NTP study. In comparison to a single occurrence of renal tubular adenoma in the control group, a total of 9 out of 50 high-dose male rats exhibited renal tubular adenoma or adenocarcinoma, as revealed following step-section analysis. The appropriateness of using step-section results in statistical analysis and comparing the incidence of post-treatment lesions with the spontaneous incidence of lesions as reported following standard examination is questionable (Hirono, 1992; Ito, 1992). Chronic nephropathy leading to hyperplasia in response to increased regenerative cell proliferation is reported to be characteristic of aging rats (NTP, 1992). As noted by the study authors, the development of the kidney lesions, which was not apparent at the interim 6- and 15-month evaluations, may possibly be a reflection of compromised detoxifying mechanisms in older animals (Dunnick and Hailey, 1992), and as such may be considered a modifying effect resulting from metabolic overload rather than specific quercetin-related toxicity (Ito, 1992). Additionally, it should be noted that not only were kidney lesions not observed prior to the 24-month evaluation, but, also, the tumors were observed only at dose levels associated with nephropathy, which is further supportive of a potential secondary response rather than a direct carcinogenic effect (Hirono, 1992). As noted in the NTP study report, in addition to the already significant age-related background incidence of kidney diseases in the male F344 rat, compound-related kidney neoplasms also are reported to occur with greater frequency in the males of this strain of rat.

For example, comparable effects related to chronic nephropathy were observed with greater severity in male rats administered hydroquinone (also a strong antioxidant) in the diet in another 2-year NTP study also conducted with the F344/N rat (NTP, 1989).

Moreover, the severity of these age-related lesions may have been further increased in the quercetin feeding study as a result of the high protein content of the NIH-07 control diet. Recently, several short- and long-term studies were conducted by the NTP in F344 rats to assess the effect of the NIH-07 diet, characterized by a protein composition of 23%, on the development of diet- and age-associated lesions compared to lower-protein (approximately 15%) non-purified diets (NTP-88, NTP-90, NTP-91, NTP-92, and NTP-2000 diets) (Rao, 1997; Rao *et al.*, 2001). Without exception, the incidence and severity of nephropathy was consistently markedly lower in male rats maintained on diets with a reduced protein composition. Accordingly, the nephropathy observed exclusively in the high-dose male test rats may have in fact been compounded by the administration of the quercetin test material in the high-protein NIH-07 vehicle diet, subsequently leading to the formation of renal lesions. Moreover, the kidney effects were not seen in female rats. Accordingly, the possible role of $\alpha_{2\mu}$-globulin-related nephropathy, which is typical of male rats, may be a contributing factor in the formation of the renal lesions in males following quercetin exposure (Ito, 1992; NTP, 1992; Rietjens *et al.*, 2005). As per the criteria established by the U.S. Environmental Protection Agency (U.S. EPA, 1991) for $\alpha_{2\mu}$-globulin-associated renal toxicity and neoplasia in male rats, the occurrence of proteinaceous casts, single-cell necrosis, and tubule hyperplasia in the male rats of this study are considered to be pathological changes consistent with $\alpha_{2\mu}$-globulin nephropathy. Although the study did not specifically assay for the presence of $\alpha_{2\mu}$-globulin, it was suggested by the study authors that differences in the deposition of the protein in male rats may have contributed to the apparent gender-related discrepancy observed with regard to the formation of kidney lesions in quercetin-fed rats (NTP, 1992). High incidences of protein casts also were observed in a 2-stage carcinogenicity study in which male F344 rats were administered quercetin in the diet at a concentration of 5% (approximately 2,000 mg/kg body weight/day) for a period of 34 weeks (Fukushima *et al.*, 1983) and enhanced proteinuria was observed at the highest dose level tested (1,133 mg/kg body weight/day) in a 21-day feeding study in male Wistar rats (Rangan *et al.*, 2002).

Most recently, Hard *et al.* (2007) re-evaluated the kidney tumors and renal histopathology of the NTP study in order to determine whether interaction with chronic progressive nephropathy was the mechanism underlying the small increase in the number of renal lesions. Chronic nephropathy leading to hyperplasia in response to increased regenerative cell proliferation has been identified as a risk factor for marginal increases in the background incidence of renal tumors and may be exacerbated by exogenous compounds. The results of the reassessment confirmed that the renal lesions in the NTP study occurred only at dose levels associated with severe chronic nephropathy (Hard *et al.*, 2007). Consistent with the criteria for the development of kidney tumors as a result of an interaction with chronic nephropathy, the majority of tumors

observed in this study were identified as small-sized adenomas, borderline with atypical tubule hyperplasia. The single case of carcinoma, along with a few adenomas and one hyperplastic focus, exhibited a distinctive phenotype characteristic of spontaneous origin. Furthermore, the re-evaluation did not reveal any cellular alterations that would be indicative of chemical-induced toxicity. Although it was previously suggested that the renal lesions observed only at the terminal evaluation may be a reflection of compromised detoxifying mechanisms in older animals (Dunnick and Hailey, 1992), the absence of any lesions at sites unaffected by chronic nephropathy does not support this hypothesis. Hard *et al.* (2007) concluded that the kidney lesions observed in the NTP bioassay were mediated by the exacerbation of chronic nephropathy (*i.e.*, increased incidences of tumor types known to be associated with late-stage chronic progressive nephropathy in the male rat). This is further substantiated by the absence of nephrotoxicity and carcinogenicity in the female rats.

In a previous 2-year feeding study, in which F344/DuCrj rats were maintained on diets containing 1.25 or 5% quercetin (calculated using mean body weights and mean food consumption data to provide approximately 427 and 497, and 1,926 and 2,372 mg/kg body weight/day for males and females, respectively) followed by an 8-week recovery period, no statistically significant histopathological effects were observed at the lower dose level (Ito *et al.*, 1989). No differences in food consumption were observed between test and control rats; however, significant reductions were observed in body weights of high-dose males and females compared to controls. High-dose males also exhibited a decrease in serum glucose levels compared to controls. The increase in relative kidney weights in high-dose males was reported to be within the normal range, and was considered to be a result of lower male and female body weights at this dose. The incidence of chronic nephropathy was elevated in both male test groups in comparison to the control group, but the increase was reported not to exceed control values reported for the laboratory and were not considered biologically significant. A numerically-greater incidence of cecal hyperplastic polyp formation was observed in both sexes at the high-doses; however, statistical significance was attained only in males and the lesions were deemed to be non-neoplastic. In females, no significant differences were observed in the incidence of cecal polyps between test and control animals. Throughout the study, none of the animals exhibited any clinical signs of toxicity and survival rates of low-dose animals were comparable to controls and higher than controls at the high-dose level (68 and 72% *versus* 56 and 66% for males and females, respectively).

Since the male cecal lesions observed by Ito *et al.* (1989) in the high-dose group were not reproduced in any other studies using similar regimes and comparable duration and no toxicologically significant effects related to the decreased body weight gains of high-dose animals were noted, the no-observed-adverse-effect level (NOAEL) for this study was presumed to be 5% (approximately 1,926 and 2,372 mg/kg body weight/day in males and females, respectively), the highest concentration of quercetin tested in this study.

In conclusion, the pivotal study establishing the safety of quercetin is the NTP study in F344/N rats following oral exposure to quercetin for a period of 2 years (Dunnick and Hailey, 1992; NTP, 1992). The NOAEL for this study was determined to be approximately 2,200 mg/kg body weight/day.

Other Relevant Short- and Long-term Toxicity Studies

With the exception of a single study which reported urinary bladder and intestinal tumors in male and female Norwegian rats following exposure to dietary quercetin (Pamukcu *et al.*, 1980), oral toxicity studies failed to demonstrate any carcinogenicity for quercetin in male and female ACI and F344 rats, ddY and A/JJms mice, and golden hamsters at concentrations of up to 10% quercetin in the diet (Saito *et al.*, 1980; Hirono *et al.*, 1981; Hosaka and Hirono, 1981; Morino *et al.*, 1982; Takanashi *et al.*, 1983). Study results are summarized in Table A2-10 (see Attachment 2).

Based on the observation that adults cows from geographical regions with natural distributions of Braken fern (BF) (*Pteridium aquilinum*) developed bladder tumors (Pamukcu *et al.*, 1976) and a number of experimental studies in several different species showing bladder, intestinal, and other tumors following treatment with BF (IARC, 1986, 1987), combined with *in vitro* study results demonstrating mutagenicity related to quercetin, a constituent of BF, Pamukcu *et al.* (1980) studied the potential carcinogenicity of quercetin. Urinary bladder tumors were reported to occur in 29% (2/7) and 17% (3/18) of male and female Norwegian albino rats receiving 0.1% quercetin in the diet (52 and 47 mg/kg body weight/day in males and females, respectively) for a period of 58 weeks, whereas intestinal tumors were observed in 6/7 males and 14/18 females. As reported previously by Boorman and Hollander (1974), it has been suggested that the discrepancy observed in the carcinogenic response between the study conducted in the Norwegian rat (Pamukcu *et al.*, 1980) and other long-term studies may be attributable to strain- or species-specific differences in tumor susceptibility, or, alternatively, variations in experimental conditions (NTP, 1992). Several authors suggested that the difference noted in the carcinogenic response between the study conducted by Pamukcu *et al.* (1980) and the remaining studies may be attributable at least in part to the differences in the basal diets used as vehicles for the administration of quercetin (Morino *et al.*, 1982; Lamson and Brignall, 2000; Okamoto, 2005). As specified by Morino *et al.* (1982), while Pamukcu *et al.* (1980) utilized a basic grain diet composed of alfalfa, cotton seed meal, corn, fish meal, malt, meat, molasses, oats, soybean meal, sunflower meal, wheat and wheat bran, a basal diet (CE-2) consisting of alfalfa, corn flour, fish meal, flour, maize, malt, milo and soybeans was employed in the Japanese studies (Saito *et al.*, 1980; Hirono *et al.*, 1981; Hosaka and Hirono, 1981; Morino *et al.*, 1982; Takanashi *et al.*, 1983). Additionally, urinary bladder tumors were not observed in 2 separate 2-stage carcinogenesis studies in which quercetin-only treated male F344 rats received up to 5% of the flavonol (approximately 2,000 to 2,500 mg/kg body weight/day) in the diet for up to 25 weeks (Fukushima *et al.*, 1983; Hirose *et al.*, 1983).

It is possible that the tumors occurred as a result of potential cross-contamination with BF, which was provided to male and female Norwegian rats in a concurrently-conducted investigation, yielding identical tumors at greater frequency. Notably, rats treated with BF-supplemented diets (Pamukcu *et al.*, 1980) and also rats treated with milk from cows fed a BF-containing diet (Pamukcu *et al.*, 1978; NAS, 1982) were reported to develop the same types of organ tumors, albeit at a higher level of incidence than those observed following treatment with quercetin. Furthermore, in an earlier preliminary study conducted by Pamukcu and others to identify the carcinogenic substance in BF, the incidence of bladder carcinoma in Swiss albino mice with quercetin pellets implanted directly into their urinary bladders (10/46; 21.7%) was comparable to that observed in the control group (10/62; 16.1%) following a 1-year period (Wang *et al.*, 1976). As noted by Hirono *et al.* (1981), dietary quercetin at concentrations of up to 5% did not display an increase in carcinogenic potential in ACI rats, a strain of rat in which both ileal and urinary bladder tumors were observed following treatment with BF (Hirono *et al.*, 1975). BF is now known to contain the carcinogen ptaquiloside, which induces ileal and bladder tumors in rats (Hirono *et al.*, 1987). Given the lack of reproducibility of the urinary and intestinal bladder tumors in all other studies of quercetin toxicity, which were conducted for longer periods of time and at higher dose levels, the possibility of cross-contamination should be considered as a possible explanation for the appearance of these tumors limited to this single study. Consequently, the Panel did not afford credence to the study conducted by Pamukcu *et al.* (1980) in the overall evaluation of quercetin safety.

In the remaining carcinogenicity studies, none of the tumors identified occurred with statistically greater incidence compared to concurrently maintained control groups. The incidence of tumor formation in F344 rats receiving 0.1% of quercetin *via* the diet (approximately 50 mg/kg body weight/day) for a period of 540 days did not differ significantly from that observed in the control group (Takanashi *et al.*, 1983). Moreover, with the exception of single incidences of lung adenoma and jejunal adenocarcinoma in male quercetin-treated rats, all other tumors identified in the test group also were identified in control animals. Quercetin administered in the diet at 5% (approximately 7,500 mg/kg body weight/day) for a period of 23 weeks to A/JJms mice did not induce a significant difference in the incidence and multiplicity of lung tumors (Hosaka and Hirono, 1981). In a 733-day golden Syrian hamster study, histopathology revealed papillomas of the forestomach and cortical adenomas of the adrenals with statistically equal frequency in the control and quercetin-test group (approximately 12,000 mg/kg body weight/day *via* the diet) (Morino *et al.*, 1982). Only a single case of adenocarcinoma of the ileum was detected in the test group, without parallel occurrence in the control group. Lesions of the cecum, colon, adrenal cortex, and testis observed by Hirono *et al.* (1981) in ACI rats at dietary quercetin concentrations of 5% (2,500 mg/kg body weight/day) and higher for at least 540 days also were observed in the control group. Likewise, with the exception of a single incidence of a heart sarcoma, not commonly observed in mice, development of leukemia, lung tumors, and forestomach and mammary gland tumors in test ddY mice (3,000 mg quercetin/kg body weight/day *via* the diet) did not differ significantly from controls (Saito *et al.*, 1980).

Although Ertürk *et al.* (1983) reported an increased incidence of liver and bile duct tumors in female F344 rats administered up to 2% quercetin in the diet (approximately 1,000 mg/kg body weight/day) over the course of the animals' lifetime, the results were presented in abstract form only and the statistical significance of the results was not specified. Likewise, pathological changes of the colon and ileum (grossly dilated cecum, intestinal mucosa atrophy, crypts and goblet cells hypertrophy, and lymphoid hyperplasia of the colonic and ileal mucosa and submucosa) were reported in another study abstract following quercetin administration in the diet at a concentration of 5% (approximately 2,500 mg/kg body weight/day) to rats for a period of 6 months (strain not specified) (Bokkenheuser and Winter, 1990). However, due to the lack of availability of full reports by these authors, the usefulness of these studies in a safety assessment of quercetin is limited.

Several initiation-promotion studies have been performed to determine specifically the effect, if any, of quercetin on chemical-induced carcinogenesis, most of which also included a quercetin control group, and as such, are relevant to the safety assessment of the flavonoid. The results of these studies are summarized in Table A2-11 (see Attachment 2).

In a 36-week long study, male F344 rats received 1% quercetin in a powdered basal diet (approximately 500 mg/kg body weight/day) (Akagi *et al.*, 1995). All surviving animals were necropsied at the end of the 36-week treatment period, and liver and kidneys were excised and weighed. Organs examined for incidence of tumor development included the liver, kidneys, lungs, esophagus, stomach, intestines, and urinary bladder. In the group administered quercetin without carcinogen treatment, no neoplastic lesions or other toxicologically significant adverse effects were observed.

Hirose *et al.* (1983) examined the potential initiating and promoting effects of quercetin on 2-stage urinary bladder carcinogenesis in groups of 25 male F344 rats. One of the groups was provided 5% dietary quercetin (approximately 2,500 mg/kg body weight/day) for the first 4 weeks and received ordinary tap water during the following 29 weeks, while another was supplemented with 5% quercetin during the final 25 weeks of the study. Histopathological examinations of the liver and kidneys were unremarkable. Moreover, no urinary bladder lesions were detected in the animals. In another study conducted to assess the potential promoting properties of a range of chemicals on urinary bladder carcinogenesis following initiation with *N*-nitroso-*n*-butyl-(4-hydroxybutyl)amine (BBN), the un-initiated quercetin-control group of male F344 rats (30/group) was provided quercetin at a dietary concentration of 5% (approximately 2,000 mg/kg body weight/day) for a period of 34 weeks (Fukushima *et al.*, 1983). The urinary pH was noted to be slightly acidic in quercetin-treated animals as early as 12 weeks into the study period; however, neither relative nor absolute urinary bladder weights were observed to differ in the quercetin-treated test group in comparison to an untreated control group, and histopathological examination of the urinary bladder was unremarkable, although protein casts of the tubules were reported to occur with high incidence in quercetin-treated rats. No tumors of

the kidneys were reported in male Syrian hamsters administered quercetin in the diet providing daily doses of up to approximately 3,600 mg/kg body weight (Zhu and Liehr, 1994). In hamsters administered up to 4,500 mg quercetin/kg body weight/day for as long as 709 days, 1 uterine leiomyoma was detected in the quercetin-only test group, without parallel incidence in the control animals (Morino *et al.*, 1982). Papillomas of the forestomach, presenting as single sessile polyps, occurred with comparable frequency in the quercetin-only treated groups and the basal-control group.

In female CD-1 mice, receiving quercetin at dietary concentrations of 2% (approximately 3,000 mg/kg body weight/day) for a period of 9 weeks, no effect was noted on the proliferative indices of colonic epithelial cells in comparison to a basal diet-treated group (Deschner *et al.*, 1991). Following dietary administration of quercetin to male F344 rats at a concentration of 500 ppm (approximately 25 mg/kg body weight/day) for 5 consecutive weeks, no symptoms of overt toxicity were reported, and histopathological examination of the liver, kidneys, lung, and heart was unremarkable (Tanaka *et al.*, 1999). Additionally, aberrant crypt foci (ACF) formation was not observed in the distal colon of the quercetin-only treated animals. Quercetin (approximately 500 mg/kg body weight/day) provided *via* the diet to male and female Sprague-Dawley rats for a period of 459 days was not associated with any incidences of intestinal tumors as revealed at study-end necropsy (Kato *et al.*, 1984). No duodenal tumors were reported in a 16-week study, in which a quercetin-only control group of male C57BL/6 mice was provided 2% quercetin (3,000 mg/kg body weight/day) in the diet without chemical-induced duodenal tumorigenicity (Matsukawa *et al.*, 2002). Likewise, in a group of 5 male Wistar rats maintained on a 1% quercetin-supplemented diet, providing approximately 500 mg quercetin/kg body weight/day, for 16 consecutive weeks, no duodenal tumors were observed (Werner *et al.*, 1985). In a 45-week 2-stage carcinogenicity study, a single occurrence of adenocarcinoma of the small intestine, but no colon tumors, were identified in a group of 12 male F344 rats receiving quercetin only at a relatively high dose level of approximately 1,680 mg/kg body weight/day (Pereira *et al.*, 1996). No colorectal tumors were observed in quercetin-only control groups male F344 rats provided up to 10 g quercetin/kg diet (approximately 450 to 900 mg quercetin/kg body weight/day) for a period of 41 weeks (Dihal *et al.*, 2006).

Following mating, groups of Wistar rats were maintained on 0 or 1% quercetin-enriched diets (approximately 0 or 500 mg/kg body weight/day) and females were allowed to deliver (Barotto *et al.*, 1998). Subsequently, pups assigned to a non-initiated saline-control group at 30 days of age were placed on the same diet as had been administered to their respective mothers [*i.e.*, 0 (saline only group) or 1% quercetin (saline-quercetin group)] for a period of 24 weeks. At the end of the study period, the pancreas and liver were excised immediately for further examination and weighed. Final body weights and relative liver weights of rats in the saline-quercetin group did not differ from values reported for the saline only group. Relative pancreas weights of females in the saline-quercetin group were significantly increased in comparison to

the saline only group; however, no pathological variations of the pancreas were identified in either sex of the saline-quercetin-treated animals.

The promotional activity of quercetin on chemical-induced organ-specific carcinogenesis was less consistent. More specifically, while quercetin was shown to enhance the formation of azoxymethane (AOM)-induced gastrointestinal tumors in the study conducted by Pereira *et al.* (1996), as well as following N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) initiation (Werner *et al.*, 1985; Matsukawa *et al.*, 2002), in other studies, quercetin demonstrated inhibitory effects on colon and gastrointestinal tumorigenesis, not only following AOM induction (Deschner *et al.*, 1991; Exon *et al.*, 1998; Tanaka *et al.*, 1999; Yang *et al.,* 2000; Volate *et al.,* 2005; Choi *et al.,* 2006; Dihal *et al.,* 2006), but also when gastrointestinal carcinogenesis was induced with methylazoxymethanol acetate (MAM) and DMBDD[1] (Kato *et al.*, 1984; Akagi *et al.*, 1995). However, the study conducted by Pereira *et al.* (1996) cannot be considered to be a true 2-stage carcinogenicity study, as AOM was administered subcutaneously 8 days following initiation of quercetin supplementation in the diet. Therefore, the higher incidence of colon carcinomas in the quercetin-treated rats may be a result of an interactive effect rather than a true potentiation of chemical-induced carcinogenicity, and as such is considered by the Panel to be of limited relevance to the assessment of quercetin safety in the human diet. Also, it should be noted that in the study evaluating the effect of quercetin on ENNG-induced duodenal tumorigenicity, all 19 animals maintained in the ENNG-control group exhibited duodenal tumors (Matsukawa *et al.*, 2002). Quercetin administration, therefore, was reportedly associated with an increase in the multiplicity and size of tumors, but not in the incidence. As noted by the Panel, the dose of the initiating agent administered, resulting in a 100% tumor incidence, was too high to evaluate the promotional potential of quercetin. The Panel deemed this study not to be appropriate for inclusion in the evaluation of the potential of quercetin to promote tumor development. Zhu and Liehr (1994) reported quercetin-related promotion of tumor formation in the renal tissues in hamsters administered subcutaneous estradiol implants. Increased incidences of pancreatic dysplastic foci were noted in rats receiving a single intraperitoneal administration of *N*-nitrosomethylurea (NMU) at 3 days of age, subsequently followed by quercetin treatment for 20 weeks, which was initiated at weaning (30 days of age), subsequent to *in utero* exposure (Barotto *et al.*, 1998; Valentich *et al.,* 2006). In contrast, quercetin exerted protective properties with respect to the formation of NMU- or 7,12-dimethylbenz[a]anthracene (DMBA)-initiated mammary gland tumors (Verma *et al.*, 1988; Pereira *et al.*, 1996; Devipriya *et al.*, 2006), N-nitroso-n-butyl-(4-hydroxybutyl)amine (BBN)-induced urinary bladder neoplasms (Fukushima *et al.*, 1983), and benzo[a]pyrene-induced blood lymphocyte damage and pulmonary precancerous pathologic changes (Jin *et al.*, 2006). In several other studies quercetin did not act to promote (Morino *et al.*, 1982; Hirose *et al.*, 1983; Ito *et al.*, 1988) nor

[1] Quercetin administered subsequently to or concurrently with a number of carcinogens, which included intraperitoneal injections of diethylnitrosamine and N-methylnitrosourea, subcutaneous injections of 1,2-dimethylhydrazine and 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine and 0.1% 2,2'-dihydroxy-di-n-propylnitrosamine provided in the drinking water, collectively termed the DMBDD treatment.

initiate (Kato *et al.*, 1985; Sato *et al.*, 1987) chemical-induced carcinogenesis in laboratory animals.

In summary, the weight of evidence from long-term toxicology and carcinogenicity studies of dietary quercetin demonstrates the lack of systemic toxicity, the non-carcinogenicity, and the lack of promoting activity of quercetin.

Reproductive and Developmental Studies

The relative binding affinity (RBA) of quercetin dihydrate was determined from solid-phase competition experiments against the human estrogen receptor α, hERα (Kuiper *et al.*, 1998). The RBA for quercetin was 0.01% relative to the natural hormone E2 (RBA = 100%) indicating very weak affinity for the receptor. More recently, high-mass matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) combined with chemical cross-linking was used to monitor the ligand-dependent dimerization of the human estrogen receptor α ligand binding domain (hERα LBD) in solution (Bovet *et al.*, 2009). Using this analytical assay quercetin was identified as a potential ER ligand with a Z-value (relative homodimer abundance) of approximately 10% compared to E2.

Dietary quercetin administration to ovariectomized female Sprague-Dawley rats (11 or 12 animals per group) at daily levels of 3.55 or 18.42 mg quercetin/animal for a period of 3 months (approximately 8.9 and 46.1 mg/kg body weight/day, respectively) did not produce any estrogenic effects (Rachoń *et al.,* 2008). Specifically, dietary supplementation with quercetin had no significant effect on pituitary luteinizing hormone (LH) gene expression, serum LH levels, uterine weight, or on the expression of uterine estrogen-regulated genes, insulin-like growth factor 1, the progesterone receptor, or complement protein 3 mRNA in comparison to controls provided a soy-free diet. Histopathological examination of the uterus revealed no signs of estrogenic stimulation or any other changes in comparison to the control group.

Drosophila melanogaster females heterozygous for a variety of *X* chromosome markers were raised on a 5% quercetin diet and then crossed individually to appropriate tester males (Schramm *et al.*, 1998). The progeny of this cross were analyzed to determine the fate of the maternal chromosomes in meiosis. This experimental design allowed identification of effects of quercetin on the number of progeny produced, the level of meiotic recombination, and the amount of *X* and 4th chromosome non-disjunction. No significant effect on either non-disjunction levels or the percentage distribution of exchange bivalents was observed. A significant increase on the number of offspring was observed with F1 and F2 generations of flies raised on a 5% quercetin diet producing >10% more progeny than flies raised on a control diet. The authors concluded that "...current or increased flavonoid intake by humans will not pose a risk to meiotic fidelity."

The protective effect of quercetin against oxidative damage of 4-nitrophenol (PNP) on cultured testicular cells of Day 18 male embryonic chickens was studied (Mi *et al.*, 2009). Embryos were treated with quercetin (1.0 µg/mL) alone (control) or in combination with PNP (10^{-7} - 10^{-5} M) for 48 hours. Oxidative damage was estimated by measuring cell morphology and viability, content of malondialdehyde (MDA) activity of superoxide dismutase (SOD) and glutathione peroxidation (GSH-Px) activity. PNP exposure at 10^{-5} M induced condensed nuclei, vacolated cytoplasm and a decrease in testicular cell viability and spermatogonial cell number. Exposure to PNP also induced lipid peroxidation, and decreased GSH-Px and SOD activity. However, simultaneous exposure of quercetin with PNP restored these parameters to the same levels as control. The study also demonstrated that *in vitro* exposure to a high concentration of quercetin (1.0 µg/mL) was not toxic to germ cells under the conditions of this study.

Evaluated *in vitro* in non-human mammalian germ cells, some variable results were reported with respect to sperm motility and viability following quercetin exposure (Nass-Arden and Breitbart, 1990; Swisher *et al.*, 1994; Trejo *et al.*, 1995). At concentrations as low as 5 µM, quercetin was reported to induce an irreversible and dose-dependent reduction in human sperm motility, as well as adversely affect sperm viability at higher concentrations (50 to 100 µM) (Khanduja *et al.*, 2001).

Examined *in vivo* in male (C57 x BALB/B)F1 mice, significant sperm abnormalities were reported at 3 and 5 weeks following a 5-day quercetin treatment period during which animals were treated with intraperitoneal doses of up to 32 mg quercetin/kg body weight/day (Rastogi and Levin, 1987). As noted by the authors, intraperitoneally administered quercetin was not metabolized by the intestinal microflora (or epithelium) prior to its contact with the germ cells, which may potentially account for the significant elevation in the percent of abnormal cells. In a study in which quercetin was provided orally at dose levels of up to 150 mg/kg body weight/day to male Swiss albino mice, flavonoid treatment did not induce any significant increases in the frequency of abnormal sperm (Nandan and Rao, 1983).

No signs of reproductive or developmental toxicity were reported in F344 rats following parental oral exposure to dietary quercetin at dose levels of up to approximately 58 and 76 mg/kg body weight/day in females and males, respectively, for a period of 64 weeks (Stoewsand *et al.*, 1984). Likewise, with the exception of a significant reduction in the weights of 20-day old fetuses at the 2 highest dose levels following single quercetin administration, and at the lowest and highest dose levels following repeat quercetin exposure, maternal Sprague-Dawley rat reproduction was not affected following administration of the flavonol by gastric intubation at doses of 2, 20, 200, or 2,000 mg/kg body weight/day on a single occasion [Gestation Day (GD) 9] or on GD 6 through GD 15 (Willhite, 1982). Furthermore, dose-dependent teratogenic anomalies related to quercetin administration were not observed. The potential germinal effects of quercetin were evaluated in inbred Swiss mice and Wistar rats, receiving 2 intraperitoneal administrations of quercetin at 24-hour intervals at dose levels of up to 400 and 300 mg/kg body

weight, respectively (Aravindakshan *et al.*, 1985). Following the 2nd injection, males were paired with 2 untreated females and placed on a 1-week mating schedule for a total of 6 consecutive pairings. At 14 to 15 days following the half-point of each 7-day mating period, females were necropsied. At dose levels of 300 mg/kg body weight/day and greater, quercetin treatment was associated with an impairment of mouse, but not rat, fertility, as well as a reduction in the mean number of implantation, which also was primarily limited to the mice.

As part of a 64-week dietary toxicity study, Stoewsand *et al.* (1984) also included an evaluation of several reproductive and developmental indices. Parameters monitored included parturition index, mean viable litter size, live birth index, 3-day survival, lactation index, birth weight, and 21-day weight. Prior to mating at 12 to 13 weeks into the study period, male and female F344 rats were maintained on powdered basal diets containing 0 (basal control), 0.1, or 0.2% quercetin (approximately 40.7 and 75.9 mg/kg body weight/day, respectively, for males and 29.0 and 57.5 mg/kg body weight/day, respectively, for females). During periods of gestation and lactation, rats were provided agar-based diets. Compared to untreated controls, no differences were observed in any of the parameters examined, including pups' birth weights.

The data summarized above demonstrates that quercetin is not a reproductive or development toxin.

Clinical and Epidemiological Studies

No adverse effects were reported in a group of 10 healthy human male and female subjects (mean age = 33.5 ± 7.5) following ingestion of single oral doses of quercetin (8.5 mg/kg body weight/day or approximately 500 mg for an average 60 kg individual) in a study demonstrating a quercetin-related increase in adenosine levels (Blardi *et al.*, 1999). With the exception of headaches experienced early in the treatment period by one subject, and a mild tingling sensation reported by another study participant throughout the trial, ingestion of a daily quercetin dose of 1,000 mg (approximately 17 mg/kg body weight/day) for a period of 1 month was not associated with any significant adverse effects in adult males (ages 26-72) diagnosed with category III-a or III-b chronic pelvic pain syndrome (Shoskes *et al.*, 1999). Administration of 3 mg quercetin/day from 1,500 mg of a guava leaf extract for 3 consecutive days did not result in any quercetin-related adverse effects in a group of 50 adults (23M, 27F; ages 20 to 55) with acute diarrheic disease (*i.e.*, infectious gastroenteritis) (Lozoya *et al.*, 2002). Laboratory test results (hematology, clinical chemistry, urinalysis) were unremarkable in groups of male and female patients (n = 86 and 84 in the 360 and 720 mg groups, respectively) with chronic venous insufficiency receiving up to 720 mg daily of the red vine leaf extract, AS 195 (*folia vitis viniferae*), containing unspecified levels of quercetin-3-*O*-β-glucuronid and isoquercetin for 12 consecutive weeks (Kiesewetter *et al.*, 2000). Most adverse effects reported by study participants consisted of mild to moderate gastrointestinal disturbances. Currently in the recruitment phase, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH) has developed a protocol for a double blind,

placebo-controlled trial investigating the potential effects of 1 or 2 grams of supplemental quercetin on glucose absorption (NLM, 2010).

In several studies conducted to investigate various potential effects of quercetin on immune function, risk factors for heart disease, or exercise-induced oxidative stress among others, the ingestion of quercetin by healthy subjects (males and females, group sizes ranging from 11 to 63; mean ages for treatment and control groups ranged from 20 to 46 years) at a daily dose of 600 to 1000 mg/day for periods of 1 to 6 weeks was not associated with any reports of adverse effects (Conquer *et al.*, 1998; Cureton *et al.*, 2009; Davis *et al.*, 2010; Dumke *et al.*, 2009; Henson *et al.,* 2008; MacRae and Mefferd, 2006; McAnulty *et al.*, 2008; Nieman *et al.,* 2007a,b,c, 2009, 2010; Quindry *et al.,* 2008); however, the incidence and/or severity of adverse events was not reported for any of these trials.

No adverse effects were reported in a 28-day cross-over study evaluating the potential effects of quercetin on blood pressure in 47 hypertensive subjects (26M, 21F) consuming 730 mg quercetin/day; however, as in the studies conducted with healthy study participants, the incidence and/or severity of adverse events was not reported (Jalili *et al.,* 2006). In another 28-day cross-over trial designed primarily to investigate the potential effects of quercetin on blood pressure, adverse effects related to the consumption of quercetin (730 mg/day) were reported not to occur in a group of male and female subjects with pre-hypertension (n=19) and stage 1 hypertension (n=22) (Edwards *et al.*, 2007).

To investigate the effects of quercetin supplementation (50, 100, or 150 mg/day) on bioavailability and potential antioxidant status, inflammation, and metabolism, 35 healthy subjects (18M/17F; mean age 26.2 years) received quercetin or placebo for 2 weeks (Egert *et al.* 2008). The study was a double-blind, parallel-group design consisting of a 2-week washout period. Quercetin supplementation resulted in a significant, dose-dependent accumulation of quercetin in plasma; however, there were no changes with respect to possible risk markers of cardiovascular disease (*e.g.*, antioxidant status, oxidized LDL, inflammation, or metabolism). No adverse effects of quercetin intake were reported (Egert *et al.* 2008). In a double-blind, randomized, placebo-controlled, crossover study to investigate the effects of quercetin supplementation on cardiovascular risk factors and biomarkers, ninety-three obese or overweight subjects (42M/51F; 25-65 years of age), with metabolic syndrome, were instructed to take a total of 6 placebo or quercetin capsules per day (representing 150 mg quercetin/day) for 6 weeks (Egert *et al.*, 2009). Changes from baseline and placebo among all of the subjects receiving quercetin supplementation included the following statistically significant decreases: systolic blood pressure, pulse rate, HDL-cholesterol concentrations, and plasma concentrations of atherogenic oxidized LDL. Quercetin supplementation had no adverse effects on blood parameters of liver and kidney function, hematology or serum electrolytes. The authors concluded that these findings provide further evidence to support the safety of quercetin and

efficacy of 150 mg quercetin/day for protection against cardiovascular disease (Egert *et al.*, 2009).

In an observational study, 5 patients (3M, 2F) 21 to 54 years of age with familial adenomatous polyposis (FAP) ingested daily capsules providing 60 mg of quercetin and 1,440 mg of curcumin for a mean duration of 6 months (Cruz-Correa *et al.,* 2006). No abnormal clinical chemistry test results (complete blood count, blood glucose, blood urea nitrogen, serum creatinine, electrolytes, and bilirubin) were reported in comparison to baseline. Two mild gastrointestinal effects, transient nausea in 1 patient at the beginning of the trial (which subsided after the 3rd day of the study without recurrence) and loose stools in another, were reported by subjects consuming test capsules; however, since the capsules contained both curcumin and quercetin, it is not possible to determine whether the gastrointestinal disturbances were related to quercetin *per se*.

A randomized, double-blind, placebo-controlled 12-week study was recentlly conducted in which subjects (male and female, ages 18 to 85 years) were randomized to consume a placebo (n = 335) or 500 (n=334) or 1,000 (n=333) mg of quercetin daily (Nieman, 2008; Shanely *et al.*, 2010). There were no significant group differences over time (pre-supplementation *versus* post-supplementation) in clinical chemistry measurements with the exception of decreases in serum creatinine and increases in glomerular filtration rate for the quercetin-treated groups ($p<0.05$), which remained within the reported normal ranges for humans (Murray *et al.*, 2000). Over the course of the study, there were no differences observed in the levels of reduced or oxidized glutathione among the 3 treatment groups. It was concluded that supplementation with 500 or 1,000 mg quercetin/day for 12 weeks was safe in this study as there were no adverse symptoms or change in blood diagnostic chemistries and GSH.

In an extensive phase I clinical trial designed to investigate potential quercetin-related anti-proliferative properties in patients not responsive to typical cancer treatments, neither dose-limiting toxicity, as determined by the onset of grade 2 renal toxicity, nor any other adverse or side effects, except for mild pain at the injection site and transient flushing immediately following treatment, were observed at quercetin dose levels of up to 11.4 mg/kg body weight (equivalent to 684 mg quercetin, based on a 60 kg individual) administered intravenously every 3 weeks (Ferry *et al.*, 1996). At the higher dose levels of up to 54.1 mg/kg body weight (equivalent to 3246 mg), more pronounced and longer-lasting pain, dyspnea, emesis, and nephrotoxicity (grades 1 to 4) were reported in test subjects. At the lowest dose level of 25.5 mg/kg body weight (equivalent to 1494 mg) used in the weekly quercetin-administration protocol, grade 2 nephrotoxicity developed in only 1 out of 6 subjects; however, more severe nephrotoxicity was observed in the first trial with 3-week intervals between quercetin treatments at this dose level. It was noted that renal toxicity was ameliorated when quercetin administration was preceded by prehydration of subjects. Regardless, taking into consideration the low bioavailability and extensive metabolism of ingested quercetin, the relevance of this study to a safety assessment

of quercetin intended for use in food is not easily defined. More specifically, based on the ADME data indicating a total (free and conjugated) plasma quercetin level of 447 µg/L following ingestion of 114 mg quercetin (Janssen *et al.*, 1998), an oral dose of much greater than 10,000 mg would be required in order to provide a plasma quercetin level comparable to that following intravenous administration of 722 mg quercetin (approximately 150 mg/L).

The safety of quercetin is corroborated by a series of epidemiological studies, which consistently demonstrate an absence of any correlation between flavonoid intakes (up to 68 mg total flavonoids/day), represented in large proportion by a high quercetin content, from the habitual diet and incidence of cancer (Hertog *et al.*, 1994, 1995; Goldbohm *et al.*, 1995; Lin *et al.*, 2006). Knekt *et al.* (1997, 2002) and Lam *et al.* (in press) have demonstrated an inverse association between total flavonoid intake (up to approximately 40 mg/day), 95% of which was determined to be quercetin, and overall cancer incidence, as well as incidence of lung cancer specifically.

Most recently, three pharmacokinetic studies have demonstrated that quercetin exposure (1500 mg/day) does not pose a risk to patients taking warfarin or digoxin, nor does it interfere with glucose levels in individuals with type 2 diabetes (Prism Research, 2010a,b,c). These studies are further summarized in the section entitled "Effects on Enzymes and Other Biochemical Parameters."

In conclusion, the human clinical studies summarized above, corroborating epidemiological studies, and recently conducted human pharmacokinetic studies demonstrate the safety of quercetin in diverse human populations.

Safety of Potential Degradation Products

The products resulting from possible quercetin degradation (*i.e.*, phloroglucinol, phloroglucinic acid, and protocatechuic acid (PCA)) are known to occur ubiquitously in nature and are not expected to result in any adverse effects on human health. No signs of toxicity were reported in rats exposed to PCA at a dietary concentration of 1000 ppm (~50 mg/kg body weight/day) for 32 weeks (Tanaka *et al.*, 1993) and there were no effects in mice given drinking water with 0.01% PCA for 60 days (Nakamura *et al.*, 2001). In a human clinical study to assess the efficacy of phloroglucinol (80 mg/day) for acceleration of labor, there were no side effects reported, and no toxic effects were noted in either the mother or fetus (Tabassum *et al.*, 2005). Additionally, data related to the metabolic fate of phloroglucinol and protocatechuic acid indicate that both compounds, much like quercetin, are subjected to extensive phase I and II metabolic reactions following absorption and are subsequently rapidly excreted (Dollo *et al.*, 1999; Xu *et al.*, 2007). Therefore, any unmitigated degradation of quercetin to these compounds is not expected to result in any adverse effects on human health.

SUMMARY

Quercegen intends to market high-purity food-grade quercetin (≥99.5%) as a food ingredient. Quercetin is a naturally-occurring polyphenolic flavonol commonly consumed in fruits and vegetables, such as onions and apples, which are the primary dietary sources of quercetin, containing the flavonol at levels as high as approximately 350 ppm (expressed as the aglycone) (Hertog *et al.*, 1992; Day and Williamson, 1999; Harnly *et al.*, 2006). In the United States, dietary consumption of the flavonol glycosides, expressed as quercetin equivalents, was estimated to be approximately 100 mg/day; however, in high-end consumers of fruits and vegetables, levels as high as 200 to 500 mg/day have been reported (Jones and Hughes, 1982). Quercegen's quercetin will be added to a variety of food products including beverages and beverage bases, grain products and pastas, processed fruits and fruit juices, and soft candies at use-levels providing up to 500 mg of quercetin/serving depending on the particular food category.

Under the conditions of intended use of the ingredient, the total population all-user mean intake of quercetin was estimated to be 205 mg/person/day or 4 mg/kg body weight/day. The heavy consumer (90th percentile) all-user intake of quercetin from all proposed food-uses was estimated to be 461 mg/person/day or 9 mg/kg body weight/day. The traditional type of intake methodology that was utilized to generate estimates of quercetin intake is generally considered to be 'worst case' as a result of several conservative assumptions made in the consumption estimates, and therefore, the actual exposure to quercetin is expected to be more restricted than calculated by this method. The all-user intake of quercetin based on all proposed food-uses was estimated for infants (ages 0 to 2); the 90th percentile intake was estimated to be

53 mg/kg body weight/day; however, the proposed uses do not include foods intended for infants since the Targeted Population is exercising adults.

For adult exercising Targeted Population consumers who would intentionally seek high-purity quercetin-containing foods and beverages, consumption is estimated at 1000 mg quercetin/day. It is possible that these adult exercising consumers who were to select high-purity quercetin-containing foods and beverages would consume more than two to four servings/day for short periods of time that would provide 1250 or 1500 mg quercetin/day. However, long-term consumption at this level is considered unlikely based on premium pricing and the limited number of target foods. The Non-targeted Population (particularly infants and children) are expected to consume high-purity quercetin at much lower levels and, in fact, their consumption is expected to be much lower than estimated by the traditional method. The safety of the anticipated maximum daily intake foreseen by the Targeted Population adult exercising consumers is supported by data summarized in the following section, Safety Assessment.

The Total Population maximum estimated daily background intake of quercetin from naturally occurring sources was 258.2 mg/day, which is within the published range of 200-500 mg/day for high-end consumers of fruits and vegetables (Jones and Hughes, 1982). Therefore, a Targeted Population consumer with a daily intake of 1000 mg high-purity quercetin, who also was high-end consumer of fruits and vegetables, may consume approximately 1250 mg quercetin/day. This daily intake is also considered safe based on the totality-of-the-evidence.

Quercetin is produced by Merck S/A (Brazil) in accordance with local cGMP, which are consistent with the ICH's guideline for good manufacturing practice for active pharmaceutical ingredients (Q7A), and meets appropriate food grade specifications. The manufacturing process for quercetin consists of extraction of the quercetin glycoside, rutin, from Fava d'Anta beans or, alternatively, Uncaria leaves, hydrolysis of the glycoside to yield the aglycone, and extensive, step-wise purification. In order to ensure a consistent product, Merck S/A has established rigorous chemical and microbiological specifications. Batch samples are routinely assayed to verify compliance with specifications.

Quercetin was reported to be mutagenic and genotoxic *in vitro*. These results have been reported for other common antioxidants; for example vitamin C and TBHQ. This may be due to their pro-oxidant activity at unrealistically high concentrations, which are not likely to be attainable *in vivo*. The *in vitro* mutagenic/genotoxic properties of quercetin have not been replicated *in vivo* following oral administration.

The formation of albumin-quercetin complexes following absorption of the aglycone may prolong its elimination and consequently increase the possibility for the compounds to dissociate and interact with cellular proteins and DNA (Skibola and Smith, 2000). However, the limited bioavailability of orally administered quercetin, extensive intestinal degradation, and post-absorption enzymatic metabolism of the absorbed portion may act in concert to limit the

potential for *in vivo* adverse effects following quercetin consumption. This supports the irrelevancy of the *in vitro* findings in the safety assessment of oral quercetin. Quercetin is extensively metabolized to non-mutagenic compounds such as 3'-*O*-methylquercetin and 4'-*O*-methylquercetin, which may explain the lack of *in vivo* mutagenicity and carcinogenicity (Zhu and Liehr, 1993). Although both animal and human studies have demonstrated that following oral consumption of quercetin as much as 60% of the dose is absorbed (as total quercetin), extensive metabolism as a result of the first-pass effect ensures that the free unconjugated quercetin aglycone circulates in plasma at extremely low concentrations. As such, short- and long-term animal studies with oral exposure are likely a more reliable and appropriate model for the assessment of the toxicity and carcinogenicity of quercetin.

In vitro evidence that quercetin inhibits monosaccharide absorption is equivocal (Kwon *et al.*, 2007; Cermak *et al.*, 2004; Strobel *et al.*, 2005; Barrenetxe *et al.*, 2006); whereas the available human study demonstrates that daily oral intake of a 1500 mg quercetin liquid formulation for five days does not impact blood glucose levels as compared to placebo in subjects with type 2 diabetes (Prism Research, 2010a).

In vitro data indicate that quercetin may induce or inhibit the activity of metabolizing enzymes belonging to the CYP450 family and/or are substrates of P-glycoprotein (P-gp). In most cases, the animal data support the *in vitro* findings when quercetin is administered orally and prior to the drug (Hsiu *et al.*, 2002; Choi *et al.*, 2004a; Choi and Han, 2004; Choi and Li, 2005; Umathe *et al.*, 2008; Bansal *et al.*, 2008; Wang *et al.*, 2004; Shin *et al.*, 2006; Li and Choi, 2009). However, the preponderance of human data do not support a conclusion that oral intake of quercetin interferes with drug bioavailability, since 5 of 7 clinical studies show no changes in drug bioavailability following repeated exposure of up to 1500 mg of quercetin/day (Rashid *et al.*, 1993; DiCenzo *et al.*, 2006; Choi *et al.*, 2004b; Kim *et al.*, 2005, 2009; Prism Research, 2010b,c).

Although quercetin possesses antioxidant properties, under certain *in vitro* conditions quercetin acts as a prooxidant (i.e. "the quercetin paradox") (Boots *et al.*, 2007). *In vivo*, however, there are a number of mechanisms in place to protect against the possible prooxidant effects of quercetin (*e.g.* low bioavailability of quercetin following oral intake, methylation, physiologic conditions, albumin binding, etc.) (Boots *et al.*, 2007; Harwood *et al.*, 2007).

As an antioxidant, GSH binds reactive quercetin and as such a potential exists for GSH depletion following quercetin exposure; however, in the human study that monitored GSH levels in >1000 subjects during an extended period of quercetin exposure (1000 mg quercetin/day for 12 weeks), there was no impact on oxidized or reduced GSH levels (Nieman, 2008; Shanely *et al.*, 2010).

Long-term toxicity and carcinogenicity studies failed to demonstrate quercetin-related toxicity or carcinogenicity (Ambrose *et al.*, 1951; Saito *et al.*, 1980; Hirono *et al.*, 1981; Hosaka and

Hirono, 1981; Morino *et al.*, 1982; Takanashi *et al.*, 1983; Stoewsand *et al.*, 1984; Ito *et al.*, 1989; NTP, 1992). A statistically significant increase in cecal non-neoplastic polyps was reported in male F344/DuCrj rats fed 5% quercetin (1,926 mg/kg body weight/day) in the diet for 2 years, but not in females exposed to 2,372 mg/kg body weight/day (Ito *et al.*, 1989). No lesions of the gastrointestinal tract were observed in the subsequently conducted 2-year NTP study (1992) in male or female F344/N rats administered quercetin *via* the diet at a level providing a similar daily dose. No statistically significant differences in intestinal tumors were observed between treated and control ddY mice provided 2% quercetin (approximately 3,000 mg/kg body weight/day) in the diet for their life span (microscopy included examination of duodenum, jejunum, ileum, cecum, and colon) (Saito *et al.*, 1980). In Syrian hamsters provided quercetin at 4% (4.8 g/kg body weight/day) or 10% (12 g/kg body weight/day) in the diet for at least 700 days, 1/20 ileal adenocarcinoma was reported in test females at the higher concentration (Morino *et al.*, 1982). Male and female ACI rats received quercetin as a dietary admixture at levels of up to 5% (approximately 2,500 mg/kg body weight/day) for 540 days and 10% (approximately 5,000 mg/kg body weight/day) for 850 days (Hirono *et al.*, 1981). At the 5% level, 1/8 cecal adenoma was reported in males and at the 10% level, 1/19 cecal adenoma and 2/19 cecal adenocarcinomas were reported in males. In 2-stage carcinogenicity studies in which quercetin-only control groups were included, no significant increases were observed in the incidence of upper or lower intestinal tumors in Syrian hamsters (Morino *et al.*, 1982), C57BL/6 mice (Matsukawa *et al.*, 2002), or F344 rats (Pereira *et al.*, 1996) administered quercetin *via* the diet for at least 16 weeks (and up to 700 days) at dose levels ranging from 1,680 to 4,500 mg/kg body weight/day. Since the male cecal lesions observed by Ito *et al.* (1989) in the high-dose group were not reproduced in any other studies using similar regimes and comparable duration and no toxicologically significant effects related to the decreased body weight gains of high-dose animals were noted, the no-observed-adverse-effect level (NOAEL) for this study was presumed to be 5% (approximately 1,926 and 2,372 mg/kg body weight/day in males and females, respectively), the highest concentration of quercetin tested in this study.

The authors of the NTP study conducted in 1992 reported a significant dose-dependent reduction in body weight gains of males and females and an increased severity of chronic nephropathy, hyperplasia, and neoplasia of the renal tubular epithelium (causing primarily benign tumors of the renal tubular epithelium) in male, but not female rats exposed to 40,000 ppm quercetin in the diet (approximately 2,200 mg/kg body weight/day) for 2 years. It was concluded by the study authors that, under the conditions of the study, there was some evidence of carcinogenic activity of quercetin in male F344/N rats based on an increased incidence of renal tubule cell adenomas, and no evidence of carcinogenic activity of quercetin in female F344/N rats (NTP, 1992). Following re-evaluation of the kidney lesions, Hard *et al.* (2007) concluded that the alterations observed in the renal tissues of male rats in this study were the result of a secondary mechanism for renal tumor development (*i.e.*, exacerbation of late-stage progressive chronic nephropathy), with no relevance for humans. In the absence of any differences in the survival rates between quercetin-treated and control animals,

morphological abnormalities, or organ-based toxicity, the body weight differences observed between high-dose test and control rats were not considered to be adverse to the health of the animals. Based on the results of the NTP study, the highest dietary concentration of quercetin tested (40,000 ppm providing 2,203 and 2,231 mg/kg body weight/day for males and females, respectively) was established as the NOAEL. This is supported by other long-term toxicity and carcinogenicity studies conducted not only in rats, but also in hamsters and mice, which demonstrated the absence of significant histopathological effects, including those of the kidneys, at comparable and in some cases higher dose levels (up to 12 g/kg body weight/day) (Ambrose *et al.*, 1951; Saito *et al.*, 1980; Hirono *et al.*, 1981; Hosaka and Hirono, 1981; Morino *et al.*, 1982; Takanashi *et al.*, 1983; Stoewsand *et al.*, 1984; Ito *et al.*, 1989). The non-neoplastic polyps of the cecum observed in male rats in a 2-year study (Ito *et al.*, 1989) were not reproduced in the later-conducted NTP study at similar dose levels (2,200 mg/kg body weight/day) (NTP, 1992).

Urinary bladder and intestinal tumors were observed in a single rat study at considerably lower doses, approximately 50 mg quercetin/kg body weight/day, and following an exposure of 58 weeks (Pamukcu *et al.*, 1980). These results were not confirmed in any of the other longer term studies performed with several-fold higher dose levels of quercetin. While the Pamucku *et al.* (1980) study specified a greater than 99% level of purity for the quercetin test material, the possibility of cross-contamination needs to be considered as a possible explanation for the unique appearance of these tumors limited to this single study, particularly since other studies of potential quercetin-related toxicity conducted for longer periods of time and at higher dose levels failed to reproduce the intestinal and urinary bladder tumors. Given the lack of reproducibility of these study results and the possibility of contamination by BF, one cannot afford credence to the Pamukcu *et al.* (1980) study in an overall evaluation of quercetin safety. The weight of evidence on the toxicity and carcinogenicity indicates that quercetin is not carcinogenic.

Additional experimental investigations have been conducted in which quercetin was tested in various 2-stage (initiation-promotion) carcinogenicity studies with various standard chemical carcinogens. A limited number of studies indicated some enhancing activity of quercetin (Werner *et al.*, 1985; Zhu and Liehr, 1994; Pereira *et al.*, 1996; Barotto *et al.*, 1998; Matsukawa *et al.*, 2002; Valentich *et al.,* 2006); most studies failed to demonstrate any quercetin-related promotion of carcinogenesis or demonstrated chemoprotective properties (Morino *et al.*, 1982; Fukushima *et al.*, 1983; Hirose *et al.*, 1983; Kato *et al.*, 1984, 1985; Sato *et al.*, 1987; Ito *et al.*, 1988; Verma *et al.*, 1988; Deschner *et al.*, 1991; Akagi *et al.*, 1995; Exon *et al.*, 1998; Tanaka *et al.*, 1999; Yang *et al.*, 2000; Devipriya *et al.*, 2006; Dihal *et al.*, 2006; Jin *et al.*, 2006). These studies further support the non-carcinogenicity of quercetin.

Clinical data related to the safety of quercetin were obtained from randomized, double blind, clinical trials (Ferry *et al.*, 1996; Shoskes *et al.*, 1999; Kiesewetter *et al.*, 2000; Lozoya *et al.*, 2002; Edwards *et al.*, 2007). One of the studies examined the short-term safety of a phytodrug

containing a standardized content of quercetin (1 mg flavonoids expressed as quercetin) (Lozoya *et al.*, 2002), while the other evaluated the safety of red vine leaf extract, which was reported to contain quercetin-3-*O*-beta-glycoside and isoquercetin (levels not specified) and was provided for a period of 12 weeks (Kiesewetter *et al.*, 2000). No compound-related adverse effects were reported in either of the studies, including the absence of any variations in laboratory tests. No adverse effects were reported in 2 efficacy trials in which quercetin was provided to study participants at dose levels of up to 1 g/day (Shoskes *et al.*, 1999; Edwards *et al.*, 2007). The safety of quercetin, administered *via* intravenous infusion at escalating doses (60 to 2,000 mg/m^2 equivalent to approximately 104 to 3,420 mg/day) at 3-week intervals, was assessed in a phase I clinical trial of cancer patients non-responsive to traditional therapy (Ferry *et al.*, 1996). At dose levels of up to 11.1 mg/kg body weight (approximately 720 mg/day) no adverse effects were noted. Nephrotoxicity was reported at higher doses (≥16.6 mg/kg body weight or approximately 1,080 mg/day). The intravenous route of administration is not appropriate for oral quercetin. Quercetin is scheduled for evaluation in a phase II clinical trial at doses of up to 2 grams (approximately 30 mg/kg body weight) (NLM, 2010). In addition to the controlled clinical trials, epidemiological findings support the absence of any relationship between flavonoid consumption from the diet (up to 68 mg/day) and an increased risk of cancer (Hertog *et al.*, 1994, 1995; Goldbohm *et al.*, 1995; Knekt *et al.*, 1997, 2002; Lin *et al.*, 2006). Most recently, three pharmacokinetic studies have demonstrated that quercetin exposure (1500 mg/day) does not interfere with the CYP450 system or P-gp transporter and, therefore, does not pose a risk to patients taking warfarin or digoxin; nor does it interfere with glucose uptake in individuals with type 2 diabetes (Prism Research, 2010a,b,c). In conclusion, the human clinical studies summarized above, corroborating epidemiological studies, and recently conducted human pharmacokinetic studies demonstrate the safety of quercetin in diverse human populations.

Based on the results of the pivotal 2-year rat feeding study (Dunnick and Hailey, 1992; NTP, 1992), a NOAEL of approximately 2,200 mg/kg body weight/day was established.

The weight of the evidence summarized above supports the safety and the proposed uses of Quercegen's high-purity quercetin.

CONCLUSION

We, the Expert Panel, have, independently and collectively, critically evaluated the data and information compiled and summarized above and conclude that the proposed uses of Quercegen's high-purity quercetin, manufactured consistent with current Good Manufacturing Practice and meeting appropriate food grade specifications as described in the dossier, are safe.

We further conclude that the proposed uses of Quercegen's high-purity quercetin, manufactured consistent with current Good Manufacturing Practice and meeting appropriate food grade specifications as described in the dossier, are Generally Recognized as Safe (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

(b)(6)

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REFERENCES

Abrahamse, S.L.; Kloots, W.J.; van Amelsvoort, J.M.M. 2005. Absorption, distribution, and secretion of epicatechin and quercetin in the rat. Nutr Res 25(3):305-317.

Ader, P.; Wessmann, A.; Wolffram, S. 2000. Bioavailability and metabolism of the flavonol quercetin in the pig. Free Radic Biol Med 28(7):1056-1067.

Aeschbacher, H.-U.; Meier, H.; Ruch, E. 1982. Nonmutagenicity *in vivo* of the food flavonol quercetin. Nutr Cancer 4(2):90-98.

Akagi, K.; Hirose, M.; Hoshiya, T.; Mizoguchi, Y.; Ito, N.; Shirai, T. 1995. Modulating effects of ellagic acid, vanillin and quercetin in a rat medium term multi-organ carcinogenesis model. Cancer Lett 94(1):113-121.

Ambrose, A.M.; Robbins, D.J.; DeEDS, F. 1951. Comparative toxicities of quercetin and quercitrin. J Am Pharm Assoc. XLI(3):119-122.

Aravindakshan, M.; Chauhan, P.S.; Sundaram, K. 1985. Studies on germinal effects of quercetin, a naturally occurring flavonoid. Mutat Res 144(2):99-106.

Bando, N.; Wakamatsu, S.; Terao, J. 2007. Effect of an excessive intake of quercetin on the vitamin E level and antioxidative enzyme activities of mouse liver under paraquat-induced oxidative stress. Biosci Biotechnol Biochem 71(10):2569-2572.

Barotto, N.N.; Lopez, C.B.; Eynard, A.R.; Fernandez, Zapico, M.E.; Valentich, M.A. 1998. Quercetin enhances pretumorous lesions in the NMU model of rat pancreatic carcinogenesis. Cancer Lett 129(1):1-6.

Barrenetxe, J.; Aranguren, P.; Grijalba, A.; Martínez-Penuela, J.M.; Marzo, F.; Urdaneta, E. 2006. Effect of dietary quercetin and sphingomyelin on intestinal nutrient absorption and animal growth. Br J Nutr 95(3):455-461.

Bieger, J.; Cermak, R.; Blank, R.; de Boer, V.C.J.; Hollman, P.C.H.; Kamphues, J.; Wolffram, S. 2008. Tissue distribution of quercetin in pigs after long-term dietary supplementation. J Nutr 138:1417-1420.

Bjeldanes, L.F.; Chang, G.W. 1977. Mutagenic activity of quercetin and related compounds. Science 197(403):577-578.

Blardi, P.; De Lalla, A.; Volpi, L.; Di Perri, T. 1999. Stimulation of endogenous adenosine release by oral administration of quercetin and resveratrol in man. Drugs Exp Clin Res 25(2&3):105-110.

Boersma. M.G.; Van Der Woude, H.; Bogaards, J.; Boeren, S.; Vervoort, J.; Cnubben, N.H.P.; Van Iersel, M.L.P.S.; Van Bladeren, P.J.; Rietjens, I.M.C.M. 2002. Regioselectivity of phase II metabolism of luteolin and quercetin by UDP-glucuronosyl transferases. Chem Res Toxicol 15(5):662-670.

Bokkenheuser, V.D.; Winter, J. 1990. Effect and metabolism of ingested quercetin in rats. Abstr Annu Meet Am Soc Microbiol 90:119 [Abstract No. D232].

Boorman, G.A.; Hollander, C.F. 1974. High incidence of spontaneous urinary bladder and ureter tumors in the brown Norway rat. J Ntl Cancer Inst 52(3):1005-1008.

Booth, A.N.; Deeds, F.; Jones, F.T.; Murray, C.W. 1956. The metabolic fate of rutin and quercetin in the animal body. J Biol Chem 223(1):251-257.

Boots, A.W.; Kubben, N.; Haenen, G.R.M.M.; Bast, A. 2003. Oxidized quercetin reacts with thiols rather than with ascorbate: Implication for quercetin supplementation. Biochem Biophys Res Commun 308(3):560-565.

Boots, A. W., Li, H., Schins, R. P., Duffin, R., Heemskerk, J. W., Bast, A. and Haenen, G. R. 2007. The quercetin paradox. Toxicology and Applied Pharmacology, 222:89-96.

Bovet, C., Plet, B., Ruff, M., Eiler, S., Granger, F., Panagiotidis, A., Wenzel, R., Nazabal, A., Moras, D. and Zenobi, R. 2009. Towards high-throughput identification of endocrine disrupting compounds with mass spectrometry. Toxicol In Vitro. 23(4):704-9.

Braune, A.; Gutschow, M.; Engst, W.; Blaut, M. 2001. Degradation of quercetin and luteolin by *Eubacterium ramulus*. Appl Environ Microbiol 67(12):5558-5567.

Brown, J.T. 1980. A review of the genetic effects of naturally occurring flavonoids, anthraquinones and related compounds. Mutat Res 75:243-277.

Brown, J.P.; Dietrich, P.S. 1979. Mutagenicity of plant flavonols in the *Salmonella*/mammalian microsome test: Activation of flavonol glycosides by mixed glycosidases from rat cecal bacteria and other sources. Mutat Res 66(3):223-240.

Bun, S.S.; Ciccolini, J.; Bun, H.; Aubert, C.; Catalin, J. 2003. Drug interactions of paclitaxel metabolism in human liver microsomes. J Chemother 15(3):266-274 [Abstract Only].

Busch, D.B.; Hatcher, J.F.; Bryan, G.T. 1986. Urine recovery experiments with quercetin and other mutagens using the Ames test. Environ Mutagen 8(3):393-399.

Canada, A.T.; Giannella, E.; Nguyen, T.D.; Mason, R.P. 1990. The productive of reactive oxygen species by dietary flavonols. Free Rad Biol Med 9:441-449.

Carbonaro, M.; Grant, G. 2005. Absorption of quercetin and rutin in rat small intestine. Ann Nutr Metab 49(3):178-182.

Caria, H.; Chaveca, T.; Laires, A.; Rueff, J. 1995. Genotoxicity of quercetin in the micronucleus assay in mouse bone marrow erythrocytes, human lymphocytes, V79 cell line and identification of kinetochore-containing (crest staining) micronuclei in human lymphocytes. Mutat Res 343(2&3):85-94.

Carver, J.H.; Carrano, A.V.; MacGregor, J.T. 1983. Genetic effects of the flavonols quercetin, kaempferol, and galangin on Chinese hamster ovary cells *in vitro*. Mutat Res 113:45-60.

CDC. 2006. Analytical and Reporting Guidelines: The National Health and Nutrition Examination Survey (NHANES). Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS); Hyattsville, Maryland. Available from: http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/nhanes_analytic_guidelines_dec_2005.pdf.

Cermak, R.; Landgraf, S.; Wolffram, S. 2004. Quercetin glucosides inhibit glucose uptake into brush-border-membrane vesicles of porcine jejunum. Br J Nutr 91(6):849-855.

Chang, T.K.; Chen, J.; Yeung, E.Y. 2006. Effect of *Ginkgo biloba* extract on procarcinogen-bioactivating human CYP1 enzymes: Identification of isorhamnetin, kaempferol, and quercetin as potent inhibitors of CYP1B1. Toxicol Appl Pharmacol 213(1):18-26.

Chen, X.; Yin, O.Q.; Zuo, Z.; Chow, M.S. 2005. Pharmacokinetics and modeling of quercetin and metabolites. Pharm Res 22(6):892-901.

Choi, E.J.; Chee, K.-M.; Lee, B.H. 2003. Anti-and prooxidant effects of chronic quercetin administration in rats. Eur J Pharmacol 482(1-3):281-285.

Choi, E.J.; Lee, B.H.; Lee, K.; Chee, K.-M. 2005. Long-term combined administration of quercetin and daidzein inhibits quercetin-induced suppression of glutathione antioxidant defenses. Food Chem Toxicol 43(5):793-798.

Choi, S.-Y.; Park, J.H.Y.; Kim, J.-S.; Kim, M.K.; Aruoma, O.I.; Sung, M.-K. 2006. Effects of quercetin and β-carotene supplementation on azoxymethane-induced colon carcinogenesis and inflammatory responses in rats fed with high-fat diet rich in ω-6 fatty acids. Biofactors 27(1-4):137-146.

Cierniak, A.; Papiež, M.; Kapiszewska, M. 2004. Modulatory effect of quercetin on DNA damage, induced by etoposide in bone marrow cells and on changes in the activity of antioxidant enzymes in rats. Rocz Akad Med Bialymst 49(Suppl. 1):167-169.

Conquer, J.A.; Maiani, G.; Azzini, E.; Raguzzini, A.; Holub, B.J. 1998. Supplementation with quercetin markedly increases plasma quercetin concentrations without effect on selected risk factors for heart disease in healthy subjects. J Nutr 128:593-597.

Crebelli, R.; Aquilina, G.; Falcone, E.; Carere, A. 1987. Urinary and faecal mutagenicity in Sprague Dawley rats dosed with the food mutagens quercetin and rutin. Food Chem Toxicol 25(1):9-15.

Crespy, V.; Morand, C.; Manach, C.; Besson, C.; Demigne, C.; Remesy, C. 1999. Part of quercetin absorbed in the small intestine is conjugated and further secreted in the intestinal lumen. Am J Physiol Gastrointest Liver Physiol 277(1, Part 1):G120-G126.

Cross, H.J.; Tilby, M.; Chipman, K.; Ferry, D.R.; Gescher, A. 1996. Effect of quercetin on the genotoxic potential of cisplatin. Int J Cancer 66(3):404-408.

Cruz-Correa, M.; Shoskes, D.A.; Sanchez, P.; Zhao, R.; Hylind, L.M.; Wexner, S.D.; Giardiello, F.M. 2006. Combination treatment with curcumin and quercetin of adenomas in familial adenomatous polyposis. Clin Gastroenterol Hepatol 4(8):1035-1038.

Cureton, K.J., Tomporowski, P.D., Singhal, A., Pasley, J.D., Bigelman, K.A., Lambourne, K., Trilk, J.L., McCully, K.K., Arnaud, M.J., and Zhao, Q. (2009) Dietary quercetin supplementation is not ergogenic in untrained men. J Appl. Physiol. 107(4):1095-104.

Czeczot, H. 1994. Mutagenic activity of quercetin in derivatives of *Escherichia coli* WP2 *uvr*A with increased permeability. Acta Biochim Pol 41(2):144-147.

Czeczot, H.; Kusztelak, J. 1993. A study of the genotoxic potential of flavonoids using short-term bacterial assays. Acta Biochim Pol 40(4):549-554.

Czeczot, H.; Tudek, B.; Kusztelak, J.; Szymczyk, T.; Dobrowolska, B.; Glinkowska, G.; Malinowski, J.; Strzelecka, H. 1990. Isolation and studies of the mutagenic activity in the Ames test of flavonoids naturally occurring in medical herbs. Mutat Res 240:209-216.

Czeczot, H.; Rahden-Staron, I.; Bilbin, M. 1993. Quercetin introduces strand breaks into bacterial DNA. Acta Biochim Pol 40(1):72-73.

da Silva, J.; Herrmann, S.M.; Heuser, V.; Peres, W.; Possa Marroni, N.; Gonzalez-Gallego, J.; Erdtmann, B. 2002. Evaluation of the genotoxic effect of rutin and quercetin by comet assay and micronucleus test. Food Chem Toxicol 40(7):941-947.

Day, A.J.; Williamson, G. 1999. Human metabolism of dietary quercetin glycosides. In: Gross, G.G.; Hemingway, R.W.; Yoshida, T. (Eds.). Plant Polyphenols 2: Chemistry, Biology, Pharmacology, Ecology. Kluwer Academic / Plenum Publishers; New York. Basic Life Sciences, Vol. 66, pp. 415-434.

Day, A.J.; Mellon, F.; Barron, D.; Sarrazin, G.; Morgan, M.R.; Williamson, G. 2001. Human metabolism of dietary flavonoids: Identification of plasma metabolites of quercetin. Free Radic Res 35(6):941-952.

Dayan, J.; Deguingand, S.; Truzman, C.; Chevron, M. 1987. Application of the SOS chromotest to 10 pharmaceutical agents. Mutat Res 187:55-66.

Davis, J.M.; Murphy, E.A.; Carmichael, M.D.; Davis, J.B.; Gangemi, J.D.; Mayer, E.P. 2007. Effect of quercetin feedings on tissue mitochondrial enzymes and performance in mice. Med Sci Sports Exerc 39(5, Suppl.):S90.

Davis, J.M., Carlstedt, C.J., Chen, S., Carmichael, M.D., and Murphy, E.A. 2010. The dietary flavonoid quercetin increases VO2 max and endurance capacity. Int. J Sport Nutr. Exerc. Metab. 20(1):56-62.

de Boer, V.C.; Dihal, A.A.; van der Woude, H.; Arts, I.C.; Wolffram, S.; Alink, G.M.; Rietjens, I.M.; Keijer, J.; Hollman, P.C. 2005. Tissue distribution of quercetin in rats and pigs. J Nutr 135(7):1718-1725.

De Santi, C.; Pietrabissa, A.; Mosca, F.; Pacifici, G.M. 2002. Methylation of quercetin and fisetin, flavonoids widely distributed in edible vegetables, fruits and wine, by human liver. Int J Clin Pharmacol Ther 40(5):207-212.

de Vries, J.H.; Hollman, P.C.; Meyboom, S.; Buysman, M.N.; Zock, P.L.; Van Staveren, W.A.; Katan, M.B. 1998. Plasma concentrations and urinary excretion of the antioxidant flavonols quercetin and kaempferol as biomarkers for dietary intake. Am J Clin Nutr 68(1):60-65.

DeEds, F. 1968. Flavonoid metabolism. In: Florkin, M.; Stotz, E.H. (Eds.). Comprehensive Biochemistry. Elsevier; Amsterdam/New York, Vol. 20, pp. 127-171.

Deschner, E.E.; Ruperto, J.; Wong, G.; Newmark, H.L. 1991. Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. Carcinogenesis 12(7):1193-1196.

Devipriya, S.; Ganapathy; V.; Shyamaladevi, C.S. 2006. Suppression of tumor growth and invasion in 9,10 dimethyl benz(α)anthracene induced mammary carcinoma by the plant bioflavonoid quercetin. Chem Biol Interact 162(2):106-113.

Dierks, E.A., Stams, K.R., Lim, H.K., Cornelius, G., Zhang, H. and Ball, B.E. 2001. A method for the simultaneous evaluation of the activities of seven major human drug-metabolizing cytochrome P450s using an in vitro cocktail of probe substrates and fast gradient liquid chromatography tandem mass spectrometry. Drug Metab Dispos. 29(1):23-29.

Dihal, A.A.; de Boer, V.C.; van der Woude, H.; Tilburgs, C.; Bruijntjes, J.P.; Alink, G.M.; Rietjens, I.M.; Woutersen, R.A.; Stierum, R.H. 2006. Quercetin, but not its glycosidated conjugate rutin, inhibits azoxymethane-induced colorectal carcinogenesis in F344 rats. J Nutr 136(11):2862-2867.

Dollo, G.; Chevanne, F.; Le Corre, P.; Chemtob, C.; Le Verge, R. 1999 . Biodisponibilité du phloroglucinol chez l'homme = [Bioavailability of phloroglucinol in man]. J Pharm Belg 54(3):75-82.

Duarte, J.; Galisteo, M.; Ocete, M.A.; Perez-Vizcaino, F.; Zarzuelo, A.; Tamargo, J. 2001. Effects of chronic quercetin treatment on hepatic oxidative status of spontaneously hypertensive rats. Mol Cell Biochem 221(1&2):155-160.

Dumke, C.L.; Nieman, D.C.; Utter, A.C., Rigby, M.D.; Quindry, J.C.; Triplett, N.T.; McAnulty, S.R.; McAnulty, L.S. Quercetin's effect on cycling efficiency and substrate utilization. Appl Physiol Nutr Metab 34:993-1000.

Dunnick, J.K.; Hailey, J.R. 1992. Toxicity and carcinogenicity studies of quercetin, a natural component of foods. Fundam Appl Toxicol 19(3):423-431.

Edwards, R.I.; Lyon, T.; Litwin, S.E.; Rabovsky, A.; Symons, J.D.; Jalili, T. 2007. Quercetin reduces blood pressure in hypertensive subjects. J. Nutr. 137:2405-2411.

Egert, S.; Wolffram, S.; Bosy-Westphal, A.; Boesch-Saadatmandi, C.; Wagner, A.E.; Frank, J.; Rimbach, G.; Müller, M.J. 2008. Daily quercetin supplementation dose-dependently increases plasma quercetin concentrations in healthy humans. J Nutr 138:1615-1621.

Egert, S.; Bosy-Westphal, A.; Seiberl, J.; Kürbitz, C.; Settler, U.; Plachta-Danielzik, S.; Wagner, A.E.; Frank, J.; Schrezenmeir, J.; Rimbach, G.; Wolffram, S.; Müller, M.J. 2009. Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentration in overweight subjects with a high-cardiovascular disease risk phenotype: a double-blinded, placebo-controlled cross-over study. Br J Nutr 102(7): 1065-1074.

Ertürk, E.; Nunoya, T.; Hatcher, J.F.; Pamucku, A.M.; Bryan, G.T. 1983. Comparison of bracken fern (BF) and quercetin (Q) carcinogenicity in rats (R). Proc Am Assoc Cancer Res Annu Meet 24:53 [Abstract No. 210].

Exon, J.H.; Magnuson, B.A.; South, E.H.; Hendrix, K. 1998. Dietary quercetin, immune functions and colonic carcinogenesis in rats. Immunopharmacol Immunotoxicol 20(1):173-190.

Ferry, D.R.; Smith, A.; Malkhandi, J.; Fyfe, D.W.; deTakats, P.G.; Anderson, D.; Baker, J.; Kerr, D.J. 1996. Phase I clinical trial of the flavonoid quercetin: Pharmacokinetics and evidence for *in vivo* tyrosine kinase inhibition. Clin Cancer Res 2(4):659-668.

Fischer, J.G.; Mikulcik, E.; Power, J. 2002. Effect of dietary quercetin on glutathione-S-transferase and glutathione reductase activities. FASEB J 16(5):A1002 [Abstract No. 735.6].

Fukushima, S.; Hagiwara, A.; Ogiso, T.; Shibata, M.; Ito, N. 1983. Promoting effects of various chemicals in rat urinary bladder carcinogenesis initiated by *N*-nitroso-*n*-butyl-(4-hydroxybutyl)amine. Food Chem Toxicol 21(1):59-68.

Gandhi, R.K.; Khanduja, K.L. 1993. Impact of quercetin consumption on phase-I and phase-II drug-metabolizing enzymes in mice. J Clin Biochem Nutr 14(2):107-112.

García-Saura, M.F.; Galisteo, M.; Villar, I.C.; Bermejo, A.; Zarzuelo, A.; Vargas, F.; Duarte, J. 2005. Effects of chronic quercetin treatment in experimental renovascular hypertension. Mol Cell Biochem 270(1&2):147-155.

Gaspar, J.; Rodrigues, A.; Laires, A.; Silva, F.; Costa, S.; Monteiro, M.J.; Monterio, C.; Rueff, J. 1994. On the metabolisms of genotoxicity and metabolism of quercetin. Mutagenesis 9(5):445-449.

Goldberg, D.M.; Yan, J.; Soleas, G.J. 2003. Absorption of three wine-related polyphenols in three different matrices by healthy subjects. Clin Biochem 36(1):79-87.

Goldbohm, R.A.; Brandt, P.A.; Hertog, M.G.L.; Brants, H.A.M.; van Poppel, G. 1995. Flavonoid intake and risk of cancer: A prospective cohort study. Am J Epidemiol 141(11, Suppl.):S61 [Abstract No. 243].

Graefe, E.U.; Wittig, J.; Mueller, S.; Riethling, A.K.; Uehleke, B.; Drewelow, H.; Pforte, G.; Jacobasch, H.; Derendorf, H.; Veit, M. 2001. Pharmacokinetics and bioavailability of quercetin glycosides in humans. J Clin Pharmacol 41:492-499.

Graf, B.A.; Milbury, P.E.; Ameho, C.; Dolnikowski, G.G.; Blumberg, J.B. 2005. Metabolism of dietary quercetin in the gastrointestinal tract, liver and kidney of the rat. Abstr Pap Am Chem Soc 229(Part 1):U59.

Graf, B.A.; Ameho, C.; Dolnikowski, G.G.; Milbury, P.E.; Chen, C.-Y.; Blumberg, J.B. 2006. Rat gastrointestinal tissues metabolize quercetin. J Nutr 136(1):39-44.

Gross, M.; Pfeiffer, M.; Martini, M.; Campbell, D.; Slavin, J.; Potter, J. 1996. The quantitation of metabolites of quercetin flavonols in human urine. Cancer Epidemiol Biomarkers Prev 5(9):711-720.

Gugler, R.; Leschik, M.; Dengler, H.J. 1975. Disposition of quercetin in man after single oral and intravenous doses. Eur J Clin Pharmacol 9(2&3):229-234.

Hang, B.Q., Wu, Y., Hang, S., Yang, Y., Wang, M.S. and Xuebao, N.Y. 1985. Effect of quercetin and rutin on the micronucleus frequency of mouse bone marrow polychromatic erythrocytes. Nanjing Yaoxueyan Xuebao. 16(3):52-55.

Hard, G.C.; Seeley, J.C.; Betz, L.J.; Hayashi, S.-M. 2007. Re-evaluation of the kidney tumors and renal histopathology in a 2-year rat carcinogenicity bioassay of quercetin. Food Chem Toxicol 45(4):600-608.

Hardigree, A.A.; Epler, J.L. 1978. Comparative mutagenesis of plant flavonoids in microbial systems. Mutat Res 58:231-239.

Harnly, J.M.; Doherty, R.F.; Beecher, G.R.; Holden, J.M.; Haytowitz, D.B.; Bhagwat, S.; Gebhardt, S. 2006. Flavonoid content of U.S. fruits, vegetables, and nuts. J Agric Food Chem 54(26):9966-9977.

Harwood, M.; Danielewska-Nikiel, B.; Borzelleca, J.F.; Flamm, G.W.; Williams, G.M.; Lines, T.C. 2007. A critical review of the data related to the safety of quercetin and lack of evidence of *in vivo* toxicity, including lack of genotoxic/carcinogenic properties. Food Chem Toxicol 45(11):2179-2205.

Hatcher, J.F.; Bryan, G.T. 1985. Factors affecting the mutagenic activity of quercetin for *salmonella typhimurium* TA98: Metal ions, antioxidants and pH. Mutat Res 148(1&2):13-23.

Hatcher, J.F.; Pamukcu, A.M.; Bryan, G.T. 1981. Quercetin (Q) and kaempferol (K) content of bracken fern (BF) and mutagenic activity in urine of rats ingesting Q, rutin (R) or BF. Proc Am Assoc Cancer Res Annu Meet 22:114 [Abstract No. 450].

He, N. and Edeki, T. 2004. The inhibitory effects of herbal components on CYP2C9 and CYP3A4 catalytic activities in human liver microsomes. Am J Ther. 11(3):206-212.

Henson, D.A.; Nieman, D.C.; Davis, J.M.; Murphy, E. A.; Carmichael, M.D.; Dumke, C.L.; Gross, S.J.; Jenkins, D.P.; Quindry, J.C.; Mcanulty, S.R.; Mcanulty, L.S.; Utter, A.C.; Mayer, E.P. 2008. Post-160-km race illness rates and decreases in granulocyte respiratory burst and salivary IgA output are not countered by quercetin ingestion. Int J Sports Med. 29(10):856-863.

Hertog, M.G.L.; Hollman, P.C.H.; Peter, C.H.; Katan, M.B. 1992. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. J Agric Food Chem 40(12):2379-2383.

Hertog, M.G.L.; Hollman, P.C.H.; van de Putte, B. 1993. Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. J Agric Food Chem 41(8):1242-1246.

Hertog, M.G.; Feskens, E.J.M.; Hollman, P.C.H.; Katan, M.B.; Kromhout, D. 1994. Dietary flavonoids and cancer risk in the Zutphen elderly study. Nutr Cancer 22(2):175-184.

Hertog, M.G.L.; Kromhout, D.; Aravanis, C.; Blackburn, H.; Buzina, R.; Fidanza, F.; Giampaoli, S.; Jansen, A.; Menotti, A.; Nedeljkovic, S.; Pekkarinen, M.; Simic, B.S.; Toshima, H.; Feskens, E.J.M.; Hollman, P.C.H.; Katan, M.B. 1995. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med 155(4):381-386.

Hirono, I. 1992. Is quercetin carcinogenic? Jpn J Cancer Res 83:312-314.

Hirono, I.; Sasaoka, I.; Shibuya, C.; Shimizu, M.; Fushimi, K.; Mori, H.; Kato, K.; Haga, M. 1975. Natural carcinogenic products of plant origin. In: Odashima, S.; Takayama, S.; Sato, H. (Eds.). Recent Topics in Chemical Carcinogenesis. University of Tokyo Press, Tokyo, Japan; GANN Monograph on Cancer Research, No. 17, pp. 205-217.

Hirono, I.; Ueno, I.; Hosaka, S.; Takanashi, H.; Matsushima, T.; Sugimura, T.; Natori, S. 1981. Carcinogenicity examination of quercetin and rutin in ACI rats. Cancer Lett 13(1):15-21.

Hirono, I.; Ogino, H.; Fujimoto, M.; Yamada, K.; Yoshida, Y.; Ikagawa, M.; Okumura, M. 1987. Induciton of tumors in ACI rats given a diet containing ptaquiloside, a bracken carcinogen. J Natl Cancer Inst 79(5):1143-1149.

Hirose, M.; Fukushima, S.; Sakata, T.; Inui, M.; Ito, N. 1983. Effect of quercetin on two-stage carcinogenesis of the rat urinary bladder. Cancer Lett 21(1):23-27.

Hollman, P.C.; de Vries, J.H.; van Leeuwen, S.D.; Mengelers, M.J.; Katan, M.B. 1995. Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. Am J Clin Nutr 62(6):1276-1282.

Hollman, P.C.; Vd, Gaag, M.; Mengelers, M.J.; Van Trijp, J.M.; de Vries, J.H.; Katan, M.B. 1996. Absorption and disposition kinetics of the dietary antioxidant quercetin in man. Free Radic Biol Med 21(5):703-707.

Hollman, P.C.; Van Trijp, J.M.; Mengelers, M.J.; de Vries, J.H.; Katan, M.B. 1997. Bioavailability of the dietary antioxidant flavonol quercetin in man. Cancer Lett 114(1&2):139-140.

Hong, Y-J.; Mitchell, A.E. 2006. Identification of glutathione-related quercetin metabolites in humans. Chem Res Toxicol 19(11):1525-1532.

Hosaka, S.; Hirono, I. 1981. Carcinogenicity test of quercetin by pulmonary-adenoma bioassay in strain A mice. GANN 72(2):327-328.

IARC. 1986. Bracken fern (Pteridium aquilinum) and some of its constituents. In: Some Naturally Occurring and Synthetic Food Components, Furocoumarins and Ultraviolet Radiation. International Agency for Research on Cancer (IARC); Lyon, France, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 40, pp. 47-65.

IARC. 1987. Bracken fern. In: Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42. International Agency for Research on Cancer (IARC); Lyon, France, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Suppl. 7, pp. 135-136.

IARC. 1999. Quercetin. In: Some Chemicals that Cause Tumours of the Kidney or Urinary Bladder in Rodents and Some Other Substances. International Agency for Research on Cancer (IARC); Lyon, France. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 73, pp. 497-515.

Ishikawa, M.; Oikawa, T.; Hosokawa, M.; Hamada, J.; Morikawa, K.; Kobayashih, H. 1985. Enhancing effect of quercetin on 3-methylcholanthrene carcinogenesis in C57Bl/6 mice. Neoplasma 32(4):435-441.

Ito, N. 1992. Is quercetin carcinogenic? Jpn J Cancer Res 83:312-313.

Ito, N.; Tsuda, H.; Tatematsu, M.; Inoue, T.; Tagawa, Y.; Aoki, T.; Uwagawa, S.; Kagawa, M.; Ogiso, T.; Masui, T.; Imaida, K.; Fukushima, S.; Asamoto, M. 1988. Enhancing effect of various hepatocarcinogens on induction of preneoplastic glutathione S-transferase placental form positive foci in rats - An approach for a new medium-term bioassay system. Carcinogenesis 9(3):387-394.

Ito, N.; Hagiwara, A.; Tamano, S.; Kagawa, M.; Shibata, M.; Kurata, Y.; Fukushima, S. 1989. Lack of carcinogenicity of quercetin in F344/DuCrj rats. Jpn J Cancer Res 80(4):317-325.

Janssen, K.; Mensink, R.P.; Cox, F.J.; Harryvan, J.L.; Hovenier, R.; Hollman, P.C.; Katan, M.B. 1998. Effects of the flavonoids quercetin and apigenin on hemostasis in healthy volunteers: Results from an *in vitro* and a dietary supplement study. Am J Clin Nutr 67(2):255-262.

Jalili, T.; Edwards, R.; Lyon, T.; Symons, J.D. 2006. Quercetin reduces blood pressure in hypertensive subjects. Circulation 114:II_890 [Abstract 4122].

JECFA. 1977. Evaluation of Certain Food Additives. 21st JECFA Session, Apr. 18-27, 1977, Geneva, Switz. World Health Organization (WHO), Joint FAO/WHO Expert Committee on Food Additives (JECFA) & Food and Agriculture Organization of the United Nations; Geneva, Switz. WHO Technical Report Series, No. 617.

JECFA. 1998. Safety Evaluation of Certain Food Additives and Contaminants. 49th Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Joint FAO/WHO Expert Committee on Food Additives (JECFA)/Food and Agriculture Organization of the United Nations (FAO) & World Health Organization (WHO), Geneva, Switz. WHO Food Additives Series, No. 40.

JECFA. 2006. Safety Evaluation of Certain Food Additives and Contaminants. 63rd Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Joint FAO/WHO Expert Committee on Food Additives (JECFA)/Food and Agriculture Organization of the United Nations (FAO) & World Health Organization (WHO), Geneva, Switz. WHO Food Additives Series, No. 54.

Jin, N.-Z.; Zhu, Y.-P.; Zhou, J.-W.; Mao, L.; Zhao, R.-C.; Fang, T.-H.; Wang, X.-R. 2006. Preventive effects of quercetin against benzo[α]pyrene-induced DNA damages and pulmonary precancerous pathologic changes in mice. Basic Clin Pharmacol Toxicol 98(6):593-598.

Johannot, L.; Somerset, S.M. 2006. Age-related variations in flavonoid intake and sources in the Australian population. Public Health Nutr 9(8):1045-1054.Jones, E.; Hughes, R.E. 1982. Quercetin, flavonoids and the life-span of mice. Exp Gerontol 17(3):213-217.

Jones, E.; Hughes, R.E. 1982. Quercetin, flavonoids and the life-span of mice. Exp Gerontol 17(3):213-217.

Jones, D.J.L.; Jukes-Jones, R.; Verschoyle, R.D.; Farmer, P.B.; Gescher, A. 2005. A synthetic approach to the generation of quercetin sulfates and the detection of quercetin 3'-o-sulfate as a urinary metabolite in the rat. Bioorg Med Chem 13(24):6727-6731.

Justesen, U.; Arrigoni, E. 2001. Electrospray ionisation mass spectrometric study of degradation products of quercetin, quercetin-3-glucoside and quercetin-3-rhamnoglucoside, produced by *in vitro* fermentation with human faecal flora. Rapid Commun Mass Spectrom 15(7):477-483.

Justino, G.C.; Santos, M.R.; Canário, S.; Borges, C.; Florêncio, M.H.; Mira, L. 2004. Plasma quercetin metabolites: structure-antioxidant activity relationships. Arch Biochem Biophys 432(1):109-121.

Kaldas, M.I.; Walle, U.K.; van der Woude, H.; McMillan, J.M.; Walle, T. 2005. Covalent binding of the flavonoid quercetin to human serum albumin. J Agric Food Chem 53(10):4194-4197.

Kato, K.; Mori, H.; Fujii, M.; Bunai, Y.; Nishikawa, A.; Shima, H.; Takahashi, M.; Kawai, T.; Hirono, I. 1984. Lack of promotive effect of quercetin on methylazoxymethanol acetate carcinogenesis in rats. J Toxicol Sci 9(4):319-325.

Kato, K.; Mori, H.; Tanaka, T.; Fujii, M.; Kawai, T.; Nishikawa, A.; Takahashi, M.; Hirono, I. 1985. Absence of initiating activity by quercetin in the rat liver. Ecotoxicol Environ Saf 10(1):63-69.

Keppler, K.; Hein, E.-M.; Humpf, H.-U. 2006. Metabolism of quercetin and rutin by the pig caecal microflora prepared by freeze-preservation. Mol Nutr Food Res 50(8):686-695.

Khaled, K.A.; El-Sayed, Y.M.; Al-Hadiya, B.M. 2003. Disposition of the flavonoid quercetin in rats after single intravenous and oral doses. Drug Dev Ind Pharm 29(4):397-403.

Khanduja, K.L.; Verma, A.; Bhardwaj, A. 2001. Impairment of human sperm motility and viability by quercetin is independent of lipid peroxidation. Andrologia 33(5):277-281.

Kiesewetter, H.; Koscielny, J.; Kalus, U.; Vix, J.-M.; Peil, H.; Petrini, O.; van Toor, B.S.J.; de Mey, C. 2000. Efficacy of orally administered extract of red vine leaf AS 195 (folia vitis viniferae) in chronic venous insufficiency (Stages I-II). Drug Res 50(I)(2):109-117.

Kim, K.A.; Park, J.Y.; Lee, J.S.; Lim, S. 2003. Cytochrome P450 2C8 and CYP3A4/5 are involved in chloroquine metabolism in human liver microsomes. Arch Pharm Res 26(8):631-637 [Abstract Only].

Kim, K.-A.; Park, P.-W.; Kim, H.-K.; Ha, J.-M.; Park, J.-Y. 2005. Effect of quercetin on the pharmacokinetics of rosiglitazone, a CYP2C8 substrate, in healthy subjects. J Clin Pharmacol 45(8):941-946.

Kimira, M.; Arai, Y.; Shimoi, K.; Watanabe, S. 1998. Japanese intake of flavonoids and isoflavonoids from foods. J Epidemiol 8(3):168-175.

Knekt, P.; Järvinen, R.; Seppänen, R.; Heliövaara, M.; Teppo, L.; Pukkala, E.; Aromaa, A. 1997. Dietary intake of flavonoids and the risk of lung cancer and other malignant neoplasms. Am J Epidemiol 146:223-230.

Knekt, P.; Kumpulainen, J.; Järvinen, R.; Rissanen, H.; Heliövaara, M.; Reunanen, A.; Hakulinen, T.; Aromaa, A. 2002. Flavonoid intake and risk of chronic diseases. Am J Clin Nutr 76(3):560-568.

Krishnamurty, H.G.; Cheng, K.-J.; Jones, G.A.; Simpson, F.J.; Watkin, J.E. 1970. Identification of products produced by the anaerobic degradation of rutin and related flavonoids by *Butyrivibrio* sp. C_3. Can J Microbiol 16(8):759-767.

Kubiak, R.; Rudek, Z. 1990. SCEs and chromosome aberrations in mammalian cells *in vitro* treated with quercetin. Acta Biol Hung 41(1-3):121-124.

Kühnau, J. 1976. The flavonoids: A class of semi-essential food components: Their role in human nutrition. World Rev Nutr Diet 24:117-191.

Kuiper, G.G.J.M, Lemmen, J.G., Carlsson, B., Corton, J.C., Safe, S.H., van der Saag, P.T., van der Burg, B. and Gustafsson, J. 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology, 139:4252-4263.

Kwon, O.; Eck, P.; Chen, S.; Corpe, C.P.; Lee, J.-H.; Kruhlak, M.; Levine, M. 2007. Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. FASEB J 21:366-377.

Lam, T.K.; Rotunno, M.; Lubin, J.H.; Wacholder, S.; Consonni, D.; Pesatori, A.C.; Bertazzi, P.A.; Chanock, S.; Burdette, L.; Goldstein, A.M.; Tucker, M.A.; Caporasp, N.E.; Subar, A.F.; Landi, M.T. in press. Dietary quercetin, quercetin-gene interaction, metabolic gene expression in lung tissue, and lung cancer risk. Carcinogenesis. In press.

Lamson, D.W.; Brignall, M.S. 2000. Antioxidants and cancer III: Quercetin. Altern Med Rev 5(3):196-208.

Lau, A.J. and Chang, T.K. 2009. Inhibition of human CYP2B6-catalyzed bupropion hydroxylation by Ginkgo biloba extract: effect of terpene trilactones and flavonols. Drug Metab Dispos. 37(9):1931-1937.

Lesser, S.; Cermak, R.; Wolffram, S. 2006. The fatty acid pattern of dietary fat influences the oral bioavailability of the flavonol quercetin in pigs. Br J Nutr 96(6):1047-1052.

Lin, J.; Zhang, S.M.; Wu, K.; Willett, W.C.; Fuchs, C.S.; Giovannucci, E. 2006. Flavonoid intake and colorectal cancer risk in men and women. Am J Epidemiol 164(7):644 651.

Llagostera, M.; Garrido, S.; Barbe, J.; Guerrero, R.; Rueff, J. 1987. Influence of S9 mix in the induction of SOS system by quercetin. Mutat Res 191(1):1-4.

Lozoya X, Reyes-Morales H, Chavez-Soto M.A., Martinez-Garcia Mdel C., Soto-Gonzalez Y., Doubova S.V. 2002. Intestinal anti-sposmodic effect of a phytodrug of *Psidium guajava folia* in the treatment of acute diarrheic disease. Journal of Ethnopharmacology 83:19-24.

MacGregor, J.T. 1979. Mutagenicity studies of flavonoids *in vivo* and *in vitro*. Toxicol Appl Pharmacol 48(1, Part 2):A47 [Abstract No. 94].

MacGregor, J.T.; Jurd, L. 1978. Mutagenicity of plant flavonoids: structural requirements for mutagenic activity in Salmonella typhimurium. Mutat Res 54(3):297-309.

MacRae, H.S. and Mefferd, K.M. (2006) Dietary antioxidant supplementation combined with quercetin improves cycling time trial performance. Int. J Sport Nutr. Exerc. Metab. 6(4):405-419.

Makena, P.S., Pierce, S. C., Chung, K.T. and Sinclair S.E. 2009. Comparative mutagenic effects of structurally similar flavonoids quercetin and taxifolin on tester strains Salmonella typhimurium TA102 and Escherichia coli WP-2uvrA. Environ Mol Mutagen. 50(6):451-459.

Manach, C.; Morand, C.; Texier, O.; Favier, M.-L.; Agullo, G.; Demigné, C.; Régérat, F.; Rémésy, C. 1995. Quercetin metabolites in plasma of rats fed diets containing rutin or quercetin. J Nutr 125(7):1911-1922.

Manach, C.; Morand, C.; Demigne, C.; Texier, O.; Regerat, F.; Remesy, C. 1997. Bioavailability of rutin and quercetin in rats. FEBS Lett 409(1):12-16.

Manach, C.; Morand, C.; Crespy, V.; Demigne, C.; Texier, O.; Regerat, F.; Remesy, C. 1998. Quercetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. FEBS Lett 426(3):331-336.

Manach, C.; Texier, O.; Morand, C.; Crespy, V.; Régérat, F.; Demigné, C.; Rémésy, C. 1999. Comparison of the bioavailability of quercetin and catechin in rats. Free Radic Biol Med 27(11&12):1259-1266.

Maruta, A.; Enaka, K.; Umeda, M. 1979. Mutagenicity of quercetin and kaempferol on cultured mammalian cells. GANN 70(3):273-276.

Matsukawa, Y.; Nishino, H.; Yoshida, M.; Sugihara, H.; Katsura, K.; Takamatsut.; Okuzumi, J.; Matsumoto, K.; Sato-Nishimori, F.; Sakai, T. 2002. Quercetin enhances tumorigenicity induced by *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine in the duodenum of mice. Environ Health Prevent Med 6(4):235-239.

McAnlis, G.T.; Mceneny, J.; Pearce, J.; Young, I.S. 1999. Absorption and antioxidant effects of quercetin from onions, in man. Eur J Clin Nutr 53(2):92-96.

McAnulty, S.R.; McAnulty, L.S.; Nieman, D.C.; Quindry, J.C.; Hosick, P.A.; Hudson, M.H.; Still, L.; Henson, D.A.; Milne, G.L.; Morrow, J.D.; Dumke, C.L.; Utter, A.C.; Triplett, N.T.; Dibarnardi, A. 2008. Chronic quercetin ingestion and exercise-induced oxidative damage and inflammation. Appl Physiol Nutr Metab 33(2):254-262.

Meltz, M.L.; MacGregor, J.T. 1981. Activity of the plant flavanol quercetin in the mouse lymphoma L5178Y TK+/-mutation, DNA single-strand break, and Balb/c 3T3 chemical transformation assays. Mutat Res 88(3):317-324.

Metodiewa, D.; Jaiswal, A.K.; Cenas, N.; Dickancaite, E.; Segura-Aguilar, J. 1999. Quercetin may act as a cytotoxic prooxidant after its metabolic activation to semiquinone and quinoidal product. Free Radic Biol Med 26(1&2):107-116.

Meyers, K.J.; Rudolf, J.L.; Mitchell, A.E. 2008. Influence of dietary quercetin on glutathione redox staus in mice. J Agric Food Chem 56:830-836.

Mi, Y., Zhang, C., Li, C., Taneda, S., Watanabe, G., Suzuki, A.K. and Taya, K. 2009. Protective effect of quercetin on the reproductive toxicity of 4-nitrophenol in diesel exhaust particles on male embryonic chickens. J Reprod Dev. In press.

Moon, J.H.; Tsushida, T.; Nakahara, K.; Terao, J. 2001. Identification of quercetin 3-*O*-β-D-glucuronide as an antioxidative metabolite in rat plasma after oral administration of quercetin. Free Radic Biol Med 30(11):1274-1285.

Morales, A.I.; Vicente-Sánchez, C.; Sandoval, J.M.S.; Egido, J.; Mayoral, P.; Arévalo, M.A.; Fernández-Tagarro, M.; López-Novoa, J.M.; Pérez-Barriocanal, F. 2006. Protective effect of quercetin on experimental chronic cadmium nephrotoxicity in rats is based on its antioxidant properties. Food Chem Toxicol 44(12):2092-2100.

Morand, C.; Crespy, V.; Manach, C.; Besson, C.; Demigne, C.; Remesy, C. 1998. Plasma metabolites of quercetin and their antioxidant properties. Am J Physiol Regul Integr Comp Physiol 275(1, Part 2):R212-R219.

Morand, C.; Manach, C.; Crespy, V.; Remesy, C. 2000a. Respective bioavailability of quercetin aglycone and its glycosides in a rat model. Biofactors 12(1-4):169-174.

Morand, C.; Manach, C.; Crespy, V.; Remesy, C. 2000b. Quercetin 3-*O*-β-glucoside is better absorbed than other quercetin forms and is not present in rat plasma. Free Radic Res 33(5):667-676.

Morino, K.; Matsukara, N.; Kawachi, T.; Ohgaki, H.; Sugimura, T.; Hirono, I. 1982. Carcinogenicity test of quercetin and rutin in golden hamsters by oral administration. Carcinogenesis 3(1):93-97.

Morrice, P.C.; Wood, S.G.; Duthie, G.G. 2000. High-performance liquid chromatographic determination of quercetin and isorhamnetin in rat tissues using beta-glucuronidase and acid hydrolysis. J Chromatogr B Biomed Sci Appl 738(2):413-417.

Mullen, W.; Boitier, A.; Stewart, A.J.; Crozier, A. 2004. Flavonoid metabolites in human plasma and urine after the consumption of red onions: analysis by liquid chromatography with photodiode array and full scan tandem mass spectrometric detection. J Chromatogr A 1058(1&2):163-168.

Murota, K.; Terao, J. 2005. Quercetin appears in the lymph of unanesthetized rats as its phase II metabolites after administered into the stomach. FEBS Lett 579(24):5343-5346.

Murota, K.; Shimizu, S.; Chujo, H.; Moon, J.H.; Terao, J. 2000. Efficiency of absorption and metabolic conversion of quercetin and its glucosides in human intestinal cell line caco-2. Arch Biochem Biophys 384(2):391-397.

Murray, C.W.; Booth, A.N. DeEds, F.; Jones, F.T. 1954. Absorption and metabolism of rutin and quercetin in the rabbit. J Am Pharm Assoc Pract Pharm Ed 43(6):361-364.

Murray, R.K.; Granner, D.K.; Mayes, P.A.; Rodwell, V.W. (Eds.). 2000. Appendix. In: Harper's Biochemistry (25th Ed.). Appleton & Lange/Prentice Hall Canada; Stamford, Conn./Toronto, pp. 867-872.

Myhrstad, M.C.; Carlsen, H.; Nordström, O.; Blomhoff, R.; Moskaug, J.Ø. 2002. Flavonoids increase the intracellular glutathione level by transactivation of the gamma-glutamylcysteine synthetase catalytical subunit promoter. Free Radic Biol Med 32(5):386-393.

Nakamura, Y.; Ishimitsu, S.; Tonogai, Y. 2000. Effects of quercetin and rutin on serum and hepatic lipid concentrations, fecal steroid excretion and serum antioxidant properties. J Health Sci (Tokyo) 46(4):229-240.

Nakamura, Y.; Torikai, K.; Ohigashi, H. 2001. Toxic dose of a simple phenolic antioxidant, protocatechuic acid, attenuates the glutathione level in ICR mouse liver and kidney. J Agric Food Chem 49(11):5674-5678.

Nakayasu, M.; Sakamoto, H.; Terada, M.; Nagao, M.; Sugimura, T. 1986. Mutagenicity of quercetin in Chinese hamster lung cells in culture. Mutat Res 174(1):79-83.

Nandan, S.D.; Rao, M.S. 1983. Lack of mutagenic effects of quercetin in the germ cells of mice. IRCS Med Sci 11(3):210.

NAS. 1982. Naturally occurring carcinogens. In: Diet, Nutrition and Cancer. National Academy of Sciences (NAS)/National Academy Press (NAP); Washington, DC, pp. 244-245. Available from: http://books.nap.edu/openbook.php?record_id=371&page=234.

Nass-Arden, L.; Breitbart, H. 1990. Modulation of mammalian sperm motility by quercetin. Mol Reprod Dev 25(4):369-373.

Ngomuo, A.J.; Jones, R.S. 1996. Genotoxicity studies of quercetin and shikimate *in vivo* in the bone marrow of mice and gastric mucosal cells of rats. Vet Hum Toxicol 38(3):176-180.

Nguyen, T.; Fluss, L.; Madej, R.; Ginther, C.; Leighton, T. 1989. The distribution of mutagenic activity in red, rose and white wines. Mutat Res 223:205-212.

Nieman, D.C. 2008 [unpublished]. Quercetin Community Trial on 1,002 Subjects. Quercetin, Upper Respiratory Tract Infection, Disease Risk Factors, and Mental Cognition: A Randomized Community Trial. Appalachian State University, Department of Health Leisure & Exercise Science, Human Performance Laboratory; Boone, North Carolina [unpublished study – preliminary results].

Nieman, D.C.; Henson, D.A.; Davis, J.M.; Murphy, E.A.; Jenkins, D.P.; Gross, S.J.; Carmichael, M.D.; Quindry, J.C.; Dumke, C.L.; Utter, A.C.; McAnulty, S.R.; McAnulty, L.S.; Triplett, N.T.; Mayer, E.P. 2007a. Quercetin's influence on exercise-induced changes in plasma cytokines and muscle and leukocyte cytokine mRNA. J Appl Physiol 103(5):1728-1735.

Nieman, D.C.; Henson, D.A.; Davis, J.M.; Dumke, C.L.; Gross, S.J.; Jenkins, D.P.; Murphy, E.A.; Carmichael, M.D.; Quindry, J.C.; McAnulty, S.R.; McAnulty, L.S.; Utter, A.C.; and Mayer, E.P. 2007b. Quercetin ingestion does not alter cytokine changes in athletes competing in the Western States Endurance Run. J Interferon Cytokine Res 27(12):1003-1011.

Nieman, D.C.; Henson, D.A.; Gross, S.J.; Jenkins, D.P.; Davis, J.M.; Murphy, E.A.; Carmichael, M.D.; Dumke, C.L.; Utter, A.C.; Mcanulty, S.R.; Mcanulty, L.S.; Mayer, E.P. 2007c. Quercetin reduces illness but not immune perturbations after intensive exercise. Med Sci Sports Exerc 39(9):1561-1569.

Nieman, D.C.; Henson, D.A.; Maxwell, K.R.; Williams, A.S.; McAnulty, S.R.; Jin, F.; Shanely, R.A.; Lines, T.C. 2009. Effects of quercetin and EGCG on mitochondrial biogenesis and immunity. Med Sci Sports Exerc 41(7):1467-1475.

Nieman, D.C., Williams, A.S., Shanely, R.A., Jin, F., McAnulty, S.R., Triplett, N.T., Austin, M.D., and Henson, D.A. 2010. Quercetin's influence on exercise performance and muscle mitochondrial biogenesis. Med. Sci. Sports Exerc. 42(2):338-45.

NLM. 2010. Investigating the Use of Quercetin on Glucose Absorption in Obesity, and Obesity with Type 2 Diabetes. Sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) & National Institutes of Health (NIH), Warren Grant Magnuson Clinical Center (CC), Bethesda, Maryland. Last update: March 12, 2010. Available from: http://clinicaltrials.gov/ct2/show/study/NCT00065676

Noteborn, H.P.; Jansen, E.; Benito, S.; Mengelers, M.J. 1997. Oral absorption and metabolism of quercetin and sugar-conjugated derivatives in specific transport systems. Cancer Lett 114(1&2):175-177.

NTP. 1989. Toxicology and Carcinogenesis Studies of Hydroquinone (CAS No. 123-31-9) in F344/N Rats and B6C3F$_1$ mice (Gavage Study). National Toxicology Program (NTP); Research Triangle Park, North Carolina. NTP Technical Report Series No. 366.

NTP. 1992. Toxicology and Carcinogenesis Studies of Quercetin (CAS No. 117-39-5) in F344/N Rats (Feed Study). National Toxicology Program (NTP); Research Triangle Park, North Carolina. NTP Technical Report Series No. 409.

Obach, R.S. 2000. Inhibition of human cytochrome P450 enzymes by constituents of St. John's Wort, and herbal preparation used in the treatment of depression. J Pharmacol Exp Ther. 294(1):88-95.

O'Leary, K.A.; Day, A.J.; Needs, P.W.; Mellon, F.A.; O'Brien, N.M.; Williamson, G. 2003. Metabolism of quercetin-7- and quercetin-3-glucuronides by an *in vitro* hepatic model: The role of human β-glucuronidase, sulfotransferase, catechol-o-methyltransferase and multi-resistant protein 2 (MRP2) in flavonoid metabolism. Biochem Pharmacol 65(3):479-491.

Ochiai, M.; Nagao, M.; Wakabayashi, K.; Sugimura, T. 1984. Superoxide dismutase acts as an enhancing factor for quercetin mutagenesis in rat-liver cytosol by preventing its decomposition. Mutat Res 129:19-24.

Okamoto, T. 2005. Safety of quercetin for clinical application (review). Int J Mol Med 16(2):275-278.

Oliveira, E.J.; Watson, D.G. 2000. *In vitro* glucuronidation of kaempferol and quercetin by human UGT-1A9 microsomes. FEBS Lett 471(1):1-6.

Olthof, M.R.; Hollman, P.C.H.; Buijsman, M.N.C.P.; Van Amelsvoort, J.M. M.; Katan, M.B. 2003. Chlorogenic acid, quercetin-3-rutinoside and black tea phenols are extensively metabolized in humans. J Nutr 133(6):1806-1814 [& Erratum 133(8):2692].

Onishi, M.; Hatcher, J.F.; Nunoya, T.; Pamukcu, A.M.; Bryan, G.T. 1982. *In vitro* metabolism of quercetin by rat liver and kidney preparations. Proc Am Assoc Cancer Res Annu Meet 23:90 [Abstract No. 353].

Pamukcu, A.M.; Price, J.M.; Bryan, G.T. 1976. Naturally occurring and braken-fern-induced bovine urinary bladder tumors. Clinical and morphological characteristics. Vet Pathol 13(2):110-122.

Pamukcu, A.M.; Erturk, E.; Yalciner, S.; Milli, U.; Bryan, G.T. 1978. Carcinogenic and mutagenic activities of milk from cows fed bracken fern (Pteridium aquilinum). Cancer Res 38(6):1556-1560.

Pamukcu, A.M.; Yalçiner, Ş.; Hatcher, J.F.; Bryan, G.T. 1980. Quercetin, a rat intestinal and bladder carcinogen present in Bracken Fern (Pteridium aquilinum). Cancer Res 40:3468-3472.

Papiez, M.A.; Cierniak, A.; Krzysciak, W.; Bzowska, M.; Taha, H.M.; Jozkowicz, A.; Piskula, M. 2008. The changes of antioxidant defense system caused by quercetin administration do not lead to DNA damage and apoptosis in the spleen and bone marrow cells of rats. Food Chem Toxicol 46(9):3053-3058.

Patel, J.; Buddha, B.; Dey, S.; Pal, D.; Mitra, A.K. 2004. In vitro interaction of the HIV protease inhibitor ritonavir with herbal constituents: changes in P-gp and CYP3A4 activity. Am J Ther 11(4):262-277.

Pereira, M.A.; Grubbs, C.J.; Barnes, L.H.; Li, H.; Olson, G.R.; Eto, I.; Juliana, M.; Whitaker, L.M.; Kelloff, G.J.; Steele, V.E.; Lubet, R.A. 1996. Effects of the phytochemicals, curcumin and quercetin, upon azoxynethane-induced colon cancer and 7,12-dimethylbenz[*a*]anthacene-induced mammary cancer in rats. Carcinogenesis 17(6):1305-1311.

Pietta, P.G.; Gardana, C.; Mauri, P.L. 1997. Identification of *Gingko biloba* flavonol metabolites after oral administration to humans. J Chromatogr B Biomed Sci Appl 693(1):249-255.

Popp, R.; Schimmer, O. 1991. Induction of sister-chromatid exchanges (SCE), polyploidy, and micronuclei by plant flavonoids in human lymphocyte cultures. A comparative study of 19 flavonoids. Mutat Res 246(1):205-213.

Prism Research. 2010a. Effect of Multiple Doses of Compound Q on Plasma Glucose Levels after Oral Glucose Tolerance Test in Patients with Type 2 Diabetes. Unpublished report for Project No. 915 (Protocol 102).

Prism Research. 2010b. Effect of Multiple Doses of Compound Q on Plasma Digoxin Disposition after a Single Oral Dose of Digoxin in Normal Healthy Volunteers Unpublished report for Project No. 916 (Protocol 103).

Prism Research. 2010c. Effect of Multiple Doses of Compound Q on Plasma Warfarin Disposition after a Single Oral Dose of Warfarin in Normal Healthy Volunteers. Unpublished report for Project No. 914 (Protocol 101).

Quindry, J.C.; McAnulty, S.R.; Hudson, M.B.; Hosick, P.; Dumke, C.; McAnulty, L.S.; Henson, D.; Morrow, J.D.; Nieman D. 2008. Oral quercetin supplementation and blood oxidative capacity in response to ultramarathon competition. Int. J Sport Nutr. Exerc. Metab. 18(6):601-616.

Rachoń, D.; Vortherms, T.; Seidlová-Wuttke, D.; Jarry, H.; Wuttke, W. 2008. Dietary quercetin does not affect pituitary lutenizing hormone (LH) expression and has no uterotropic effects in ovariectomized Sprague-Dawley rats. Food Chem Toxicol 46(2):513-518.

Rahden-Staron, I.; Czeczot, H.; Szumilo, M. 2001. Induction of rat liver cytochrome P450 isoenzymes CYP 1A and CYP 2B by different fungicides, nitrofurans, and quercetin. Mutat Res 498:57-66.

Rajaraman, G.; Yang, g.; Chen, J.; Chang, T.K.H. 2009. Modulation of CYP1B1 and CYP1A1 gene expression and activation of aryl hydrocarbon receptor by Ginkgo biloba extract in MCF-10A human mammary epithelial cells. Can J Physiol Pharmacol 87(9):674-83.

Rangan, G.K.; Wang, Y.; Harris, D.C.H. 2002. Dietary quercetin augments activator protein-1 and does not reduce nuclear facto-κB in the renal cortex of rats with established chronic glomerular disease. Nephron 90:313-319.

Rao, G.N. 1997. New nonpurified diet (NTP-2000) for rodents in the National Toxicology Program's toxicology and carcinogenesis studies. J Nutr 127(5, Suppl.):842S-846S.

Rao, G.N.; Morris, R.W.; Seely, J.C. 2001. Beneficial effects of NTP-2000 diet on growth, survival, and kidney and heart diseases of Fischer 344 rats in chronic studies. Toxicol Sci 63:245-255.

Rastogi, P.B.; Levin, R.E. 1987. Induction of sperm abnormalities in mice by quercetin. Environ Mutagen 9(1):79-86.

Rechner, A.R.; Kuhnle, G.; Bremner, P.; Hubbard, G.P.; Moore, K.P.; Rice-Evans, C.A. 2002. The metabolic fate of dietary polyphenols in humans. Free Radic Biol Med 33(2):220-235.

Rietjens, I.M.C.M.; Boersma, M.G.; van der Woude, H.; Jeurissen, S.M.F.; Schutte, M.E.; Alink, G.M. 2005. Flavonoids and alkenylbenzenes: Mechanisms of mutagenic action and carcinogenic risk. Mutat Res 574(1&2):124-138.

Rimm, E.C.; Katan, M.B.; Ascherio, A.; Stampfer, M.J.; Willett, W.C. 1996. Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. Ann Intern Med 125(5):384-389.

Rueff, J.; Laires, A.; Borba, H.; Chaveca, T.; Gomes, M.; Halpern, M. 1986. Genetic toxicology of flavonoids: The role of metabolic conditions in the induction of reverse mutation, SOS function and sister-chromatid exchanges. Mutagenesis 1(3):179-183.

Rueff, J.; Laires, A.; Gaspar, J.; Borba, H.; Rodrigues, A. 1992. Oxygen species and the genotoxicity of quercetin. Mutat Res 265(1):75-81.

Ruiz, M.J.; Fernández, M.; Estela, J.M.; Asensi, M.; Mañes, J.; Picó, Y. 2006. Short-term oral toxicity of quercetin and pterostibene in Swiss mice. Toxicol Lett 164(Suppl.): S275-S276 [Abstract No. P22-15].

Sahu, R.K.; Basu, R.; Sharma, A. 1981. Genetic toxicological of some plant flavonoids by the micronucleus test. Mutat Res 89(1):69-74.

Saito, D.; Shirai, A.; Matsushima, T.; Sugimura, T.; Hirono, I. 1980. Test of carcinogenicity of quercetin, a widely distributed mutagen in food. Teratog Carcinog Mutagen 1(2):213-221.

Sampson, L.; Rimm, E.; Hollman, P.C.; de Vries, J.H.; Katan, M.B. 2002. Flavonol and flavone intakes in US health professionals. J Am Diet Assoc 102(10):1414-1420.

Sato, H.; Takahashi, M.; Furukawa, F.; Miyakawa, Y.; Hasegawa, R.; Toyoda, K.; Hayashi, Y. 1987. Initiating potential of 2-2-furyl)-3- (5-nitro-2-furyl) acrylamide (AF- 2), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and 3,3,4,5,7-pentahydroxyflavone (quercetin) in two-stage mouse skin carcinogenesis. Cancer Lett 38(1&2):49-56.

Schimmer, O.; Hafele, F.; Kruger, A. 1988. The mutagenic potencies of plant extracts containing quercetin in *Salmonella typhimurium* TA98 and TA100. Mutat Res 206(2):201-208.

Schramm, D.D., Collins, H.E. Hawley, R.S. and German, J.B. 1998. Unaltered meiotic chromosome segregation in Drosophila melanogaster raised on a 5% quercetin diet. Food Chem Toxicol. 36(7):585-9.

Schulz, H.U.; Schürer, M.; Bässler, D.; Weiser, D. 2005a. Investigation of the bioavailability of hypericin, pseudohypericin, hyperforin and the flavonoids quercetin and isorhamnetin following single and multiple oral dosing of a hypericum extract containing tablet. 55(1):15-22.

Schulz, H.U.; Schürer, M.; Bässler, D.; Weiser, D. 2005b. Investigation of pharmacokinetic data of hypericin, pseudohypericin, hyperforin and the flavonoids quercetin and isorhamnetin revealed from single and multiple oral dose stduies with a hypericum extract containing tablet in healthy male volunteers. 55(10):561-568.

Schwarz, D., Kisselev, P. and Roots, I. 2003. St. John's wort extracts and some of their constituents potently inhibit ultimate carcinogen formation from benzo[a]pyrene-7,8-dihydrodiol by human CYP1A1. Cancer Res. 63(22):8062-8068.

Seino, Y.; Nagao, M.; Yahagi, T.; Sugimura, T.; Yasuda, T.; Nishimura, S. 1978. Identification of a mutagenic substance in a spice, sumac, as quercetin. Mutat Res 58(2&3):225-229.

Sengupta, B.; Sengupta, P.K. 2003. Binding of quercetin with human serum albumin: A critical spectroscopic study. Biopolymers 72(6):427-434.

Sergent, T.; Dupont, I.; Van der Heiden, E.; Scippo, M.L.; Pussemier, L.; Larondelle, Y.; Schneider, Y.J. 2009. CYP1A1 and CYP3A4 modulation by dietary flavonoids in human intestinal Caco-2-cells. Toxicol Lett 191(2-3):216-22.

Shali, N.A.; Curtis, C.G.; Powell, G.M.; Roy, A.B. 1991. Sulphation of the flavonoids quercetin and catechin by rat liver. Xenobiotica 21(7):881-893.

Shanely, R.A., Knab, A.M., Nieman, D.C., Jin, F., McAnulty, S.R. and Landram, M.J. 2010. Quercetin supplementation does not alter antioxidant status in humans. Free Radical Research 44(2):224-231.

Shoskes,D.A.; Zeitlin, S.I.; Shahed, A.; Rajfer, J. 1999. Quercetin in men with category III chronic prostatitis: A preliminary prospective, double-blind, placebo-controlled trial. Urology 54(6): 960-963.

Si, D., Wang, Y., Zhou, Y.H., Guo, Y., Wang, J., Zhou, H., Li, Z.S. and Fawcett, J.P. 2009. Mechanism of CYP2C9 inhibition of flavones and flavonols. Drug Metab Dispos. 37(3):629-634.

Silberberg, M.; Morand, C.; Manach, C.; Scalbert, A.; Remesy, C. 2005. Co-administration of quercetin and catechin in rats alters their absorption but not their metabolism. Life Sci 77(25):3156-3167.

Skibola, C.F.; Smith, M.T. 2000. Potential health impacts of excessive flavonoid intake. Free Radical Biology and Medicine 29(3&4):375-383.

Stelzig, D.A.; Ribeiro, S. 1972. Metabolism of quercetin and tricin in the male rat. Proc Soc Exp Biol Med 141(1):346-349.

Stoewsand, G.S.; Anderson, J.L.; Boyd, J.N.; Hrazdina, G.; Babish, J.G.; Walsh, K.M.; Losco, P. 1984. Quercetin: A mutagen, not a carcinogen, in Fischer rats. J Toxicol Environ Health 14(2&3):105-114.

Strobel, P.; Allard, C.; Perez-Acle, T.; Calderon, R.; Aldunate, R.; Leighton, F. 2005. Myricetin, quercetin and catechin-gallate inhibit glucose uptake in isolated rat adipocytes. Biochem J 386(3):471-478.

Sullivan, M.; Follis, R.H. (Jr.); Hilgartner, M. 1951. Toxicology of podophyllin. Proc Soc Exp Biol Med 77:269-272.

Suzuki, S.; Takada, T.; Sugawara, Y.; Muto, T.; Kominami, R. 1991. Quercetin induces recombinational mutations in cultured cells as detected by DNA fingerprinting. Jpn J Cancer Res 82(10):1061-1064.

Swisher, D.K.; Harris, R.D.; Tague, R.M.; Seyler, D.E. 1994. Effects of quercetin on rabbit sperm. Biol Reprod 50(Suppl. 1):107 [Abstract No. 211].

Tabassum, S.; Afridi, B.; Aman, Z. 2005. Phloroglucinol for acceleration of labor: double blind, randomized controlled trial. J Pak Med Assoc 55:270-273.

Taj, S.; Nagarajan, B. 1996. Inhibition by quercetin and luteolin of chromosomal alterations induced by salted, deep-fried fish and mutton in rats. Mutat Res 369:97-106.

Takanashi, H.; Aiso, S.; Hirono, I.; Matsushima, T.; Sugimura, T. 1983. Carcinogenicity test of quercetin and kaempferol in rats by oral administration. J Food Saf 5(2):55-60.

Tanaka, T.; Kojima, T.; Suzui, M.; Mori, H. 1993. Chemoprevention of colon carcinogenesis by the natural product of a simple phenolic compound protocatechuic acid: suppressing effects on tumor development and biomarkers expression of colon tumorigenesis. Cancer Res 53:3908-3913.

Tanaka, T.; Kawabata, K.; Honjo, S.; Kohno, H.; Murakami, M.; Shimada, R.; Matsunaga, K.; Yamada, Y.; Shimizu, M. 1999. Inhibition of azoxymethane-induced aberrant crypt foci in rats by natural compounds, caffeine, quercetin and morin. Oncol Rep 6(6):1333-1340.

Trejo, R.; Valadéz-Salazar, A.; Delhumeau, G. 1995. Effects of quercetin on rat testis aerobic glycolysis. Can J Physiol Pharmacol 73(11):1605-1615.

U.S. FDA. 1993. Appendix I. Table 14. Conversion table for test chemical treatment doses used in PAFA. In: Priority Based Assessment of Food Additives (PAFA) Database. Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition (CFSAN); Washington, DC, p. 58.

U.S. FDA. 2007. Title 21. Subchapter B--Food for human consumption. Part 101 – Food Labeling. Section §101.12—Reference amounts customarily consumed per eating occasion. In: CFR. Code of Federal Regulations. U.S. Government, Food and Drug Administration, U.S. (FDA).

Ueno, I.; Nakano, N.; Hirono, I. 1983. Metabolic fate of [14C] quercetin in the ACI rat. Jpn J Exp Med 53(1):41-50.

Ueno, I.; Kohno, M.; Haraikawa, K.; Hirono, I. 1984. Interaction between quercetin and superoxide radicals. Reduction of the quercetin mutagenicity. J Pharmacobiodyn 7(11):798-803.

Umezawa, K.; Matsushima, T.; Sugimura, T.; Hirakawa, T.; Tanaka, M.; Katoh, Y.; Takayama, S. 1977. *In vitro* transformation of hamster embryo cells by quercetin. Toxicol Lett 1(3):175-178.

U.S. EPA. 1991. Alpha$_{2u}$-Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in the Male Rat. Peer Review Workshop, Nov. 13-14, 1990, Gaithersburg, Maryland. U.S. Environmental Protection Agency, (U.S. EPA), Risk Assessment Forum; Washington, DC, pp. 81-97 [EPA/625/3-91/019F].

USDA. 2000. 1994-1996, 1998 Continuing Survey of Food Intakes by Individuals (CSFII) and Diet and Health Knowledge Survey (DHKS) (On CD-ROM). U.S. Department of Agriculture (USDA); Riverdale, Maryland. PB2000-500027 Supercedes PB98-500457.

USDA. 2009a. *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2003-2004.* Riverdale (MD): U.S. Department of Agriculture (USDA). Available from: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release.

USDA. 2009b. *What We Eat In America: National Health and Nutrition Examination Survey (NHANES): 2005-2006.* U.S. Department of Agriculture (USDA); Riverdale, Maryland. Available from: http://www.ars.usda.gov/Services/docs.htm?docid=15044.

USP. 2007. Microbiological tests. In: United States Pharmacopeia & National Formulary 2007 (30th Ed.). U.S. Pharmacopeia (USP) Convention Inc.; Rockville, Maryland [Online version].

Valentich, M.A.; Eynard, A.R.; Barotto, N.N.; Diaz, M.P.; Bongiovanni, G.A. 2006. Effect of the co-administration of phenobarbital, quercetin and mancozeb on nitrosomethylurea-induced pancreatic tumors in rats. Food Chem Toxicol 44(12):2101 2105.

van der Hoeven, J.C.; Bruggeman, I.M.; Debets, F.M. 1984. Genotoxicity of quercetin in cultured mammalian cells. Mutat Res 136(1):9-22.

Verma, A.K.; Johnson, J.A.; Gould, M.N.; Tanner, M.A. 1988. Inhibition of 7,12-dimethylbenz(*a*)anthracene-and *N*-nitrosomethylurea-induced rat mammary cancer by dietary flavonol quercetin. Cancer Res 48(20):5754-5758 [Erratum, 1989, 49(4):1073].

Volate, S.R.; Davenport, D.M.; Muga, S.J.; Wargovich, M. 2005. Modulation of aberrant crypt foci and apoptosis by dietary herbal supplements (quercetin, curcumin, silymarin, ginseng and rutin). Carcinogenesis 26(8):1450-1456.

von Moltke, L.L., Weemhoff, J.L., Bedir, E., Khan, I.A., Harmatz, J.S., Goldman, P. and Greenblatt, D.J. 2004. Inhibition of human cytochromes P450 by components of Ginkgo biloba. J Pharm Pharmacol. 56(8):1039-1044.Walsky, R.L., Gaman, E.A. and Obach, R.S. 2005. Examination of 209 drugs for inhibition of cytochrome P450 2C8. J Clin Pharmacol. 45(1):68-87.

Vrijsen, R.; Michotte, Y.; Boeye, A. 1990. Metabolic activation of quercetin mutagenicity. Mutat Res 232(2):243-248.

Walgren, R.A.; Walle, U.K.; Walle, T. 1998. Transport of quercetin and its glucosides across human intestinal epithelial Caco-2 cells. Biochem Pharmacol 55(10):1721-1727.

Walle, T.; Otake, Y.; Walle, U.K.; Wilson, F.A. 2000. Quercetin glucosides are completely hydrolyzed in ileostomy patients before absorption. J Nutr 130(11):2658-6661.

Walle, T.; Walle, U.K.; Halushka, P.V. 2001. Carbon dioxide is the major metabolite of quercetin in humans. J Nutr 131(10):2648-2652.

Walsky, R.L., Astuccio, A.V. and Obach, R.S. 2006. Evaluation of 227 drugs for in vitro inhibition of cytochrome P450 2B6. J Clin Pharmacol. 46(12):1426-1438.

Wang, L.; Morris, M.E. 2005. Liquid chromatography-tandem mass spectroscopy assay for quercetin and conjugated quercetin metabolites in human plasma and urine. J Chromatogr B 821(2):194-201.

Wang, C.Y.; Chiu, C.W.; Pamukcu, A.M.; Bryan, G.T. 1976. Identification of carcinogenic tannin isolated from Bracken fern (*Pteridium aquilinum*). J Natl Cancer Inst 56(1):33 36.

Wang, F.M.; Yao, T.W.; Zeng, S. 2003a. Determination of quercetin and kaempferol in human urine after orally administrated tablet of ginkgo biloba extract by HPLC. J Pharm Biomed Anal 33(2):317-321.

Wang, F.M.; Yao, T.W.; Zeng, S. 2003b. Disposition of quercetin and kaempferol in human following an oral administration of Ginkgo biloba extract tablets. Eur J Drug Metab Pharmacokinet 28(3):173-177.

Watson, W.A. 1982. The mutagenic activity of quercetin and kaempferol in *Drosophila melanogaster*. Mutat Res 103(2):145-147.

Weldin, J.; Jack, R.; Dugaw, K.; Kapur, R.P. 2003. Quercetin, an over-the-counter supplement, causes neuroblastoma-like elevation of plasma homovanillic acid. Pediatr Dev Pathol 6(6):547-551.

Werner, B.; Sprecher, W.; Mitschke, H. 1985. Quercetin und dünndarmkrebs. [Quercetin and intestinal tumours an experimental study]. Verdauungskrankheiten 3(3):113-115.

Wiczkowski, W.; Nèmeth, K.; Buciński, A.; Piskula, M.K. 2003. Bioavailability of quercetin from flesh scales and dry skin of onion in rats. Pol J Food Nutr Sci 12(Spec. Iss. 1):95-99.

Willhite, C.C. 1982. Teratogenic potential of quercetin in the rat. Food Chem Toxicol 20(1):75-77.

Xu, M.; Zhang, Z.; Fu, G.; Sun, S.; Sun, J.; Yang, M.; Liu, A.; Han, J.; Guo, D. 2007. Liquid chromatography-tandem mass spectrometry analysis of protocatechuic aldehyde and its phase I and II metabolites in rat. J Chromatogr B 856(1&2):100-107.

Yang, K.; Lamprecht, S.A.; Liu, Y.; Shinozaki, H.; Fan, K.; Leung, D.; Newmark, H.; Steele, V.E.; Kelloff, G.J.; Lipkin, M. 2000. Chemoprevention studies of the flavonoids quercetin and rutin in normal and azoxymethane-treated mouse colon. Carcinogenesis 21(9):1655 1660.

Yang, C.-Y.; Hsiu, S.-L.; Wen, K.-C.; Lin, S.-P.; Tsai, S.-Y.; Hou, Y.-C.; Chao, P.-D.L. 2005. Bioavailability and metabolic pharmacokinetics of rutin and quercetin in rats. Yao Wu Shi Pin Fen Xi 13(3):244-250.

Yoshida, M.A.; Sasaki, M.; Sugimura, K.; Kawachi, T. 1980. Cytogenetic effects of quercetin on cultured mammalian cells. Proc Jpn Acad Ser B 56(7):443-447.

Young, J.F.; Nielsen, S.E.; Haraldsdottir, J.; Daneshvar, B.; Lauridsen, S.T.; Knuthsen, P.; Crozier, A.; Sandstrom, B.; Dragsted, L.O. 1999. Effect of fruit juice intake on urinary quercetin excretion and biomarkers of antioxidative status. Am J Clin Nutr 69(1):87-94.

Zhu, B.T.; Liehr, J.G. 1993. Rapid methylation of the mutagenic flavonoid quercetin: A potential mechanism for its inactivation and lack of carcinogenicity *in vivo*. Proc Am Assoc Cancer Res Annu Meet 34:155 [Abstract No. 924].

Zhu, B.T.; Liehr, J.G. 1994. Quercetin increases the severity of estradiol-induced tumorigenesis in hamster kidney. Toxicol Appl Pharmacol 125(1):149-158.

Zhu, B.T.; Ezell, E.L.; Liehr, J.G. 1994. Catechol-*O*-methyltransferaase-catalyzed rapid *O*-methylation of mutagenic flavonoids. J Biol Chem 269(1):292-299.

Zou, L., Harkey, M.R. and Henderson, G.L. 2002. Effects of herbal components on cDNA-expressed cytochrome P450 enzyme catalytic activity. Life Sci. 71(13):1579-1589.

ATTACHMENT 1

CURRICULA VITAE OF EXPERT PANEL MEMBERS

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ATTACHMENT 2

TABLES A2-1 THROUGH A2-11
SUMMARIZING RESULTS OF THE TOXICOLOGICAL STUDIES

Table A2-1 Effects of Quercetin on Plasma Glucose Levels in Normoglycemic and Diabetic Rats

Quercetin dose (mg/kg bw)	Route/Frequency of quercetin dosing (if repeated report duration)	Serum/plasma quercetin levels (ng/mL)	Time of diabetes induction compared to quercetin administration	Effects of quercetin treatment on plasma glucose levels in diabetic rats	Effects of quercetin treatment on plasma glucose levels in control rats	Reference
Streptozocin-Induced Diabetic Rats						
10, 15 (dissolved in 60% ethanol)	IP, repeated (once per day for 10 days)	N/D	2 weeks before	Decreased and normalized	No change	Vessal *et al.*, 2003
10 (suspended in 0.5% sodium carboxy methylcellulose)	Oral, repeated (once per day for 4 weeks)	N/D	4 weeks before	No change	No change	Anjaneyulu and Chopra, 2005
27 (suspended in 0.2% Tween)	IP, repeated (once per day for 8 weeks)	N/D	5 days before	No change	No change	Dias *et al.*, 2005
50	Oral, repeated (once per day for 3 weeks)	N/D	2 weeks before	Decreased	No change	Kato *et al.*, 2008
Streptozocin-Induced Diabetic Mice						
150 or 750 (in the diet)	Oral, repeated (once per day for 2 weeks)	2127 (in the 150 mg/kg body weight group) 6235 (in the 750 mg/kg body weight group)	1 week before	Decreased	N/D	Kobori *et al.*, 2009

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Table A2-2 Summary of Studies Conducted to Assess GSH Status in Animals Following Quercetin Administration

Species	Dose	Route	Duration	Special Conditions	Results[a]	Reference
Mouse (Swiss NMRI; M; 20 to 25 g)	1, 3, or 9 µg/mL (0.25, 0.75, and 2.25 mg/kg bw/day)	Oral (water)	8 weeks	Normal diet	↑ hepatic and pulmonary GST [9] and GSH [3 and 9]	Gandhi and Khanduja (1993)
Mouse (Swiss Webster; M; 25 to 30 g)	1 mg/day (36.4 mg/kg bw/day)	Oral (diet)	7 days	Normal diet	<u>In tissue:</u> ↑ GSH in liver (sig.) and plasma, no Δ in heart; No Δ in GSSG levels (liver, heart, plasma); ↑ in liver GSH:GSSG (non-sig.; 40%), no Δ in heart and plasma; ↓ levels of protein-GSH mixed disulfide in liver and plasma, no Δ in heart. <u>In mitochondria:</u> No Δ in liver GSH or GSSG, or heart GSH; ↑ in heart GSSG; ↓ in liver and heart GSH:GSSG ratio; ↓ levels of protein-GSH mixed disulfide in liver, ↑ in heart.	Meyers *et al.* (2008)
Rat [Wistar Kyoto (WKY); M]	10 mg/kg bw/day	Gavage	5 weeks	None	↓ liver GSH levels compared to WKY-control; No Δ in GPx, GR, or MDA.	Duarte *et al.* (2001)
Rat [Spontaneously hypertensive (SH)[b]; M]				Hypertensive rats	↓ liver GSH levels compared to SH control, but ↑ (non-sig.) compared to WKY-control and WKY-Qu; ↓ in MDA; ↑ in GPx, no Δ in GR.	
Rat (Wistar; M)	10 mg/kg bw/day	Gavage	5 weeks	None	No Δ in TBARS and NO_x, liver GST, GPx, or GR; ↓ in liver GSH.	García-Saura *et al.* (2005)
Rat (2K1C Goldblatt)				Hypertensive rats	↓ in plasma TBARS and NO_x; No Δ in liver GSH, GST, and GR; ↑ in liver GPx.	

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Table A2-2 Summary of Studies Conducted to Assess GSH Status in Animals Following Quercetin Administration

Species	Dose	Route	Duration	Special Conditions	Results[a]	Reference
Rat (Sprague-Dawley; M)	2 or 20 mg/day (8 or 80 mg/kg bw/day)	Gavage	4 weeks	Normal diet	No Δ in serum or liver vitamin E levels [2 or 20] ↓ in liver MDA [2 or 20] ↓ in liver GSH levels [2 or 20]; ↓ in liver GR levels [sig. at 20] No Δ in GPx	Choi *et al.* (2003)
				Vitamin E-free diet	Compared to vitamin E-free vehicle: ↑ in serum and liver vitamin E levels [2 or 20] ↓ in liver MDA [2 or 20] ↓ in liver GSH levels [2 or 20]; ↓ in liver GR levels [sig. at 20] No Δ in GPx	
Rat (Sprague-Dawley; M)	20 mg/day (80 mg/kg bw/day)	Gavage	6 weeks	Quercetin only	↑ in serum and liver (sig) vitamin E; ↓ in liver GSH and GR levels; ↓ in liver MDA No Δ in liver GPx.	Choi *et al.* (2005)
				With daidzein	↑ liver GSH, GPx, and GR levels compared to Qu-only	

Δ = change; ↓ - decreased; ↑ = increased; GPx = Glutathione peroxidise; GR = Glutathione reductase; GSH = reduced glutathione; GSSG = glutathione disulfide or oxidized fructathione; GST = glutathione transferase; i.p = intraperitoneal; M = Male; MDA = Malondialdehyde; non-sig = non-significant; NO_X = Nitrates plus nitrites; Qu = Quercetin; SH = Spontaneously hypertensive; sig = significant; TBARS = Thiobarbituric acid reactive substances.
[a] Values in brackets indicate the dose at which the effect was observed.
[b] Shows evidence of oxidative stress.

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Table A2-3 Quercetin Effect on the Pharmacokinetics of CYP3A or P-gp Substrate Drugs in Different Species

Quercetin dose (mg/kg bw)	Route/Frequency and Duration	Drug	Drug dose (mg/kg bw)	Route/Frequency and Duration	Increase in Drug AUC (%)[a,b]	Reference
Rat						
50 (in glycofurol)	Oral; single	Cyclosporin	10 (in olive oil)	Oral; single	43% decrease	Hsiu *et al.*, 2002
2, 10, 20 (suspension in water and tween80)	Oral; single (2, 10, 20 mg/kg bw); repeated (10 and 20 mg/kg bw for 3 days)	Paclitaxel	40 (in suspension)	Oral; single (20 min after quercetin administration); repeated (3 days after quercetin administration)	Single quercetin treatment: 77 (2 mg/kg bw); 161 (10 mg/kg bw); 210 (20 mg/kg bw) Repeated quercetin treatment: 219 (10 mg/kg bw); 230 (20 mg/kg)	Choi *et al.*, 2004a
2.5, 7.5, 15 (in distilled water)	Oral; single	Tamoxifen	10 (in distilled water)	Oral; single	35 (2.5 mg/kg bw); 61 (7.5 mg/kg bw)	Shin *et al.*, 2006
			2 (in saline)	IV; single	No change	
10 (suspension in 0.5% methylcelulose)	Oral; single	Pioglitazone	10 (0.1 M citrate buffer)	Oral; single (1 hour after quercetin administration)	58	Umathe *et al.*, 2008
			5 (0.1 M citrate buffer)	IV; single (1 hour after quercetin administration)	21	
20 (suspension in water and tween80)	Oral; single	Irinotecan	80	Oral; single (2 hours after quercetin administration)	29	Bansal *et al.*, 2008
			20	IV; single (2 hours after quercetin administration)	No change	
0, 1, 5, 15 (dissolved in distilled water)	Oral; single	Etoposide	9 (in saline)	Oral; single	42 (5 mg/kg bw), 53-fold (15 mg/kg bw)	Li and Choi, 2009
			3 (in saline)	IV; single	No change	
Rabbit						
5, 15 (dissolved in distilled water)	Oral; single	Verapamil	10 (dissolved in distilled water)	Oral; single	No change	Choi and Han, 2004
			10 (dissolved in distilled water)	Oral; single (30 min after quercetin administration)	46 (5 mg/kg bw); 72 (15 mg/kg bw)	

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Table A2-3 Quercetin Effect on the Pharmacokinetics of CYP3A or P-gp Substrate Drugs in Different Species

Quercetin dose (mg/kg bw)	Route/Frequency and Duration	Drug	Drug dose (mg/kg bw)	Route/Frequency and Duration	Increase in Drug AUC (%)[a,b]	Reference
2, 10, 20 (suspension in distilled water)	Oral; single	Diltiazem	15 (dissolved in distilled water)	Oral; single	No change	Choi and Li, 2005
			15 (dissolved in distilled water)	Oral, single (0.5 hour after quercetin administration)	75 (2 mg/kg bw); 143 (10 mg/kg bw; 176 (20 mg/kg bw)	
			5 (dissolved in saline)	IV; single	No change	
Pig						
50 (in glycofurol)	Oral; single	Cyclosporin	10 (in olive oil)	Oral; single	42% decrease	Hsiu *et al.*, 2002
40 (in glycofurol)	Oral; single	Digoxin	0.02 (in distilled water)	Oral; single	170	Wang *et al.*, 2004

Abbreviations: AUC = area under the serum concentration-time curve; bw = body weight; IV = intravenous.
[a] The AUC reported reflects the area under the plasma concentration-time curve from time zero to the last point.
[b] Numbers in parentheses represent dose levels at which the effects were observed.

Table A2-4 Summary of *In Vitro* Mutagenicity/Genotoxicity Studies on Quercetin in Prokaryotic Test Systems

Test System	Type	Metabolic Activation	Concentration(s) Tested	Result	Reference
Salmonella typhimurium TA100	Mut	+S9	5 µg/plate[1]	Positive	Stoewsand *et al.*, 1984
		-S9	18 µg/plate[1]		
S. typhimurium TA98	Mut	+/-S9	100 nmol/plate (30.2 µg/plate)	Positive	Hatcher and Bryan, 1985
S. typhimurium TA98	Mut	-S9 or +X/XO + SOD	0.1 or 0.2 µmol/plate	Positive	Ueno *et al.*, 1984
		+X/XO		Positive[2]	
S. typhimurium TA98	Mut	+/-S9	Up to 500 µg/plate	Positive	Ochiai *et al.*, 1984
S. typhimurium TA98	Mut	+/-S9, S100	15 µg/plate	Positive	Vrijsen *et al.*, 1990
S. typhimurium TA98	Mut	+/-S9	2.5 to 7.5 µg/plate	Positive	Nguyen *et al.*, 1989
S. typhimurium TA98	Mut	+/-S9, S100	Up to 80 nmol/plate (24.2 µg/plate)	Positive	Rueff *et al.*, 1986
S. typhimurium TA98	Mut	-S9	1.6 to 100 µg/plate	Positive[3]	Cross *et al.*, 1996
S. typhimurium TA98	Mut	+/-S9, S100, SOD, CAT	33 nmol (9.97 µg/plate)	Positive	Rueff *et al.*, 1992
S. typhimurium TA98	Mut	+X/XO	33 nmol (9.97 µg/plate)	Negative	Rueff *et al.*, 1992
S. typhimurium TA102	Mut	NS	10 to 500 µg/plate	Negative	Tieppo *et al.*, 2007
S. typhimurium TA102	Mut	+/-S9	25 to 250 µM	Positive	Makena *et al.*, 2009
S. typhimurium TA100	Mut	+/-S9	Up to 500 µg/plate	Positive	Czeczot, 1994
S. typhimurium TA100 and TA102	Mut	-S9	1.6 to 100 µg/plate	Negative	Cross *et al.*, 1996
S. typhimurium TA97 and TA1537	Mut	-S9	Up to 50 µg/plate	Positive	Busch *et al.*, 1986
S. typhimurium TA98 and TA100	Mut	+/-S9	1.5 to 150 µg/plate	Positive	Crebelli *et al.*, 1987

Table A2-4 Summary of *In Vitro* Mutagenicity/Genotoxicity Studies on Quercetin in Prokaryotic Test Systems

Test System	Type	Metabolic Activation	Concentration(s) Tested	Result	Reference
S. typhimurium TA98 and TA100	Mut	+/-S9	Up to 50 µg/plate	Positive	Bjeldanes and Chang, 1977
S. typhimurium TA98, TA100, and TA1537	Mut	+/-S9	Up to 500 µg/plate	Positive	Hardigree and Epler, 1978
S. typhimurium TA1535 and TA1538	Mut	NS	NS	Negative/weakly positive	Hardigree and Epler, 1978
S. typhimurium TA98, TA100, TA1537, and TA1538	Mut	+/-S9	20 µg/plate	Positive	Brown and Dietrich, 1979
S. typhimurium TA97, TA98, TA100, TA102, and TA1538	Mut	+/-S9	Up to 100 µg/plate	Positive	Czeczot *et al.*, 1990
S. typhimurium TA1538	Mut	+S9	Up to 100 µg/plate	Positive	Bjeldanes and Chang, 1977
S. typhimurium TA1535	Mut	NS	NS	Negative	Bjeldanes and Chang, 1977
S. typhimurium TA1535	Mut	+/-S9	Up to 100 µg/plate	Negative	Czeczot *et al.*, 1990
S. typhimurium TA1538, TA1534, TA1978, TA94, and D3052	Mut	-S9	1.5 to 150 µg/plate	Negative	Crebelli *et al.*, 1987
S. typhimurium TA98 and TA100	Mut	+/-S9	0.3 to 1,000 µg/plate	Positive	NTP, 1992
S. typhimurium TA98 and TA100	Mut	+/-S9	Up to 100 µg/plate	Positive	Seino *et al.*, 1978
S. typhimurium TA98 and TA100	Mut	+/-S9	Up to 100 µg/plate	Positive	Schimmer *et al.*, 1988
S. typhimurium TA98 and TA100	Mut	+/-S9	13 to 3,310 nmol/plate (3.9 to 993 µg/plate)	Positive	MacGregor and Jurd, 1978
S. typhimurium TA1538 *uvr*B$^-$ and TA1978 *uvr*B$^+$	Repair assay	+/-S9	50 to 200 µg/plate	Negative	Czeczot and Kusztelak, 1993
Bacilus subtilis 168	For. Mut	+/-S9	NS	Negative	MacGregor, 1979 [abstract]
B. subtilis hcr-9	For. Mut	+S9	NS	Negative	MacGregor, 1979 [abstract]

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Table A2-4 Summary of *In Vitro* Mutagenicity/Genotoxicity Studies on Quercetin in Prokaryotic Test Systems

Test System	Type	Metabolic Activation	Concentration(s) Tested	Result	Reference
Escherichia coli K-12 PQ37*uvr*A	SOS chromotest	+/-S9	2.5 to 60 µg/mL	Weakly positive	Czeczot *et al.*, 1993
E. coli PQ37	SOS chromotest	+/-S9, S100	Up to 70 nmol/plate (21 µg/plate)	Positive	Rueff *et al.*, 1986
E. coli PQ37	SOS chromotest	+/-SOD[4]	16.5 nmol (4.99 µg/plate)	Positive[5]	Rueff *et al.*, 1992
E. coli PQ37	SOS chromotest	+/-S9	Up to 1,000 µg/mL	Positive	Dayan *et al.*, 1987
E. coli PQ30	SOS chromotest (*sfiA* gene)	+/-S9	Up to 80 nmol (24.2 µg/plate)	Positive[6]	Llagostera *et al.*, 1987
E. coli GC2375 and UA4163	SOS chromotest (*recA* and *umuC* gene, respectively)	+/-S9	Up to 80 nmol (24.2 µg/plate)	Weakly positive[7]	Llagostera *et al.*, 1987
E. coli K-12 PQ37	SOS chromotest	+/-S9	Up to 20 µg/plate	Weakly positive	Czeczot and Kusztelak, 1993
E. coli K-12 343/113	Rev. Mut (*nad* and *arg* locus)	+S9	Up to 2,000 µg/mL	Positive	Hardigree and Epler, 1978
E. coli K-12 343/113	For. Mut (*gal* locus)	+/-S9	NS	Negative	Hardigree and Epler, 1978
E. coli WP-2 *uvrA*	Mut	+/-S9	25 to 250 µM	Positive	Makena *et al.*, 2009
E. coli WP2 *uvrA* and IC2486 *uvrA* (with and without pKM101)	Mut	+/-S9	Up to 500 µg/plate	Positive	Czeczot, 1994
E. coli IC188 and IC203	Mut	NS	10 to 500 µg/plate	Negative	Tieppo *et al.*, 2007
E. coli MD322-PQ37 *ser*B, *leu*$^+$, *dna*C	SSB	+/-S9	50 µg/mL	Positive	Czeczot *et al.*, 1993

Mut = mutation; -S9 = without metabolic activation; +S9 = with metabolic activation; X/XO = xanthine/xanthine oxidase; SOD = superoxide dismutase; CAT = catalase; NS = not specified; For. Mut. = forward mutation; Rev. Mut. = reverse mutation; SSB = DNA single strand breaks.

[1] The lowest concentration at which a statistically significant response was observed.
[2] Although positive compared to controls, fewer revertants compared to -S9.
[3] Positive at 3.13 µg/plate and greater.
[4] Examined at pH 6.0 and 7.4.
[5] Positive response was greater at pH 6.0 in comparison to at pH 7.4. SOD did not affect the mutagenicity at pH 6.0, but increased the mutagenicity at 7.4.
[6] Enhanced activity in the absence of S9.
[7] No difference in activity with and without S9.

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Table A2-5 Summary of *In Vitro* Mutagenicity/Genotoxicity Studies on Quercetin in Eukaryotic Test Systems

Test System	Type	Metabolic Activation	Concentration(s) Tested	Result	Reference
Saccharomyces cerevisiae D4	Gene conversion	-S9	Up to 10 mg/2 mL of incubation mix	Positive[1]	Hardigree and Epler, 1978
Saccharomyces cerevisiae XΛ4-8C*p*⁻	For. and Rev. Mut	+/-S9	NS	Negative	Hardigree and Epler, 1978
BALB/c 3T3 mouse cells	CTA	-S9	5 to 20 µg/mL	Negative[2]/weakly positive	Meltz and MacGregor, 1981
Mouse lymphoma L5178Y cells	*tk* locus	-S9	8.9 to 44.7 µg/mL	Negative[3]/weakly positive	van der Hoeven *et al.*, 1984
		+S9	8.9 and 26.8 µg/mL	Negative	
Mouse lymphoma L5178Y cells	*tk* locus	+/-S9	10 to 50 µg/mL	Positive[4]	Meltz and MacGregor, 1981
Mouse lymphoma L5178Y cells	HPRT locus	+/-S9	8.9 to 44.7 µg/mL	Negative	van der Hoeven *et al.*, 1984
Mouse lymphoma L5178Y cells	SSB	-S9	10 to 20 µg/mL	Positive	Meltz and MacGregor, 1981
C57BL/6 mouse BMT-11 tumor cells	DNA finger-printing[5]	-S9	55 µM (16.6 µg/mL)	Positive	Suzuki *et al.*, 1991
C3H/He mouse FM3A tumor cells	DNA finger-printing[5]	-S9	55 µM (16.6 µg/mL)	Positive	Suzuki *et al.*, 1991
Chinese hamster Don6 and B131 fibroblasts	SCE	-S9	1.0 to 15.0 µg/mL	Positive	Yoshida *et al.*, 1980
Chinese hamster Don6 and B131 fibroblasts	CA	-S9	1.0 to 15.0 µg/mL	Positive	Yoshida *et al.*, 1980
Chinese hamster ovary-AT3-2 cells	Mut (*tk* locus)	-S9	6 to 15 µg/mL	Positive	Carver *et al.*, 1983
	Mut (*hgprt, aprt,* and ATPase) loci)			Negative	
Chinese hamster ovary cells	SCE	-S9	2.5 to 20 µg/mL	Weakly positive[6]	Kubiak and Rudek, 1990
Chinese hamster ovary cells	SCE	-S9	0.67 to 20.0 µg/mL	Positive	NTP, 1992
		+S9	2.0 to 45.0 µg/mL		

Table A2-5 Summary of *In Vitro* Mutagenicity/Genotoxicity Studies on Quercetin in Eukaryotic Test Systems

Test System	Type	Metabolic Activation	Concentration(s) Tested	Result	Reference
Chinese hamster ovary-AT3-2 cells	SCE	-S9	5 to 15 µg/mL	Negative	Carver *et al.*, 1983
		+S9	6 to 15 µg/mL	Weakly positive (highest concentration only)	
Chinese hamster ovary cells	CA	-S9	2.5 to 20 µg/mL	Positive	Kubiak and Rudek, 1990
Chinese hamster ovary cells	CA	-S9	7.6 to 50.0 µg/mL[7]	Negative[8]/positive	NTP, 1992
		+S9	25.2 to 75.0 µg/mL[9]	Positive	
Chinese hamster ovary-AT3-2 cells	CA	-S9	6 to 15 µg/mL	Positive	Carver *et al.*, 1983
		+S9	NS	Negative	
V79 Chinese hamster cells	CA	+/-S9	8.9 to 65 µM (2.7 to 19.6 µg/mL)	Positive[10]	Gaspar *et al.*, 1994
Engineered V79 Chinese hamster cells with CYP 1A1, 1A2, or 2B1	CA	-S9	8.9 to 65 µM (2.7 to 19.6 µg/mL)	Positive	Gaspar *et al.*, 1994
Chinese hamster lung cells	DTR	-S9	Up to 200 µg/mL	Positive	Nakayasu *et al.*, 1986
Syrian golden hamster embryo cells	CTA	-S9	5 and 10 µg/mL	Positive	Umezawa *et al.*, 1977
V79 Chinese hamster lung cells	Mut[11]	-S9	10 to 50 µg/mL	Positive[12]	Maruta *et al.*, 1979
		+S9	50 to 200 µg/mL	Positive	
V79 Chinese hamster lung cells	HPRT locus	+/-S9	4.5 to 89.4 µg/mL	Negative	van der Hoeven *et al.*, 1984
V79 Chinese hamster lung cells	SCE	+/-S9	0.9 to 22.3 µg/mL	Negative	van der Hoeven *et al.*, 1984
V79 Chinese hamster lung cells	MN	-S9	3 to 22 µg/mL	Positive	Caria *et al.*, 1995
		+S9	3 to 22 µg/mL	Negative[13]/positive	
Rat hepatocytes	UDS	-S9	Up to 20 µM (~6 µg/mL)	Negative	Cross *et al.*, 1996
Rat hepatocytes	DNA repair test	-S9	10 µM (3.02 µg/mL)	Negative	Kato *et al.*, 1985

Table A2-5 Summary of *In Vitro* Mutagenicity/Genotoxicity Studies on Quercetin in Eukaryotic Test Systems

Test System	Type	Metabolic Activation	Concentration(s) Tested	Result	Reference
Rat liver nuclei	DNA unwinding assay	-S9	20 to 100 µM (~ 6 to 30 µg/mL)	Positive	Sahu and Washington, 1991
Rat H4IIE hepatoma cells	Comet assay	Metabolically active cell line	50 to 250 µmol/L	Positive[14]	Watjen *et al.*, 2005
Human HE2144 fibroblasts	SCE	-S9	1.0 to 5.0 µg/mL	Positive	Yoshida *et al.*, 1980
Human HE2144 fibroblasts	CA	-S9	1.0 to 5.0 µg/mL	Positive[15]	Yoshida *et al.*, 1980
Human lymphocytes	MN	+/-S9	3 to 22 µg/mL	Positive[16]	Caria *et al.*, 1995
Human lymphocytes	MN	-S9	Up to 50 µg/mL	Positive	Popp and Schimmer, 1991
Human lymphocytes	CA	-S9	5 to 12.5 µg/mL	Positive[17]	Yoshida *et al.*, 1980
Human lymphocytes	SCE	-S9	5 to 12.5 µg/mL	Positive[17]	Yoshida *et al.*, 1980
Human lymphocytes	SCE	+/-S9	Up to 4.5 x 10^{-4} mmol/plate (0.14 µg/plate)	Positive[4]	Rueff *et al.*, 1986
Human lymphocytes	SCE	-S9	5 to 50 µg/mL	Weakly positive[18]	Popp and Schimmer, 1991
Human lymphocytes	Comet assay	+/-S9	Up to 500 µM (151 µg/mL)	Positive	Basaran *et al.*, 1999 [abstract]
Human sperm cells	Comet assay	+/-S9	Up to 500 µM (151 µg/mL)	Negative	Basaran *et al.*, 1999 [abstract]

-S9 = without metabolic activation; +S9 = with metabolic activation; NS = not specified; CTA = cell transformation assay; SSB = DNA single-strand breaks; SCE = sister chromatid exchange; CA = chromosome aberrations; Mut = mutation; DTR = diphtheria toxin-resistant mutants; MN = micronuclei; UDS = unscheduled DNA synthesis

[1] Non-concentration-dependent at the higher concentrations (4 to 10 mg).
[2] Negative up to 12.5 µg/mL, positive at 15 µg/mL and greater.
[3] Negative at 8.9 µg/mL, positive at 17.8 µg/mL and greater.
[4] Mutagenicity decreased with metabolic activation.
[5] Mutations due to recombinations detected.
[6] Increase was not statistically significant.

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[7] Delayed harvest protocol employed to offset toxicity.
[8] Negative at 7.6 µg/mL, positive at 10.1 µg/mL and greater
[9] Standard harvest time.
[10] A decrease in the mutagenic response was observed with the addition of S9. Statistical significance was attained at 32.5 and 42.3 µM with S9 in comparison to test performed at same concentrations without S9
[11] Induction of 8-azaguanine-resistant mutation.
[12] Without metabolic activation, positive response observed only with a 2-day incubation period. No mutagenic activity following only a 1-hour incubation, at concentrations as high as 200 µg/mL.
[13] Negative up to 11 µg/mL, positive at the highest concentration (22 µg/mL).
[14] Cytotoxicity; EC_{50} = 35 ± 4 µmol/L
[15] Positive at the highest concentration tested (5 µg/mL).
[16] CREST-negative (kinetochore-negative) micronuclei.
[17] Positive at 8 µg/mL and greater.
[18] Positive at 20 µg/mL and above. Additionally, cytotoxic at 50 µg/mL in 1 of 2 trials.

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Table A2-6 Summary of *In Vitro* Mutagenicity/Genotoxicity Studies with Quercetin Derivatives and Metabolites

Test System (Substance)	Type	Metabolic Activation	Concentration(s) Tested	Result	Reference
S. typhimurium TA1535, TA1538, TA97, TA98, TA100 and TA102 (isorhamnetin)	Mut	+/-S9	5 to 500 µg/plate	Negative	Czeczot *et al.*, 1990
S. typhimurium TA98 (isorhamnetin extracted from urine of quercetin-treated rats)	Mut	+/-S9	NS	Negative	Onishi *et al.*, 1982 [abstract]
S. typhimurium TA98 (unidentified quercetin metabolite extracted from urine of quercetin-treated rats)	Mut	-S9	NS	Positive	Onishi *et al.*, 1982 [abstract]
		+S9		Negative	
S. typhimurium TA98 and TA100 (hydroxyphenylacetic acid, 3,4-dihydroxyphenylacetic acid, homovanillic acid, and a quercetin mono-methyl ether extracted from urine of quercetin-treated rat)	Mut	NS	NS	Negative	Hatcher *et al.*, 1981
S. typhimurium TA98 (3-*O*-methylquercetin and 3',4'-, 3,5-, 3,7-, 3,3'-di-*O*-methylquercetin, and pentamethylquercetin)	Mut	+/-S9	166 to 1,660 nmol/plate	Negative	MacGregor and Jurd, 1978
S. typhimurium TA98 (isorhamnetin)	Mut	-S9	166 to 443 nmol/plate	Equivocal[1]	
		+S9		Positive[2]	
S. typhimurium TA98 [4'-methylquercetin (tamarixetin) and 7,4'-*O*-dimethylquercetin]	Mut	-S9	166 to 1,660 nmol/plate	Negative	
		+S9		Positive[2]	
S. typhimurium TA100 (3-*O*-methylquercetin and 3',4'-methylquercetin)	Mut	+/-S9	166 to 1,660 nmol/plate	Negative	
S. typhimurium TA98 and TA100 [rhamnetin (7-methylquercetin) and 5,7-di-*O*-methylquercetin]	Mut	+/-S9	166 to 1,660 nmol/plate	Positive[2]	
S. typhimurium TA1538 [rhamnetin (7-methylquercetin)]	Mut	+/-S9	NS	Negative	Brown and Dietrich, 1979
S. typhimurium TA98, TA100, and TA1537 [rhamnetin (7-methylquercetin)]	Mut	+/-S9	100 µg/plate	Positive[2]	

Mut = mutation; +/-S9 = with or without metabolic activation; NS = not specified.
[1] Less than double the spontaneous mutation frequency at the lower concentration (166 nmol/plate) and 2.2-fold greater than the spontaneous mutation frequency at the higher concentration (443 nmol/plate).
[2] Mutagenic response reduced in comparison to quercetin.

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Table A2-7 Summary of *In Vitro* Mutagenicity/Genotoxicity Studies Using Urine, Fecal, and Plasma Samples of Quercetin-treated Rats

Test System	Type	Metabolic Activation	Quercetin Dose in Rats (mg/kg body weight)	Result	Reference
Urinary Samples/Extracts					
S. typhimurium TA100 (samples)	Mut	-S9	50 or 100	Negative	Stoewsand *et al.*, 1984[1]
		+S9	100	Positive	
S. typhimurium TA98 (extract)	Mut	+S9, +DE, or S9 + DE	500, 1,000, or 2,000[2]	Positive[3]	Crebelli *et al.*, 1987
S. typhimurium TA98 and TA100 (sample)	Mut	NS	1,000[2]	Positive	MacGregor, 1979 [abstract]
S. typhimurium TA98 and TA100	Mut	NS	NS	Negative	Hatcher *et al.*, 1981
Fecal Samples/Extracts					
S. typhimurium TA100 (sample)	Mut	-S9	1,000	Positive	Stoewsand *et al.*, 1984[1]
		+S9	500 or 1,000	Negative	
S. typhimurium TA98 (extract)	Mut	+/-DE	2,000[2]	Positive[4]	Crebelli *et al.*, 1987
Plasma Samples					
S. typhimurium TA98	Mut	+/-DE	1,000[5] or 2,800 [6]	Negative	Crebelli *et al.*, 1987

Mut = mutation; -S9 = without metabolic activation; +S9 = with metabolic activation; DE = deconjugating enzymes
[1] 0, 0.1 or 0.2% (~0, 50, and 100 mg/kg body weight/day, respectively) quercetin provided in the diet to Fisher 344 rats for a period of 64 weeks.
[2] Administered perorally or intraperitoneally.
[3] Following peroral administration, mutagenicity lasted for only 24 hours, whereas administered intraperitoneally, mutagenicity lasted for 48 hours.
[4] Feces samples mutagenic on the 1st and 2nd day following peroral and intraperitoneal treatment, respectively.
[5] Single gavage administration.
[6] Continuous feeding of 1.125 mg for 48 hours (approximately 2,800 mg/kg body weight/day).

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Table A2-8 Summary of *In Vivo* Mutagenicity/Genotoxicity Studies

Species and Test System	Type	Duration	Concentration or Dose (route of administration)	Result	Reference
Fly					
Drosophila melanogaster	RSLLT	18 hours	4.12 to 16.5 x 10^{-2} M (13 to 50 mg/mL)	Negative[1]/ positive	Watson, 1982
Drosophila melanogaster	MCS	7 days	5% diet	Negative	Schramm *et al.*, 1998
Mouse					
Mice (M) bone marrow cells	MN	Single dose[2]	0, 1, 20, 50, 80, 120, 160, 500, or 1,000 mg/kg bw (p.o.)	Negative	Aeschbacher *et al.*, 1982
ddY Mice (M)	MN	Single dose[3]	0, 125, 250, 500, or 1000 mg/kg bw (i.p.)	Negative	Hayashi *et al.*, 1988
CD-1 mice (M, F) bone marrow cells	MN	Single dose[3]	0, 2.78, 27.9, 279, or 558 mg/kg bw (i.p.)	Negative	Caria *et al.*, 1995
Mice (M) bone marrow cells	MN	2x (24-hour interval)[4]	0, 200, or 400 mg/kg bw (i.p.)	Negative	Ngomuo and Jones, 1996
Swiss-Webster mice (M, F)	MN	2x (24-hour intervals)	0, 625, 1,250, or 2,500 mg/kg bw (i.p.)	Negative/ positive[5]	da Silva *et al.*, 2002
Mice bone marrow erythrocytes	MN	Single dose	Up to 1 g/kg bw (p.o. or i.p.)	Negative	MacGregor, 1979 [abstract]
Swiss mice (M)	MN	2x (24-hour interval)	0, 200, 400, or 800 mg/kg bw (i.p.)	Positive	Sahu *et al.*, 1981
Mice (M) peripheral erythrocytes	MN	2x (3-day interval)[6]	0 or 400 mg/kg bw (i.p.)	Negative	Ngomuo and Jones, 1996
Mice	MN	7x (24-hour intervals)	0 or 100 mg/kg bw (i.p.)	Negative	Hang *et al.*, 1985
C57Bl/6 mice (F)	SCE	Single dose	100 mg/animal (gavage)	Negative	Ishikawa *et al.*, 1985
Mice (M)	HMA	2x (1-hour interval)[7]	0, 160, 320, or 500 mg/kg bw (p.o.)	Negative	Aeschbacher *et al.*, 1982
Swiss mice (M)[8]	DLT	2x (24-hour interval)	0, 200, 300, or 400 mg/kg bw (i.p.)	Negative[9]	Aravindakshan *et al.*, 1985
Swiss albino mice (M)	AS	Single dose[10]	0, 50, 100, or 150 mg/kg bw (p.o.)	Negative	Nandan and Rao, 1983
Swiss-Webster mice (M, F)	Comet Assay	2x (24-hour interval)	0, 625, 1,250, or 2,500 mg/kg bw (i.p.)	Positive	da Silva *et al.*, 2002
Rat					
Wistar rat bone marrow erythrocytes	MN	7 days	1% (diet)	Negative	Taj and Nagarajan, 1996

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Table A2-8 Summary of *In Vivo* Mutagenicity/Genotoxicity Studies

Species and Test System	Type	Duration	Concentration or Dose (route of administration)	Result	Reference
Wistar (M), bone marrow cells	MN	14 days	0 or 50 mg/kg bw/d (i.p)	Negative	Tieppo *et al.*, 2007
Wistar (M), bone marrow cells	MN	Single dose	0, 200, 632, or 2,000 mg/kg body weight (gavage)	Negative	Utesch *et al.*, 2008
Wistar rat bone marrow stem cells	CA	7 days	1% (diet)	Negative	Taj and Nagarajan, 1996
Wistar rat gastric mucosal cells	UDS	Single dose	0, 50, 100, 400, or 800 mg/kg bw (p.o.)	Negative	Ngomuo and Jones, 1996
Wistar rats (M)[8]	DLT	2x (24-hour interval)	0, 200, or 300 mg/kg bw (i.p.)	Negative	Aravindakshan *et al.*, 1985
Wistar (M), hepatocytes	UDS	Single dose	0, 800, or 2,000 mg/kg body weight (gavage)	Negative	Utesch *et al.*, 2008
Wistar rat bone marrow cells	Comet Assay	3 days	0 or 135 mg/kg bw/d (gavage)	Negative	Cierniak *et al.*, 2004
Wistar (M), blood, liver, and lung cells	Comet assay	14 days	0 or 50 mg/kg bw/d (i.p)	Negative	Tieppo *et al.*, 2007

RSLLT = recessive sex-linked lethal test; M =male; MCS = Meiotic Chromosome Segregation; MN = micronucleus test; p.o. = oral; F = female; i.p. = intraperitoneal; SCE = sister chromatid exchange; HMA = host-mediated assay with *S. typhimurium* TA98; DLT = dominant lethal test; AS = abnormal sperm; CA = chromosomal aberrations; UDS = unscheduled DNA synthesis.

[1] Negative at 4.12×10^{-2} M (12,500 µg/mL), positive at 8.25×10^{-2} (25,000 µg/mL) and greater.
[2] Administered at 6 or 30 hours prior to necropsy.
[3] Animals killed 24 hours following treatment.
[4] Animals killed 30 hours after 1st treatment.
[5] Positive only at 1,250 mg/kg bw.
[6] Smears obtained 24 hours after 1st administration, and for 7 days thereafter.
[7] Administered at 0 and 1 hour, 2 and 3, and 11 and 12 hours before administration of tester organism.
[8] 7-day mating schedule; males treated, females untreated.
[9] A statistically significant reduction in male fertility at 300 and 400 mg/kg bw and in female pregnancy rates at 300 mg/kg bw. These effects may be possibly attributed to cytotoxicity, oligospermia, and physiological impairment of the fertilization ability of the sperm in the case of reduced male fertility, and preimplantation losses, as opposed to genetic factors, could have contributed to the decline in decreased number of mean implantations in females.
[10] Mice killed at weekly intervals for up to 5 weeks post-treatment.

Table A2-9 Incidence of Renal Tubule Lesions in F344/N Rats in the 2-Year Feed Study of Quercetin Conducted by NTP

	0 ppm (control)	1,000 ppm	10,000 ppm	40,000 ppm
Males				
Initial Evaluation (Single Sections)				
Hyperplasia	1/50 (2%)	2/50 (4%)	3/50 (6%)	4/50 (8%)
Adenoma	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adenocarcinoma	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Adenoma or Adenocarcinoma[1]	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
Evaluation of Step Sections				
Hyperplasia	2/50 (4%)	2/50 (4%)	6/50 (12%)	8/50 (16%)
Adenoma	1/50 (2%)	2/50 (4%)	7/50 (14%)	6/50 (12%)
Combined[2]				
Hyperplasia	3/50 (6%)	3/50 (6%)	8/50 (16%)	11/50 (22%)
Adenoma	1/50 (2%)	2/50 (4%)	7/50 (14%)	8/50 (16%)
Adenoma or Adenocarcinoma	1/50 (2%)	2/50 (4%)	7/50 (14%)	9/50 (18%)*
Females				
Initial Evaluation (Single Sections)				
Hyperplasia	1/49 (2%)	1/49 (2%)	3/50 (6%)	1/50 (2%)
Adenoma[3]	0/49 (0%)	0/49 (0%)	1/50 (2%)	0/50 (0%)
Evaluation of Step Sections				
Hyperplasia	1/49 (2%)	-[4]	-	3/50 (6%)
Adenoma	1/49 (2%)	-	-	0/50 (0%)
Combined				
Hyperplasia	2/49 (4%)	-	-	4/50 (8%)
Adenoma	1/49 (2%)	-	-	0/50 (0%)

* $p \leq 0.01$ pair-wise comparison between control and dose group.
[1] Historical incidence for 2-year NTP feed studies with untreated control groups 4/499 (0.8% ± 1.1%).
[2] Where the combined incidence of lesions identified in the single and step section evaluations is less than the addition of both, it is because the same lesion was identified in both examinations.
[3] Historical incidence for 2-year NTP feed studies with untreated control groups 1/499 (0.2% ± 0.6%).
[4] Step-sections not conducted at the 1,000 and 10,000 ppm dose levels.

Table A2-10 Summary of Carcinogenicity Study Results with Quercetin Administered in the Diet

Species	Duration	Doses	Results	Reference
F344 rats (M & F)	2-years	0, 1,000, 10,000 or 40,000 ppm Qu [51, 517 or 2,203 (M) and 53, 525 or 2,231 mg/kg bw/d (F)]	Qu (40,000 ppm): reduced growth rate; renal tubule hyperplasia [11/50 (M); 4/50 (F)]: renal tubule adenoma or adenocarcinoma [9/50 (M); adenoma only 4/50 (F)]	NTP, 1992
F344/DuCrj rats (M & F)	2-years	0, 1.25 or 5% Qu [427 or 1,926 (M) and 497 or 2,372 mg/kg bw/d (F)]	Qu (5%): reduced growth rate; cecum hyperplasic polyp [11/50 (M); 2/50 (F)], adenoma [1/50 (M)], adenocarcinoma [2/50 (M)]; colon adenoma [2/50 (F)]; urinary bladder transitional cell hyperplasia [1/50 (M); 3/50 (F)], papillomatosis [1/50 (M); 1/50 (F)]	Ito *et al.*, 1989
Norwegian rats (M & F)	58 weeks	0 or 0.1% Qu [52 (M) and 47 (F) mg/kg bw/d]	Qu (0.1%): reduced growth rate in test rats; intestinal tumors [(6/7 (M); 14/18 (F)] and bladder tumors[1] [2/7 (M); 3/18 (F)].	Pamukcu *et al.*, 1980
		0 or 33% BF [11 (M) and 10 (F) mg/kg bw/d]	BF (33%): reduced growth rate; intestinal [7/8 (M), 10/11 (F)] and urinary bladder tumors [6/8 (M); 8/11 (F)].	
F344 rats (F)	Life-long exposure	0, 1, or 2% Qu (0, 500, and 1,000 mg/kg bw/d)	Qu (0, 1, and 2%): liver preneoplastic foci (6/15, 20/21, 12/14); hepatomas (0/15, 5/21, 9/14); hepatocarcinomas (0/15, 0/21, 1/14); and, bile-duct tumors (1/15, 13/21, 11/14).	Ertürk *et al.*, 1983 [abstract]
Rats (strain not specified)	6-months	0 or 5% Qu (2,500 mg/kg bw/d)	Qu (5%): cecal dilation; intestinal mucosa atrophy; crypt and goblet cell hypertrophy; colon and ileum (mucosa and submucosa) lymphoid hyperplasia.	Bokkenheuser and Winter, 1990 [abstract]
F344 rats (M & F)	540 days	0 or 0.1% Qu (50 mg/kg bw/d)	Qu (0.1%): No differences in the type and incidence of tumors between test and control animals.	Takanashi *et al.*, 1983
ACI rats (M & F)	540 days	0, 1, or 5% Qu (500 and 2,500 mg/kg bw/d)	Qu (5%): body weights (M only) significantly reduced; cecal adenoma [1/8 (M)].	Hirono *et al.*, 1981
	850 days	0 or 10% Qu (5,000 mg/kg bw/d)	Qu (10%): cecal adenoma [1/19 (M)] and adenocarcinoma [2/19 (M)].	
ddY mice (M & F)	~ 842 days (life-long exposure)	0 or 2% Qu (3,000 mg/kg bw/d)	Qu (2%): Tumor incidence of Qu-test group comparable to controls; liver [4/38 (M)], uterine [3/35 (F)], ovary [1/35 (F)] and salivary gland [2/35 (F)] tumors, and malignant spindle cell heart sarcoma [1/38 (M)].	Saito *et al.*, 1980
A/JJms mice (M & F)	23 weeks	0 or 5% Qu (7,500 mg/kg bw/d)	Qu (5%): No difference in lung tumor incidence and multiplicity between Qu-test and control groups.	Hosaka and Hirono, 1981
Syrian gold hamsters	733 days	0 or 10% Qu (12,000 mg/kg bw/d)	Qu (10%): No difference in the incidence of forestomach papillomas and adrenal cortical adenomas; ileal adenocarcinoma [1/20 (F)].	Morino *et al.*, 1982

M = male; F = female; Qu = quercetin; BF = Bracken fern
[1] Frequency of bladder tumors significantly reduced compared to BF test group.

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Table A2-11 Summary of Two-Stage Carcinogenicity and Anticarcinogenicity Study Results of Quercetin Administered in Conjunction with Known Carcinogens

Species	Initiator	Test compound (dose, route, treatment duration)		Conclusion	References
		Initiation	Promotion		
Unspecified target organ					
Hamster	Croton oil	Qu (4,500 mg/kg bw/d; diet; 709 days) or basal diet (701 days)		No clear evidence for 2-stage carcinogenesis (forestomach, intestine, urinary bladder, and others).	Morino *et al.*, 1982
		Qu (1,300 mg/kg bw/d; 351 days)	Basal diet (350 days)		
		Qu (1,300 mg/kg bw/d; 351 days)	Croton oil (1%; 350 days)		
		Basal diet (350 days)	Croton oil (1%; 351 days)		
Urinary bladder					
Rat	BHBN	Water (4 wks)	Qu (2,500 mg/kg bw/d; diet; 25 wks)	Absence of quercetin-related promoting activity and lack of quercetin-associated carcinogenicity in the bladder. No effects in liver and kidneys.	Hirose *et al.*, 1983
		Qu (2,500 mg/kg bw/d; diet; 4 wks)	Water (29 wks)		
			BHBN (0.001%) (29 wks)		
		Basal diet (4 wks)	BHBN (0.001%; 29 wks)		
		BHBN (0.01%; 4 wks)	Qu (2,500 mg/kg bw/d; diet) or basal diet (25 wks)		
Rat	BBN	Water (4 wks)	Qu (2,000 mg/kg bw/d; diet; 34 wks)	No changes in the urinary bladder.	Fukushima *et al.*, 1983
		BBN (water, 4 wks)	Qu (2,000 mg/kg bw/d; diet; 34 wks)	↓ bladder PN hyperplasia and papilloma; Incidences of simple hyperplasia.	
Kidneys					
Hamster	Estradiol	Qu (0, 360, or 3,600 mg/kg bw/d; diet; 5.5 months)		Absence of tumor formation.	Zhu and Liehr, 1994
		Qu (0, or 3,600 mg/kg bw/d; diet; 6.3 months)			
		Estradiol (2 x 25 mg; s.c.)[1]	Qu (0, 360, or 3,600 mg/kg bw/d; diet 2 wks + 5.5 months)	All estradiol-treated rats exhibited tumors ↑ mean no. of tumors/hamster in all Qu-estradiol groups and ↑ abdominal metastases in Qu-estradiol compared to estradiol-only group.	
			Qu (0 or 3,600 mg/kg bw/d; diet; 2 wks + 6.3 months)	↑ abdominal metastases in Qu-estradiol compared to estradiol-only group.	

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Table A2-11 Summary of Two-Stage Carcinogenicity and Anticarcinogenicity Study Results of Quercetin Administered in Conjunction with Known Carcinogens

Species	Initiator	Test compound (dose, route, treatment duration)		Conclusion	References
		Initiation	Promotion		
Gastrointestinal Tract					
Mouse	AOM	Saline[2]	Qu (0 or ~3,000 mg/kg bw/day; diet; 8 weeks)	Qu-induced non s.s. ↑ in FAD	Yang *et al.*, 2000
		AOM[2] (↑'ing doses of 5 to 10 mg/kg bw/week; s.c.; 6 weeks)		Qu-induced ↓ in AOM-induced FAD	
Mouse	AOM (azoxy-methanol)	Qu (3,000 mg/kg bw/d; diet; 9 weeks)		No effects.	Deschner *et al.*, 1991
		Qu (0 or 3,000 mg/kg bw/d; diet) + AOM (weekly injections) (9 weeks)		Qu-induced ↓ in no. of cells in S-phase and in no. of FAD in colonic tissue.	
		AOM[3] (weekly injections; 3x)	Qu (0, 150, 750, or 3,000 mg/kg bw/d; diet; 48 weeks)	Qu-induced ↓ (4-fold) in colonic tumors.	
Mouse	ENNG	Qu (3,000 mg/kg bw/day; diet; 16 wks)		No duodenal tumors detected.	Matsukawa *et al.*, 2002
		ENNG (water; 4 wks)	Qu 0, 300, or 3,000 mg/kg bw/d (diet; 16 wks)	↑ in size and average number of duodenal tumors.	
Rat	AOM	AOM[4] (15 mg/kg bw; s.c.; weekly injection; 2x)	Basal diet (up to 41 weeks)	All AOM-treated rats developed ACF mainly in the distal colon (at 8 wks post-AOM treatment) Qu-induced ↓ (dose-dependent) colorectal tumor incidence	Dihal *et al.*, 2006
			Qu (4.5 to 9, 45 to 90, or 450 to 900 mg/kg bw/d; diet; up to 41 weeks)		
		Vehicle (s.c.; weekly injection; 2x)	Basal diet (up to 41 weeks)	Absence of colorectal tumors.	
			Qu (4.5 to 9, 45 to 90, or 450 to 900 mg/kg bw/d; diet; up to 41 weeks)		
Rat	AOM	Qu (1,680 mg/kg bw/d; diet; 46 weeks)		Adenocarcinoma (small intestine) (1/12).	Pereira *et al.*, 1996
		AOM (30 mg/kg bw; s.c.)[5]	Qu (840 or 1,680 mg /kg bw/d; diet; 46 weeks)	↑ incidence and no. of colonadenocarcinomas, but not adenomas. No effect observed on the small intestine.	
Rat	AOM	Qu (0 or 25 mg/kg bw/d; diet; 5 wks)		No adverse effect (no histopathology in liver, kidneys, lungs and heart, and no ACF).	Tanaka *et al.*, 1999
		AOM (s.c.) (1/week; 3 wks)[6]	Basal diet (5 wks)	Qu-induced ↓ in ACF.	
			Qu (25 mg/kg bw/d; diet; 3 + 2 wks)[7]		

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Table A2-11 Summary of Two-Stage Carcinogenicity and Anticarcinogenicity Study Results of Quercetin Administered in Conjunction with Known Carcinogens

Species	Initiator	Test compound (dose, route, treatment duration)		Conclusion	References
		Initiation	Promotion		
Rat	AOM	Qu (100 mg/kg bw; diet; 5x/week; 7 wks)		No difference in multiplicity and incidence of ACFs in Qu+AOM compared to AOM-only rats.	Exon *et al.*, 1998
		AOM (s.c.) (2x; on Days 2 and 9 of 7-wk feeding period)	Qu (100 mg/kg bw; diet; 5x/week; 7 wks)		
Rat	AOM	Saline (1/wk for 2 wks)	Basal diet	Absence of ACF formation in the colon.	Volate *et al.*, 2005
			Qu (3,000 mg/kg bw/d; diet; initiated at 7 wks of age for 4 wks)		
		AOM (1/wk for 2 wks)[8]	Basal diet	↓ in incidence of ACF formation in AOM-Qu compared to AOM-control.	
			Qu (1,500 mg/kg bw/d; diet; initiated at 7 wks of age for 4 wks)		
			Qu (3,000 mg/kg bw/d; diet; initiated at 7 wks of age for 4 wks)		
Rat	AOM	AOM (1/wk for 2/wks)[9]	Basal diet (up to 35 wks)	No effect on ACF formation or tumor incidence at 8 and 33 wks after last AOM administration, respectively.	Choi *et al.*, 2006
			Qu (250 mg/kg bw/d; diet, initiated at 5 wks of age for up to 35 wks)		
Rat	ENNG	Qu (500 mg/kg bw/d; diet; 16 wks)		Absence of duodenal tumor formation.	Werner *et al.*, 1985
		Qu (500 mg/kg bw/d) + ENNG (120 mg/L; water) (16 wks)		↑ incidence of duodenal tumors (not s.s.)	
Rat	MAM	Saline (1/wk for 3 wks)	Basal diet (459 d)	Absence of tumor formation.	Kato *et al.*, 1984
			Qu (500 mg/kg bw/d; diet; 459 d)		
		MAM (1/wk for 3 wks)	Basal diet (459 d)	↓ colonic adenomas in MAM-Qu compared to MAM-control.	
			Qu (500 mg/kg bw/d; diet; 459 d)		
Rat	DMBDD[10]	Qu (0 or 500 mg/kg bw/d; diet; 36 weeks)		Absence of tumors in liver, kidney, lungs, esophagus, stomach, intestine, bladder.	Akagi *et al.*, 1995
		Qu (500 mg/kg bw/d; diet) + DMBDD (4 wks)		Qu fully inhibited small intestinal tumors (promotional phase).	
		DMBDD (4 wks)	Qu (500 mg/kg bw/d; diet; 31 wks)	↓ incidence and number of total small intestinal tumors.	

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Table A2-11 Summary of Two-Stage Carcinogenicity and Anticarcinogenicity Study Results of Quercetin Administered in Conjunction with Known Carcinogens

Species	Initiator	Test compound (dose, route, treatment duration)		Conclusion	References
		Initiation	Promotion		
Liver and Pancreas					
Rat	PB	Qu (50 or 500 mg/kg bw/d; i.p.; 6 days)		No hepatic lesions.	Kato *et al.*, 1985
		Qu (50 or 500 mg/kg bw/d; i.p.; 6 days)	PB (diet; 467 days)		
Rat	NMU	Saline (single i.p.; 3 days of age)	Basal diet	↑ pancreas wts (relative) Qu-sal (F) compared to sal-control group ↓ bw Qu-NMU (M+F) compared to sal-control and NMU-control ↑ pancreas and liver wts (relative) of Qu-NMU (M+F) compared to Qu-sal and NMU-controls ↑ DYF of Qu-NMU (M & F) compared to NMU-control; carcinoma *in situ* observed in 12% and 1 microcarcinoma Qu-NMU rats.	Barotto *et al.*, 1998
			Qu (500 mg/kg bw/d; 30 days of age; 20 wks)		
		NMU (single i.p.; 3 days of age)	Basal diet		
			Qu (500 mg/kg bw/d; diet; initiated at 30 days of age for 20 wks)		
Rat	NMU; PB	Saline (single i.p.; 3 days of age)	Basal diet	↑ DYF of Qu-NMU (M & F) and Qu + PB-NMU (M only) compared to NMU-control. ↑ DYF of Qu-NMU (M & F) compared to Qu + PB-NMU. ↑ DYF of Qu-NMU (M) compared to Qu-NMU (F) (observed in 80 and 54%, respectively). ↑ DYF of Qu + PB-NMU (M) compared to Qu + PB-NMU (F) (44 and 25%, respectively). Carcinoma *in situ* was observed in 3 M (20%) of the NMU-quercetin group	Valentich *et al.*, 2006
			PB (50 mg/kg bw/d; diet; initiated at 30 days of age for 20 wks)		
		NMU (single i.p.; 3 days of age)	Basal diet		
			PB (50 mg/kg bw/d; diet; initiated at 30 days of age for 20 wks)		
			Qu (500 mg/kg bw/d; diet; initiated at 30 days of age for 20 wks)		
			Qu + PB (500 and 25 mg/kg bw/d, respectively; diet; initiated at 30 days of age for 20 wks)		
			Qu (3,000 mg/kg bw/d; diet; initiated at 7 wks of age for 4 wks)		
			Qu (1,500 mg/kg bw/d; diet; initiated at 7 wks of age for 4 wks)		
Rat	DEN	DEN[11] (200 mg/kg bw; single i.p.)	Qu (1,250 mg/kg bw/d; diet; 6 weeks)	No change in GST-P^{+} foci compared to DEN-only group.	Ito *et al.*, 1988

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