Table 6.4.2.2-1 Summary of *In Vivo* Mutagenicity/Genotoxicity Studies

Species and Test System	Type	Duration	Concentration or Dose (route of administration)	Result	Reference
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, 2.79, 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	0.1% (diet)	Negative	Taj and Nagarajan, 1996
Wistar rat (M) bone marrow cells	MN	14 days	0 or 50 mg/kg bw/d (i.p)	Negative	Tieppo *et al.*, 2007
Wistar rat (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	0.1% (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 rat (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

Table 6.4.2.2-1 Summary of *In Vivo* Mutagenicity/Genotoxicity Studies

Species and Test System	Type	Duration	Concentration or Dose (route of administration)	Result	Reference
Wistar rat (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 x 10^{-2} M (12,500 µg/mL), positive at 8.25 x 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 1hour, 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

6.4.2.3 Summary for Short-Term Tests for Genotoxicity

Although the majority of results from numerous *in vitro* investigations of quercetin-related mutagenicity and genotoxicity were positive, *in vivo* results, for orally administered quercetin consistently tested negative for mutagenic activity in somatic cells (MacGregor, 1979 [abstract]; Aeschbacher *et al.*, 1982; Ishikawa *et al.*, 1985; Ngomuo and Jones, 1996; Taj and Nagarajan, 1996; Cierniak *et al.*, 2004; Utesch *et al.*, 2008); *in vivo* results following intraperitoneal administration were positive in some cases (Sahu *et al.*, 1981; da Silva *et al.*, 2002). However, increased frequency of micronuclei was not observed in an additional study in mice receiving either single oral or intraperitoneal administrations of quercetin at comparable doses (up to 1 g/kg body weight) (MacGregor, 1979 [abstract]; Hayashi *et al.*, 1988). Additionally, quercetin tested negative in the MN assay (mice and rats), DLT test (mice and rats), and Comet assay (rat) following intraperitoneal administration (Aravindakshan *et al.*, 1985; Caria *et al.*, 1995; Ngomuo and Jones, 1996; Hang *et al.*, 1985; Tieppo *et al.*, 2007). The quercetin-related mutagenicity observed *in vitro* at high concentrations in the presence of oxygen is believed to be mediated *via* a prooxidant mechanism, similar to the mechanism underlying the *in vitro* mutagenic and genotoxic properties characteristic of other strong antioxidants, such as ascorbic acid (JECFA., 1998, 2006). Although quercetin under certain *in vitro* experimental conditions may have prooxidant properties, several *in vivo* protective mechanisms (e.g., GSH conjugation, other antioxidants, pre-absorption microbial degradation in the intestine, and post-absorption metabolism and conjugation)

act together to limit its potential to act as a genotoxic substance *via* a prooxidant mechanism (Harwood *et al.*, 2007).

It has been suggested that the formation of the 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, although positive results were observed with *in vitro* test systems exposed to quercetin, the limited absorption of quercetin and almost complete metabolism in the intestinal tract should be considered when extrapolating these results as indicators of potential quercetin-induced genotoxicity in humans (Gross *et al.*, 1996). More specifically, as discussed in Section 6.2.2, quercetin is extensively metabolized to non-mutagenic compounds such as 3'-*O*-methylquercetin and 4'-*O*-methylquercetin, which may contribute to the absence of quercetin-induced *in vivo* mutagenicity and carcinogenic activity (Zhu and Liehr, 1993 [abstract]). As such, short- and long-term animal studies with oral exposure are more reliable and appropriate for the assessment of the actual carcinogenic potential of quercetin in food.

6.4.3 Subchronic and Chronic Studies

The toxicity of quercetin has been studied extensively in numerous short and long-term toxicity studies. Consequently, a considerable amount of information has been reported in the scientific literature with respect to quercetin safety. Of the key safety studies is a 2-year rat feeding study, which was conducted by the National Toxicology Program (NTP) (Dunnick and Hailey, 1992; NTP, 1992). Although emphasis has been placed on this study with respect to the safety assessment of quercetin, the results of other relevant short- and long-term toxicity studies also are presented in support of the safety of this naturally-occurring flavonoid.

With the exception of reductions in hemoglobin and hematocrit levels (statistical significance not reported), no variations were observed in a number of other standard toxicological parameters including body weight, food and water consumption, biochemistry, and organ weights in male and female Swiss mice administered quercetin in the diet at dose levels of 0 (control) 30, 300, or 3,000 mg/kg body weight/day for a period of 28 days in comparison to a control group (n=5/group) (Ruiz *et al.*, 2006). The variations observed in the hematological parameters were within historical limits for this mouse strain. The results of study-end histopathological examination of various organs were reported to be unremarkable.

Groups of 20 to 30 Swiss mice (sex not specified) were administered approximately 25 mg quercetin/day as 0.5% of the diet (corresponding to an average of 950 mg quercetin/kg body weight/day for the entire study period when the growth of the animals is taken into consideration) for a period of 28 days (Barrenetxe *et al.*, 2006). At the end of the study period, the animals were killed, plasma samples were collected for clinical chemistry, and organs were extracted and weighed. No significant differences were observed in body weight gain, organ weights (jejunum, pancreas, colon, spleen, and liver; study did not indicate whether these were relative or absolute), biochemical parameters, or intestinal histopathology of quercetin-treated mice in comparison to a control group. Although small intestinal enzymatic activity was altered in the group provided dietary quercetin, the physiological implications of this effect were stated by the authors to be unclear.

In a 21-day dietary feeding study, designed specifically to determine the efficacy of quercetin to suppress renal cortical nuclear factor (NF)-κB and tubulointerstitial injury, adult male Wistar rats with (n = 27) or without (control groups; n = 15) doxorubion hydrochloride-induced Adiamycin nephropathy (AN) were provided 0, 0.5, or 2% quercetin (Rangan *et al.*, 2002). Animals were pair-fed, and body weights and food consumption were monitored daily. On the day immediately preceding scheduled necropsy, urine and serum samples were collected to determine levels of urinary protein and serum creatinine, albumin, and cholesterol concentrations, as well as creatinine

clearance. At necropsy, kidneys were removed and weighed, and prepared for further histopathological examination. Based on calculations of food intake, rats receiving dietary concentrations of 0.5 and 2% quercetin consumed 285 and 1,133 mg quercetin/kg body weight/day, respectively. All animals survived the entire duration of the study period and no variations in final body weight, body weight gain, kidney weights and food intake were reported in the quercetin control groups compared to the basal diet control group. Urinalysis, however, revealed a dose-dependent, statistically significant increase in proteinuria in high-dose quercetin-only treated rats. In comparison to the basal-diet control group, quercetin-treatment was associated with an enhancement in renal cortical activator protein (AP)-1 DNA binding activity, albeit not at levels of statistical significance, and malondialdeyde (MDA) production ($p = 0.03$) at both quercetin dose levels. Therefore, under the conditions of this study, the no-observed-adverse-effect level (NOAEL) was 285 mg quercetin/kg body weight/day in rats.

In a 5-week study evaluating the potential effects of quercetin treatment on experimental renovascular hypertension in rats, quercetin administered to a control group of normotensive male Wistar rats at daily dose levels of 10 mg/kg body weight by gavage (2 mg/rat daily) did not induce any body, or relative kidney or heart weight variations in comparison to untreated controls (8/group) (García-Saura *et al.*, 2005). Plasma levels of thiobarbituric acid (TBARS) and nitrates plus nitrites (NO_x), markers of oxidative liver stress, in quercetin-treated rats were comparable to controls. With the exception of a slight reduction in liver GSH levels in quercetin-treated rats, no significant changes were observed in liver glutathione-*S*-transferase (GST), glutathione peroxidase (GPx), and glutathione reductase (GR) activity between test and control rats. Excretion of protein in the urine of quercetin-treated rats was also comparable to that of controls. In the same study, groups of renovascular hypertensive male Wistar rats were treated with 0 (control) or 10 mg quercetin/kg body weight/day for a period of 5 weeks (11/group). In comparison to normotensive rats, hypertensive rats exhibited increased systolic blood pressure, cardiac and renal tissue weights, and excretion of protein in the urine. Treatment of hypertensive rats with quercetin significantly attenuated the increases in urinary excretion of protein (to levels comparable to those in the normotensive control group), relative heart and left ventricle weights, and systolic blood pressure observed in untreated hypertensive rats. The authors concluded that quercetin demonstrated antihypertensive and antioxidant effects in the renovascular hypertensive rat model.

Quercetin or its glycoside, rutin, were administered orally to groups of 5 male Wistar rats for a period of 22 days at dose levels of 0 (control), 10, 50, 100, 200, 500, or 1,000 mg/kg body weight/day, respectively (Nakamura *et al.*, 2000). Neither the aglycone nor the glycoside affected body weight gain, food intake, relative and absolute liver weights, and dry fecal weight. Hepatic and serum lipid concentrations of quercetin-treated rats did not differ from those observed in the control group. Excretion of fecal neutral

steroids in rats administered quercetin remained comparable to controls, while the excretion of a select number of fecal bile acids (murodeoxycholic acid, deoxycholic acid, isodeoxycholic acid, α-muricholic acid) was significantly enhanced and reduced in the case of 12-ketolithocholic acid. As such, based on the results of this study 1,000 mg quercetin/kg body weight/day was determined to be the NOAEL.

Although daily quercetin administration at a dose level of 10 mg/kg body weight to normotensive male Wistar Kyoto rats (WKR) for a period of 5 weeks *via* gastric intubation did not significantly affect liver GPx activity compared to untreated controls, it did significantly enhance GPx activity in spontaneously hypertensive (SHR) rats, which were lower compared to WKR control and test rats (Duarte *et al.*, 2001). The increase in GPx activity in SHR rats was paralleled by a significant reduction in MDA levels following quercetin treatment, levels of which in control SHR rats were significantly higher compared to WKR rats. Hepatic levels of GR were similar among all groups following the treatment period. The WKR control rats demonstrated significantly lower liver GSH levels compared to SHR control rats. Following the 5-week quercetin treatment period, GSH levels were significantly reduced in both strains of rats in comparison to control respective values; however, differences observed in levels of GSH between the 2 strains of rats were no longer statistically significant. Finally, although quercetin treatment did not affect the blood pressure of WKR rats, a 12%, statistically significant reduction was observed in the blood pressure of SHR rats.

The potential effect of dietary quercetin on hepatic antioxidant activity was evaluated in female BALB/c mice (n=5/group) fed a diet containing 0 (control) or 0.1% quercetin [providing approximately 0 and 150 mg/kg body weight/day, respectively (U.S. FDA, 1993)] for a period of 40 days (Bando *et al.,* 2007). Urine was collected during the last 3 days of treatment and was analyzed for levels of 8-hydroxydeoxyguanosine (8-OHdG), a marker of oxidative stress. At the end of the treatment period, the animals were killed and their livers were excised. Supplementation with quercetin significantly reduced hepatic α-tocopherol levels relative to the control group. Hepatic GPx and SOD activities were higher in quercetin-treated rats in comparison to the control group; however, only the latter reached statistical significance. In comparison to the control group, urinary 8-OHdG content was elevated in the quercetin group, but did not reach statistical significance.

Male Swiss NMRI mice (10/group) were administered quercetin at concentrations of 0, 1, 3, or 9 µg/mL in the drinking water for a period of 8 weeks in order to assess the effect of quercetin on phase I and II enzymes in the hepatic and pulmonary tissues (Gandhi and Khanduja, 1993). Food and water were provided *ad libitum* for the duration of the study. Body weights, and food and water consumption were monitored on a weekly basis. All animals were necropsied at study termination, and the lungs and liver removed for

further examination. Water consumption measurements revealed total average intakes of 0.36, 1.10, and 3.28 mg quercetin (approximately 0.3, 0.9, and 2.6 mg/kg body weight/day, respectively) at the 1, 3, and 9 µg quercetin/mL concentrations, respectively. At the end of the study period, no statistically significant variations were reported with respect to the final body weights, absolute liver and lung weights, and the postmitochondrial supernatant (PMS) protein content of the respective organ tissues. Levels of pulmonary arylhydrocarbon hydroxylase (AHH), cytochrome P-450 (CYP-450), cytochrome b_5, and cytochrome *c* reductase remained unaffected in test animals in comparison to controls. Similarly, with the exception of a slight increase in hepatic cytochrome *c* reductase levels in low-dose animals, no other variations were reported in phase I enzymes of test animals. Levels of the liver or pulmonary deconjugative enzyme UDP-glucuronyltransferase (UDP-GT) were also comparable between the test and control groups. In contrast, a statistically significant increase was observed in levels of GST and GSH in the liver at the mid- and high-dose levels (0.9 and 2.6 mg/kg body weight/day, respectively), as well as in the lungs; however, the increase in pulmonary GST was limited to the high-dose level only. The enhanced presence of GST and GSH in the lung and liver tissue may be indicative of a protective mechanism and possibly responsible for the absence of quercetin-related carcinogenesis generally observed *in vivo*.

Alternatively, in another study, levels of liver GST and GR were reportedly not affected by quercetin provided in the diet at 0.2 or 1% [approximately 100 and 500 mg/kg body weight/day, respectively (U.S. FDA, 1993)] to male Sprague-Dawley rats for a period of 6 weeks (Fischer *et al.*, 2002 [abstract]). At the higher dose level (500 mg/kg body weight/day), enhanced GST activity was observed in both the proximal and distal colon mucosa, whereas increased GR activity was restricted to the distal colon mucosa. Additionally, high-dose rats exhibited increased antioxidant capacity as revealed by the Ferric Reducing Ability of Plasma (WKR) assay.

Choi *et al.* (2003, 2005) conducted a study to assess the potential for quercetin-related prooxidant activity in male Sprague-Dawley rats following 4- to 6-week oral treatments with 2 or 20 mg quercetin/day (approximately 8 and 80 mg/kg body weight/day, respectively). Quercetin was observed to significantly decrease hepatic levels of GSH in normal diet and vitamin E-deprived rats, while concomitantly increasing, albeit only slightly and not at levels of statistical significance, the activity of GPx. Liver GR activity was significantly reduced in a dose-dependent fashion in normal and vitamin E-deprived rats administered 20 mg quercetin/day compared to respective controls. Moreover, administration of quercetin at the low- and high-dose level significantly increased serum and liver vitamin E levels in vitamin E-deprived rats compared to vehicle-treated vitamin E-deprived rats, and reduced hepatic MDA levels in normal and vitamin E-deprived rats compared to respective vehicle-treated controls. When quercetin (20 mg/day) was

provided to rats in combination with daidzein (20 mg/day) (a soy flavonoid), the reduction in liver GSH levels and GR activity was prevented.

Gavage administration of quercetin to male Wistar rats at a dose level of 135 mg/kg body weight/day every 24 hours for 3 consecutive days did not significantly enhance the activity of oxidative scavengers SOD and catalase in comparison to control group values (Cierniak *et al.*, 2004).

In a 9-week study designed to evaluate the potential protective effect of quercetin on cadmium-induced nephrotoxicity, groups of 15 male Wistar rats were administered 0 (vehicle control, saline only) or 50 mg/kg body weight/day of quercetin intraperitoneally 5 times a week (Morales *et al.,* 2006). Blood and urine samples were collected for measurements of markers of renal toxicity including N-acetyl-β-d-glucosaminidase (NAG), alkaline phosphatase, γ-glutamyl-transpeptidase (GGT), and creatinine. Plasma TBARS levels were also determined. The animals were killed and kidneys were removed for the measurement of SOD and GR activities and for histopathology examination. No evident signs of nephrotoxicity were observed in the quercetin-treated group as determined by the absence of changes in renal SOD or GR activities, plasma TBARS, or urinary or plasma markers of renal toxicity in comparison to the vehicle control group. Conversely, treatment of rats with cadmium significantly decreased plasma levels of TBARS, increased NAG, ALP, and GGT activities, and induced proteinuria, microalbuminuria, and glucosuria. Co-treatment with quercetin attenuated these cadmium-induced changes.

Exon *et al.* (1998) conducted a 7-week feeding study in 4-month-old outbred Sprague-Dawley rats to specifically evaluate the potential effect of quercetin on immune function. Groups of 8 female rats were allocated to either a test group administered quercetin in the diet 5 times per week at a dose level of 100 mg/kg body weight, or a concurrent vehicle control group. Water was provided *ad libitum* throughout the study period. Weight determinations were conducted twice weekly. At the end of the 7-week treatment period, rats were killed, the spleens excised and weighed, and colons removed for histopathological examinations. Additionally, immune function assays, including evaluation of antibody production, delayed-type hypersensitivity (DTH), and natural killer (NK) cell cytotoxicity, were performed immediately following study termination. A significant increase in the natural killer cell cytotoxicity was observed in the quercetin-treated rats. The remaining parameters of immune system function evaluated in the test group were comparable to controls. Likewise, relative to the control group, no variations were observed in the rate of body weight gain and the relative spleen weights of rats receiving quercetin. Indices measured as a reflection of colonic epithelial cell proliferation (*i.e.*, labeling index, proliferation index, and height of colonic crypts) were comparable among the groups. Two other groups of rats, a quercetin-

treated (100 mg/kg body weight) test and a vehicle control group, received subcutaneous azoxymethane (AOM) injections (20 mg/kg body weight) on 2 separate occasions (on Days 2 and 9) throughout the study period in order to evaluate quercetin as a possible anticarcinogen. In contrast to the enhanced NK activity observed in quercetin-treated rats relative to rats in the vehicle control, AOM-initiated rats receiving quercetin exhibited a significant reduction in the NK response compared to the AOM controls, which in turn did not differ from the vehicle control. Furthermore, no differences were observed in the distribution of pre-neoplastic colon lesions [*i.e.*, aberrant crypt foci (ACF)], as well as in the multiplicity and incidence of the ACFs in quercetin-treated, AOM-initiated rats compared to AOM controls. Several other studies, details of which are provided in Section 6.4.5, were conducted to assess specifically the effect of quercetin on chemical-induced carcinogenesis.

Groups of 3 to 5 male and female weanling albino rats (strain not specified) were administered 0 (basal diet control), 0.25, 0.5, or 1% quercetin [approximately 0, 125, 250, and 500 mg/kg body weight/day, respectively (U.S. FDA, 1993)] in the diet for a period of approximately 410 days (Ambrose *et al.*, 1951). Throughout the treatment period, water and food were available *ad libitum*, and body weight and food consumption were determined on a weekly basis. Hematology was conducted on Days 70, 280, and 400 into the study period. At necropsy, adrenals, kidneys, spleen, liver, heart and testes were weighed, and subsequently subjected to histological evaluation, along with thyroid, lung, pancreas, stomach, small intestine, bladder and female reproductive organ tissue samples. Body weight gain did not vary among the test groups and was comparable to the control animals. Similarly, no differences were reported in food consumption and absolute organ weights of test rats compared to controls. Microscopic evaluation did not reveal any pathological abnormalities in any of the examined tissue samples. 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.

For a period of 64 weeks, groups of male and female Fischer 344 rats were maintained on diets supplemented with 0 (basal control), 0.1, or 0.2% quercetin (Stoewsand *et al.*, 1984). In total, male and female rats were estimated to consume 7.3 and 5.2 g (approximately 40.7 and 29.0 mg/kg body weight/day, respectively), respectively, at the lower dietary concentration and 13.6 and 10.3 g (approximately 75.9 and 57.5 mg/kg body weight/day, respectively), respectively, at the higher dietary concentration. At 12 to 13 weeks into the study period, female and male rats at the same dose levels were allowed to breed, and various developmental and reproductive parameters were monitored (for results and discussion see Section 6.4.6). At study onset, until gestation and lactation, and thereafter, beginning on Day 42 of the study period, basal diets were provided *ad libitum* in powdered form. During gestation and lactation rats received agar-

based, solid diets. Blood serum samples were assayed for levels of α-fetoprotein, a possible marker for hepatocarcinogenesis, at 8 weeks into the treatment period. Limited blood chemistry profiles [*i.e.*, hemoglobin, blood urea nitrogen (BUN), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), packed red blood cell volume] were obtained 1 week prior to study termination. At the end of the study period, tissues were subject to histopathological examination. Body weight gain and survival rate were comparable among all groups. No compound-related changes were observed in α-fetoprotein levels. Blood chemistry revealed significant alterations in serum GOT and BUN levels in males at the high-dose level (approximately 75.9 mg/kg body weight/day). Lesions affecting the spleen, thyroid, skin, and inflammation of the adipose tissue were observed in rats at both dose levels. The lesions were reported to be consistent with those typically observed in aging F344 rats and also occurred in the control group. The only observed malignant tumor consisted of a uterine sarcoma reported in a low-dose female (approximately 29.0 mg/kg body weight/day). As reported by the authors, sarcomas of the uterus occur in 0.5% of older F344 rats. 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.

In the comprehensive NTP (1992) study, groups of 70 F344/N rats/sex/dose were administered 0 (control), 1,000, 10,000, or 40,000 ppm quercetin in the diet for a period of 104 weeks. Based on mean body weights and food consumption data reported in the study, rats were exposed to daily quercetin doses of 51, 517, and 2,203 mg/kg body weight/day for males and 53, 525, and 2,231 mg/kg body weight/day for females at the respective test concentrations. At each dose level, randomly selected groups of 10 rats/sex were necropsied at 6 and 15 months for interim evaluation. Food and water were provided *ad libitum* throughout the study period. Animals were observed twice daily, and clinical signs of toxicity were recorded weekly for the first 13 weeks and monthly thereafter. Food consumption was monitored on a weekly basis and weight determinations were conducted at study initiation, at weekly intervals for the first 14 weeks and, subsequently, once every 4 weeks throughout the study period. Evaluation of clinical pathology consisting of hematology, clinical chemistry, and urinalysis was limited to animals scheduled for interim evaluation. Blood samples were drawn immediately before animals were killed, whereas 24-hour pooled urine samples were collected 1 week prior to necropsy. Organ weights (brain, liver, and right kidney) were also obtained from animals at Months 6 and 15. All surviving animals, as well as those found moribund and those selected for interim evaluation were subject to necropsy. Major organs and tissues were examined macroscopically and prepared for further microscopic examination. Complete histology, including assessment of all gross lesions, was performed on all animals from the control and high-dose (40,000 ppm) groups, and all animals dying prior to study termination. Additionally, at 6 months the large and small

intestines and uterus of mid-dose (10,000 ppm) rats were examined microscopically, and at 15 months the large intestine of the low-dose (1,000 ppm) group and the large and small intestines and stomach of the mid-dose group were subject to histopathological examination. In the remaining low- and mid-dose animals the major organs, including the kidney, liver, tongue, and urinary bladder among others, were evaluated.

Survival of rats was not affected by compound administration and food consumption of test animals was reported to be comparable to that of controls. A compound-related decrease in body weight was observed in males and females, which reached statistical significance at the high-dose level. At week 25, the decrease in body weight for high dose males was 5% and in females it was 10%. From week 65 to the end of the study, the decrease in body weight among high dose males ranged from 6% to 13%, and in females it ranged from 13% to 15%. Yellowish discoloration of the hair coat, affecting particularly the perineal area was observed in the mid- and high-dose test groups, possibly occurring as a result of urinary or fecal excretion of quercetin and/or its metabolites.

At both interim evaluations, statistically significant increases in organ weight to body weight ratios for kidney (9% to 16%) and liver (7% to 16%) weights in both sexes of rats receiving 40,000 ppm were reported compared to controls. In females, the increase was deemed to be a secondary change, appearing in response to the reduction in body weight. With the exception of a significant reduction in sorbitol dehydrogenase (SDH) activity in high-dose males, clinical hematology and chemistry were generally unremarkable, while urinalysis revealed presence of calcium oxalate crystals in 7 of the 10 high-dose males. Rats exhibited yellow-brown pigmentation of several tissues, which was dose- and time-dependent in the incidence and severity, and occurred with greatest prevalence in the glandular stomach and distal segment of the small intestine. However, no incidence of either neoplastic or nonneoplastic lesions was reported. Pathological effects identified were limited primarily to the kidneys, parathyroid and mammary glands, tongue, uterus, and gastrointestinal tract, and are discussed in section 6.4.4.

In another 2-year feeding study, quercetin (Tokyo Kasei Co. Ltd.; purity >99.4%) was incorporated into the diets of male and female F344/DuCrj rats (50/sex/group) at concentrations of 0 (control), 1.25 (low dose), or 5% (high dose) (Ito *et al.*, 1989). Subsequently, all rats were observed for an additional 8 weeks, during which time animals received the basal diet. Average quercetin intakes were calculated to be 427 and 497 mg/kg body weight/day for males and females, respectively, receiving 1.25%, quercetin and 1,926 and 2,372 mg/kg body weight/day for males and females, respectively, provided 5% quercetin in the diet. Animals were observed daily for clinical signs of toxicity, and body weight determinations were conducted weekly for the first 14 weeks, and once every two weeks thereafter. Two days prior to each weighing, urine

samples were collected from 10 rats in every group. Blood samples for hematological and serum chemistry analyses were obtained at Week 112 when all surviving rats were necropsied. Animals were subjected to gross examination and a wide-range of tissue samples was subject to histopathological evaluation. Additionally, the luminar surfaces of the intestines and urinary bladder were examined microscopically. Brain, heart, liver, spleen, kidneys, and testes/ovaries were weighed and organ-to-body weight ratios determined.

Significant reductions in body weights of male and female high-dose rats were reported throughout the entire duration of the study, while only occasional decreases were noted in the low-dose animals. Final mean body weights were decreased by 9% and 7% for males and females in the high-dose group, respectively, as compared to controls at study termination. Body weight variations were not accompanied by any changes in food consumption. No overt, clinical signs of toxicity were noted, and mortality rates of all groups were comparable. Urinalysis and hematology results were unremarkable, while serum chemistry revealed a significant decrease in glucose levels of high-dose males. The decrease was considered to be related to the apparent growth retardation. A dose-dependent increase in relative brain weights was observed in males and females, reaching statistical significance at the high-dose level compared to controls. In high-dose males, relative kidney weights were also significantly elevated and demonstrated dose-dependency. However, the weights were within the normal range for this specific strain of rats and were thus also considered to be related to the reduction in body weights. Pathological effects identified were limited primarily to the cecum, liver and urinary bladder, and are discussed in section 6.4.4.

6.4.3.1 Summary for Subchronic and Chronic Studies

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. Based on the absence of any adverse effects observed in rats following dietary administration of quercetin, a no-observed-effect level (NOEL) of 500 mg quercetin/kg body weight/day, the highest dose level tested, was established by Ambrose *et al.* (1951) and a no-observed-adverse-effect level (NOAEL) of 76 mg quercetin/kg body weight/day, the highest dose level tested, was demonstrated by Stoewsand *et al.* (1984). 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 more recent 2-year studies.

6.4.4 Carcinogenicity Studies

With the exception of a single study, which reported urinary bladder and intestinal tumors in Norwegian rats following exposure to dietary quercetin at a concentration of 0.1% (Pamukcu *et al.*, 1980), subsequent oral carcinogenicity studies produced negative results for enhanced induction of any organ tumors in 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; NTP, 1992). It has been suggested that the discrepancy in the carcinogenic responses observed between the Pamukcu *et al.* (1980) rat study and other long-term studies may be due to strain or species differences attributable to strain or species differences in tumor susceptibility, variations in experimental conditions (NTP, 1992), or cross-contamination of the diet with bracken fern from a concurrently-conducted study (Harwood *et al.*, 2007). Study results are summarized in Table 6.4.4-1 and presented in detail below.

Table 6.4.4-1 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]

Table 6.4.4-1 Summary of Carcinogenicity Study Results with Quercetin Administered in the Diet

Species	Duration	Doses	Results	Reference
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.

In the extensive NTP (1992) study, groups of 70 F344/N rats/sex/dose were administered 0 (control), 1,000, 10,000, or 40,000 ppm quercetin in the diet for a period of 104 weeks. Using mean body weights and food consumption data reported in the study, rats were exposed to daily quercetin doses of 51, 517, and 2,203 mg/kg body weight/day for males and 53, 525, and 2,231 mg/kg body weight/day for females at the respective concentrations. Complete study design details are provided in section 6.4.3.

Pathological effects identified were limited primarily to the kidneys, parathyroid and mammary glands, tongue, uterus, and gastrointestinal tract. In the kidneys, increased incidence of renal tubule hyperplasia and renal tubule neoplasms was observed in the high-dose males (40,000 ppm). Table 6.4.4-2 summarizes the incidence of renal tubular lesions observed. A dose-related increase in the incidence of renal tubule hyperplasia was observed in males. The hyperplasia was characterized by focal lesions, which induced slight tubule dilation, and consisted of cells that ranged from normal in appearance to enlarged polygonal cells resembling adenomas. Based on the results of the initial evaluation and the more detailed step-section analysis, in total, 9 adenomas or adenocarcinomas were detected. The adenomas were small (400 to 800 µm), exhibited a prominent microtubular pattern, and were expansile, compressing the surrounding

cells. The adenocarcinomas were 0.7 cm in diameter, expansile, and composed of variable-sized tubule-like structures, which were filled with cells that often contained necrotic centers. In contrast, no evidence of a compound-related, increased incidence of kidney neoplasms was observed in quercetin-treated females. Furthermore, in addition to the increase in the development of the proliferative kidney lesions in the high-dose males, an increase in the severity of nephropathy, characterized by a spectrum of lesions distinct from those of the tubular hyperplasia, was also noted compared to controls. The nephropathy was accompanied by a dose-dependent increase in the incidence of renal pelvic transitional epithelial hyperplasia and parathyroid gland hyperplasia. Parathyroid gland hyperplasia occurred with greater frequency and severity in animals with marked nephrotoxicity, typical of secondary renal hyperparathyroidism.

Table 6.4.4-2 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%)

Table 6.4.4-2 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**
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.

Additional findings reported included a single incidence of squamous cell papilloma of the tongue in a high-dose male, while squamous cell carcinoma affecting the tongue was identified in two high-dose females. In comparison, 4 of 500 female NTP historical controls were reported to develop such lesions spontaneously, and these lesions in the test animals were deemed to be not quercetin-related. A significant and dose-related reduction in mammary gland fibroadenomas was observed in females provided 10,000 and 40,000 ppm quercetin in the diet (controls: 29/50; 1,000 ppm: 27/50; 10,000 ppm: 16/50; 40,000 ppm: 9/50). An increase in the frequency of uterine stromal polyps was noted in females at the mid-dose level (control: 7/50; 1,000 ppm: 9/50; 10,000 ppm: 16/50; 40,000 ppm: 11/50). Additionally, a single incidence of uterine stromal sarcoma was identified in a mid-dose female, which was not considered to be compound-related. A significant and dose-dependent accumulation of a fine granular yellow to light-brown pigment was reported in the gastrointestinal tract, with heavier deposition observed in the proximal segments of the tract, including the stomach, and to a smaller degree in the distal portions such as the duodenum and colon. Overall, under the conditions of the study and specifically based on the increase in the incidence of renal tubule cell adenomas in the high-dose males, the NTP concluded that "there was *some evidence of carcinogenic activity* of quercetin in male F344/N rats", but "*no evidence of carcinogenic activity* of quercetin in female F344/N rats" receiving up to 40,000 ppm (NTP, 1992).

In another 2-year feeding study, quercetin (Tokyo Kasei Co. Ltd.; purity >99.4%) was incorporated into the diets of male and female F344/DuCrj rats (50/sex/group) at concentrations of 0 (control), 1.25 (low dose), or 5% (high dose) (Ito *et al.*, 1989). Subsequently, all rats were observed for an additional 8 weeks, during which time animals received the basal diet. Average quercetin intakes were calculated to be 427 and 497 mg/kg body weight/day for males and females, respectively, receiving 1.25%, quercetin and 1,926 and 2,372 mg/kg body weight/day for males and females,

respectively, provided 5% quercetin in the diet. Complete study design details are provided in section 6.4.3.

Pathological effects identified were limited primarily to the cecum, liver and urinary bladder. Microscopic evaluations revealed an increase in the incidences of elevated areas of the cecum were reported in males in the high-dose group relative to controls. Increases in the occurrence of hyperplastic cecal polyps were also detected in males (11) as well as in females (2) maintained on 5% quercetin; however, statistical significance was attained only in males and the lesions were deemed to be non-neoplastic. Furthermore, cecal adenoma (1) and adenocarcinoma (2) were observed in 5% males, while colon adenoma was reported in two females at the same dose level. In comparison to controls, which did not exhibit any cecal or colon lesions, results were not statistically significant. Sporadic urinary bladder abnormalities were limited to a single case of transitional cell hyperplasia in males at both dose levels, and in three high-dose females. Papillomatosis of the urinary bladder was observed in a single high-dose male and female. None of these changes was statistically significant in comparison to the respective male and female control groups. A dose-independent increase in malignant lymphoma/leukemia was reported in males treated at the low-dose level. Although, males in both treatment groups exhibited increased incidences of chronic nephropathy compared to controls in this study, values were within the normal range for the laboratory. All other histopathological variations consisting of non-neoplastic lesions were deemed as common occurrences in aged rats, and in some instances incidences of lesions recorded in 5% group treated rats were in fact decreased compared to controls, as were incidences of foci of hepatic cellular alteration (females), bile duct hyperplasia (males), and mammary gland fibroadenomas (females).

Based on the observation that cows feeding on bracken 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 quercetin for potential carcinogenic activity. Groups of male and female albino rats of a Norwegian strain were provided BF or pure quercetin in the diet for a period of 58 weeks. BF comprised 33% of the diet, and contained 0.57 g of quercetin and 1.1 g of kaempferol in the form of the glycosides astragalin, isoquercetin, rutin, and tiliroside per kg of dried BF. The dietary concentration of BF resulted in estimated cumulative intakes of 1.8 and 1.6 g quercetin/rat and 3.4 and 3.1 g kaempferol/rat for males and females, respectively. Therefore, male (n = 8) and female (n = 11) rats were exposed to quercetin at daily dose levels of approximately 11 and 10 mg/kg body weight, respectively. The other test group (7M, 18F) received 0.1% quercetin (Sigma Chemical Co; purity >99%) in the diet, such that, in total, males were estimated to consume 8.4 g

quercetin and females 7.6 g quercetin over the duration of the study period and approximately 52 and 47 mg/kg body weight/day, respectively. A concurrent control group (9M, 10F) was maintained on the basic diet. Food and water were available *ad libitum* to both control and test animals. At the end of the study period or following earlier death, rats were necropsied and their bladders distended. Samples of several organs (stomach, intestine, kidney, urinary bladder, lungs, liver, heart, brain, and any organs with gross abnormalities) were prepared for histological examination. Relative to the control group, no variations were observed in either of the test groups with respect to the survival rate. Weight gain was comparable among all groups up until weeks 20 to 24 of the study period; however, thereafter the growth rate of rats in both of the test groups was significantly reduced compared to the control animals. At necropsy, intestinal and bladder neoplasms were identified in the test groups, but not in the control group. Although tumors were observed as early as 34 weeks into the study period in the BF-group, quercetin-fed rats were tumor-free until Week 53 of the study. In the BF-group, 7/8 males and 10/11 females developed intestinal tumors and 6/8 males and 8/11 females exhibited bladder tumors. Likewise, in the quercetin-group, intestinal tumor formation was observed in 6/7 males and 14/18 females. The intestinal tumors of the quercetin group were reported to occur in the ileal segment of the small intestine and, histopathologically, were classified as adenomas (4), fibroadenomas (7), or adenocariconmas (9) with mesenteric metastases (3), which in most cases penetrated all layers of the intestinal wall. The incidence of bladder tumors [2/7 (M); 3/18 (F)] was significantly lower in the quercetin-fed group compared to the BF-group. Histologically, however, the neoplasms were indistinguishable and consisted of papillary and sessile transitional cell carcinoma, with one occurrence of associated fibromyxoma. It was concluded by the authors that the abnormalities of the intestinal and bladder epithelium observed in both test groups were attributable to quercetin exposure. The authors suggested that the difference in the incidence of bladder tumor formation observed between the 2 test groups was possibly due to the absence of thiamine (vitamin B_1)-supplementation of the diets provided to quercetin-fed rats, which was demonstrated to significantly increase the incidence of urinary bladder tumors in BF-treated rats in a previous study (Pamukcu *et al.*, 1970).

In another study, thiamine supplementation did not affect the incidence of urinary bladder tumor development in female Sprague-Dawley (SD) rats receiving BF in the diet (Ertürk *et al.*, 1983 [abstract]). In a parallel investigation of life-long exposure to quercetin, administered in the diet at concentrations of 0 (control), 1, or 2% [approximately 0, 500, and 1,000 mg/kg body weight/day, respectively (U.S. FDA, 1993)] to groups of 25 female F344 rats, receiving concomitantly weekly subcutaneous injections of 2 mg thiamine for 18 months, variations of the liver were observed. Liver preneoplastic foci were reported in rats at both dose levels (1%: 20/21 and 2%: 12/14), as were incidences of hepatoma (1%: 5/21 and 2%: 9/14) and bile duct tumors (1%:

13/21 and 2%: 11/14); however, hepatic preneoplastic lesions (6/15) and bile-duct neoplasms (1/15) were also identified in control rats. Additionally, a single occurrence of hepatocarcinoma was detected in a high-dose rat (1,000 mg quercetin/kg body weight/day). The statistical significance was not reported.

Pathological changes of the ileum and colon were also observed by Bokkenheuser and Winter (1990 [abstract]) in a 6-month rat study (strain not specified) during which test animals were administered 5% quercetin in the diet [approximately 2,500 mg/kg body weight/day (U.S. FDA, 1993)]. Specifically, dilation of the cecum, atrophy of the intestinal mucosa, and hypertrophy of crypts and goblet cells were observed at necropsy. Additionally, the mucosa and submucosa of the colon and ileum exhibited lymphoid hyperplasia.

The potential for dietary quercetin (purity 99%) to induce carcinogenesis in rats was re-examined in Fisher 344 rats receiving 0.1% [approximately 50 mg/kg body weight/day (U.S. FDA, 1993)] of the flavonol in the diet for a period of 540 days (Takanashi *et al.*, 1983). The test and control groups consisted of 15 and 16 rats/sex, respectively. All surviving animals were necropsied at study termination or earlier at the onset of moribundity. Tissue samples (not specified) were prepared for histological evaluation. No early deaths were reported in the quercetin test group. Incidence of tumor formation in test animals did not differ significantly from that observed in the control group. 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 were also observed in control animals (*i.e.*, testicular interstitial cell tumors, adrenal cortical adenoma, pituitary adenoma).

Hirono *et al.* (1981) confirmed the absence of *in vivo* carcinogenic potential of quercetin in another rat study in which groups of ACI inbred rats were provided 1 or 5% quercetin (10 rats/sex, and 8 male and 9 female rats, respectively) or 5% rutin (11 males and 10 females) in the diet for a period of 540 days. Additionally, groups of 20 male and 20 female rats received 10% [5,000 mg/kg body weight/day (U.S. FDA, 1993)] quercetin or rutin for 850 consecutive days. All animals were subjected to necropsy and their tissues examined histologically, including those found in moribund condition or that died prior to study completion. In the 540-day study, body weights of male rats in the high-dose quercetin group (2,500 mg quercetin/kg body weight/day) were significantly reduced compared to controls. Most rats survived for at least 300 days into the study period, and no statistically significant differences in the survival rates among groups were noted. All groups exhibited a comparable tumor incidence and most tumors detected in the quercetin-test groups (adrenal cortical adenoma, pheochromocytoma, and testis interstitial cell tumor) were also identified in controls. A single occurrence of cecal adenoma affecting a high-dose quercetin-treated male (2,500 mg quercetin/kg body

weight/day) provided the only exception. In the longer-term study, no differences in body weights were observed. Survival of rats in the quercetin group spanned at least 349 days, at which point a total of 37 male and female rats were alive. As in the former trial, tumors (adrenal cortical adenoma, pituitary adenoma, testis interstitial cell tumors, and uterus adenocarcinoma) occurred with comparable frequency in the quercetin-fed group compared to the control group. Only isolated incidences of adenoma (1) and adenocarcinoma (2) of the cecum were observed in quercetin-fed male rats without parallel occurrence in the controls. The authors suggested that the inconsistency observed between the results obtained in this study and those of Pamukcu *et al.* (1980) may possibly be attributed to strain differences. Furthermore, limited absorption, extensive metabolism, and excretion, as well as possible pure initiator activity, were pointed out as potential factors contributing to the lack of carcinogenicity noted *in vivo*, in spite of the well-documented mutagenic activity observed *in vitro*.

Evaluated in 38 male and 35 female ddY mice maintained on a quercetin-supplemented diet for the duration of their life-span (approximately 842 days), no statistically significant differences were reported in incidence of tumor formation compared to untreated controls (Saito *et al.*, 1980). Quercetin-treated mice received 2% of the aglycone in the diet [approximately 3,000 mg quercetin/kg body weight/day (U.S. FDA, 1993)], which together with water were provided *ad libitum*. Animals were weighed and examined (scope of examination not specified) weekly, and all dead and moribund animals were subjected to necropsy. A wide range of organs, including any organs exhibiting gross variations, was examined microscopically. Body weights of quercetin-fed mice remained comparable to those of controls for the duration of the study period. Animals in both groups were reported to develop leukemia, as well as tumors of the lungs, forestomach, mammary glands, adrenals and soft tissues. However, in quercetin-treated mice, metastasis to the heart and bronchial cavity were reported to occur respectively in 2 cases of lung adenocarcinoma, and the tumor of the forestomach was determined to be a malignant squamous cell carcinoma *versus* a papilloma in the control animal. Furthermore, formation of liver (4 M) (*i.e.*, hemangioma, hepatocellular adenoma, hemangioendothelial sarcoma, and hepatocellular carcinoma), uterine (3), ovary (1), and salivary gland (2F) tumors was limited to the quercetin-test group. Additionally, a malignant spindle cell sarcoma of the heart was identified in a quercetin-fed male. The authors noted this tumor to be generally considered as atypical in mice.

As a variety of carcinogens have been reported to be associated with lung tumor formation in strain A mice (Shimkin and Stoner, 1975), the potential effect of quercetin specifically on lung tumor development was investigated in this strain of mice (A/JJms) (Hosaka and Hirono, 1981). Mice (23M, 24F) were administered quercetin in the diet at 5% [approximately 7,500 mg/kg body weight/day (U.S. FDA, 1993)] for a period of 23 weeks, while a concurrently-maintained control group (21M, 27F) received an

unmodified basal diet. At study termination or earlier in a single case of premature death (77th day, control mouse), animals were necropsied, and lungs removed and sections prepared for histopathological examination. Lung tumor-bearing animals occurred with similar incidence and multiplicity in the test (3/23M and 1/24F) and control (2/21M and 1/27F) group. More specifically, tumors occurred as single nodules and were not observed to metastasize.

Two studies were conducted in non-inbred Syrian golden hamsters to determine the potential carcinogenicity, as well as possible initiator activity of quercetin (see Section 6.4.5 for a discussion of the study designed to assess potential initiator activity of quercetin) (Morino *et al.*, 1982). In the carcinogenicity study, groups of 20 hamsters per sex were administered 0 (basal diet control group) or 10% quercetin or rutin, resulting in estimated dose levels of approximately 12 g quercetin and 10 g rutin per kg body weight/day, for a period of 733 days. Survival of male control hamsters and all animals in the test groups exceeded 70%; however, the survival of female controls was only 55%. Body weight measurements did not reveal any significant differences in the growth rates of any of the animals. Following necropsy and histopathological evaluation of selected tissues, papillomas of the forestomach and cortical adenomas of the adrenal were observed consistently in all 3 groups, without any statistical variance in incidence. Additionally, an adenocarcinoma of the ileum was identified in a single quercetin-fed female hamster and leiomyoma of the uterus was detected in a female in the rutin-treated group. The results of this study failed to demonstrate any carcinogenic potential associated with the oral intake of quercetin.

See "6.4.7 Discussion for Chronic Toxicity and Carcinogenicity" for supporting discussion and rationale on the totality-of-scientific-evidence indicating that quercetin is not a carcinogen and is not a promoter of tumor development.

6.4.5 Two-Stage Carcinogenicity Studies

Several initiation-promotion studies have been performed to determine the potential effect of quercetin on chemically-induced carcinogenesis. Most studies have included a quercetin control group, and as such are relevant to the safety determination of the flavonoid. The results of these studies are summarized in Table 6.4.5-1.

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. In total, 6 different treatment regiments were tested. For the first 4 weeks, group 1 received 5% quercetin [approximately 2,500 mg/kg body weight/day (U.S. FDA, 1993)] in the diet and, subsequently, 0.01% *N*-butyl-*N*-(-4-hydroxybutyl)nitrosamine (BHBN) in the drinking water for a period of 29 weeks, while group 2, which was also provided 5% dietary quercetin for the first 4 weeks, received ordinary tap water during the following 29

weeks. Administration of the basal diet for 4 weeks to rats of group 3 was subsequently followed by treatment with drinking water containing 0.001% BHBN for a period of 29 weeks. Rats in groups 4 and 5 received 0.01% BHBN in the drinking water for 4 weeks. Thereafter, 5% quercetin and basal diet, respectively, were provided for 25 weeks. After receiving ordinary tap water for 4 weeks, the diet of group 6 rats was supplemented with 5% quercetin during the final 25 weeks. Animals in groups 1 through 3 and 4 through 6 were necropsied at 33 and 29 weeks, respectively, following initiation of the study. Bladders, as well as kidneys and livers were prepared for detailed microscopic evaluation. No statistically significant differences were observed between the weight gain and final body weights of quercetin-treated rats (groups 1, 2, 4, and 6) and those in the BHBN-only groups (groups 3 and 5). Histopathological examinations of the liver and kidneys did not reveal any variations among any of the groups. No urinary bladder lesions were detected in any of the group 2 or 6 animals, which were treated only with dietary quercetin for 4 and 25 weeks, respectively. Furthermore, none of the animals in groups 1 and 3 developed any urinary bladder neoplasms. Absence of tumors in these groups was reported to be indicative of a lack of quercetin-initiating activity. Urinary bladder lesions, classified as either papillary or nodular hyperplasia, or papilloma, were reported in groups 4 and 5 with comparable incidence. Therefore, no promoting activity could be ascribed to quercetin administration in the diet on the basis of these results. Overall, the results were suggestive of a lack of quercetin-associated carcinogenicity in the urinary bladders of F344 rats.

Another study was conducted to assess the potential promoting properties of a range of chemicals on urinary bladder carcinogenesis in male F344 rats following initiation with *N*-nitroso-*n*-butyl-(4-hydroxybutyl)amine (BBN) provided in the drinking water for a period of 4 weeks (Fukushima *et al.*, 1983). Groups of rats (30/group) were provided quercetin, one of the test compounds evaluated in the study, at a dietary concentration of 5% for a period of 34 weeks following BBN-initiation. A concurrently maintained quercetin-control group received water without BBN during the 4-week initiation period. Food and water were provided *ad libitum* throughout the study period. Body weights and food and water consumption were recorded weekly, and animals were examined once daily for overt signs of clinical toxicity. A thorough physical examination was conducted once per week. Urine samples were collected from at least 10 rats in each group at Weeks 12, 24, and 36 for urinary sedimentation tests, and measurements of pH and osmolality. Additional parameters of urinalysis, including bilirubuin, urobilinogen, protein, glucose, and ketone body levels, were examined only at study termination (*i.e.*, 36 weeks). Animals were necropsied at study termination or earlier with the onset of moribundity. Relative and absolute kidney weights were determined, and renal organs (*i.e.*, urinary bladder, urethra, ureters, and kidneys) and gross lesions were subjected to microscopic evaluation. Based on the estimates of daily food intake, rats ingested approximately 2,000 mg quercetin/kg body weight/day. In both the uninitiated and BBN-

treated quercetin-groups of rats, no variations were reported with respect to food consumption or final body weights, as well as absolute and relative kidney weights in comparison to the basal diet and BBN-only control groups (60 rats/control group), respectively. Compared to the respective control groups, the urinary pH was noted to be slightly acidic in quercetin-treated animals as early as 12 weeks into the study period; however, all other urinary parameters monitored were comparable between the test and control groups. Neither relative nor absolute urinary bladder weights were observed to differ in either of the quercetin-treated test groups in comparison to the corresponding control group. In the quercetin-only treated group, histopathological examination of the urinary bladder was unremarkable. Although not at levels of statistical significance, quercetin provided to BBN-initiated animals was reported to reduce the incidence of papillary or nodular hyperplasia and papilloma of the urinary bladder compared to the BBN-initiated-only rat control group. However, incidences of simple hyperplasia of the urethra were reported in BBN-initiated quercetin-treated rats. Additionally, protein casts of the tubules were reported to occur with high incidence in quercetin-treated rats.

The potential of quercetin to increase estradiol-induced tumorigenesis in the kidneys of male Syrian hamsters was investigated by Zhu and Liehr (1994). Tumor induction was evaluated in groups of hamsters receiving a laboratory chow, or the same lab chow supplemented with 0.3 or 3% quercetin [approximately 360 and 3,600 mg/kg body weight/day, respectively (U.S. FDA, 1993)] for 2 weeks prior to single subcutaneous implantation of 25 mg estradiol. Subsequently, hamsters continued on their respective diets for a period of 5.5 months. Estradiol administration was repeated at 3 months following the first estradiol treatment. Additional groups of hamsters following the same estradiol-dosing schedule were maintained on the 3% quercetin diet for 6.3 months after the initial estradiol application. No tumors of the kidneys were reported in all quercetin-only treated groups, as well as in untreated controls. In contrast, all animals receiving estradiol-only (5.5 months: 6/6; 6.3 months: 12/12) and estradiol in combination with 3% quercetin (5.5 months 6/6; 6.3 months: 12/12), as well as 7 of 8 animals treated with estradiol and maintained on the 0.3% quercetin diet (only 5.5 months) exhibited tumors. A statistically significant increase was observed in the mean number of large tumors observed per hamster in all quercetin- and estradiol-combined treatment groups compared to the respective estradiol-only groups. Furthermore, in the 6.3-month quercetin and estradiol group, 6 of the 12 tumor bearing animals developed abdominal metastases, compared to only 3 of the 12 in the estradiol-only group ($p < 0.05$). Metastases were also observed in 2 animals in each of the quercetin and estradiol 5.5 month-treatment groups (0.3 and 3% quercetin) and in none of the animals in the estradiol-only group. Examination of renal and hepatic enzyme activity revealed that quercetin treatment at the 3% dietary concentration was associated with a significant enhancement of the renal NADPH-dependent estradiol-4-hydroxylase activity compared

to estradiol-only controls and a reduction in the rate of COMT-mediated *O*-methylation of 2- and 4-hydroxyestradiol.

Yang *et al.* (2000) evaluated the potential effects of dietary supplementation with quercetin or its glycoside, rutin, on the colon of saline- and AOM-treated male and female CF1 mice. Six-week-old mice were divided into 3 dietary groups (10/sex/group) and provided a basal diet (control) or the basal diet supplemented with either 2% quercetin or 4% rutin *ad libitum* for a period of 8 weeks. Treatment groups consumed approximately 3,000 or 6,000 mg/kg body weight of quercetin and rutin, respectively (U.S. FDA, 1993). After 2 weeks of dietary treatment, 10 mice from each group (5/sex) were administered weekly subcutaneous injections of either 0.9% saline or AOM for a period of 6 weeks. A total of 3 animals, 2 from the quercetin group and the other from the rutin group, died during the study period due to unknown reasons (no further details were provided). All surviving animals were killed one week after the last saline or AOM injection (*i.e.,* at 14 weeks of age) and the colon was excised for histopathology. Endpoints including cell proliferation, apoptotic cell death, cyclin D1 expression, and focal areas of dysplasia (FAD) were evaluated. Comparing saline-treated groups, supplementation with quercetin resulted in a non-significant increase in the number of FAD relative to the control group (in 2/9 and 0/10 mice, respectively). FAD development was not observed in any mice fed the rutin-containing diet. Treatment of rats receiving the AOM injection with either quercetin or rutin resulted in significantly reduced incidence of AOM-induced development of FAD. FAD incidence rates for the AOM-treated control, quercetin, and rutin groups were 10/10, 5/9, and 6/9, respectively. Relative to the respective AOM- or saline-control groups, both quercetin and rutin supplementation significantly increased the number of colonic epithelial cells per crypt column, indicative of colonic crypt cell proliferation.

In a study designed to assess the potential inhibitory effect of quercetin on azoxymethanol-induced colonic neoplasia in mice, Deschner *et al.* (1991) did not note any adverse effects with respect to body weight gain or any other overt symptoms of toxicity in female CF1 mice receiving up to 2% quercetin [approximately 3,000 mg/kg body weight/day (U.S. FDA, 1993)] for up to 50 weeks. Among saline control groups, quercetin (2%) administration over a 9-week treatment period did not affect any of the proliferative indices of colonic epithelial cells, compared to the basal diet-only treated group. Co-administration of 2% quercetin together with weekly AOM injections revealed a significant reduction in the number of cells identified in the S-phase per crypt in comparison to AOM-treated rats maintained on the quercetin-free basal diet. Furthermore, compared to the AOM-control group, AOM-injected mice receiving quercetin at the 2% dietary level exhibited a significantly reduced number of focal areas of dysplasia (FAD) in 500 µm of distal colonic tissue. In a longer trial, quercetin was provided to mice at concentrations of 0, 0.1, 0.5, or 2% [approximately 0, 150, 750, and

3,000 mg/kg body weight/day, respectively (U.S. FDA, 1993)] for a period of 48 successive weeks following the 1st of 3 AOM injections. At the highest-dose level, a statistically significant 4-fold inhibition of AOM-induced colonic tumors was observed compared to the AOM-control group. Overall, based on the results of the study, the authors proposed that quercetin acts to inhibit colonic neoplasia at the promotional stage of carcinogenesis.

Quercetin was also demonstrated to exert a protective effect with respect to AOM-induced ACF formation in male F344 rats (Tanaka *et al.*, 1999). One week prior to initiation of a series of weekly subcutaneous AOM injections for a period of 3 weeks, groups of 9 or 8 rats were administered quercetin in the diet at 0 and 500 ppm [approximately 25 mg quercetin/kg body weight/day (U.S. FDA, 1993)], respectively, for a total of 5 consecutive weeks. Two other concurrently maintained groups of 7 and 6 rats each also received 0 and 500 ppm of quercetin in the diet, respectively, without intervening AOM injections. At study termination all animals were necropsied, and the large intestine, liver, kidneys, lungs and heart were subjected to microscopic examination. No variations were observed in final body weights or absolute and relative liver weights, and all groups exhibited comparable food consumption. Animals did not present any symptoms of clinical toxicity, and histopathological examination of the liver, kidneys, lungs and heart was unremarkable. ACF formation was not observed in the distal colon of the uninitiated quercetin group. Moreover, quercetin treatment was associated with a statistically significant reduction in the number of ACF, as well as in the number of aberrant crypts per focus in AOM-initiated rats, compared to AOM-initiated rats maintained on quercetin-free basal diets. Quercetin treatment of AOM-induced rats induced a significant decrease in colonic mucosa and blood total polyamine levels relative to levels observed in AOM-only treated animals, which were significantly higher compared to untreated controls. In comparison to the AOM-only treated group, quercetin co-administration significantly reduced the number of cells positive for proliferative cell nuclear antigen (PCNA) in ACF crypts, as well as in normal-appearing crypts.

Inhibition of AOM-induced colorectal carcinogenesis was demonstrated in male F344 rats administered quercetin *via* the diet (Dihal *et al.,* 2006). Groups of 42 rats were provided diets supplemented with 0, 0.1, 1, or 10 g quercetin/kg diet for a period of 41 weeks resulting in dose levels of approximately 0 (control), 4.5 to 9, 45 to 90, and 450 to 900 mg quercetin/kg body weight/day, respectively. Beginning 2 weeks after initiation of the dietary treatment, 30 rats from each group received single weekly subcutaneous AOM injections (15 mg/kg body weight/week) for 2 consecutive weeks (remaining 12 rats/group were treated with vehicle only). Eight weeks after the second AOM injection, 6 to 8 rats/group were killed and ACF development in the colon was examined. ACF were observed to form mainly in the distal colon and were noted in all AOM-treated

animals. The number of ACF observed in quercetin-treated groups was comparable to the control group; however, upon study completion (*i.e.,* 38 weeks post-AOM treatment), quercetin treatment was associated with a dose-dependent decrease in colorectal tumor incidence relative to the control AOM-treated rats, with statistical significance reached at the highest dose level (*i.e.*, tumor incidence in AOM-treated control and quercetin low-, mid-, and high-dose groups was 50, 41, 36, and 20%, respectively). No colorectal tumors were observed in any of the AOM-untreated rats fed either the control or quercetin-supplemented diets.

In another study utilizing the AOM-induced rat colon cancer model, groups of 10 male F344 rats were fed diets containing 0 (positive control), 15,000, or 30,000 ppm quercetin [providing 0, 1,500, and 3,000 mg/kg body weight/day, respectively (U.S. FDA, 1993)] for a period of 5 weeks (Volate *et al.*, 2005). After 1 week on the experimental diets, the rats were injected intraperitoneally with 15 mg AOM/kg body weight/week for 2 consecutive weeks. Additional groups of rats (5/group) maintained on diets containing 0 or 30,000 ppm quercetin [0 and 3,000 mg/kg body weight/day, respectively (U.S. FDA, 1993)] were injected with saline rather than AOM and were included in the study as negative controls. Supplementation with quercetin did not result in ACF formation in the colon of saline-treated rats while in the AOM-treated rats, a significant dose-dependent decrease in the total number of ACF relative to the positive control group was observed.

To determine the potential effect of quercetin on AOM-induced colon carcinogenesis, male Sprague-Dawley rats (27/group) were fed diets containing 0 (control) or 0.5% quercetin, providing 0 and 250 mg quercetin/kg body weight/day, respectively (U.S. FDA, 1993), for a period of 35 weeks (Choi *et al.*, 2006). At Weeks 2 and 3 of the study, the animals were intraperitoneally injected with 15 mg/kg body weight of AOM. Twelve rats/groups were killed at 8 weeks after the second AOM injection (*i.e.*, Week 10) to assess colonic ACF formation. The remaining animals were killed at Week 35 and were examined for colon tumour formation. Dietary treatment with quercetin had no significant effect on ACF formation or tumour incidence when compared to the control group.

In groups of male and female SD rats, quercetin did not induce a promoting effect on methylazoxymethanol acetate (MAM)-induced intestinal tract carcinogenesis (Kato *et al.*, 1984). Two groups of rats received weekly MAM injections for a period of 3 weeks, and, thereafter, were maintained for up to 459 days on a basal diet or a basal diet containing 1% quercetin [approximately 500 mg quercetin/kg body weight/day (U.S. FDA, 1993)]. Two control groups, which received saline injections, were maintained on either the basal diet or a quercetin-enriched diet for the duration of the 459-day study period. Body weight gain was comparable among all groups throughout the treatment period and animals in the saline-treated control groups generally exhibited a higher survival rate

than MAM-treated animals. Examination following scheduled necropsy or earlier revealed no incidences of tumor formation in both saline control groups, including the 1% quercetin group. Incidences of tumor formation and types of tumor identified in the MAM and MAM-quercetin groups were comparable. Furthermore, administration of quercetin in the diets of MAM-initiated rats was associated with a statistically significant reduction in the incidence of colonic adenomas compared to MAM-initiated rats maintained on the basal diet.

In contrast, Matsukawa *et al.* (2002) noted significant increases in the size of duodenal tumors and in the average number of the tumors in C57BL/6 mice provided quercetin at dietary concentrations of 0.2 and 2%, respectively [approximately 300 and 3,000 mg/kg body weight/day, respectively (U.S. FDA, 1993)] for a period of 16 weeks following induction with *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (ENNG) administered in the drinking water for a period of 4 weeks, in comparison to mice treated only with ENNG. It should be noted that all 19 animals maintained in the ENNG-only control group exhibited duodenal tumors (100% incidence of duodenal tumorigenicity). However, no tumor incidences were reported in the concurrently maintained control group (n = 20) provided 2% quercetin in the diet without chemical-induced duodenum tumorigenicity. 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 (U.S. FDA, 1993) for 16 consecutive weeks, no duodenal tumors were observed (Werner *et al.*, 1985). As in the mouse study, concomitant administration of 1% quercetin in the diet and ENNG in the drinking water for the entire duration of the 16-week study period was associated with a marked, but not statistically significant, increase in the incidence of duodenal tumors (28/33) relative to a group administered only ENNG in the drinking water and maintained on a basal diet (22/33).

In a study conducted by Akagi *et al.* (1995) the potential effects of quercetin administered subsequently to or concurrently with a number of carcinogens, collectively termed the DMBDD treatment, which included intraperitoneal injections of diethylnitrosamine (100 mg/kg body weight x 1) and *N*-methylnitrosourea (20 mg/kg body weight x 4), subcutaneous injections of 1,2-dimethylhydrazine (40 mg/kg body weight x 4), and 0.05% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (2 weeks) and 0.1% 2,2'-dihydroxy-di-*n*-propylnitrosamine (2 weeks) provided in the drinking water, was investigated in male F344 rats. The DMBDD treatment was administered to rats during a 4-week multiple-initiation period. Quercetin was provided in a powdered basal diet at a concentration of 1% [approximately 500 mg quercetin/kg body weight/day (U.S. FDA, 1993)] to groups of 15 animals beginning either 1 day prior to commencement of the DMBDD treatment and continuing throughout the 4 weeks of DMBDD treatment, or alternatively, 1 week after completion of the DMBDD treatment and continuing for the remainder of the 36-week test period. Additionally, 2 separate groups of 10 animals

each received quercetin or the basal diet without DMBDD treatment for the entire duration of the 36-week treatment period. All surviving animals were necropsied at the end of the 36-week treatment period, and liver and kidneys 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. In comparison to a DMBDD-only treated group, quercetin administered during initiation or promotion did not significantly affect final average body weight or relative liver and kidney weights. With respect to tumor development, quercetin administered during the promotion phase fully inhibited development of small intestinal tumors (adenomas and carcinomas), and demonstrated a tendency to reduce the incidence and number of total small intestinal tumors, albeit not at levels of statistical significance, when administered during the initiation phase. No other variations in the formation of lesions of the examined organs in comparison to the DMBDD-only treated group were noted.

The potential chemopreventive effects of quercetin on AOM-induced colon tumors and 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced mammary tumors was investigated in male F344 and female Sprague-Dawley rats, respectively (Pereira *et al.*, 1996). In the former trial, quercetin was administered in the diet to groups of 36 rats at concentrations of 16.8 or 33.6 g quercetin/kg diet [approximately 840 and 1,680 mg/kg body weight/day, respectively (U.S. FDA, 1993)] for a week prior to subcutaneous AOM initiation (30 mg/kg body weight). Quercetin administration was subsequently continued for 45 weeks. A concurrently maintained group of rats (n = 12) received the high-dose quercetin treatment (*i.e.*, 1,680 mg/kg body weight/day) for the entire duration of the 46-week treatment period without AOM initiation. No statistically significant final body weight variations were reported among any of the quercetin-treated groups compared to the AOM control group (n = 12). Quercetin provided to rats without AOM initiations was not associated with any incidences of colon tumor formation, and only a single incidence of adenocarcinoma of the small intestine was identified. In contrast, in rats treated with AOM, quercetin was associated with a dose-dependent statistically significant increase in the incidence and number per rat of colon adenocarcinomas at both dose levels of the flavonoid in comparison to the AOM control group. In an accompanying study, examining specifically the effect of quercetin on AOM-induced ACF, administration of the flavonoid at 30 g/kg diet [approximately 1,500 mg/kg body weight/day (U.S. FDA, 1993)] for a period of 10 weeks, during which 15 mg AOM/kg body weight was administered subcutaneously on 2 separate occasions (Days 8 and 15), did not induce a significant enhancing effect. Furthermore, no incidences of ACF were reported in a group treated with quercetin (30 g/kg diet) only for 10 consecutive weeks.

In the DMBA-induced mammary tumors experiment, dietary quercetin administration at 10 or 20 g/kg diet [approximately 500 and 1,000 mg/kg body weight/day (U.S. FDA, 1993)] was initiated 1 week prior to DMBA treatment *via* gavage (12 mg), and continued thereafter for a period of 100 days (Pereira *et al.*, 1996). Although not at levels of statistical significance, quercetin at both dose levels reduced the incidence of animals exhibiting mammary tumors, as well as tumor multiplicity. Additionally, a significant increase was observed in the tumor latency of high-dose animals. Verma *et al.* (1988) also observed a statistically significant and dose-dependent reduction in the incidence and multiplicity of palpable mammary tumors in female Sprague-Dawley rats, initiated with a single intragastric administration of DMBA one week following initiation of dietary 2 or 5% quercetin treatment [approximately 1,000 and 2,500 mg/kg body weight, respectively (U.S. FDA, 1993)]. Although not as effective as the inhibition of DMBA-induced tumors, quercetin also inhibited induction of mammary tumors initiated with a single intravenous administration of *N*-nitrosomethylurea (NMU). Furthermore, no palpable mammary tumors were detected in an uninitiated group of rats, maintained on a 2% quercetin-supplemented diet and no variations in body weight gain were reported among any of the test groups.

Wistar rats were mated and the dams subsequently randomly segregated into 1 of 2 groups, namely the control or test group, which were provided 0 or 1.0% quercetin in the diet [approximately 500 mg quercetin/kg body weight/day (U.S. FDA, 1993)], respectively (Barotto *et al.*, 1998). Litters of each group received either a single intraperitoneal injection of 50 mg NMU/kg body weight in 0.9% sodium chloride or 0.9% sodium chloride at 3 days of age. At 30 days of age, pups were placed on the same diets as had been administered to their respective mothers. Thus, the study consisted of 4 different treatment groups: (i) a saline-control group (10M, 9F); (ii) a saline-quercetin group (10M, 9F); (iii) a NMU-control group (9/sex); and (iv) a NMU-quercetin group (14M, 12F). Rats received food and water *ad libitum* throughout the study period, and were examined daily for overt signs of toxicity. With the exception of rats exhibiting sudden weight loss or found in a moribund state, all rats were killed on the 24th week 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-control group. Relative pancreas weights of females in the saline-quercetin group were significantly increased in comparison to the saline-control group; however, no pathological variations of the pancreas were identified in either sex of the quercetin-saline treated animals. Significant reductions in body weights of quercetin-NMU males and females compared to the NMU-control and quercetin-saline groups were observed. Relative pancreas and liver weights of males in the NMU-quercetin group were significantly elevated compared to both the quercetin-saline and NMU-control groups. In females of the NMU-quercetin group, relative pancreas and liver weights were significantly increased relative to the

quercetin-saline and NMU-quercetin groups, respectively. Although no difference was noted in the occurrence of multicentrical focal acinar cell hyperplasia (FACH) of the pancreatic tissue in NMU-quercetin rats compared to NMU-control group, a significant increase was observed in the incidence of dysplastic foci (DYF) in male and female NMU-quercetin rats. In comparison to female rats, DYF development was generally enhanced in males, albeit not at levels of statistical significance. Furthermore, 4 of the 12 NMU-quercetin males exhibiting severe DYF. Carcinoma *in situ* was observed in 12% of the NMU-quercetin test animals, with males demonstrating greater susceptibility (21%). An isolated occurrence of microcarcinoma was also reported in this group of rats. Microscopic evaluation of the lesions revealed an increase in mitosis and a concomitant decrease in apoptosis in the NMU-quercetin group. Overall, the authors concluded that quercetin demonstrated a promoting and progressing effect in the rat model of NMU-induced pancreatic carcinogenesis.

More recently, another study conducted by Barotto and others evaluated the effect of dietary phenobarbital (PB) administration in combination with quercetin on NMU-induced pancreatic tumors in rats (Valentich *et al.*, 2006). Pregnant Wistar dams were administered a standard diet containing 0 (control) or 10,000 ppm quercetin [approximately 0 or 500 mg quercetin/kg body weight/day (U.S. FDA, 1993)], beginning on Gestation Day 10 and throughout the lactation period. Litters of the control group received either a single intraperitoneal injection of 50 mg NMU/kg body weight in 0.9% sodium chloride (NMU treatment) or 0.9% sodium chloride (saline treatment) at 3 days of age, whereas all litters of the quercetin-treated group received the NMU treatment. Once pups were 30 days of age, they were weaned onto diets containing 0 or 10,000 ppm quercetin (*i.e.*, same diet that had been administered to their respective mothers), with or without the addition of 500 ppm PB [approximately 25 mg/kg body weight/day (U.S. FDA, 1993)]. Thus, the study consisted of 6 different treatment groups: (i) a saline-control group (10M, 9F); (ii) a saline-PB group (10M, 4F); (iii) a NMU-control group (15M, 12F); (iv) a NMU-PB group (15M, 10F); (v) a NMU-quercetin group (15M, 13F); and (vi) a NMU-quercetin + PB group (8M, 9F). Food and water were provided *ad libitum* throughout the study period, and clinical observations were made daily. Unless rats exhibited sudden weight loss or were found in a moribund state, they were killed at 24 weeks of age. The pancreas was removed immediately for further macro- and microscopic examinations. Macroscopically, the pancreas of all NMU-treated rats exhibited surface irregularity due to the occurrence of 1 to 3 mm nodules, which were yellowish to pink in color. No significant differences were noted in the occurrence of FACH of the pancreatic tissue among any of the NMU-treated groups. In comparison to the NMU-control group, significant increases were observed in the incidence of DYF in the NMU-PB group (both sexes), NMU-quercetin group (both sexes), and NMU-quercetin + PB group (males and total only). Significant differences were also noted between rats in the NMU-quercetin and NMU-quercetin + PB groups, with fewer

incidences of DYF observed in male and female rats of the latter group (*i.e.*, co-treatment with PB demonstrated a protective activity). Likewise, a significantly lower incidence of DYF was observed in the NMU-quercetin + PB group (males and total) compared to the NMU-PB group. Within the NMU-quercetin group, the incidence of DYF was significantly greater in males (80% or 12/15) relative to the females (54% or 7/13). Conversely, in the NMU-quercetin + PB group, the incidence of DYF development in female rats (44% or 4/9) was significantly higher than that of the males (25% or 2/8). Carcinoma *in situ* was observed in 3 male rats (20%) of the NMU-quercetin group, whereas none of the rats in the NMU-quercetin + PB group developed carcinoma *in situ* (1 male rat in the NMU-PB group developed this lesion). The findings of this study further supported the authors' previous conclusion that quercetin demonstrated a promoting and progressing effect in the rat model of NMU-induced pancreatic carcinogenesis.

Quercetin was among 112 different compounds evaluated for hepatocarcinogenic potential by determining the effect of the compound on the induction of preneoplastic GST placental form positive (GST-P^+) foci following initiation with a single intraperitoneal dose of diethyl nitrosamine (DEN) in male Fischer rats (Ito *et al.*, 1988). Quercetin administration in the diet at a concentration of 25,000 ppm, equivalent to approximately 1,250 mg/kg body weight/day (U.S. FDA, 1993), was initiated 2 weeks following DEN treatment and continued thereafter for a period of 6 weeks. At the end of the treatment period, livers were excised and the incidence of GST-P^+ foci was determined. The GST-P^+ foci formation in quercetin-treated rats was comparable to that observed in the DEN control group.

Jin *et al.* (2006) demonstrated the chemoprotective effect of orally administered quercetin against benzo[a]pyrene (B[a]P)-induced pulmonary precancerous changes in ICR mice. Groups of male and female mice (5/sex/group) were administered quercetin at dose levels of 0 (negative control), 0 (positive control), 500, 1,000, or 2,000 mg/kg body weight/day by gavage for a period of 90 days. After 1 week of treatment, animals in all groups, except the negative control group, were intraperitoneally administered benzo[a]pyrene at a dose level of 100 mg/kg body weight. Upon completion of the 90-day treatment period, peripheral blood samples were collected, following which mice were killed and their lungs were excised for histopathological examination. DNA damage was assessed in the peripheral blood cells under the conditions of the Comet assay. In comparison to the B[a]P-untreated control group, the comet tail length was significantly increased in samples from all B[a]P-induced groups of mice, including quercetin-treated groups; however, the DNA damage was significantly lower in quercetin-treated mice compared to the positive control of B[a]P-induced mice. Furthermore, the quercetin-related protective effect against B[a]P-induced DNA damage was demonstrated to be dose-dependent, such that significant differences were

observed between successive quercetin dose levels, with the exception of the mid- and high-dose level in females, where the difference was only marginal. Microscopically, pulmonary pathological changes, including squamous epithelial metaplasia and atypical hyperplasia were noted in all benzo[a]pyrene-treated rats; however, the severity of the changes were significantly reduced in all quercetin-treated groups relative to the positive control group.

The potential chemoprotective effect of weekly intraperitoneal injections of quercetin on DMBA-induced mammary tumors in female Wistar albino rats was evaluated by Devipriya *et al.* (2006). In a preliminary study, groups of 6 rats with DMBA-induced mammary tumors were administered quercetin intraperitoneally at dose levels of 0, 15, 25, 35, or 45 mg/kg body weight (the duration of exposure during this dose-finding study was not reported). Quercetin treatment at all dose levels was associated with a reduction in tumor volume (statistical significance not reported), with the 25 mg/kg body weight dose level determined to be the effective dose. Subsequently, 2 groups of rats (6/group) with DMBA-induced mammary tumors were either treated with 5 weekly intraperitoneal injections of 25 mg quercetin/kg body weight (on Days 1, 7, 14, 21, and 28) or were left untreated (control group). Two additional groups of normal rats (*i.e.*, not pre-exposed to DMBA) were also treated in a similar manner for comparative purposes. Treatment of DMBA-induced rats with quercetin significantly reduced the serum level of carcinoembyronic antigen (CEA), a reported potent marker of tumor growth and invasion. The serum activities of acid phosphatase (increased levels are reported to be characteristic of invasive or malignant breast carcinoma) and cathepsin D (overexpression of cathepsin D in breast cancer cells is associated with increased risk of metastasis) were also significantly reduced with quercetin treatment. Compared to the DMBA control group, quercetin treatment of DMBA-induced rats significantly reduced levels of serum, but not tissue activities of lysosomal enzymes (activity decreases in growing tumors, but increases during tumor regression), β-D-galactosidase, β-D-glucosidase, and sialidase, with the exception of β-D-glucuronidase which was decreased in serum, but increased in tissue. Based on these results, the authors suggested a potential role of quercetin in the treatment of primary and invasive mammary tumors.

A lack of quercetin-related initiating activity in the liver, as evidenced by the absence of preneoplatic and neoplastic hepatic lesions, was demonstrated in inbred ACI rats administered 50 or 500 mg quercetin/kg body weight/day intraperitoneally for 6 consecutive days and subsequently maintained on basal or phenobarbital-enriched diets for a period of 467 days (Kato *et al.*, 1985).

Sato *et al.* (1987) did not observe any tumor-initiating activity in the 2-stage mouse skin carcinogenesis model following topical application of 100 mg quercetin/mouse twice

weekly for a period of 5 weeks, with or without subsequent 47-week treatment with the promoter 12-*O*-tetradecanoyl phorbol-13-acetate (TPA).

Administered as a single 20 mg intramuscular dose, quercetin did not induce any incidences of local subcutaneous sarcomas in a group of 10 C57Bl/6 female mice examined over the course of a 420-day observation period (Ishikawa *et al.*, 1985). In contrast, quercetin administered intramuscularly (0.5 to 20 mg) in combination with 0.1 or 1.0 mg 3-methylcholanthrene (MCA) or provided in the diet for the duration of the mice's lifespan, at a concentration of 0.1 or 1.0% [approximately 50 and 500 mg quercetin/kg body weight, respectively (U.S. FDA, 1993)], following initiation with 0.1 mg MCA was reported to enhance carcinogenesis (affected organs not specified), as evidenced by significantly reduced latency periods, survival times, as well as increased occurrence of metastasis.

Morino *et al.* (1982) evaluated the potential inititation properties of quercetin in a series of major organs. In total, 5 different treatment protocols were examined. Except for group 1, which was maintained on a diet supplemented with 4% quercetin (approximately 4.5 g/kg body weight/day) for 709 days, the treatment periods of all other groups (groups 2 to 4), including that of the basal control group (group 5), were 701 days in duration. In groups 2 and 3, hamsters received 1% quercetin (approximately 1.3 g/kg body weight/day) for the first 351 days, and basal diet or 1% croton oil, a potent promoter, respectively, thereafter. Group 4 was provided the basal diet for 351 days, followed by 1% croton oil in the diet for the remaining 350 days. No significant differences were reported in the survival of animals in any of the groups and no statistical variability was observed in the body weights of males. Body weights of groups 1, 2, and 3 females were slightly increased compared to groups 4 and 5 for the first 351 days of the study period. Subsequently, females in groups 1 through 4 exhibited lower body weights relative to group 5, the basal diet controls. With the exception of 1 uterine leiomyoma in group 1 and 1 adrenal cortical adenoma in a male of the control group, all other tumors observed in groups 1, 2, and 5 were forestomach papillomas occurring as single sessile polyps. Tumors were induced with similar incidence in all 3 groups. In contrast, some multiple sessile polyps of the forestomach were identified in groups 3 and 4. Furthermore, the incidence of papillomas in group 3 was significantly elevated in comparison to group 5 ($p < 0.05$); however, no difference was observed between group 3 and 4. Consequently, the authors concluded that the increase in papillomas was not quercetin-related. Expansive, but not invasive growth was noted to be characteristic of some of the forestomach papillomas. The results of this study did not provide clear evidence for quercetin-related 2-stage carcinogenesis.

See "6.4.7 Discussion for Chronic Toxicity and Carcinogenicity" for supporting discussion and rationale on the totality-of-scientific-evidence indicating that quercetin is not a carcinogen and is not a promoter of tumor development.

Table 6.4.5-1 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 or 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.	

Table 6.4.5-1 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		
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.	
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	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	Vehicle (s.c.; weekly injection; 2x)	Basal diet (up to 41 weeks)	Absence of colorectal tumors.	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)		
		AOM[4] (15 mg/kg bw; s.c.; weekly	Basal diet (up to 41 weeks)	All AOM-treated rats developed ACF mainly in	

Table 6.4.5-1 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		
		injection; 2x)	Qu (4.5 to 9, 45 to 90, or 450 to 900 mg/kg bw/d; diet; up to 41 weeks)	the distal colon (at 8 wks post-AOM treatment) Qu-induced ↓ (dose-dependent) colorectal tumor incidence.	
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]		
Rat	AOM	Qu (100 mg/kg bw; diet; 5x/week; 7 wks)		No difference in colonic epithelial cell proliferation.	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)	No difference in multiplicity and incidence of ACFs in Qu+AOM compared to AOM-only rats.	
Rat	AOM	Saline (1/wk for 2 wks)[8]	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 5 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 5 wks)		
			Qu (3,000 mg/kg bw/d; diet; initiated at 7 wks of age for 5 wks)		
Rat	AOM	AOM (1/wk for 2/wks)[9]	Basal diet (up to 35 wks)	No effect on ACF formation or tumour	Choi *et al.*, 2006

Table 6.4.5-1 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		
			Qu (250 mg/kg bw/d; basal diet, initiated at 5 wks of age for up to 35 wks)	incidence at 8 and 33 wks after last AOM administration, respectively.	
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	
Liver and Pancreas					
Rat	PB	Qu (50 or 500 mg/kg bw/d; i.p.; 6 days)	Basal diet (467 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.	Barotto *et al.*, 1998
			Qu (500 mg/kg bw/d; diet; 30 days of age; 20 wks)		
		NMU (single i.p.;	Basal diet	↓ bw Qu-NMU (M+F)	

Table 6.4.5-1 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		
		3 days of age)	Qu (500 mg/kg bw/d; diet; initiated at 30 days of age for 20 wks)	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% of QU-NMU rats; 1 microcarcinoma in a Qu-NMU rat.	
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

Table 6.4.5-1 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		
Mammary glands					
Rat	DMBA; NMU	Qu (1,000 mg/kg bw/d; diet; 20 wks)		No palatable tumors identified.	Verma *et al.*, 1988
		DMBA[5] (gavage) or NMU[5] (i.v.) (single)	Qu [1,000 (w/ DMBA only) or 2,500 mg/kg bw/d; diet; 20 wks]	Qu + DMBA ↓ (dose-dependent) incidence and multiplicity of tumors. Qu + NMU ↓ induction of tumors; less effective than w/ DMBA.	
Rat	DMBA	DMBA[5] (12 mg; gavage)	Qu (500 or 1,000 mg/kg bw/d; diet; 107 days)	Qu ↓ incidence and multiplicity of mammary tumors (not s.s.) & ↑ tumor latency.	Pereira *et al.*, 1996
Rat	DMBA	DMBA	Qu (0 or 25 mg/kg bw/week; i.p.; 5 weeks)	Qu ↓ tumor volume. Qu ↓ serum activities of acid phosphatase and cathepsin D and serum level of lysosomal enzymes and carcinoembryonic antigen	Devipriya *et al.*, 2006
Lung					
Mouse	B[a]P	B[a]P[12] (100 mg/kg bw; i.p., single)	Qu (0, 500, 1,000, or 2,000 mg/kg bw/d; gavage; 90 days)	Qu + B[a]P ↓(dose-dependent) comet cell tail and DNA damage in peripheral blood lymphocytes. Qu ↓ severity of squamous epithelial metaplasia and atypical hyperplasia observed in the lungs.	Jin *et al.*, 2006
Skin carcinogenesis model					
Mouse	TPA	Qu (100 mg/animal; topical application; 2/week; 5 weeks)		Absence of tumor initiating activity.	Sato *et al.*, 1987
		Qu (100 mg/animal; topically; 2/week; 5 weeks)	TPA (topically; 47 weeks)		
Mouse	MCA	Qu (20 mg; single i.m.) (420-day observation period)		Absence of local subcutaneous tumors without MCA initiation.	Ishikawa *et al.*, 1985
		Qu (0.5 to 20 mg) + MCA (0.1 or 1.0 mg; single i.m.)		Enhanced MCA-induced carcinogenesis with quercetin.	
		MCA (0.1 mg; single i.m.)	MCA (0.1 mg; single i.m.)		

ACF= Aberrant crypt foci; AOM = azoxymethane; BBN = N-nitroso-n-butyl-(4-hydroxybutyl)amine; B[a]P = benzo[a[pyrene; BHBN = *N*-butyl-*N*-(-4-hydroxybutyl)nitrosamine; DEN = diethylnitrosamine; DYF = Dysplastic foci; DMBA = 7,12-dimethylbenz[a]anthracene;

ENNG = N-ethyl-N'-nitro-N-nitrosoguanidine F = female; FAD = focal areas of hyperplasia; GST-P$^+$ = glutathione *S*-transferase placental form-positive; i.m. = intramuscular; i.p. = intraperitoneal; i.v. = intravenous; M = male; MAM = methylazoxymethanol acetate; MCA = 3-methylcholanthrene; NMU = *N*-nitrosomethylurea; PB = phenobarbital; PN = papillary or nodular; Qu = quercetin; s.c. = subcutaneous; s.s. = statistically significant; TPA = 12-*O*-tetradecanoyl phorbol-13-acetate.

[1] Quercetin treatment was initiated 2 weeks before estradiol administration and continued thereafter for 5½ months. Estradiol administration repeated at 3 months following 1st injection.

[2] Quercetin treatment started 2 weeks prior to carcinogen treatment

[3] Quercetin treatment started 1 week before 1st injection

[4] Quercetin treatment started after 1st injection.

[5] Quercetin treatment started 1 week prior to carcinogen treatment.

[6] AOM injections administered for the 1st 3 weeks of the total 5-week treatment period.

[7] Quercetin treatment initiated 1 week prior to 3-week AOM treatment.

[8] Quercetin started 1 weeks prior to 1st AOM/saline injection.

[9] Quercetin started 2 weeks prior to 1st AOM injection.

[10] "DMBDD treatment": i.p. injections of diethylnitrosamine (100 mg/kg body weight x 1) and *N*-methylnitrosourea (20 mg/kg body weight x 4), s.c. injections of 1,2-dimethylhydrazine (40 mg/kg body weight x 4), and 0.05% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (2 weeks) and 0.1% 2,2'-dihydroxy-di-*n*-propylnitrosamine (2 weeks) in water.

[11] Quercetin treatment started 2 following DEN initiation.

[12] Quercetin treatment started 1 week prior to carcinogen treatment.

6.4.6 Developmental and Reproductive Toxicity 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.

Potential sperm abnormalities related to quercetin exposure were investigated *in vitro* in several different mammalian cells (Nass-Arden and Breitbart, 1990; Swisher *et al.*, 1994 [abstract]; Trejo *et al.*, 1995; Khanduja *et al.*, 2001). Although the percent of motile rabbit sperm was not affected in the presence of quercetin, decreases were observed in the percent of progressively motile sperm, path velocity, and percent linearity (Swisher *et al.*, 1994 [abstract]). The authors suggested that this may have been a result of quercetin-induced alterations at the mitochondrial level. Inhibition of quercetin-induced aerobic glycolysis in rat germ cells was suggested to have contributed to the apparent decrease in the cells' viability in the presence of the flavonoid (Trejo *et al.*, 1995). In sperm obtained from ram, quercetin induced an inhibitory effect on the sperm motility, but only in the first 2 hours of exposure (Nass-Arden and Breitbart, 1990). Thereafter (3 to 4 hours), an increase in sperm motility was observed compared to untreated control cells. In comparison to the control cells, which exhibited a high degree of lipid peroxidation after 3.5 hours of incubation, quercetin markedly arrested lipid peroxidation in the cells. As such, quercetin may exert a protective effect on the fertilizing potential of spermatozoa. However, in human cells, quercetin did not affect lipid peroxidation, and, furthermore, induced an irreversible and dose-dependent reduction in sperm motility at concentrations as low as 5 μM and at higher concentrations (50 to 100 μM) in viability (Khanduja *et al.*, 2001). A decline in the activity of the Ca^{2+}-ATPase was deemed responsible for reduced sperm function.

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.

Examined *in vivo* in male (C57 x BALB/B) F1 mice, significant sperm abnormalities were reported following 3- and 5-week quercetin treatment periods (Rastogi and Levin, 1987). In particular, groups of 10 mice were administered quercetin intraperitoneally at dose levels of 3.2, 6.4, 16, or 32 mg/kg body weight/day for a period of 5 days. At each dose level, 5 mice were killed at 3 and 5 weeks following the last intraperitoneal injection. The cauda epididymides were removed and sperm smears prepared. The authors noted an appearance of toxic effects at the highest dose level evaluated. At 3 weeks, the percentage of abnormal cells was significantly increased at every non-toxic dose level (*i.e.*, up to 16 mg/kg body weight/day) in comparison to the spontaneous occurrence of abnormal sperm cells in untreated controls. Likewise, evaluated 2 weeks later, with the exception of the lowest dose level, evaluation of the sperm smears revealed a significant increase in the percentage of abnormal cells. Furthermore, the enhanced incidence of abnormal spermatozoa was accompanied by a significant reduction in the sperm count and an increase in the testicular weight. As noted by the authors, however, provided intraperitoneally, quercetin was not metabolized in the intestinal microflora prior to its contact with the germ cells, which may potentially account for the significant elevation in percent of abnormal cells.

In a study in which quercetin was provided orally at dose levels of 0 (control group), 50, 100, or 150 mg/kg body weight/day to male Swiss albino mice, the flavonoid did not induce any significant increases in the frequency of abnormal sperm (Nandan and Rao, 1983). At each dose level several mice were necropsied at weekly intervals over a period of 5 weeks, corresponding to different stages of spermatogenesis. The authors

noted that the apparent lack of quercetin-induced sperm abnormalities following oral exposure may be related to its inability to penetrate the blood-testicular barrier.

Dietary administration of quercetin to ovariectomized female Sprague-Dawley rats (11 or 12 animals per group) at daily levels of 3.55 or 18.42 mg quercetin [approximately 8.9 and 46.1 mg/kg body weight/day, respectively (U.S. FDA, 1993)] for a period of 3 months did not produce any estrogenic effects (Rachoń *et al.,* 2008). Animals fed a soy-free diet without quercetin served as negative controls, whereas 2 additional groups fed diets providing 0.07 or 0.20 mg estradiol-3 benzoate/animal/day were included as positive controls. 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 (IGF-1), the progesterone receptor, or complement protein 3 mRNA in comparison to controls provided the soy-free diet. Histopathological examination of the uterus revealed no signs of estrogenic stimulation or any other changes as compared to the control group.

Groups of pregnant Sprague-Dawley CD rats received quercetin administrations *via* oral gavage at dose levels of 0, 2, 20, 200, or 2,000 mg/kg body weight on a single occasion during embryonic development [Gestation Day (GD) 9] or daily during major organogenesis (GD 6 to 15) (Willhite, 1982). Concurrently maintained vehicle control groups were provided corn oil. Dams were killed on GD 20, and fetuses removed and weighed. The number of live fetuses and resorption sites were recorded. Fetuses were examined for gross developmental malformation and rib anomalies. Throughout the treatment period, females did not exhibit any clinical signs of toxicity. The authors noted that a substantial portion of the administered test material passed unaltered through the gastrointestinal tract and was subsequently excreted in the feces. With respect to the reproductive parameters evaluated, no variations were reported in the mean number of fetuses and resorption rate of test dams in comparison to the vehicle control group. Statistically significant variations were limited to a reduction in the mean weight of the 20-day old fetuses observed in groups receiving single administration at the 200 and 2,000 mg quercetin/kg body weight dose levels, as well as in groups receiving repeated treatments at the 2 and 2,000 mg/kg body weight dose levels. Macroscopic examination of control fetuses was generally unremarkable with the exception of a single animal exhibiting several abnormalities deemed as spontaneous congenital malformations. In test animals, incidences of wavy ribs were observed in 2 fetuses in the single administration 2 mg/kg body weight group and in one 20 mg/kg body weight fetus. In groups receiving multiple administrations, fusion of ribs was reported in two fetuses at the highest dose level (2,000 mg/kg body weight). Unilateral testicular agenesis (2) and renal hypoplasia (1) in fetal rats obtained from the 2 and 200 mg quercetin/kg body weight/day litters, respectively, were noted following dissection of the fetus. The former

was also reported in one fetus from the corn oil control group. Overall, no dose-dependent teratogenic anomalies related to quercetin administration were observed.

Aravindakshan *et al.* (1985) evaluated the potential germinal effects of quercetin in inbred Swiss mice and Wistar rats. Groups of male mice and rats received two intraperitoneal injections of quercetin at 24-hour intervals at dose levels of 0, 200, 300 or 400 mg/kg body weight and 0, 200, or 300 mg/kg body weight, respectively. Following the second i.p. injection, males were paired with two untreated females and placed on a one-week mating schedule for a total of six consecutive pairings. At 14 to 15 days following the half-point of each 7-day mating period, females were necropsied. The percentage of pregnant females was determined and the uterine contents were examined for live and dead implantations. Additionally in rats, the number of corpora lutea as a determinant of pre-implantation losses was counted. Aside from statistically insignificant marginal differences, pregnancy rate, and the number of live, total and dead implantations in the low-dose female mice (200 mg quercetin/kg body weight/day) were comparable to controls. At the mid-dose level (300 mg quercetin/kg body weight/day), a statistically significant decrease was observed in the pregnancy rate. Slight reductions were also noted in the mean number of total and live implantations at every mating, except for the first week; however, statistical significance was attained only in the third week of mating. Likewise, at the highest dose level evaluated in mice (400 mg/kg body weight), pregnancy rates were reduced at every week, although not significantly except for Weeks 1 and 4 through 6. The lack of statistical significance, however, was attributed to the relatively small sample sizes at the highest dose level. Declines in the mean total and live number of implantations were also identified at every week in the high-dose group; however, due to the smaller sample size, statistical significance was reached only with respect to total implantations and only at Week 5. Post-implantation losses recorded in the mid-dose mice were comparable to controls. Similarly, mice in the high-dose group did not demonstrate a tendency for increased numbers of dead implantations. In rats, pregnancy rates of test dams were comparable to those of control animals, with the exception of Week 1 and Weeks 1 and 2 at the low- and high-dose levels, respectively, during which, slight but statistically significant reductions were observed. Significant reductions in total and live implantations were limited to Weeks 1 and 5 in rats of the low- and high-dose groups, respectively. At neither dose level were variations observed with regard to pre- and post-implantation losses. Overall, quercetin treatment was associated with an impairment of mouse, but not rat, fertility, possibly attributable to non-genetic factors such as cytotoxicity, oligospermia, or physiological impairment of the sperm's fertilization ability. The authors also noted that non-genetic factors should not be discounted, pending further investigation, in explaining the reductions observed in the mean number of implantations also predominantly limited to treated mice.

As part of a 64-week dietary toxicity study (see Section 6.4.2), 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 PND 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.

6.4.6.1 Summary for Developmental and Reproductive Toxicity Studies

The data summarized above demonstrate that orally administered quercetin is not a reproductive or development toxicant based on the following totality-of-evidence:

- Relative Binding Affinity for quercetin relative to the natural hormone E2 indicated very weak affinity for the receptor
- Either *in vitro* quercetin exposure or intraperitoneal injection in mice was required for manifestation of potential sperm abnormalities
- 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
- Dietary quercetin administration to ovariectomized female Sprague-Dawley rats 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
- 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
- Administration of two intraperitoneal injections of quercetin at 24-hour intervals to rats and mice (up to 300 or 400 mg/kg body weight, respectively) was associated with an impairment of mouse, but not rat, fertility
- 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
- 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
- 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

6.4.7 Discussion for Chronic Toxicity and Carcinogenicity

Enhanced proteinuria was observed in a 21-day toxicity study in high-dose (1,133 mg/kg body weight/day) quercetin-treated male Wistar rats, 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 acid excretion, no significant effects were reported in a group of male Wistar rats administered quercetin in the diet at dose levels of up to 1,000 mg/kg body weight/day for 22 consecutive days (Nakamura *et al.*, 2000). No biologically significant adverse 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 (Barrenetxe *et al.*, 2006) or 3,000 mg/kg body weight (Ruiz *et al.*, 2006). Exon *et al.* (1998) noted increased natural killer cell activity in Sprague-Dawley rats provided 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, as well as spleen weight were unaffected by compound administration. Significantly decreased liver α-tocopherol content and increased hepatic SOD activity were observed in female BALB/c mice fed a diet containing 0.1% quercetin (150 mg/kg body weight/day) over the course of a 40-day period, whereas no significant changes in hepatic GPx activity or urinary 8-OHdG content were observed relative to the control group (Bando *et al.,* 2007). In comparison to the control group, significant increases in the levels of hepatic and pulmonary GST and GSH levels were noted in an 8-week mouse study in which the test animals 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 *via* gavage) normotensive rats compared to untreated controls, but no changes in GPx and GR levels. While a slight reduction in liver GSH levels in male Wistar rats was also reported following daily gavage administrations of 10 mg quercetin/kg body weight in a 5-week study, no variations were noted in liver GST, GPx, and GR activities and levels of TBARS and NO_x (García-Saura *et al.*, 2005). Choi *et al.* (2003, 2005) reported increased liver GPx activity and reductions in hepatic GSH and MDA levels and increased GR activity in Sprague-Dawley rats treated daily with up to 80 mg quercetin/kg body weight 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 2% or 500 mg/kg body weight/day), no changes in activities of the enzymes in the liver were reported (Fischer *et al.*, 2002 [abstract]). Levels of oxidative scavenger enzymes (SOD and catalase) were not increased in Wistar rats following 3 days of daily quercetin gavage administrations at dose levels of 135 mg/kg body weight (Cierniak *et al.*, 2004). In comparison to a vehicle-treated control group, no signs of nephrotoxicity were observed in a 9-week study wherein male Wistar rats were intraperitoneally administered 50 mg quercetin/kg body weight/day 5 times a week (Morales *et al.,* 2006). In a study conducted by Stoewsand *et al.* (1984),

statistically significant reductions in levels of GOT and BUN were observed in male rats receiving approximately 76 mg quercetin/kg body weight/day in the diet for a period of 64 weeks. These variations in clinical chemistry values were not accompanied by any histopathological changes. No adverse effects were reported in a 410-day feeding study in which groups of male and female rats were provided up to 1.0% (approximately 500 mg quercetin/kg body weight/day) in the diet (Ambrose *et al.*, 1951).

In the 2-year rat feeding study conducted by the NTP, no statistically significant adverse effects were reported at dietary quercetin levels of up to 10,000 ppm (calculated using mean body weights and 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, as well as to a smaller degree in 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 or 2,203 and 2,231 mg/kg body weight/day in 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-nutritive 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 females in the high-dose group 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. The reduction in food consumption at the highest dose level is not unexpected in light of the characteristic bitterness of quercetin. 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.

With the exception of male-specific nephropathy, the variations in body weights occurred in the absence of any physiological or morphological abnormalities. Most notably, the reduction in body weights had no effect on 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 test 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 and may be beneficial to the health of animals.

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. In comparison to a single occurrence of adenoma in the control group, a total of 9 out of 50 high-dose male rats exhibited renal tubule 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, 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, treatment-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 (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 neoplastic lesions. Moreover, the kidney effects were not confirmed 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 were also 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. The re-evaluation 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.

In male hamsters, no kidney lesions were observed following administration of quercetin at concentrations of up to 3% (approximately 3,600 mg/kg body weight/day) in the diet for approximately 6 months (Zhu and Liehr, 1994). Likewise, the absence of renal tissue neoplasms was confirmed by Saito *et al.* (1980) in a life-time exposure mouse study in which test animals received 2% quercetin in the diet, providing dose levels of approximately 3,000 mg/kg body weight/day. Overall tumor incidence in the test group was reported to be comparable to that observed in controls. Furthermore, in another 2-year feeding study, in which F344/DuCrj rats were maintained on diets containing 1.25 or 5% quercetin (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). 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 a concomitant decrease in male and female body weights at this dose level. Likewise, although the incidence of chronic nephropathy was elevated in both male test groups in comparison to the control group, the increase was reported not to exceed control values reported for the laboratory and were not considered biologically significant.

Pamucku *et al.* (1980) demonstrated an increased incidence of ileal and urinary bladder tumors in Norwegian rats maintained on 0.1% (52 and 47 mg/kg body weight/day in males and females, respectively) quercetin in a basic grain diet. However, an extensive 2-year rat feeding study conducted by the NTP (Dunnick and Hailey, 1992; NTP, 1992), and several other carcinogenicity studies in which quercetin was provided in the diets of laboratory animals, including different strains of rats, at dose levels of up to 10%, did not confirm the urinary bladder or intestine as possible target organs for quercetin-related toxicity (Saito *et al.*, 1980; Hirono *et al.*, 1981; Hosaka and Hirono, 1981; Takanashi *et al.*, 1983; Ito *et al.*, 1989). No urinary bladder tumors were observed in two separate 2-stage carcinogenesis studies in which male F344 rats receiving up to 5% quercetin (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 possible cross-contamination with BF, which was provided to 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). BF is now known to contain the carcinogen ptaquiloside, which induced 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 with BF should be considered as a possible explanation for the appearance of these tumors limited to this single study. Additionally, Boorman and Hollander (1974) reported a high incidence of spontaneously occurring urinary bladder and ureter tumors in the brown Norway male rat, which is further suggestive of a strain-specific susceptibility rather than a quercetin-related effect.

Furthermore, in none of the carcinogenesis studies were tumors of any other organs occurring with statistically greater incidence compared to concurrently maintained control groups identified. Lesions of the cecum, colon, adrenal cortex, and testis observed by Hirono *et al.* (1981) in ACI rats at quercetin concentrations of 5% and higher were also 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, forestomach and mammary gland tumors in the test animals (3,000 mg quercetin/kg body weight/day) did not differ significantly from controls (Saito *et al.*, 1980). Examined in F344 rats in a 64-week study in which quercetin was provided in the diet 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), selected specifically to parallel the dose levels administered in the study by Pamucku *et al.* (1980), the neoplastic lesions reported were typical of aging rats and were not attributed to quercetin exposure *per se* (Stoewsand *et al.*, 1984).

Several authors suggested that the difference noted in the carcinogenic response between the study conducted by Pamukcu *et al.* (1980) and other studies (Saito *et al.*, 1980; Hirono *et al.*, 1981; Hosaka and Hirono, 1981; Morino *et al.*, 1982; Takanashi *et al.*, 1983) may be attributable, at least in part, to the compositional 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).

In female F344 rats administered up to 2% quercetin in the diet (approximately 1,000 mg/kg body weight/day), Ertürk *et al.* (1983 [abstract]) reported an increased incidence of liver and bile duct tumors. The results, however, were presented in abstract form only and the statistical significance of the results was not specified. Likewise, pathological changes of the colon and ileum 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 [abstract]). Due to the unavailability of full reports by these authors, the usefulness of these studies in a safety assessment of quercetin is limited.

Notably, with the exception of one 2-stage carcinogenicity study in which a single incidence of adenocarcinoma of the small intestine was observed in a group of 12 rats receiving a relatively high dose of quercetin (*i.e.*, 1,680 mg/kg body weight/day) for a period of 46 weeks (Pereira *et al.*, 1996), none of the other numerous mouse, rat, and hamster studies performed in which a 'quercetin-only' control group was included, ranging between 4 weeks and 2 years in duration, demonstrated increased incidences of ACF formation or tumors of the gastrointestinal tract at oral dose levels of up to 4,500 mg quercetin/kg body weight/day (Morino *et al.*, 1982; Kato *et al.*, 1984; Werner *et al.*, 1985; Deschner *et al.*, 1991; Akagi *et al.*, 1995; Exon *et al.*, 1998; Tanaka *et al.*, 1999; Matsukawa *et al.*, 2002; Volate *et al.*, 2005). Yang *et al.* (2000) noted only a slight and not statistically significant increase in the incidence of FAD in mice provided quercetin in the diet (3,000 mg/kg body weight/day) for a period of 8 weeks compared to basal diet controls. Likewise, incidences of tumor formation in other examined organs (*i.e.*, urinary bladder, kidneys, liver, pancreas, mammary glands, skin, and other unspecified organs) in mice, rats, and hamsters of the quercetin-only treated control groups following administration of the aglycone *via* oral and non-oral routes (up to 4,500 mg quercetin/kg body weight/day) were comparable to those in the basal diets or vehicle control groups (Morino *et al.*, 1982; Fukushima *et al.*, 1983; Hirose *et al.*, 1983; Ishikawa *et al.*, 1985; Kato *et al.*, 1985; Sato *et al.*, 1987; Verma *et al.*, 1988; Deschner *et al.*, 1991; Zhu and Liehr, 1994; Barotto *et al.*, 1998).

The promotional activity of quercetin on chemically-induced organ-specific carcinogenesis was less consistent. More specifically, while quercetin was shown to enhance the formation of AOM-induced colon tumors in the study conducted by Pereira

et al. (1996), as well as following 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 MAM and DMBDD (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 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. 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 NMU at 3 days of age, 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 DMBA-initiated mammary gland tumors (Verma *et al.*, 1988; Pereira *et al.*, 1996; Devipriya *et al.*, 2006), 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 initiate (Kato *et al.*, 1985; Sato *et al.*, 1987) chemically-induced carcinogenesis in laboratory animals. Collectively, the totality-of-scientific-evidence indicates that quercetin is not a carcinogen and is not a promoter of tumor development.

6.5 Human Studies

6.5.1 Clinical Studies

Quercetin was administered to 10 healthy volunteers (7M, 3F; mean age = 33.5 ± 7.5) as a single oral 8.5 mg/kg body weight dose in order to investigate the potential induction of adenosine release (Blardi *et al.*, 1999). Blood samples were collected immediately before quercetin administration and at 15, 30, 60, and 120 minutes after ingestion of quercetin. Adenosine levels were reported to increase steadily from a mean baseline value of 210.7 nM to a peak value of 328.5 nM reached at 30 minutes post-treatment administration. No adverse effects were reported in any of the subjects.

A prospective, randomized, double-blind, placebo-controlled study was conducted to evaluate the potential effect of quercetin on category III chronic prostatitis syndromes (Shoskes *et al.*, 1999). A group of 30 men was initially enrolled in the study, diagnosed with category III-a or III-b chronic pelvic pain syndrome and randomized into a quercetin-test or placebo-control group (15/group). Subjects in the test group were provided 500 mg encapsulated quercetin twice daily for a period of 1 month (approximately 16.7 mg/kg body weight/day for a 60 kg individual). Additionally, a follow-up, unblind, open-label study was performed with 17 additional men, ingesting 2 capsules daily of Prosta-Q, a quercetin supplement (500 mg quercetin/capsule), also containing bromelain and papain, which are reported to enhance bioflavonoid absorption. In the first study, 2 placebo subjects withdrew early from the study due to increased level of discomfort associated with their existing condition. The mean symptom score for patients taking quercetin was reported to improve from a mean pretreatment value of 21.0 to 13.1 in the test group, representative of a mean improvement of 35%. An improvement of at least 25% was noted by 67% of all quercetin-exposed subjects. In comparison, the mean symptom score improved by only 1.4 points in the placebo group (20.2 to 18.8). Side-effects reported during quercetin-treatment included onset of a headache after each of the first few doses in one subject and a mild tingling sensation affecting the extremities of another patient following each dose. The latter resolved with treatment cessation at the end of the study period. In the open-label study, 82% of participants reported an improvement of at least 25%, with the mean symptom score determined to be 14.6 at the end of the trial compared to a mean score of 25.1 at the beginning of the study. Additionally, examinations of the prostatic fluid in 4 patients revealed a substantial decrease in levels of 8-iso-prostaglandin $F_{2\alpha}$ (Iso P) from a baseline level of 1,365 pg/mL to 179 pg/mL.

The safety and efficacy of a phytodrug derived from *Psidium guajava folia* was evaluated in a randomized, double-blind study in a group of adults with acute diarrheic disease (*i.e.*, infectious gastroenteritis) (Lozoya *et al.*, 2002). The quercetin test group consisted of 50 patients (23M, 27F; ages 20 to 55) receiving 500 mg capsules of a standardized *P.*

guajava leaf extract (QG-5®) containing 1 mg of flavonoids expressed as quercetin, 3 times daily (every 8 hours) for 3 consecutive days. The placebo group (n = 50) was provided capsules similar in taste and appearance. In the quercetin test group, a significant reduction in the incidence of abdominal pain was reported after the second day of treatment. Test capsules were generally well accepted and tolerated, and no secondary effects related to treatment administration were reported. Constipation occurred in both control and quercetin-exposed test subjects (10 and 8%, respectively), and relapse at the end of the 3-day study period was observed in 8% and 6% of the patients in the quercetin and placebo groups, respectively (Lozoya *et al.*, 2002).

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 1 to 6 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).

Eleven (11) elite male cyclists (age not specified) completed a randomized, double-blind, crossover study to test the effects of daily antioxidant supplementation containing essential vitamins plus 600 mg quercetin (300 mg, twice daily, FRS® beverage supplement), and antioxidant supplementation minus quercetin (FRS® minus quercetin). The authors concluded that after 6 weeks of quercetin supplementation, high-intensity cycling time trial performance was significantly ($p \leq 0.05$) improved through enhancement of power output. The authors did not report any adverse effects following 6 weeks of daily quercetin supplementation (MacRae and Mefferd, 2006).

In a between-subject study design, 40 trained male cyclists (mean age range 26-29 years) were randomly assigned to quercetin (n=20) or placebo (n=20) groups and, under double-blind methods, ingested 2 Tang beverages daily for a total quercetin intake of 1000 mg/day, or placebo, for 3 weeks before, 3 days during, and 2 weeks after the subjects cycled for 3 hours/day at approximately 57% of the maximal watts attained during graded maximal protocol (W_{max}). Blood and saliva samples were collected before and after each of the 3 exercise sessions, and muscle biopsies were obtained before and after the first and third exercise session. Quercetin treatment did not impact performance data (i.e., power, heart rate, oxygen consumption, cadence) when average over the 3, 3-hour trials. Quercetin exposure did not affect the following measures of immune or muscle function: natural killer (NK) cell activity, phytohemagglutinin (PHA)-stimulated lymphocyte proliferation, polynuclear oxidative-burst activity, salivary IgA, nuclear factor-kappaB (NF-κB) content, cytokine mRNA, cyclooxygenase-2 (COX-2) mRNA expression, plasma lipid or antioxidant capacity, oxidative damage, cycling

efficiency or fuel (*i.e.*, fat and carbohydrate) utilization. Quercetin exposure did significantly ($p \leq 0.05$) reduce the post-exercise incidence of upper respiratory tract infections (URTI), as well as blood leukocyte interleukin (IL)-8 and IL-10 mRNA. No adverse effects were reported in any of the subjects (Dumke *et al.*, 2009; McAnulty *et al.*, 2008; Nieman *et al.*, 2007a,c).

In a between-subject study design, 63 runners (both male and female elite runners; mean age range 44-46 years) were randomly assigned to quercetin or placebo groups and, under double-blind methods, ingested 4 soft chews daily for a total quercetin intake of 1000 mg/day, or placebo, for 3 weeks before, during, and 2 weeks after an ultramarathon (160 km). Thirty-nine (n=18 quercetin; n= 21 placebo) of the 63 runners completed the race and provided blood and saliva samples the morning before the race and 15-30 minutes postrace. Quercetin intake did not affect the performance measures of time to complete the race. With respect to immunological endpoints, quercetin did not impact post-race illness rates, leukocyte subset counts, granulocyte respiratory burst, salivary immunoglobulin (Ig) A, plasma cytokine levels, messenger RNA (mRNA) expression, or muscle soreness. Additionally, quercetin did not impact antioxidant capacity or oxidative damage. No adverse effects were reported in any of the subjects (Henson *et al.*, 2008; Nieman *et al.*, 2007b; Quindry *et al.*, 2008).

In a between-subject study design similar to the one above, 39 trained cyclists (males and females; mean age range 26-28 years) were randomly assigned to quercetin (n=13), quercetin plus epigallocatechin 3-gallate (EGCG), isoquercetin and polyunsaturated fatty acids (n=14), or placebo (n=12) groups and, under double-blind methods, ingested 4 soft chews daily for a total quercetin intake of 1000 mg/day, 1000 mg/day quercetin plus 120 mg/day of EGCG, or placebo, for 2 weeks before, 3 days during, and 1 week after the subjects cycled for 3 hours/day at approximately 57% of the maximal watts attained during graded maximal protocol (W_{max}). Blood, saliva, and muscle biopsy samples were collected before and after the 2 week supplementation, and immediately after the exercise trial on the third day. With respect to mitochondrial biogenesis, immunity and inflammation, quercetin did not have an impact. Under the conditions of the study, quercetin exposure did not impact exercise performance. No adverse effects were reported in any of the subjects (Nieman *et al.*, 2009).

Twelve (12) untrained volunteers (males and females; mean age 22 years) completed a randomized, double-blind, crossover study to test the performance effects of daily supplementation of 1000 mg quercetin (500 mg, twice daily, Tang beverage), or placebo. Quercetin or placebo was administered for a period of 7 days. All participants were involved in regular activity and considered to be fit, but were not highly trained. Baseline maximal oxygen uptake (VO_{2max}) and cycle ergometer-ride times to fatigue were established. On Day 7, a VO_{2max} test was conducted, and 24 hours later (Day 8),

participants performed a ride to fatigue. The authors concluded that 7 days of quercetin supplementation can increase endurance. Daily quercetin supplementation did not result in any reported adverse effects in untrained participants (Davis *et al.*, 2010).

In a between-subject study design, 30 untrained males (mean age range 22-23 years) completed a randomized, double-blind, pretest/post-test control group study to determine the ergogenic effects of daily supplementation of 1000 mg quercetin (250 mg, 4 times daily, sports hydration beverage), or placebo. Quercetin (n=15) or placebo (n=15) was administered for a period of 7-16 days. All participants were healthy and recreationally active, but not endurance-trained. The following parameters were assessed before and after quercetin supplementation: a noninvasive measure of muscle oxidative capacity; peak oxygen uptake ($V0_{2peak}$); metabolic and perceptual responses to submaximal exercise; work performed on a 10-minute maximal-effort cycling ergometer test following the submaximal cycling; and voluntary and electrically-evoked strength loss following cycling. During the 10-minute trial, which followed a 60-minute ergometer ride at 50% $V0_{2peak}$, work performance was assessed as an increase in pedaling speed. Following the 10-minute trial, $V0_2$, % $V0_{2peak}$, heart rate, and RPE were also recorded. Seven to 16 days of quercetin exposure did not impact pretreatment-to-posttreatment changes in measures of muscle oxidative capacity, $V0_{2peak}$, substrate utilization and perception of effort during submaximal exercise, total work done during the 10-minute maximal effort cycling trial, or voluntary and electrically-evoked strength loss. The authors concluded that short-duration, chronic dietary quercetin supplementation in untrained men does not improve metabolic or physical performance under the test conditions employed in this study. Daily quercetin supplementation for 7-16 days did not result in any reported adverse effects in the untrainted participants (Cureton *et al.*, 2009).

Twenty-six (26) young adult males (mean age 20 years) completed a randomized, double-blind, crossover study to test the performance effects of daily supplementation of 1000 mg quercetin (500 mg, twice daily, PowerAde), or placebo. Quercetin or placebo was administered for a period of 2 weeks prior to performance evaluations. Participants were healthy, but as a prerequisite, required to be physically sedentary during the previous 6 months (*e.g.*, not physically active during work, less than 2 exercise sessions a week during the last 6 months, etc). Participants provided blood and muscle biopsy samples during pre- and post-supplementation periods, and were given 12-minute time trials on 15% graded treadmills following 60-minute moderate exercise preloads at 60% VO_{2max}. During the time trials, the net change in distance achieved was significantly ($p \leq 0.05$) greater following quercetin exposure compared to placebo. Skeletal muscle mRNA expression for four genes related to mitochondrial biogenesis was increased but did not differ significantly between the quercetin and placebo groups. Daily quercetin

supplementation for 2 weeks did not result in any reported adverse effects in the untrained participants (Nieman *et al.*, 2010).

In a double-blind study undertaken to assess the potential of quercetin to positively affect risk factors for heart disease, the study authors did not note any adverse effects in 27 healthy male and female subjects (mean age 42.0 ± 2.6 years) consuming a rice flour placebo (n=14) or a quercetin-containing supplement (n=13) providing 1 g of quercetin/day for 28 consecutive days; however, it was not specified whether subjects were specifically monitored for the occurrence of any side effects (Conquer *et al.*, 1998). Treatment with quercetin did not significantly alter serum total, LDL or HDL cholesterol, or triglyceride levels. 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). Similarly, no adverse effects were reported in a 28-day double-blind, placebo-controlled, crossover study involving 47 hypertensive (26M, 21F) subjects administered 730 mg quercetin/day; however, as in the Conquer *et al.* (1998) trial, the authors did not state whether the subjects were monitored for the occurrence of any side effects (Jalili *et al.*, 2006 [abstract]). In comparison to a 28-day placebo treatment phase, treatment with quercetin significantly reduced the diastolic blood pressure, mean arterial pressure, and rate pressure product of the hypertensive subjects. Fasted plasma concentrations of total cholesterol, HDL, LDL, triglycerides, and glucose levels were comparable after quercetin and placebo treatment phases. In another 28-day, randomized, double-blind, placebo-controlled, crossover trial designed primarily to investigate the potential effects of quercetin on blood pressure, male (n=13) and female (n=6) subjects with prehypertension and stage 1 hypertension (n=22; 13M, 9F) ingested a placebo or 730 mg quercetin/day (Edwards *et al.*, 2007). The authors specifically noted that no adverse effects were reported during either phase of the trial. Ingestion of quercetin had no effect on the blood pressure of the prehypertensive subjects; however, for stage 1 hypertensive subjects there were significant decreases in systolic, diastolic, and mean arterial pressure (MAP). Quercetin consumption did not result in any significant changes in body weight, body mass index, or heart rate in any of the subjects.

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) were exposed to 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). Egert *et al.* (2009) designed a

double-blind, randomized, placebo-controlled, crossover study to investigate the effects of quercetin supplementation on cardiovascular risk factors and biomarkers as well as to assess the safety of daily quercetin exposure. The placebo or quercetin treatment periods consisted of 6 weeks each, separated by a 5-week washout period. Ninety-three obese or overweight subjects (42M/51F; 25-65 years of age), with metabolic syndrome traits, were instructed to take a total of 6 placebo or quercetin capsules per day (representing 150 mg quercetin/day). Mean fasting plasma quercetin concentrations statistically significantly increased during quercetin treatment. Changes from baseline and placebo among all of the subjects receiving quercetin supplementation included the following statistically significant ($p \leq 0.05$) 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).

Kiesewetter *et al.* (2000) conducted a 12-week, randomized, double-blind, placebo-controlled, parallel-group study to investigate the safety and efficacy of a red vine leaf extract AS 195 (*folia vitis viniferae*) containing quercetin-3-*O*-β-glucuronide and isoquercetin (quercetin-3-*O*-β-glycoside) as the primary active ingredients (concentrations not specified) in patients with stage I and II chronic venous insufficiency. Of the 260 male and female patients initially enrolled at the beginning of the study, 219 completed the protocol (87, 86, 84, in the placebo, 360 mg, and 720 mg roup, respectively). Participants were allocated to a placebo group or 1 of 2 treatment groups, receiving 2 gelatin capsule daily, containing 160 or 360 mg red vine leaf extract AS 195, (360 or 720 mg vine leaf extract AS 195/day), respectively. Female patients comprised more than 65% of the population of each of the 3 study groups and the mean age range among the 3 groups was 55.8-59.3 years of age. The 12-week treatment period was preceded and followed by 2-week run-in and follow-up periods. Study parameters were evaluated at baseline (visits 1 and 2), after 6 and 12 weeks of treatment (visits 3 and 4, respectively), and 2 weeks after study discontinuation (visit 5). Hematology, clinical chemistry, and urinalysis were performed on the first visit and subsequently at treatment termination (Week 12). Additionally, sitting blood pressure and heart rate readings were taken at each visit, and an electrocardiogram (ECG) was recorded at visits 1 and 4. Lower leg volume was the principal efficacy parameter monitored. Generally, treatments were well tolerated and the tolerability was described as good or satisfactory. Adverse events were rare (13/88 in the placebo group, 10/87 at 360 mg and 2/85 at 720 mg), of mild to moderate severity and limited to gastrointestinal disorders, and to a smaller extent, infections, headaches, and musculoskeletal system disorders. A total of 4 incidents were classified as severe, 3 of which occurred in the placebo group and only 1 in the group receiving 360 mg red vine leaf extract AS 195 (leg hematoma following

minor trauma). Single occurrences of mild constipation and hair thinning at the lower dose level were considered by the authors to be casually related to treatment with the test compound. None of the laboratory tests revealed any compound-related variations. With regard to the compound's therapeutic properties, administration of the red vine leaf extract AS 195 at either dose level was associated with a substantial reduction in the lower leg volume in comparison to pre-treatment values.

In an observational study, 5 patients (3M, 2F) between the ages of 21 to 54 years old with familial adenomatous polyposis (FAP) ingested daily capsules providing 60 mg of quercetin and 1,440 mg of curcumin/day for a mean duration of 6 months (durations ranged from 3 to 9 months) (Cruz-Correa *et al.,* 2006). FAP is a hereditary form of colorectal cancer that is characterized by the development of hundreds of colorectal adenomas in adolescence. Endoscopic examinations of the rectum were conducted before treatment initiation (baseline) and at 3-month intervals thereafter. Co-treatment with quercetin and curcumin appeared to be effective in reducing the number and size of adenomatus polyps following treatment in comparison to baseline. The authors reported no abnormal clinical chemistry test results (complete blood count, blood glucose, blood urea nitrogen, serum creatinine, electrolytes, and bilirubin) as determined at monthly examinations. Two mild gastrointestinal effects, transient nausea in 1 patient at the beginning of the trial (which subsided after the third day of the study without recurrence) and loose stools in another patient (for a period of 5 days during the study), were reported by subjects consuming the quercetin 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 phase I clinical trial was conducted in 51 (26M, 25F) cancer patients, 23 to 78 years of age (median: 55 years), non-responsive to conventional cancer-therapy (Ferry *et al.*, 1996). During the first part of the trial, quercetin was administered intravenously once every 3 weeks, in escalating doses beginning with 60 mg quercetin/m^2 (equivalent to 1.6 mg quercetin/kg body weight based on 60 kg individual). Doses were progressively increased, with 2,000 mg/m^2 (equivalent to 54.1 mg quercetin/kg body weight based on 60 kg individual) being the highest dose administered. Groups of patients were treated at each dose level in the absence of grade 3 or 4 toxicity. Incidence of dose-limiting toxicity, defined as grade 3 or 4 general toxicity or grade 2 renal, cardiac or neurotoxicity, marked the maximum tolerated dose. Following baseline determinations, patients were subsequently examined weekly (clinical exam, blood count, and serum chemistry). Additionally, blood and urine samples were collected for estimation of pharmacokinetic parameters. The number of patients and number of courses administered at each dose level are summarized in Table 6.5.1-1. As myelosuppression was not apparent following treatment administration at 3-week intervals, additional groups of patients were treated weekly with 945 or 1,400 mg quercetin/m^2. During

weekly treatments, renal toxicity was extensively monitored by determining 24-hour creatinine clearances before and after quercetin administration.

Table 6.5.1-1 Summary of Study Protocol and Renal Toxicity at Each Dose Level (Ferry *et al.*, 1996)

Dose (mg/m^2)	Dose (mg/kg bw)[a]	No. of patients	No. of courses	Grade of Nephrotoxicity				
				0	1	2	3	4
3-Week Intervals								
60	1.6	3	9	3	0	0	0	0
120	3.2	3	8	3	0	0	0	0
200	5.4	3	5	3	0	0	0	0
300	8.1	3	7	3	0	0	0	0
420	11.4	3	8	3	0	0	0	0
630	17.0	5	10	4	0	0	1	0
945	25.5	8	11	3	3	1	1	0
1,400	37.8	5	8	3	1	0	0	0
2,000	54.1	1	1	1	0	0	0	0
1,700	45.9	3	3	0	1	1	0	1
Weekly								
945	25.5	6	8	5	0	1	0	0
1,400	37.8	8	18	3	1	1	0	1

[a]Body weight to surface area conversion factor: assume 60 kg adult; surface area = 1.6 m^2; conversion factor = 37 (Derelanko, 2008).

Patients (n = 3) at the lowest dose level examined (60 mg/m^2) reported mild local pain at the site of injection, which lasted for the duration of the injection and for a few seconds thereafter, as well as a brief period of flushing of the entire body and sweating lasting for approximately 3 minutes. The latter was not accompanied by a reduction in blood pressure. At doses of 945 mg quercetin/m^2 (equivalent to 25.5 mg/kg body weight) and greater, the pain intensified and persisted for longer periods of time after injection. In comparison, in 2 subjects receiving the vehicle solution alone without test material, administered at the same rate of injection as that used to administer quercetin, no side-effects were observed. At 1,400 and 2,000 mg/m^2 (equivalent to 37.8 and 54.1 mg/kg body weight, respectively), patients experienced dyspnea for up to 5 minutes following treatment. The severity of the condition was found unacceptable at the higher dose level, and consequently patients in the subsequent group were treated with 1,700 mg quercetin/m^2 (equivalent to 45.9 mg/kg body weight). At 1,700 mg quercetin/m^2, severe emesis was reported within minutes of quercetin administration. Co-administration of an antiemetic was not effective at controlling the vomiting. Additionally, treatment of 3 patients at this dose level (1700 mg/m^2) was associated with grade 1, 2, and 3 renal toxicity, respectively. In the patient exhibiting grade 4 renal toxicity, serum creatinine levels rose to 2,145 μM, 11 days after treatment. None of the patients that experienced

renal toxicity had evidence of nephritis, infection, or obstructive uropathy on an ultrasound scan of the abdomen.

In total, 18 weekly courses of quercetin at the 1,400 mg/m^2 dose level were administered, with 4 patients receiving 3 or more courses of quercetin. One patient developed acute renal failure, requiring peritoneal dialysis, while 2 other subjects exhibited grade 1 and 2 renal toxicity, respectively. The subsequent group of 6 subjects was treated weekly at the 945 mg quercetin/m^2 dose level. Only 1 subject developed grade 2 renal toxicity. However, in the initial 3-week interval protocol, 5 incidences of renal toxicity were reported in the subjects at the 945 mg/m^2 dose level, 1 of which was classified as grade 3 toxicity. Overall, an approximately 20%, transient and reversible reduction in creatinine clearance was observed post-quercetin administration. In the group of 5 patients receiving 630 mg quercetin/m^2 once every 3 weeks, grade 3 renal toxicity was induced in 1 subject, with serum creatinine levels increasing to 420 µM by Day 10. In spite of the apparent renal toxicity, the patient agreed to continue therapy, and subsequently was retreated at 945 mg/m^2, preceded and followed by hydration with normal saline and 5% dextrose, respectively. No signs of renal toxicity were reported. Similarly, administration of 945 mg quercetin/m^2, following prehydration of patients in a parallel trial combining carboplatin and quercetin treatment, did not affect the 24-hour creatinine clearance. Incidences of renal toxicity at each dose level of quercetin are presented in Table 6.5.1-1.

In a patient (quercetin dose level not specified) with a history of hypertension, ensuing grade 1 renal toxicity, as evidenced by a substantial reduction in creatinine clearance, was associated with grade 3 hypertension. Within 2 weeks of quercetin administration, creatinine levels normalized and the blood pressure was controlled with medication. Two other patients, both treated with 945 mg quercetin/m^2, died of cardiovascular failure 24 hours and 16 days after receiving a second course of quercetin treatment, respectively. The patient dying within 24 hours of treatment was reported to have a prior history of angina. In the other patient, heart failure was associated with concurrent grade 3 nephrotoxicity. Aortic dissection, extending beyond the renal arteries, was noted at postmortem examination.

Although anti-tumor activity consistent with the World Health Organization's criteria for tumor response was not attained in any of the treated patients, positive results indicative of possible anti-proliferative activity were observed in 2 patients. In 1 subject diagnosed with cisplatin refractory ovarian cancer, a sustained reduction in serum CA 125 levels was observed following initial treatment with 2 courses of 420 mg quercetin/m^2. Further reduction was noted with 3 additional courses of quercetin treatment at 500 mg/m^2, administered together with carboplatin AUC 4. In the second patient with metastatic hepatocellular carcinoma, quercetin treatment at the 60 mg/m^2 dose level was

associated with a sustained decrease in levels of α-fetoprotein and alkaline phosphatase. Additionally, lymphocyte tyrosine kinase activity was monitored in a total of 11 patients before and for 16 hours after quercetin administration. Prior to treatment administration, phosphotyrosine bands were observed in all patients. In 2 of 11 patients, phosphotyrosine-banding patterns were unchanged up to 60 minutes after quercetin administration. At 2 hours post-treatment, inhibition of phosphotyrosine banding occurred in 5/5 patients. In 1 patient, inhibition of phosphotyrosine banding was apparent up to 16 hours after treatment. This was the first reported Phase I clinical trial with the novel class of antitumor agents, tyrphostins, in humans.

Evaluated at less than 60 minutes following treatment in 8 patients, the phosphotyrosine banding pattern remained unchanged. However, at 1 hour post-treatment administration, phosphotyrosine bands of M_W 45,000 and 60,000 were attenuated or eliminated in all 7 examined patients, 5 of which had not been evaluated previously and 2 of which had exhibited bands at less than 60 minutes. Bands continued to be absent when re-evaluated in 5 subjects at 2 hours post-treatment.

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 to conduct a double blind, placebo-controlled phase II trial investigating the potential effects of 1 or 2 grams of coadministered quercetin on glucose absorption in 16 obese non-diabetic and 16 obese Type 2 diabetic subjects. An estimated study completion date is not given (NLM, 2010).

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 daily a placebo (n = 335) or 500 (n=334) or 1,000 (n=333) mg of quercetin (Nieman, 2008; Shanely *et al.*, 2010). Subjects were stratified by gender, age, and body mass index (BMI) during study recruitment to ensure that these various subgroups were represented in the study. Both diseased and disease-free subjects were included in the study, and subjects were monitored for disease status and medication use. Pregnant and lactating women were excluded from the study; however, there were no other exclusion criteria. Quercetin was provided twice daily in soft chews. Blood samples were obtained from study participants prior to treatment initiation and at the end of the 12-week study period for assessment of plasma quercetin levels and a series of clinical chemistry (BUN, ALT, AST, ALK, bilirubin, calcium, sodium, potassium, albumin, creatinine, and glucose) and hematology (hemoglobin and hematocrit) parameters. The glomerular filtration rate (GFR) was also monitored.

Plasma quercetin levels were significantly increased in the quercetin-500 and quercetin-1000 groups compared to the placebo group, and varied widely among subjects; however, the interindividual variation was not influenced by age, gender, or BMI.

Comparison of the pre- and post-treatment changes between the placebo group and the test groups revealed small increases in sodium and potassium levels in the low-, but not high-dose quercetin group. 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). Red blood cell glutathione levels (reduced and oxidized state) were monitored monthly in a subgroup (placebo n = 25; 500 mg quercetin n = 28; 1,000 mg quercetin n = 27; sex not specified). Over the course of the study, there were no differences observed in the levels of reduced or oxidized glutathione among the 3 treatment groups.

Of the 21 subjects who did not complete the study (drop-outs were evenly distributed among groups), 12 did not comply with the study protocol and 9 reported adverse effects (3 in the placebo group and 6 in the quercetin groups). No consistent pattern of adverse events was reported. Monthly symptom logs revealed no differences among groups in gastrointestinal, skin, headache, mental, allergy, energy, or pain symptoms, or in overall well-being. Mean arterial blood pressure (MAP) decreased in the quercetin-treated groups compared to placebo. The authors stated that the decrease in MAP may be attributed to a decrease in sodium reabsorption, increased urine output, and increased nitric oxide availability through the elevation of nitric oxide synthase activity. 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.

Recently, 2 *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). The methodology and results of each study are highlighted below and summarized in more detail in Section 6.3.2 of this dossier.

To examine the effect of multiple doses of quercetin on plasma warfarin disposition after a single dose of warfarin in healthy individuals, a randomized, placebo-controlled, two-way crossover clinical study was conducted. Pharmacokinetics of (S)- and (R)-warfarin were monitored for 10 days following co-administration with quercetin. In total, 16 male subjects (20-50 years of age) participated in the study. During the first study phase, subjects were randomized to study assignment groups and given 20 minutes to consume a 20-ounce beverage containing quercetin (1500 mg), or the same formulation without quercetin (placebo), daily for Study Days 1-4. On Study Day 5, the subjects received quercetin or placebo and 10 mg of warfarin. Subjects continued taking quercetin or placebo daily for 9 days following warfarin administration. After a 5-day

washout period, the exposure conditions were reversed. To establish pharmacokinetic parameters for warfarin, and to evaluate clinical chemistry and hematology, blood samples were obtained prior to warfarin administration, and at the following intervals post-warfarin: 2, 6, 24, 48, 72, 96, 120, 144, 168, and 240 hours. Fifteen of the 16 subjects completed the study. Six subjects reported a total of 9 adverse events during the study. All adverse events were mild in severity and all had resolved by the follow-up period (30 days following the last dose). The safety parameters measured in this study did not appear to be influenced by administration of quercetin or warfarin. 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).

An open-label, within-subject clinical study was conducted in healthy male and female subjects (n=14; 20-55 years of age) to assess whether quercetin co-administration affects efflux transport of digoxin by P-glycoprotein. For the first dose, digoxin (0.5 mg) was administered orally following a single dose of placebo. After a 1-week washout, quercetin (1500 mg), formulated as a 20-ounce beverage, was administered to subjects for 5 days prior to administration of the second 0.5 mg dose of digoxin. The pharmacokinetics of digoxin were monitored for 7 additional days. Blood samples were collected prior to digoxin administration. Following digoxin administration, blood samples were obtained at the following intervals to assure an adequate sampling period: 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours. Five subjects reported a total of 9 adverse events; however, no adverse event was reported by more than 1 subject. All adverse events were mild or moderate in intensity and had resolved by the end of the study. There were no serious adverse events reported. One subject discontinued because of the adverse events of tachycardia, pyrexia, and cough, all of which were considered by the investigator as unlikely to be related to the study drug. 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 were no safety concerns, and 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).

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 (n=20; 33-69 years of age). 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. Plasma glucose concentrations were obtained pre-glucose load and at 0.5, 1, 2, 3 and 4 hours following quercetin consumption. For each OGTT, 200 ml of a glucose solution (75 g) was consumed within 10 minutes. Nine (45%) subjects reported a total of 16 adverse events during the study. Headache, reported by 5 subjects, was the most common adverse effect. Adverse events were mild in intensity with the exception of a headache in 1 subject that was moderate in intensity. 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 (Prism Research, 2010a).

6.5.2 Epidemiological Cancer Studies

The potential association between flavonoid intake and 5-year cancer incidence and mortality was evaluated in a group of 738 elderly Dutch men (65 to 84 years of age) without a history of cancer (Hertog *et al.*, 1994). The dietary history of the participants as recorded in 1985 and re-evaluated 5 years later revealed a total flavonoid intake of 25.9 mg/day at baseline and only slightly higher intake in surviving men re-examined in 1990 (26.4 mg/day). Quercetin was determined to be the predominant flavonoid, accounting for 63% of the total flavonoid intake (16.4 mg quercetin/day). Kaempferol comprised 32% of the total flavonoid intake. Flavonoids were reportedly derived primarily from the consumption of vegetables and fruits, and tea. At the end of the follow-up period, 75 men had developed cancer and 34 died of the disease. Generally, men presenting with cancer were older and smoked more heavily. Both risk factors were shown to be significantly related to cancer incidence. In contrast, flavonoid consumption was not associated with all-cause cancer incidence, or specifically with tumors of the alimentary and respiratory tract, or lung cancer. In fact, flavonoids specifically derived from vegetables and/or fruits were inversely associated with cancer development.

In another prospective cohort study, total flavonoid intake was estimated in a total of 120,852 male and female Dutch study participants, in whom the incidence of cancer was followed up 4.3 years later (Goldbohm *et al.*, 1995 [abstract]). Mean daily flavonoid intake was estimated at 28 mg of which half was derived from tea and a quarter from allium vegetables (*e.g.*, onions), both of which are rich sources of quercetin. Although initial evaluation demonstrated an inverse relationship between flavonoid intake and lung and stomach cancers, the association disappeared once dietary confounders were taken into consideration. The relative risks for development of stomach, large-bowel, lung, or breast cancer were close to unity.

Knekt *et al.* (1997) conducted an extensive study in order to investigate the potential association between flavonoid intake and the incidence of cancer. A total of 9,959 Finnish men and women without a prior history of cancer were included in this study and followed for a period of 24 years (1967 to 1991). Intakes of 5 flavonoids, including quercetin, from the habitual diet were estimated *via* the dietary history method. Total flavonoid intake ranged from 0 to 41.4 mg per day, with a mean daily intake estimated to be 4.0 mg. On average, quercetin was reported to contribute 95% to the total flavonoid intake, with apples and onions identified as the primary dietary flavonoid sources. At follow-up, a total of 995 new cancer cases were reported, of which 151 were lung cancer. The relative risk of overall, non-site specific cancer was determined to be 0.80 between subjects in the highest and lowest quartiles of flavonoid intake (0.75 and 0.85 for males and females, respectively). Following adjustments for sex and age, a strong inverse association was demonstrated between flavonoid intakes and lung cancer incidence as supported by a relative risk factor of 0.54. The association was reported to be stronger in younger individuals and in non-smokers, but was independent of antioxidant vitamin C or E, or β-carotene consumption. Moreover, an association was also noted between apple consumption and risk of lung cancer development. Overall, therefore, the authors identified an inverse association between the intake of flavonoids, represented in 95% by quercetin, and incidences of all-site cancers, as well as lung cancer. The authors did note, however, that the concomitant presence of other biologically active compounds in flavonoid-rich foods, which also may impact on cancer etiology, cannot be discounted.

Average mean flavonoid intake was reported not to be related to the development of colorectal, lung, and all-cause cancer mortality in a study of 16 cohorts from 7 countries (Hertog *et al.*, 1995). A positive relationship was identified between flavonoid intake and stomach cancer; however, the effect was no longer significant once ascorbic acid consumption and smoking were accounted for. Depending on the particular cohort, the mean intakes of total flavonoids ranged between 2.6 and 68.2 mg/day. Quercetin intake was highly correlated to total flavonoid intake, and the relationship between quercetin and disease mortality paralleled that observed for total flavonoid intake.

Lin *et al.* (2006) analyzed dietary intake data collected from 2 prospective cohort studies involving 71,976 female nurses and 35,425 male health professionals from the U.S. population to assess the possibility of an inverse association between flavonoid intake and colorectal cancer risk. Based on dietary data collected in 1990, 1994, and 1998, mean intakes of flavonoids were determined to be 21.7 and 22.4 mg/day for females and males, respectively, of which quercetin accounted for 15.9 and 16.8 mg/day, respectively. Tea, onions, apples, broccoli, and tomatoes were reported to be the main dietary sources of flavonoids. Between 1990 and 2000, 498 women and 380 men of the surveyed population had developed colorectal cancer. No association was identified

between total flavonoid intake and colorectal cancer incidence. Additional adjustments for risk factors related to the development of colorectal cancer (*e.g.*, smoking, alcohol consumption, level of physical activity, *etc.*) did not significantly change the results. Analysis of individual flavonoid intakes including intakes of quercetin, kaempferol, and myricetin, also did not reveal an association with respect to colorectal cancer risk.

Lam *et al.* (in press) investigated the association between quercetin intake and risk of lung cancer in an Italian population. In total, there were 1822 subjects at risk for lung cancer and 1991 controls. Individuals consuming the most fruits and vegetables had a 30% lower risk of lung cancer compared to the lowest quintile of intake. Those who consumed the most quercetin-rich foods relative to those who consumed the least quercetin-rich foods had a 53% lower risk of lung cancer. The inverse associations of quercetin-rich foods were similar in men and women and were strongest in those who smoked more than 20 cigarettes/day (Lam *et al.*, in press).

6.5.3 Summary

No adverse effects were reported in a group of 10 human male and female subjects 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 into 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 1000 mg (16.7 mg/kg body weight/day for a 60 kg individual) for a period of 1 month was not associated with any significant adverse effects (Shoskes *et al.*, 1999). Likewise, administration of 3 mg quercetin/day from 1500 mg of a guava leaf extract for 3 consecutive days did not result in any treatment-related adverse effects (Lozoya *et al.*, 2002). Laboratory test results (hematology, clinical chemistry, urinalysis) were unremarkable in a group of male and female patients 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. 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 1 to 6 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). Likewise, no adverse effects were reported in a 28-day crossover 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, it was not indicated whether subjects were monitored specifically for the occurrence of side effects (Jalili *et al.,* 2006 [abstract]). In another 28-day crossover 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). In an intervention study conducted to investigate the effects of quercetin at doses of up to 150 mg/day in healthy subjects, despite the reported dose-dependent increase in plasma quercetin levels, there were no associated benefits on biomarkers of oxidative stress, serum chemistry, inflammation, or metabolism. Additionally, no adverse effects were reported (Egert *et al.*, 2008). In a study to investigate the safety of daily quercetin supplementation as well as its impact on cardiovascular endpoints, quercetin (150 mg/day) was administered to obsese or overweight subjects for 6 weeks and revealed no adverse effects on blood parameters of liver or kidney function, hematology or serum electrolytes (Egert *et al.*, 2009).

In an observational study in which patients with FAP ingested daily capsules providing 60 mg of quercetin and 1,440 mg of curcumin for a mean duration of 6 months, 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 (Cruz-Correa *et al.,* 2006). Although 2 mild incidences of gastrointestinal effects were reported by subjects consuming the test capsules, since the capsules contained both curcumin and quercetin, it is not possible to determine whether the gastrointestinal disturbances were related to quercetin *per se*.

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 (approximately 684 mg quercetin for 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 (approximately 3,246 mg for a 60 kg individual), 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 (approximately 1,530 mg for a 60 kg individual) 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 absorption, distribution, metabolism, excretion (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).

Quercetin supplementation was also shown to be safe in a 12-week study in which subjects received 500 or 1,000 mg quercetin/day (Nieman, 2008; Shanely *et al.*, 2010). No test substance-related effects were observed in adverse symptom reporting and clinical chemistry measurements, with the exception of creatinine and glomerular filtration rate; however, these remained within normal ranges for humans. Quercetin supplementation also resulted in a slight, but significant decrease in MAP.

No safety concerns were reported in three recently-conducted 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.

In addition to the human clinical studies, the safety of quercetin is corroborated by epidemiological studies, which consistently demonstrate an absence of any correlation between quercetin intakes or flavonoid intakes, 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 [abstract]; Lin *et al.*, 2006). Moreover, Knekt *et al.* (1997, 2002) and Lam *et al.* (in press) have demonstrated an inverse association between flavonoid intake (95% quercetin) and overall cancer incidence, as well as incidence of lung cancer specifically.

6.6 Safety of Quercetin Degradation Products

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 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.

In vitro, protocatechuic acid (PCA) did not exhibit adverse effects on normal human cell lines; however, 48 hours of PCA exposure at concentrations of up to 8 µmol/L induced apoptosis in the following cancer cell lines: breast, lung, liver, cervical, and prostate (Yin *et al.*, 2009). Exposure at concentrations of 2-8 µmol/L effectively increased DNA fragmentation, decreased mitochondrial membrane potential, lowered NA^+-K^+-ATPase activity, elevated caspase-3 and caspase-8 activities, and suppressed production certain growth factors. As apoptosis serves a key role in anticancer therapies, these data support the efficacy of PCA as an anticancer agent to retard the progression of an already established malignancy without harming normal cells (Yin *et al.*, 2009).

The chemopreventive properties of dietary PCA were investigated in male F344 rats (Tanaka *et al.*, 1993). The study was designed to determine if PCA administration impacted biomarkers of azoxymethane (AOM)-induced colon carcinogenesis. AOM injections started at 7 weeks of age, and dietary administration of PCA coincided with either the initiation or postinitiation phase of AOM-induced carcinogenesis. The animals were fed a diet containing PCA at dose levels of 250, 500, and 1000 ppm for either 4 weeks (starting at 6 weeks of age; initiation phase) or 28 weeks (starting at 1 week after the final AOM injection; postinitiation phase). Animals in a third group were fed a diet containing 1000 ppm PCA throughout the experiment (32 weeks). All animals were terminated at the same time. The results of the study indicated that all 3 PCA dose levels administered during the initiation or postinitiation phase inhibited the development of AOM-induced colonic tumors; there was both a reduction in tumors and multiplicity. The reductions were most pronounced at the highest dose (1000 ppm), and depending on the dosing paradigm, the effects at 500 ppm were significantly different from control values. Additionally, following 32 weeks of PCA exposure at a dietary concentration of 1000 ppm (~50 mg/kg body weight/day), no signs of toxicity were reported (Tanaka *et al.*, 1993).

The modifying effect of PCA (50 and 500 mg/kg i.p.) on GSH levels in ICR mouse liver and kidney tissue were examined following acute and subchronic exposure (Nakamura *et al.*, 2001). In the acute study, the mice were administered PCA (50 or 500 mg/kg, i.p.) and sacrificed 6 hours later. In the subchronic study, the mice were given drinking water containing PCA (0.01 or 0.1%) for 60 days. During the acute study, exposure to 500 mg/kg PCA led to a significant decline of liver and kidney GSH levels. Additionally, a single dose of 500 mg/kg PCA also resulted in significant hepatotoxicity and nephrotoxicity as indicated by an increase in ALT and AST levels. The only reported effect at 50 mg/kg was an increase in AST levels. During subchronic exposure via drinking water at PCA doses of up to 0.1% (~250 mg/kg/day, FDA, 1993), all animals remained healthy throughout the 60-day experiment. There were no body weight or liver weight differences; however, there was a significant increase in ALT activity and GSH levels among the animals exposed to 0.1%. There were no reported effects at a water concentration of 0.01% PCA. Moreover, there were no other signs of overt toxicity (Nakamura *et al.*, 2001).

A double-blind, randomized, placebo-controlled study was conducted with 100 patients in the active phase of uncomplicated labor to assess the efficacy of phloroglucinol for acceleration of labor (Tabassum *et al.*, 2005). Fifty patients received 2 doses of phloroglucinol (40 mg each) separated by 60 minutes. The average duration of the active phase of first stage labor was shortened by approximately 2 hours in patients receiving phloroglucinol. There were no side effects, and no toxic effects were noted in either the mother or fetus. Additionally, 7% of the patients had pregnancy induced hypertension, 2% had diabetes mellitus, and 4% had previous cesarean section; therefore, in patients with the above complications, no toxic effects were noted. Overall, phloroglucinol had only positive effects in this study (Tabassum *et al.*, 2005).

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. In support of this perspective, from the published *in vitro* and *in vivo* literature, and the estimated potential exposure (*i.e.,* 2.55 mg/kg body weight/day in the 90th percentile of the quercetin-exposed population), quercetin degradation products would not likely pose a health risk. The estimated potential exposure for the 90th percentile is more than 10 times lower than the dose level at which adverse effects were reported in the 60-day drinking water study summarized above (~250 mg/kg body weight/day).

7.0 OVERALL CONCLUSIONS PERTAINING TO SAFETY

Quercetin [CAS RN 117-39-5], a naturally occurring flavonol, is the aglycone of quercitrin, rutin, and other glycosides belonging to a broad group of pigmented substances of plant origin known as flavonoids (Merck, 2006). Quercetin is produced by the Natural Products division of Quercegen Agronegócios 1 Ltda. (formerly Merck S/A, Brazil) in accordance with current good manufacturing practice, 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. Assay result not less than 99.5% for quercetin by HPLC analysis must be achieved to meet specification. In a 60-month long-term bulk stability study of quercetin dry powder stored in plastic bags at 30±2°C and 70±5% humidity, a 24-month stability study of quercetin dry powder stored in plastic bags at 30±2°C and 75±5% humidity, and 6-month stability studies with quercetin dry powder stored in plastic bags at 30±2°C and 70±5% or 40±2°C and 75±5% humidity, quercetin was shown to be stable under all these conditions.

Quercegen's high-purity food-grade 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. Depending on the particular food category, quercetin is proposed for use as a food ingredient at levels up to 500 mg of quercetin/serving. Under the conditions of intended use of the ingredient, the total population all-user mean intake of quercetin determined by the traditional approach 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 of quercetin was estimated to be 461 mg/person/day or 9 mg/kg body weight/day. For completeness of the data, an all-user intake of quercetin based on all proposed food-uses was also estimated for the infant population (ages 0 to 2), providing an intake level estimate of 53 mg/kg body weight/day for the 90th percentile. It should be noted, however, that the proposed food-uses specified for the addition of quercetin to food are not intended to be marketed to infants since the Targeted Population is exercising adults. Therefore, it is expected that the actual exposure of infants as well as children to quercetin-enriched food products will be highly limited. Accordingly, on a body weight basis, the estimated 90th percentile all-user intake of quercetin in infants and children is considered to be a gross over-estimate of the actual exposure expected in infants from the addition of quercetin to food.

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 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.

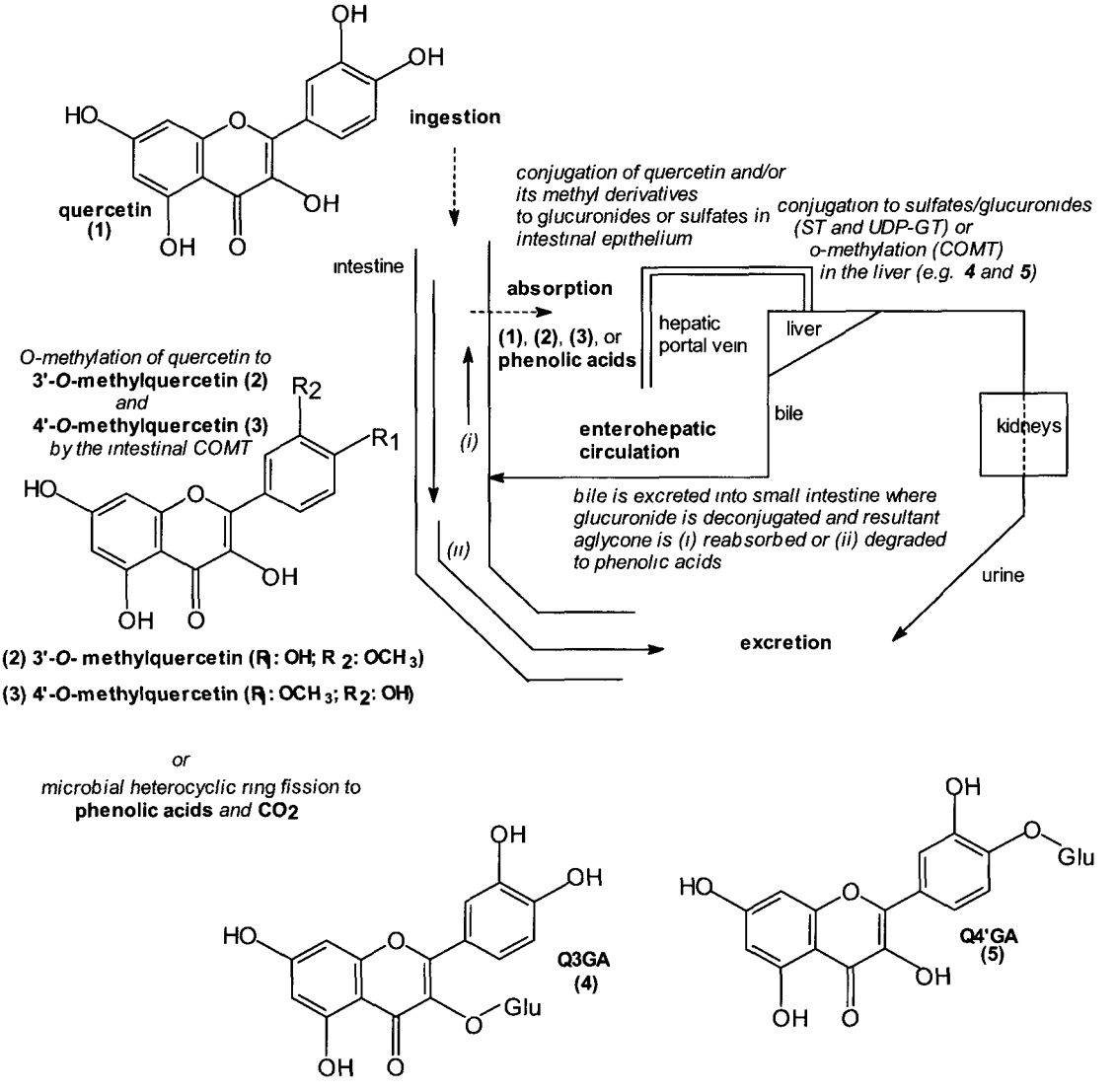
Commonly-consumed fruits and vegetables, such as onions and apples, are the primary dietary sources of quercetin, containing the flavonol at levels as high as approximately 350 ppm (expressed as the aglycone) (Day and Williamson, 1999). In the United States, consumption of the flavonol glycosides, expressed as quercetin equivalents, from the habitual diet was estimated to be at levels up to 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). The estimated mean and 90th percentile all-user intakes of quercetin from naturally occurring sources by the total U.S. population were 5.9 and 14.7 mg/person/day, respectively. These values were generated using the available data on the quercetin content of foods from the flavonoid database combined with food intake data from the NHANES 2003-2004 database (CDC, 2006; USDA, 2009a). Additionally, a maximum quercetin 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 the estimates.

The Total Population maximum estimated daily background intake of quercetin from naturally occurring sources (Table 5.1.2-1) 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.

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 7.0-1), act in concert to limit the bioavailability of quercetin *in vivo* 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 7.0-1 **Schematic Representation of the Absorption, Metabolism, and Excretion of Quercetin in Mammals**



Adapted from Day and Williamson (1999)

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

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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 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).

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.

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. 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). 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.

Additional experimental investigations have been conducted in which quercetin was tested in various 2-stage (initiation-promotion) carcinogenicity studies with various standard chemical carcinogens. 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.

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.

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).

Clinical data relating to the safety of quercetin were obtained from randomized, double-blind, clinical trials (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). 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.

The products resulting from possible quercetin degradation (*i.e.*, phloroglucinol, phloroglucinic acid, and 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.

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.

8.0 ACCEPTABLE DAILY INTAKE (ADI)

8.1 Background

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*.

8.2 Totality-of-Evidence

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 8.2-1 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 8.2-1 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.

8.3 Summary

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.

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9.0 BASIS FOR THE GRAS DETERMINATION

9.1 Regulatory Framework

The regulatory framework for determining whether a substance can be considered generally recognized as safe (GRAS) in accordance with section 201(s) (21 U.S.C. § 321(s)) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 301 et. Seq.) ("The Act"), is set forth at 21 CFR 170.30, which states:

General recognition of safety may be based only on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food. General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food.

General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety through scientific procedures shall ordinarily be based upon published studies which may be corroborated by unpublished studies and other data and information.

These criteria are applied below in an analysis of whether the use of quercetin as a food additive for selected Food Categories (i.e. beverages and beverage bases, grain products and pastas, processed fruits and fruit juices, and soft candies) is GRAS based upon scientific procedures.

9.2 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) subsequently 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*.

9.3 Comparison of the Estimated Daily Intake to the Acceptable Daily Intake

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 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 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 5.1.2-1) 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 conclusion, 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.

9.4 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. The following issues have been resolved with details provided in 1.0 Introduction and elsewhere in more detail throughout this document:

- This GRAS notification is for chemically defined pure Quercetin (≥99.5%).
- An extensive discussion supporting the absence of compound-related toxicity in the rat kidneys following chronic exposure to quercetin has been made and the re-evaluation of the kidney lesion in the NTP study by Hard *et al.* (2007) has been provided previously to FDA for its consideration.
- Rationale has been provided explaining why body weight reductions in long-term toxicity studies should not be considered an adverse effect in the case of quercetin.
- Rationale has been provided explaining why quercetin does not produce adverse effects *in vivo* despite its known anti- and prooxidant properties.
- Rationale has been provided explaining why GSH depletion, particularly in diseased sub-populations with below normal GSH levels (*e.g.*, Alzheimer's patients) should not be of concern.
- Data are provided on quercetin stability in relevant food matrices since quercetin is known to autooxidize under certain pH conditions, which could result in the generation of reactive oxygen species (ROS).

- A discussion is provided on the naturally occurring levels of quercetin in the diet and the USDA database has been used to calculate exposure estimates for quercetin from natural occurrence for comparison to intakes from addition to food (2- to 3-fold higher levels from addition as an ingredient would not be a concern).
- With respect to the potential for inhibitory effects of quercetin on intestinal glucose transporter 2 (GLUT2), the potential hyperglycemic effect of quercetin has been addressed.
- Scientific evidence has been provided regarding the potential for quercetin to inhibit CYP450 enzyme systems. Additional clinical data are now available that further confirm the safety in humans.
- Scientific evidence has been provided regarding the lack of effects of quercetin on glucose levels in the diabetic population. Additional clinical data are now available that further confirm the safety in humans.
- GRAS based upon scientific procedures has been established rather than any reliance on history of use due to natural occurrence of quercetin.

9.5 Conclusion: GRAS Determination

The safety of quercetin has been evaluated 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.

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.

Finally, these data and information are known and accepted by a consensus of qualified experts in the general scientific community. Thus, this information base not only assures

that the intended uses of quercetin described in this notice are safe, but also comprises common knowledge that quercetin is also generally recognized as safe under its intended conditions of use.

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APPENDIX A-1

BATCH ANALYSIS RESULTS

MERCK

Certificate of Analysis

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Date of print: 2007.03 20

4.100969020 - QU995 FOOD GRADE QP FA

Batch MM 2-6050

TEST	SPECIFICATION	RESULT
IDENTITY (HPLC)	CONFORMS	CONFORMS
ASSAY (HPLC, CALC. ON DRIED SUBSTANCE)	>= 99.5 %	99.8 %
SOLUBILITY		
2%; ETHANOL	<= 15 NTU	2 NTU
1%; DMF	CLEAR	CLEAR
As (ARSENIC)	<= 1 ppm	< 1 ppm
Cd (CADMIUM)	<= 1 ppm	< 1 ppm
Co (COBALT)	<= 1 ppm	< 1 ppm
Hg (MERCURY)	<= 1 ppm	< 1 ppm
Ni (NICKEL)	<= 1 ppm	< 1 ppm
Pb (LEAD)	<= 1 ppm	< 1 ppm
RELATED SUBSTANCES (TLC; 1%; 5µL)	<= 1 %	< 1 %
SULFATED ASH	<= 0.15 %	0.13 %
WATER (ACCORDING TO KARL FISCHER)	<= 4.0 %	1.3 %
SULFATE (SO4)	<= 1000 ppm	< 1000 ppm
CHLORIDE (Cl)	<= 1000 ppm	< 1000 ppm
DENSITY	>= 0.25 g/mL	0.32 g/mL
ETHANOL	<= 3000 ppm	2388 ppm

000615

MERCK

Certificate of Analysis

MICROBIOLOGICAL PURITY

AEROBIC BACTERIA	<= 5000 CFU/g	320 CFU/g
YEASTS AND MOULDS	<= 500 CFU/g	290 CFU/g
PATHOGENIC BACTERIA		
SALMONELLA SPECIES	ABSENT/10g	ABSENT
E. COLI	ABSENT/10g	ABSENT
STAPHYLOCOCCUS AUREUS	ABSENT/10g	ABSENT
PSEUDOMONAS AERUGINOSA	ABSENT/10g	ABSENT

CAS: (117-39-5)

STORAGE CONDITIONS:

MAX. TEMPERATURE: 40°C / MAX. HUMIDITY: 75%

ADDITIONAL INFORMATION:

PRESERVE CLOSED AND PROTECTED FROM LIGHT

MANUFACTURING DATE: MAR/06

RETEST DATE: MAR/11

MOISES E. DE CARVALHO JUNIOR

QUALITY ASSURANCE

THIS DOCUMENT HAS BEEN PRODUCED ELETRONICALLY AND IS VALID WITHOUT A SIGNATURE

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Certificate of Analysis

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4.100969020 - QU995 FOOD GRADE QP FA

Batch MM 2-6051

TEST	SPECIFICATION	RESULT
IDENTITY (HPLC)	CONFORMS	CONFORMS
ASSAY (HPLC, CALC. ON DRIED SUBSTANCE)	>= 99.5 %	100.0 %
SOLUBILITY		
2%; ETHANOL	<= 15 NTU	2 NTU
1%; DMF	CLEAR	CLEAR
As (ARSENIC)	<= 1 ppm	< 1 ppm
Cd (CADMIUM)	<= 1 ppm	< 1 ppm
Co (COBALT)	<= 1 ppm	< 1 ppm
Hg (MERCURY)	<= 1 ppm	< 1 ppm
Ni (NICKEL)	<= 1 ppm	< 1 ppm
Pb (LEAD)	<= 1 ppm	< 1 ppm
RELATED SUBSTANCES (TLC; 1%; 5µL)	<= 1 %	< 1 %
SULFATED ASH	<= 0.15 %	0.14 %
WATER (ACCORDING TO KARL FISCHER)	<= 4.0 %	1.0 %
SULFATE (SO4)	<= 1000 ppm	< 1000 ppm
CHLORIDE (Cl)	<= 1000 ppm	< 1000 ppm
DENSITY	>= 0.25 g/mL	0.31 g/mL
ETHANOL	<= 3000 ppm	2385 ppm

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MICROBIOLOGICAL PURITY

AEROBIC BACTERIA	<= 5000 CFU/g	< 10 CFU/g
YEASTS AND MOULDS	<= 500 CFU/g	20 CFU/g
PATHOGENIC BACTERIA		
SALMONELLA SPECIES	ABSENT/10g	ABSENT
E. COLI	ABSENT/10g	ABSENT
STAPHYLOCOCCUS AUREUS	ABSENT/10g	ABSENT
PSEUDOMONAS AERUGINOSA	ABSENT/10g	ABSENT

CAS: (117-39-5)

STORAGE CONDITIONS:

MAX. TEMPERATURE: 40°C / MAX. HUMIDITY: 75%

ADDITIONAL INFORMATION:

PRESERVE CLOSED AND PROTECTED FROM LIGHT

MANUFACTURING DATE: OCT/06

RETEST DATE: OCT/11

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4.100969020 - QU995 FOOD GRADE QP FA

Batch MM 2-6052

TEST	SPECIFICATION	RESULT
IDENTITY (HPLC)	CONFORMS	CONFORMS
ASSAY (HPLC, CALC. ON DRIED SUBSTANCE)	>= 99.5 %	99.8 %
SOLUBILITY		
2%; ETHANOL	<= 15 NTU	3 NTU
1%; DMF	CLEAR	CLEAR
As (ARSENIC)	<= 1 ppm	< 1 ppm
Cd (CADMIUM)	<= 1 ppm	< 1 ppm
Co (COBALT)	<= 1 ppm	< 1 ppm
Hg (MERCURY)	<= 1 ppm	< 1 ppm
Ni (NICKEL)	<= 1 ppm	< 1 ppm
Pb (LEAD)	<= 1 ppm	< 1 ppm
RELATED SUBSTANCES (TLC; 1%; 5µL)	<= 1 %	< 1 %
SULFATED ASH	<= 0.15 %	0.08 %
WATER (ACCORDING TO KARL FISCHER)	<= 4.0 %	1.4 %
SULFATE (SO4)	<= 1000 ppm	< 1000 ppm
CHLORIDE (Cl)	<= 1000 ppm	< 1000 ppm
DENSITY	>= 0.25 g/mL	0.37 g/mL
ETHANOL	<= 3000 ppm	793 ppm

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MICROBIOLOGICAL PURITY

AEROBIC BACTERIA	<= 5000 CFU/g	10 CFU/g
YEASTS AND MOULDS	<= 500 CFU/g	10 CFU/g
PATHOGENIC BACTERIA		
SALMONELLA SPECIES	ABSENT/10g	ABSENT
E. COLI	ABSENT/10g	ABSENT
STAPHYLOCOCCUS AUREUS	ABSENT/10g	ABSENT
PSEUDOMONAS AERUGINOSA	ABSENT/10g	ABSENT

CAS: (117-39-5)

STORAGE CONDITIONS:

MAX. TEMPERATURE: 40°C / MAX. HUMIDITY: 75%

ADDITIONAL INFORMATION:

PRESERVE CLOSED AND PROTECTED FROM LIGHT

MANUFACTURING DATE: OCT/06

RETEST DATE: OCT/11

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4.100969020 - QU995 FOOD GRADE QP FA

Batch MM 2-6053

TEST	SPECIFICATION	RESULT
IDENTITY (HPLC)	CONFORMS	CONFORMS
ASSAY (HPLC, CALC. ON DRIED SUBSTANCE)	>= 99.5 %	100.0 %
SOLUBILITY		
2%; ETHANOL	<= 15 NTU	2 NTU
1%; DMF	CLEAR	CLEAR
As (ARSENIC)	<= 1 ppm	< 1 ppm
Cd (CADMIUM)	<= 1 ppm	< 1 ppm
Co (COBALT)	<= 1 ppm	< 1 ppm
Hg (MERCURY)	<= 1 ppm	< 1 ppm
Ni (NICKEL)	<= 1 ppm	< 1 ppm
Pb (LEAD)	<= 1 ppm	< 1 ppm
RELATED SUBSTANCES (TLC; 1%; 5µL)	<= 1 %	< 1 %
SULFATED ASH	<= 0.15 %	0.08 %
WATER (ACCORDING TO KARL FISCHER)	<= 4.0 %	1.8 %
SULFATE (SO4)	<= 1000 ppm	< 1000 ppm
CHLORIDE (Cl)	<= 1000 ppm	< 1000 ppm
DENSITY	>= 0.25 g/mL	0.40 g/mL
ETHANOL	<= 3000 ppm	424 ppm

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MICROBIOLOGICAL PURITY		
AEROBIC BACTERIA	<= 5000 CFU/g	< 10 CFU/g
YEASTS AND MOULDS	<= 500 CFU/g	< 10 CFU/g
PATHOGENIC BACTERIA		
SALMONELLA SPECIES	ABSENT/10g	ABSENT
E. COLI	ABSENT/10g	ABSENT
STAPHYLOCOCCUS AUREUS	ABSENT/10g	ABSENT
PSEUDOMONAS AERUGINOSA	ABSENT/10g	ABSENT

CAS: (117-39-5)

STORAGE CONDITIONS:

MAX. TEMPERATURE: 40°C / MAX. HUMIDITY: 75%

ADDITIONAL INFORMATION:

PRESERVE CLOSED AND PROTECTED FROM LIGHT

MANUFACTURING DATE: OCT/06

RETEST DATE: OCT/11

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APPENDIX A-2

METHODS OF ANALYSIS

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QU995 Analytical methodology	Page: 1/6
4 Control of Substance	Date: 05.10.2007

Analytical Methodology

QU995

Date of issue: 05 October 2007

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QU995 Technical Information	Page: 2/ 6
4 Control of Substance	Date: 30.03.2007

4.2 Analytical Procedures:

DESCRIPTION:

Yellow or greenish-yellow, fine powdered odorless or with slight characteristic odor.

IDENTITY

Done by HPLC, together with the assay test. RT must be similar to standard quercetin.

ASSAY (HPLC)

Chromatographic conditions
Column: SUPERSPHER 100 RP-18 endcapped 4 µ 250 x 4 mm CAT. 16858
Eluent: Water adjusted for pH 2,8 with phosphoric acid : acetonitrile mixture (60:40)
Flow rate: 1,0mL/min
Detector: UV
Wavelength: 259 nm
Retention Time: aprox. 4 minutes
Volume injected: 20µL
Standard mass: aprox. 25mg to 25mL Ethanol
Dilution: 5mL to 50mL with eluent
Concentration of the measure: aprox. 100µg/mL
Temperature: 25°C (environmental temperature)

Calculations:
Assay (%) = Asp x Wst x (100 – LODst) x P / Ast x Wsp x (100 – LODsp)

Where:
Asp = sample area
Ast = standard area
Wst = standard mass (mg)
Wsp = sample mass (mg)
P = Standard Assay on the *dried basis*
LODsp = sample loss on drying (%)
LODst = standard loss on drying (%)

APPEARANCE IN ETHANOL 2 % (w/v)

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Dissolve 1,0 g of the fine powdered sample with 50 mL of ethanol (anhydrous) in a 125 mL Erlenmeyer and heat in water-bath. Transfer to the turbidimeter tube and read. The solution is considered clear if the read is lower or equal to 15 NTU.

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APPEARANCE IN DMF 1% (w/v)

Dissolve 0,5 g of the fine powdered sample with 50 mL of dimethylformamide (DMF) in a 125 mL Erlenmeyer and heat in water-bath. The solution obtained is clear.

ARSENIC (As), CADMIUM (Cd), COBALT (Co), MERCURY (Hg), NICKEL (Ni) and LEAD (Pb)

All these heavy metals are analysed by Atomic Absorption.

RELATED SUBSTANCES

Test done by TLC.

Stationary phase: Silica gel 60 F254 HPTLC pre-coated plates.
Mobile phase: buthanol R, formic acid R, water R, methyl ethyl ketone R, ethyl acetate R (5:10:10:30:50 V/V/V/V/V)

Conditions: simple development, saturated chamber, over a path of 7cm.
Test solution: Dissolve 0,1 g of the sample to be analyzed in ethanol 96% and dilute to 10.0 ml with the same solvent (consider the sample loss on drying).
Reference solutions:
A. Dissolve 0,1 g of Quercetin standard in ethanol 96% and dilute to 10.0 ml with the same solvent.
B. Dissolve 0,005 g of Isoquercitroside in methanol and dilute to 100.0 ml with the same solvent.
C. Dissolve 0,005 g of Rutin in methanol and dilute to 100.0 ml with the same solvent.
Application: apply separately 10µL of the test solution and then 10 µL of each reference solution (A, B and C) using an 8mm line.
Detection: dry the plate at 110°C for 5 minutes and then spray with $AlCl_3$ 1% solution (in ethanol 96%). Dry again for 10 minutes at 110°C and examine under UV (254 and 360 nm).
Results:
- Principal spot: Rf aprox. 0,9;
- Isoquercitroside spot: Rf aprox. 0,53;
- Rutin spot: Rf aprox. 0,27.

The spots obtained with the test solution are similar in position, colour and size to the spots in the chromatogram obtained with the reference solution.

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SULPHATED ASH

Ignite a platinum, porcelain or quartz crucible at 600 ± 50° C for 30 min, allow to cool in a dessicator over silica gel and weigh. Place 1.0 g of the substance to be examined in a crucible and weigh. Moisten the substance to be examined with a small amount of sulphuric acid 96% (usually 1 ml) and heat gently at as low a temperature as practicable until the sample is thoroughly charred. After cooling, moisten the residue with a small amount of sulphuric acid R, heat gently until white fumes are no longer evolved and ignite at aprox. 700°C until the residue is completely incinerated. Ensure that flames are not produced at any time during the procedure. Allow the crucible to cool in a dessicator over silica gel, weigh it again and calculate the mass of the residue.

If the mass of the residue so obtained exceeds the prescribed limit, continue the ignition, as previously, to constant mass, unless otherwise prescribed.

WATER

Karl Fisher (K.F.):
Determined on 0.100 g.

In a Karl Fisher recipient add 30mL of the solvent (Merck Article 106009, Methanol) and titrate with Karl Fisher solution (Merck Article 188005). Weight exactly the substance, register this weight and titrate with the titration solution.

Calculations:
%Water = a (mL) * K.F. Factor (mg/mL) * 100 / exact sample mass (mg)
Karl Fisher solution consumption = a (mL)

SULPHATE

Weight 1,0g of Quercetin sample, add 29ml of acidified water (water is acidified with 1mL of HCl 10%) and add 0,05g of activated charcoal. Agitate for 5 minutes, filter in paper (110 mm).
Add 1mL of chloridric acid (HCl) 3N and 3mL of barium chloride ($BaCl_2$) TS, complete the volume to 50,0mL with water, agitate for 10 minutes and compare the turbidity with a reference solution of the same volume, which passed through the same procedure described above, but had 1,0mL of sulphuric acid (H_2SO_4) 0,01N added to its content (standard solution limit – 0,1 %). Compare the turbidity of the test solution with the reference solution.

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CHLORIDE

Weight 1,0g of Quercetin sample, add 29ml of acidified water (water is acidified with 1mL of HNO_3 10%) and add 0,05g of activated charcoal. Agitate for 5 minutes, filter in paper (110 mm).
To the filtrate, add 1mL of nitric acid (HNO_3) and 1mL of silver nitrate ($AgNO_3$), complete the volume to 50,0mL with water, agitate for 10 minutes and compare the turbidity with a reference solution of the same volume, which passed through the same procedure described above, but had 2,8mL of chloridric acid (HCl) 0,01N added to its content (standard solution limit – 0,1 %). Compare the turbidity of the test solution with the reference solution.

DENSITY

Follow as described in SOP 41/g/1213 on its current version.

ETHANOL – GAS CHROMATOGRAPHY

External Standard – Headspace System

Sample preparation
Add 1.00 g of Quercetin sample in a 100 mL volumetric flask, dissolve and complete to volume with 1N NaOH solution which contains 20% of anhydrous Na_2SO_4.
Standard preparation (stock)
Add 1.0 mL of ethanol in a 100 mL volumetric flask and complete to volume with 1N sodium hydroxide solution.
Standard dilution
Transfer 1.0 mL of the standard solution prepared to a 100 mL volumetric flask and complete to volume with 1N sodium hydroxide solution.
Standard solution
Transfer 1.0 mL of the diluted standard solution to a 100 mL volumetric flask and complete to volume with 1N NaOH solution which contains 20% of anhydrous Na_2SO_4.
Blank
Add 10 mL of 1N sodium hydroxide solution with 20% of sodium sulphate anhydrous to a headspace flask. Prepare only one blank.
Procedure
Transfer 10 mL of sample solution and 10 mL of each of the standard solution to headspace flasks.
Inject the standards and the sample three times.
Calculations
Ethanol % (w/v) = A * 0,79 / P * W
Where:
A = area / height average from the sample injections
P = weight / height average from standard injections

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4 Control of Substance	Date: 30.03.2007

W = sample mass in g

MICROBIOLOGICAL TESTS

All microbiological tests follow Merck General Method MG 029, on its current version, which complies with USP Monograph for Microbiology Tests.

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Pages 000630-000646 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

APPENDIX A-3

STABILITY DATA

ANEX I - RESULTS TABLE

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LONG TERM STABILITY TESTING REPORT

QUALITY ASSURANCE COORDINATION

REPORT Nº:
RELD 01/09

PROJECT	Quercetina	RESPONSIBLE	CCGQ
GOAL	Long term evaluation of ethanolic process	PACKAGING	plastic bags
BATCH SIZE	960Kg	VALID BY	6/3/2008
LOT #	MM02-3028	QUANT./ANALYSIS	125g
MANUF. DATE	6/3/2003	TOTAL # SAMPLES	15
CONDITIONS	UR% = 70 ±5% / TEMPERATURA 30°C ±2°C	STUDY BEGIN	12/9/2003

PRODUCT	MANUFACTURING	VALID DATE	LOT	STUDY STATUS
Quercetin	30 06 2003	30 06 2008	MM02-3028	(X) Concluded on 17/12/2008

	Analysis date	dezembro-03	março-04	junho-04	setembro-04	dezembro-04	junho-05	janeiro-06	dezembro-06	dezembro-07	dezembro-08	ESTATISTICA					
Analytical Results	Specification	Begin	3º Mês	6º Mês	9º Mês	12º Mês	18º Mês	24º Mês	36º Mês	48º Mês	60º Mes						
DESCRIPTION	Fine yellow to light green powder, whith subtil characteristic smel	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE						
CONTENT (HPLC DRY BASIS)	no less than 98,0%	98 8%	98 7%	98 4%	98 2%	98 1%	99 3%	99 3%	98 8%	100 3%	100 3%	99.0%	0.70%	0.79%	100.50%	99.10%	1.10%
IDENTITY (HPLC)	CORRESPONDS	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE						
CHLORIDE	no more than 1000 ppm	< 1000 ppm	-	-	-	-	< 1000 PPM	-	-	-	< 1000 PPM						
SULPHATE	no more than 1000 ppm	< 1000 ppm	-	-	-	-	< 1000 PPM	CORRESPONDE	-	-	< 1000 PPM						
ARSENIC (As)	no more than 1 ppm	< 1 ppm	-	-	-	-	-	-	-	-	-						
CADMIUN (Cd)	no more than 1 ppm	< 1 ppm	-	-	-	-	-	-	-	-	-						
COBALT (Co)	no more than 1 ppm	< 1 ppm	-	-	-	-	-	-	-	-	-	0.03%	0.010%	52.70%	0.05%	0.01%	0.02%
MERCURY (Hg)	no more than 1 ppm	< 1 ppm	-	-	-	-	-	-	-	-	-	3.90%	1.20%	33.49%	6.40%	2.40%	2.00%
NICKEL (Ni)	no more than 1 ppm	< 1 ppm	-	-	-	-	-	-	-	-	-						
LEAD (Pb)	no more than 1 ppm	< 1 ppm	-	-	-	-	-	-	-	-	-	0.36	0.03	0.07	0.36	0.32	0.03
SOLUBILITY 2% (p/v) ETHANOL	clear solution	CORRESPONDE	-	-	-	-	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE	864	89	0	913	815	49
SOLUBILITY 1% (p/v) ETHANOL	clear solution	CORRESPONDE	-	-	-	-	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE						
SOLUBILITY 1% (p/v) DMF	clear solution	CORRESPONDE	-	-	-	-	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE	CORRESPONDE						
SULPHATED ASHES	no more than 0 15%	0 01%	-	-	-	-	0 02%	0 05%	0 04%	-	0 01%						
WATER KF	no more than 4 0%	2 4%	2 7%	2 7%	3 7%	3 6%	3 6%	4 0%	4 7%	6 4%	5 8%						
CORRELATED SUBSTANCES (TLC 1%, microL)	no more than 1%	CORRESPONDE	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%						
DENSITY	no less than 0 25 g/mL	0 32	-	-	-	-	0 34	0 38	0 38	-	0 37						
ETHANOL	no more than 3000 ppm	913	-	-	-	-	-	-	-	-	815	99.13%	0.11%	0.11%	99.30%	99.00%	0.15%
AEROBICS COUNT	no more than 5000 UFC/g	< 10 UFC g	-	-	-	-	< 10 UFC/g	< 10 UFC/g	< 10 UFC/g	< 10 UFC/g	< 10 UFC/g						
YEAST AND MOULD COUNT	no more than 500 UFC/g	< 10 UFC/g	-	-	-	-	< 10 UFC/g	< 10 UFC/g	< 10 UFC/g	< 10 UFC/g	< 10 UFC/g						
Salmonella	Absent/10g	AUSENTE/ 10g	-	-	-	-	AUSENTE/ 10g	AUSENTE/ 10g	AUSENTE/ 10g	AUSENTE/ 10g	AUSENTE/ 10g						
E coli	Absent/10g	AUSENTE 10g	-	-	-	-	AUSENTE/ 10g	AUSENTE/ 10g	AUSENTE/ 10g	AUSENTE/ 10g	AUSENTE/ 10g						
S aureus	Absent/10g	AUSENTE 10g	-	-	-	-	AUSENTE/ 10g	AUSENTE/ 10g	AUSENTE/ 10g	AUSENTE/ 10g	AUSENTE/ 10g						
P aeruginosa	Absent/10g	AUSENTE 10g	-	-	-	-	AUSENTE/ 10g	AUSENTE/ 10g	AUSENTE/ 10g	AUSENTE/ 10g	AUSENTE/ 10g						
CONTENT HPLC "AS IS"	≥ 94%	N/A	99%	99%	99%	99%	99%	99%	99%	99%	99%						
PARTICLE SIZE 20 MESH	retain no more than 2%	N A	-	-	-	-	0 00%	0 00%	-	-	0%						
PARTICLE SIZE 200 MESH	retain no mores than 5%	N A	-	-	-	-	0 10%	0 40%	-	-	0%						
PARTICLE SIZE 80 MESH	goes through no less than 87%	N/A	-	-	-	-	100%	100%	-	-	100%						

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STABILITY STUDY - LONG TERM

COUNTRY: *Brasil (Climatic Zone III and IV)*
PRODUCT: *QU995 FOOD GRADE QP FA*
PACKAGING SYSTEM: *Plastic bags*
STUDY PURPOSE: *Follow-up stability study*
BATCH NUMBER: *MM 2-6023*
ARTICLE NUMBER: *4.10096.9020*
TEMPERATURE: *30+ 2°C / Humidity 75+5 %*
MANUFACTURING DATE: March/*2006*
STUDY STARTING DATE: May/*2006*

TESTS	SPECIFICATIONS	INITIAL	3°MONTH	6°MONTH	9°MONTH	12°MONTH	18°MONTH	24°MONTH
Description	Yellow or greenish-yellow, crystalline powder, odorless or with slight characteristic odor	Corresponds	Corresponds	Corresponds	Corresponds	Corresponds	Corresponds	Corresponds
Assay (HPLC)	Not less than 99,5%	100,1	100,9	99,6	99,7	100,4	100,4	100,1
Identity (HPLC)	Corresponds	Corresponds	Corresponds	Corresponds	Corresponds	Corresponds	Corresponds	Corresponds
Related Substances	Not more than 1%	Corresponds	Corresponds	Corresponds	Corresponds	Corresponds	Corresponds	Corresponds
Chloride	Not more than 1000ppm	Corresponds	*	*	*	*	*	*
Sulphate	Not more than 1000ppm	Corresponds	*	*	*	*	*	*
Arsenic (As)	Not more than 1ppm	Corresponds	*	*	*	*	*	*
Cadmium (Cd)	Not more than 1ppm	Corresponds	*	*	*	*	*	*
Cobalt (Co)	Not more than 1ppm	Corresponds	*	*	*	*	*	*
Mercury (Hg)	Not more than 1ppm	Corresponds	*	*	*	*	*	*
Nickel (Ni)	Not more than 1ppm	Corresponds	*	*	*	*	*	*
Lead (Pb)	Not more than 1ppm	Corresponds	*	*	*	*	*	*
Sulphated Ash	Not more than 0,15%	0,03	*	0,05	0,02	0,02	*	0,10
Water (K.F)	Not more than 4,0 %**	1,3	2,0	2,2	2,5	3,1	4,0	4,1
Solubility								
Ethanol 2%	Not more than 15 NTU	4	1	1	1	1	1	5
DMF 1%	Clear solution	Corresponds	Corresponds	Corresponds	Corresponds	Corresponds	Corresponds	Corresponds
Density	Not more than 0.25 g/mL	0,33	0,33	0,31	0,34	0,32	*	0,30
Ethanol (GC)	Not more than 3000ppm	2194,00	*	*	*	*	*	*
Microbiology	Corresponds	Corresponds	*	*	*	*	*	Corresponds

* Unnecessary analysis According to specific SOP.
** 'Inhouse' release specification

000649

ANEX I - RESULTS TABLE

LONG TERM STABILITY TESTING REPORT

QUALITY ASSURANCE COORDINATION

REPORT Nº:

PROJECT	Quercetin Food Grade	RESPONSIBLE	Quality Assurace
GOAL	Process validation building 04	PACKAGING	PE bags inside carboard box. Silica
BATCH SIZE	960Kg	VALID BY	Feb 14
LOT #	MM 02-9046	QUANT./ANALYSIS	125g
MANUF. DATE	Febuary 09	TOTAL # SAMPLES	13
CONDITIONS	Moisture% = 70 ±5% / TEMPERATURE:30°C ±2°C	STUDY BEGIN	8/14/2009

PRODUCT	PACKAGING	VALID DATE		LOT		Study Status
Quercetin	Jul 09	Feb 14		MM 02-9046		Concluded on ____/____/____
	Analysis date	Aug 09	Sept 09	Oct 09	novembro-09	Feb 10
Analytical Results	**Specification**	**Begin**	**1st Month**	**2nd Month**	**3rd Month**	**6th Month**
ARSENIC (As)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
ASPECT IN DMF 1% P/V	CORRESPONDS	CORRESPONDS	N/A	N/A	CORRESPONDS	CORRESPONDS
ASPECT IN ETHANOL 2% P/V	Max. 15 NTU	5	N/A	N/A	6	6
CADMIUM (Cd)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
LEAD (Pb)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
ASHES	Max. 0,15%	0 10%	N/A	N/A	0 09%	0.09%
CHLORIDE	Max. 1000 PPM	< 1000	N/A	N/A	< 1000	< 1000
COBALT (Co)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
AEROBICS COUNT	Max. 5000 UFC/g	< 10	N/A	N/A	< 10	< 10
YEAST AND MOLD COUNT	Max. 500 UFC/g	5	N/A	N/A	< 10	< 10
APARENT DENSITY	Min. 0,25 g/mL	0 31	N/A	N/A	0.30	0.29
DESCRIPTION	CORRESPONDS	CORRESPONDS	CORRESPONDS	CORRESPONDS	CORRESPONDS	CORRESPONDS
E. COLI	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
ETHANOL	Max. 3000 PPM	279	N/A	N/A	630	0
IDENTITY (HPLC)	CORRESPONDS	CORRESPONDS	N/A	N/A	CORRESPONDS	CORRESPONDS
MERCURY (Hg)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
NICKEL (Ni)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
LOSS ON DRYING	Max. 4,0%	0.5%	0.9%	1.5%	1.9%	2.5%
P. AEROGINOSA	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
SALMONELA SP	ABSENT/ 10G	ABSENT	N/A	N/A	ABSENT	ABSENT
STAPHYLOCOCCUS AUREUS	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
CORRELATED SUBSTANCES (TLC)	Max. 1%	< 1%	< 1%	< 1%	< 1%	< 1%
SULPHATE	Max. 1000 PPM	< 1000	N/A	N/A	< 1000	< 1000
WATER (KF)	Max. 4,0%	0.8%	1.2%	1.9%	2.7%	3.2%
QUERCETIN (HPLC)	Min. 99,5%	100 0%	100 3%	100 2%	100.2%	99.9%

000650

ANEX I - RESULTS TABLE

LONG TERM STABILITY TESTING REPORT

REPORT N°:

QUALITY ASSURANCE COORDINATION

PROJECT	Quercetin Food Grade	RESPONSIBLE	Quality Assurace
GOAL	Process validation building 04	PACKAGING	PE bags inside carboard box. Silica
BATCH SIZE	960Kg	VALID BY	Feb 14
LOT #	MM 02-9047	QUANT./ANALYSIS	125g
MANUF. DATE	July 09	TOTAL # SAMPLES	
CONDITIONS	Moisture% = 70 ±5% / TEMPERATURE:30°C ±2°C	STUDY BEGIN	8/14/2009

PRODUCT	PACKAGING	VALID DATE		LOT		Study Status
Quercetin	Jul 09	Feb 14		MM 02-9047		Concluded on ____/____/____
	Analysis date	Aug 09	Sept 09	Oct 09	novembro-09	Feb 10
Analytical Results	**Specification**	**Begin**	**1st Month**	**2nd Month**	**3rd Month**	**6th Month**
ARSENIC (As)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
ASPECT IN DMF 1% P/V	CORRESPONDS	CORRESPONDS	N/A	N/A	CORRESPONDS	CORRESPONDS
ASPECT IN ETHANOL 2% P/V	Max. 15 NTU	5	N/A	N/A	5	5
CADMIUM (Cd)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
LEAD (Pb)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
ASHES	Max. 0,15%	0 02%	N/A	N/A	0.09%	0.05%
CHLORIDE	Max. 1000 PPM	< 1000	N/A	N/A	< 1000	< 1000
COBALT (Co)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
AEROBICS COUNT	Max. 5000 UFC/g	< 10	N/A	N/A	< 10	< 10
YEAST AND MOLD COUNT	Max. 500 UFC/g	< 10	N/A	N/A	< 10	10
APARENT DENSITY	Min. 0,25 g/mL	0 31	N/A	N/A	0 26	0 27
DESCRIPTION	CORRESPONDS	CORRESPONDS	CORRESPONDS	CORRESPONDS	CORRESPONDS	CORRESPONDS
E. COLI	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
ETHANOL	Max. 3000 PPM	407	N/A	N/A	630	0
IDENTITY (HPLC)	CORRESPONDS	CORRESPONDS	N/A	N/A	CORRESPONDS	CORRESPONDS
MERCURY (Hg)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
NICKEL (Ni)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
LOSS ON DRYING	Max. 4,0%	0.6%	1.2%	1.8%	2.2%	2.8%
P AEROGINOSA	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
SALMONELA SP	ABSENT/ 10G	ABSENT	N/A	N/A	ABSENT	ABSENT
STAPHYLOCOCCUS AUREUS	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
CORRELATED SUBSTANCES (TLC)	Max. 1%	< 1%	< 1%	< 1%	< 1%	< 1%
SULPHATE	Max. 1000 PPM	< 1000	N/A	N/A	< 1000	< 1000
WATER (KF)	Max. 4,0%	1.1%	1.4%	2.2%	3.2%	3.5%
QUERCETIN (HPLC)	Min. 99,5%	99 8%	100 2%	99.7%	100 3%	100.1%

000651

ANEX I - RESULTS TABLE

LONG TERM STABILITY TESTING REPORT

REPORT Nº:

QUALITY ASSURANCE COORDINATION

PROJECT	Quercetin Food Grade	RESPONSIBLE	Quality Assurace
GOAL	Process validation building 04	PACKAGING	PE bags inside carboard box. Silica
BATCH SIZE	960Kg	VALID BY	Feb 14
LOT #	MM 02-9048	QUANT./ANALYSIS	125g
MANUF. DATE	July 09	TOTAL # SAMPLES	
CONDITIONS	Moisture% = 70 ±5% / TEMPERATURE:30°C ±2°C	STUDY BEGIN	8/14/2009

PRODUCT	PACKAGING	VALID DATE		LOT		Study Status
Quercetin	Jul 09	Feb 14		MM 02-9048		Concluded on ____/____/____
	Analysis date	Aug 09	Sept 09	Oct 09	novembro-09	Feb 10
Analytical Results	**Specification**	**Begin**	**1st Month**	**2nd Month**	**3rd Month**	**6th Month**
ARSENIC (As)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
ASPECT IN DMF 1% P/V	CORRESPONDS	CORRESPONDS	N/A	N/A	CORRESPONDS	CORRESPONDS
ASPECT IN ETHANOL 2% P/V	Max. 15 NTU	6	N/A	N/A	7	7
CADMIUM (Cd)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
LEAD (Pb)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
ASHES	Max. 0,15%	0 03%	N/A	N/A	0.05%	0.04%
CHLORIDE	Max. 1000 PPM	< 1000	N/A	N/A	< 1000	< 1000
COBALT (Co)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
AEROBICS COUNT	Max. 5000 UFC/g	< 10	N/A	N/A	< 10	< 10
YEAST AND MOLD COUNT	Max. 500 UFC/g	< 10	N/A	N/A	< 10	< 10
APARENT DENSITY	Min. 0,25 g/mL	0 3	N/A	N/A	0 32	0.31
DESCRIPTION	CORRESPONDS	CORRESPONDS	CORRESPONDS	CORRESPONDS	CORRESPONDS	CORRESPONDS
E. COLI	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
ETHANOL	Max. 3000 PPM	375	N/A	N/A	630	0
IDENTITY (HPLC)	CORRESPONDS	CORRESPONDS	N/A	N/A	CORRESPONDS	CORRESPONDS
MERCURY (Hg)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
NICKEL (Ni)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
LOSS ON DRYING	Max. 4,0%	0.5%	1.1%	1.6%	2.1%	2.8%
P. AEROGINOSA	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
SALMONELA SP	ABSENT/ 10G	ABSENT	N/A	N/A	ABSENT	ABSENT
STAPHYLOCOCCUS AUREUS	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
CORRELATED SUBSTANCES (TLC)	Max. 1%	< 1%	< 1%	< 1%	< 1%	< 1%
SULPHATE	Max. 1000 PPM	< 1000	N/A	N/A	< 1000	< 1000
WATER (KF)	Max. 4,0%	1.2%	1.3%	2.0%	3.1%	3.2%
QUERCETIN (HPLC)	Min. 99,5%	100 4%	100 4%	99.8%	100.6%	100.2%

000652

ANEX I - RESULTS TABLE

ACCELERATED STABILITY TESTING REPORT

REPORT Nº:

QUALITY ASSURANCE COORDINATION

PROJECT	Quercetin Food Grade	RESPONSIBLE	Quality Assurace
GOAL	Process validation building 04	PACKAGING	PE bags inside carboard box. Silica
BATCH SIZE	960Kg	VALID BY	Feb 14
LOT #	MM 02-9046	QUANT./ANALYSIS	125g
MANUF. DATE	Febuary 09	TOTAL # SAMPLES	13
CONDITIONS	Moisture% = 75 ±5% / TEMPERATURE:40°C ±2°C	STUDY BEGIN	8/14/2009

PRODUCT	PACKAGING	VALID DATE		LOT		Study Status
Quercetin	Jul 09	Feb 14		MM 02-9046		Concluded on _____/_____/_____
	Analysis date	Aug 09	Sept 09	Oct 09	Nov 09	Feb 10
Analytical Results	**Specification**	**Begin**	**1st Month**	**2nd Month**	**3rd Month**	**6th Month**
ARSENIC (As)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
ASPECT IN DMF 1% P/V	CORRESPONDS	CORRESPONDS	N/A	N/A	CORRESPONDS	CORRESPONDS
ASPECT IN ETHANOL 2% P/V	Max. 15 NTU	5	N/A	N/A	6	5
CADMIUM (Cd)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
LEAD (Pb)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
ASHES	Max. 0,15%	0 10%	N/A	N/A	0.09%	0.09%
CHLORIDE	Max. 1000 PPM	< 1000	N/A	N/A	< 1000	< 1000
COBALT (Co)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
AEROBICS COUNT	Max. 5000 UFC/g	< 10	N/A	N/A	< 10	5
YEAST AND MOLD COUNT	Max. 500 UFC/g	5	N/A	N/A	< 10	< 10
APARENT DENSITY	Min. 0,25 g/mL	0 31	N/A	N/A	0.30	0.29
DESCRIPTION	CORRESPONDS	CORRESPONDS	CORRESPONDS	CORRESPONDES	CORRESPONDS	CORRESPONDS
E. COLI	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
ETHANOL	Max. 3000 PPM	279	N/A	N/A	630	630
IDENTITY (HPLC)	CORRESPONDS	CORRESPONDS	N/A	N/A	CORRESPONDS	CORRESPONDS
MERCURY (Hg)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
NICKEL (Ni)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
LOSS ON DRYING	Max. 4,0%	0.5%	1.0%	1.5%	1.8%	2.3%
P. AEROGINOSA	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
SALMONELA SP	ABSENT/ 10G	ABSENT	N/A	N/A	ABSENT	ABSENT
STAPHYLOCOCCUS AUREUS	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
CORRELATED SUBSTANCES (TLC)	Max. 1%	< 1%	< 1%	< 1%	< 1%	< 1%
SULPHATE	Max. 1000 PPM	< 1000	N/A	N/A	< 1000	< 1000
WATER (KF)	Max. 4,0%	0.8%	1.3%	2.2%	2.5%	2.7%
QUERCETIN (HPLC)	Min. 99,5%	100 0%	99 6%	99.9%	100.0%	99 7%

ANEX I - RESULTS TABLE

ACCELERATED STABILITY TESTING REPORT

REPORT Nº:

QUALITY ASSURANCE COORDINATION

PROJECT	Quercetin Food Grade	RESPONSIBLE	Quality Assurace
GOAL	Process validation building 04	PACKAGING	PE bags inside carboard box. Silica
BATCH SIZE	960Kg	VALID BY	Feb 14
LOT #	MM 02-9047	QUANT./ANALYSIS	125g
MANUF. DATE	July 09	TOTAL # SAMPLES	
CONDITIONS	Moisture% = 75 ±5% / TEMPERATURE:40°C ±2°C	STUDY BEGIN	8/14/2009

PRODUCT	PACKAGING	VALID DATE		LOT		Study Status
Quercetin	Jul 09	Feb 14		MM 02-9047		Concluded on ____/____/____
	Analysis date	Aug 09	Sept 09	Oct 09	novembro-09	Feb 10
Analytical Results	**Specification**	**Begin**	**1st Month**	**2nd Month**	**3rd Month**	**6th Month**
ARSENIC (As)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
ASPECT IN DMF 1% P/V	CORRESPONDS	CORRESPONDS	N/A	N/A	CORRESPONDS	CORRESPONDS
ASPECT IN ETHANOL 2% P/V	Max. 15 NTU	5	N/A	N/A	5	5
CADMIUM (Cd)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
LEAD (Pb)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
ASHES	Max. 0,15%	0 02%	N/A	N/A	0.12%	0 04%
CHLORIDE	Max. 1000 PPM	< 1000	N/A	N/A	< 1000	< 1000
COBALT (Co)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
AEROBICS COUNT	Max. 5000 UFC/g	< 10	N/A	N/A	< 10	< 10
YEAST AND MOLD COUNT	Max. 500 UFC/g	< 10	N/A	N/A	< 10	5
APARENT DENSITY	Min. 0,25 g/mL	0 31	N/A	N/A	0.27	0.3
DESCRIPTION	CORRESPONDS	CORRESPONDS	CORRESPONDS	CORRESPONDS	CORRESPONDS	CORRESPONDS
E. COLI	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
ETHANOL	Max. 3000 PPM	407	N/A	N/A	630	400
IDENTITY (HPLC)	CORRESPONDS	CORRESPONDS	N/A	N/A	CORRESPONDS	CORRESPONDS
MERCURY (Hg)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
NICKEL (Ni)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
LOSS ON DRYING	Max. 4,0%	0.6%	1.1%	1.6%	2.1%	2.8%
P. AEROGINOSA	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
SALMONELA SP	ABSENT/ 10G	ABSENT	N/A	N/A	ABSENT	ABSENT
STAPHYLOCOCCUS AUREUS	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
CORRELATED SUBSTANCES (TLC)	Max. 1%	< 1%	< 1%	< 1%	< 1%	< 1%
SULPHATE	Max. 1000 PPM	< 1000	N/A	N/A	< 1000	< 1000
WATER (KF)	Max. 4,0%	1.1%	1.3%	2.6%	2.9%	3.5%
QUERCETIN (HPLC)	Min. 99,5%	99 8%	100.4%	100.0%	99 9%	100.1%

000654

ANEX I - RESULTS TABLE

ACCELERATED STABILITY TESTING REPORT

REPORT Nº:

QUALITY ASSURANCE COORDINATION

PROJECT	Quercetin Food Grade	RESPONSIBLE	Quality Assurace
GOAL	Process validation building 04	PACKAGING	PE bags inside carboard box. Silica
BATCH SIZE	960Kg	VALID BY	Feb 14
LOT #	MM 02-9048	QUANT./ANALYSIS	125g
MANUF. DATE	July 09	TOTAL # SAMPLES	
CONDITIONS	Moisture% = 75 ±5% / TEMPERATURE:40°C ±2°C	STUDY BEGIN	8/14/2009

PRODUCT	PACKAGING	VALID DATE		LOT		Study Status
Quercetin	Jul 09	Feb 14		MM 02-9048		Concluded on ____/____/____
	Analysis date	Aug 09	Sept 09	Oct 09	novembro-09	Feb 10
Analytical Results	**Specification**	**Begin**	**1st Month**	**2nd Month**	**3rd Month**	**6th Month**
ARSENIC (As)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
ASPECT IN DMF 1% P/V	CORRESPONDS	CORRESPONDS	N/A	N/A	CORRESPONDS	CORRESPONDS
ASPECT IN ETHANOL 2% P/V	Max. 15 NTU	6	N/A	N/A	7	6
CADMIUM (Cd)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
LEAD (Pb)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
ASHES	Max. 0,15%	0 03%	N/A	N/A	0 05%	0.07%
CHLORIDE	Max. 1000 PPM	< 1000	N/A	N/A	< 1000	< 1000
COBALT (Co)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
AEROBICS COUNT	Max. 5000 UFC/g	< 10	N/A	N/A	< 10	< 10
YEAST AND MOLD COUNT	Max. 500 UFC/g	< 10	N/A	N/A	< 10	< 10
APARENT DENSITY	Min. 0,25 g/mL	0 3	N/A	N/A	0.29	0.31
DESCRIPTION	CORRESPONDS	CORRESPONDS	CORRESPONDS	CORRESPONDS	CORRESPONDS	CORRESPONDS
E. COLI	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
ETHANOL	Max. 3000 PPM	375	N/A	N/A	630	0
IDENTITY (HPLC)	CORRESPONDS	CORRESPONDS	N/A	N/A	CORRESPONDS	CORRESPONDS
MERCURY (Hg)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
NICKEL (Ni)	Max. 1 PPM	< 1	N/A	N/A	N/A	N/A
LOSS ON DRYING	Max. 4,0%	0.5%	1.1%	1.6%	2.0%	3.0%
P. AEROGINOSA	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
SALMONELA SP	ABSENT/ 10G	ABSENT	N/A	N/A	ABSENT	ABSENT
STAPHYLOCOCCUS AUREUS	ABSENT/G	ABSENT	N/A	N/A	ABSENT	ABSENT
CORRELATED SUBSTANCES (TLC)	Max. 1%	< 1%	< 1%	< 1%	< 1%	< 1%
SULPHATE	Max. 1000 PPM	< 1000	N/A	N/A	< 1000	< 1000
WATER (KF)	Max. 4,0%	1.2%	1.3%	2.2%	2.4%	3.8%
QUERCETIN (HPLC)	Min. 99,5%	100 4%	100.2%	99.9%	100.9%	100.1%

000655

Stability Evaluation of a High-Purity Quercetin with and without Vitamin C and Niacinamide

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Abstract

Stability of a high-purity quercetin with and without vitamin C and niacinamide (QB_3C) was evaluated under an accelerated condition–that is, a recognized storage model condition. Results indicate that quercetin is degraded only by oxygen in air under storage conditions in neutral (pH 7.5) and acidic (pH 3.2) media, with the degradation rate reaching a plateau as oxygen is reduced. However, the combination formula (QB_3C) is quite stable and no degradation occurred under the accelerated condition, which is extrapolated to 12 months at room temperature (25°C).

Keywords

Quercetin • Vitamin C • Oxidative degradation • Arrhenius equation

Introduction

Quercetin **1** has been found to be one of the most active of the

flavonoids in various studies and many medicinal plants owe much of their activity to their high quercetin content [1,2]. Quercetin has demonstrated significant anti-inflammatory activity because of direct inhibition of several initial processes of inflammation [3] in addition to its demonstrated antioxidative capacity [4].

In the course of study of the effect of dietary quercetin on endurance capacity during exercise in mice, we have found that a combination of a high purity quercetin (QU995) with niacinamide and vitamin C (QB_3C) significantly increases physiological endurance during treadmill exercise under forced conditions in mice [5].

Several human clinical studies have evaluated the effect of the QB_3C combination in comparison with quercetin alone [6-9]. In addition, our pharmacokinetics study of the QB_3C combination using rats demonstrated that blood concentration of quercetin is significantly enhanced using QB_3C when compared to administration of quercetin alone [10].

Since then a number of dietary supplements based on the unique QB_3C combination have been designed [11-12]. This situation prompted us to study the stability of the combination when stored under varying conditions.

Very recently a study of diluted quercetin solutions in contact with air indicated that oxidative degradation processes take place in moderately basic media (pH = 8-10) at ambient temperature in the absence of any radical initiators, without enzymatic catalysis or irradiation of the reaction media by light [13]. However, this study did not directly reflect the actual storage model condition because air was continuously bubbled through the aqueous solution for 3.5 hrs.

We describe here a stability study of high-purity quercetin in

combination with vitamin C and niacinamide in an aqueous medium under an accelerated condition, 75°C for 12 days; that is a recognized storage model condition.

Results and Discussion

Three different quercetin compositions (n = 5 per group) were prepared as shown in Table 1. Each sample (50 mL) in a glass bottle with a lid was placed in a dark thermostatic oven at 75°C. The samples were analyzed on days 1, 3, 6, 9, and 12, respectively, by HPLC.

Sample	Ingredient	Quercetin conc. %	pH
A	quercetin, vitamin C and niacinamide (QB_3C)	0.1% DI water	3.3
B	quercetin	0.1% DI water	7.5
C	quercetin and H_3PO_4	0.1% DI water	3.2

Tab. 1. Quercetin compositions in the samples used for stability study

The study results are summarized in Table 2. Quercetin in combination with vitamin C and niacinamide (Sample A) did not show any decomposition under these conditions. In a neutral condition (sample B, pH = 7.5) quercetin's content was reduced about 18% when heated for 12 days at 75°C. According to HPLC and LC/MS profiles, three major degradation products were identified as 3,4-dihydroxy benzoic acid **3**, 2,4,6-trihydroxy benzoic acid **4** and 1,3,5-trihydroxy benzene **5**, respectively. The structures were confirmed by direct comparison with commercially available certified samples. The degradation products identified were the same products as when quercetin was treated by flow-through aeration over the course of 3.5 hrs [13].

Quercetin formulated at low pH (pH 3.2) gave similar degradation products to those at pH 7.5, although the total degradation rate was reduced by about 10%. Interestingly the quercetin degradation rates in both samples B and C were significantly retarded at day 9 and the degradation reached a plateau on day 12. The degradation products did not increase with treatment for a more prolonged period. It is assumed that all the degradation products **3**, **4** and **5** (above) were produced from an intermediate **2** that is formed by oxidative decarboxylation, although intermediate **2** was not actually detected. These results indicate that the quercetin decomposition is initiated by oxygen under the accelerated condition, thus the decomposition ratio depends only on the concentration of oxygen in the air of the reaction media.

Sample	A (%)	B (%)	C (%)
Day 0	100	100	100
Day 1	98.7 ± 1.2	94.2 ± 1.3	97.0 ± 0.5
Day 3	100.9 ± 1.1	90.7 ± 1.6	95.9 ± 1.4
Day 6	99.0 ± 1.2	85.8 ± 0.5	93.1 ± 1.7
Day 9	99.9 ± 2.1	82.9 ± 2.3	91.3 ± 3.3
Day 12	100.6 ± 0.7	81.3 ± 1.1	90.5 ± 0.4

Tab. 2. Quercetin content change under an accelerated condition. All values are expressed as means ± S.D. (n = 5)

If, as the data indicate, a second order reaction is initiated between quercetin and oxygen as shown in Figure 1, we can apply the Arrhenius equation for the temperature dependence of the rate constant of the decomposition reaction [14]. The degradation rate constant may be roughly calculated by this approximation [15]. This accelerated condition can be extrapolated to 12 months at room temperature (25°C). The present study

showed that quercetin is mostly degraded by oxygen under a recognized storage model condition, and quercetin can be stored without degradation for at least one year in combination with vitamin C and niacinamide (Sample A). The fact that quercetin is stable in the clinically effective formula (QB_3C) is of practical importance, which may be explained in terms of values of oxidation potentials of quercetin and vitamin C (0.11 and 0.03 Epa (V) vs. SCE, respectively) [16]. Further work is in progress to monitor the long-term stability of quercetin in aqueous media in the presence of natural antioxidants with various oxidation potentials and regular storage conditions at room temperature.

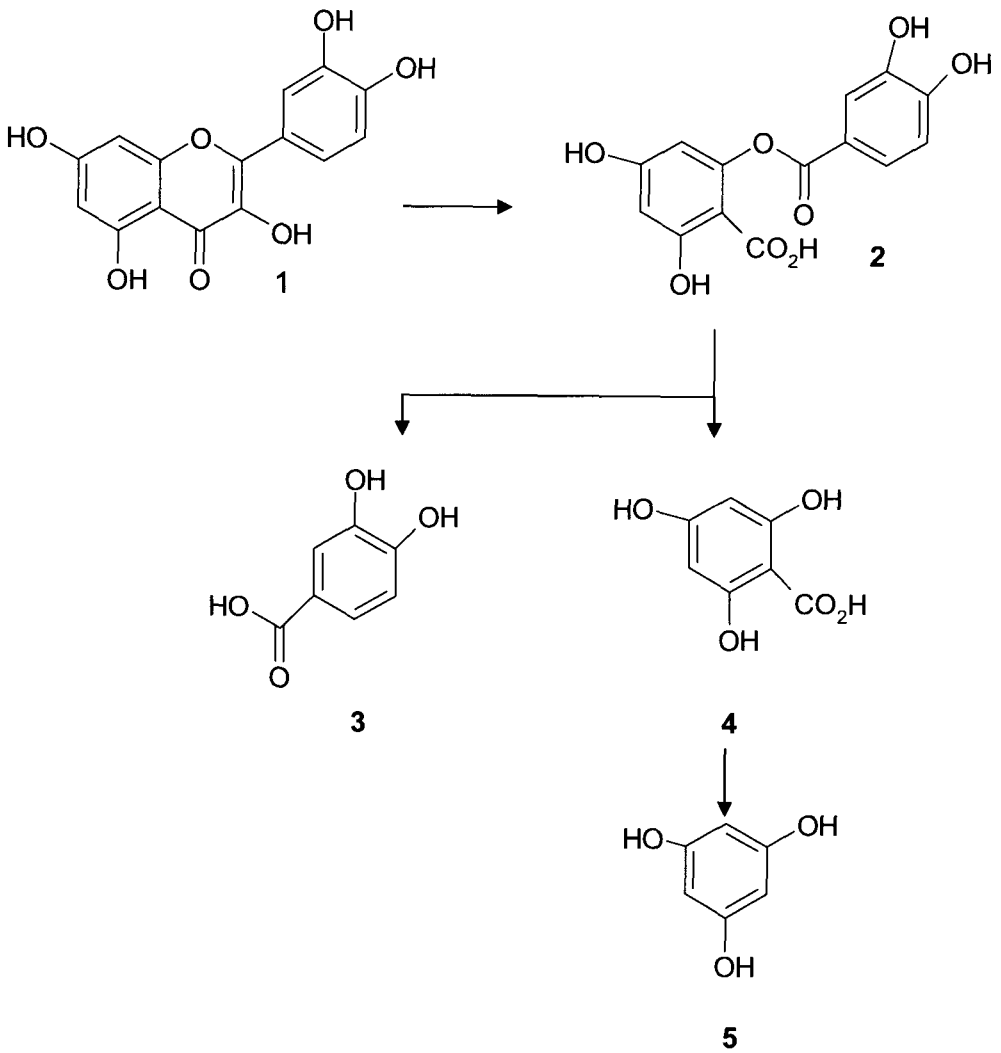

Fig. 1. Possible scheme of oxidative degradation of quercetin

Experimental

Material and Methods

A high purity quercetin (100%; QU995) was obtained from Merck & Co. Vitamin C (99.5%), niacinamide (99.5%), 3,4-dihydroxy benzoic acid (100%), 2,4,6-trihydroxy benzoic acid (91.4%) and 1,3,5-trihydroxy benzene (99.9%)

were purchased from Sigma Aldrich (St. Louis, MO). Quercetin was formulated based on the most biologically active formulation; the ratio of quercetin, vitamin C and niacinamide was 1:1:0.04 by weight [10].

Analysis of Quercetin

HPLC was performed with a Hitachi 7000 series liquid chromatography system. Analytical separations were achieved using an Inertsil C18 column (4.6 mm x 150 mm, 5um; GL Sciences, Japan). The column was eluted at 30°C, 1 mL/min. Mobile phase A was 0.1% aqueous acetic acid; mobile phase B was acetonitrile with 0.1% acetic acid. Elution regime was: isocratic for 0.5 min with 90% mobile phase A and 10% mobile phase B. This was followed by linear gradient to 60% phase A, 40% mobile phase B over 1 min. After isocratic regime at 60% mobile phase A for 7 min, a linear gradient was applied over 1 min to reach 10% mobile phase A, 90% mobile phase B. The system was maintained isocratic at 10% mobile phase A, 90% mobile phase B for 1 min before re-equilibration at 90% mobile phase A, 10% mobile phase B before the next injection. Detection was carried out with a Hitachi L-7400 UV detector at 375 nm.

Analysis of Degradation Products

Analytical separations were achieved using an Inertsil C18 column (4.6 mm x 250 mm, 5 µm; from GL Sciences, Japan). The column was eluted at 1 mL/min at room temperature with 90% aqueous 0.03% (v/v) trifluoroacetic acid and 10% acetonitrile. A linear gradient from 10 to 100% acetonitrile over the course of 45 min was applied. An injection volume of 20 µL was used. Detection was carried out with a Hitachi L-7450A Diode Array Detector measuring spectra between 200 and 500 nm. The resulting

chromatograms were based on detection at 254 nm. LC/MS analyses were conducted using a Dionex U3000 HPLC/PDA/MSQ system (Sunnyvale, CA). Mass spectra were obtained by full scans of 50-1000 m/z at both negative and positive electrospray ionization (ESI). The cone voltage was 50 V and probe temperature was 600°C. The HPLC conditions were unchanged.

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Interim Report of Quercetin Stability Study 2

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Aug 12, 2008

1. Background

This study was conducted based on protocol for the determination of the effect of different ratios of ascorbic acid under pH 3 and 6 on the stability of quercetin. The accelerated condition (75°C for 12 days) was selected from a typical extrapolation table for 1 year (365 days) at room temperature below

Table 1: Extrapolation table of temp vs. time for 1 year storage at room temperature based on the Arrhenius equation

Temp (°C)	Time (Day)
20-25 (average room temp)	365
30-35	182-183
40-45	90-91

50-55	45-46
60-65	22-23
70-75	11-12

2. Protocol

Eight different quercetin compositions (n = 3 per group) were prepared as shown below. Each sample (0.05% of quercetin; 25mg of quercetin in 50 mL of water) in a glass bottle with a lid was placed in a dark thermostatic oven at 75°C. The samples were analyzed on days 1, 3, 6, 9, and 12, respectively, by HPLC.

1a: Quercetin in buffer pH = 3

2a: Quercetin + Ascorbic acid (1:0.1) in buffer pH = 3

3a: Quercetin + Ascorbic acid (1:0.5) in buffer pH = 3

4a: Quercetin + Ascorbic acid (1:1) in buffer pH = 3

1b: Quercetin in buffer pH = 6

2b: Quercetin + Ascorbic acid (1:0.1) in buffer pH = 6

3b: Quercetin + Ascorbic acid (1:0.5) in buffer pH = 6

4b: Quercetin + Ascorbic acid (1:1) in buffer pH 6

3. Results and discussion

The study results are summarized in Table 2. Quercetin in combination with ascorbic acid with 1: 1 ratio at pH = 3 (Sample 4a) was most stable under these conditions and quercetin's content was reduced about 8% when heated for 12 days at 75°C. On the other hand, in weakly acidic condition (sample 4b, pH = 6) quercetin's content was reduced about 22%. The quercetin degradation rates in samples 4a and 4b, were significantly retarded at day 9 and the degradation reached a plateau on day 12. This tendency is the same as that observed in our previous study. It is assumed that a ratio of ascorbic acid is critical for quercetin stabilization in this condition. According to HPLC profiles, three major degradation products were identified as 3, 4-dihydroxy benzoic acid, 2, 4, 6-trihydroxy benzoic acid and 1,3,5-trihydroxy benzene, respectively. The structures were confirmed by direct comparison with commercially available certified samples. The degradation products identified were the same products as when quercetin was treated by flow-through aeration over the course of 3.5 hrs in a literature. It is assumed that all the degradation products were produced from an intermediate that is formed by oxidative decarboxylation, although intermediate was not actually detected. These results indicate that the quercetin decomposition is initiated by oxygen under the accelerated condition, thus the decomposition ratio depends only on the concentration of oxygen in the air of the reaction media.

4. Conclusion

Our previous experiment (quercetin : ascorbic acid = 1: 1 at pH = 2.8-3.0) showed no degradation of quercetin under the same accelerated conditions (75°C for 12 days). The critical difference between the previous condition and the present one is concentrations of quercetin and ascorbic acid in each test bottle. The previous one is 0.1% (50mg of quercetin in 50ml of water). The present one is 0.05% (25 mg of quercetin in 50mL of water). In other words, in the present experiment, the ratio oxygen: quercetin and the ratio oxygen : ascorbic acids are two times higher than those in the previous experiment. We selected this concentration (0.05%) because the concentration is more analogous to that of actual beverages with quercetin and ascorbic acid formula (0.05%, 250mg each in 500mL). The present result together with the previous data clearly shows that quercetin's stability only depends on the amount of oxygen in water. Two possible ways can be suggested for making a stable quercetin beverage formula. One is to add more ascorbic acid than 1:1 while keeping 0.05% quercetin concentration in beverage (250 mg in 500mL). The other is to minimize air in beverage by deaeration prior to production. In the present experiment, all buffers were sonicated for 15 min before use, to minimize differences in concentration of air among samples.

I. Quercetin Dihydrate Content: 1a Samples (Qu in buffer pH3)

Day #	Average Weight (mg)	Average Quercetin Conc (mg/mL)	Average Peak Area	Average Quercetin (mg/sample)	Average Recovery (%)	Average Recovery SD	**Avg corrected Recovery (%)**	pH
0	24.99	0.0986	3184776	24.7	**98.7**	1.5	**100.0**	3.21
1	25.17	0.0956	3029087	23.9	**94.9**	1.0	**96.2**	3.23
3	25.13	0.0918	2847222	22.9	**91.3**	0.3	**92.5**	3.21
6	25.09	0.0881	2730765	22.0	**87.8**	0.8	**89.0**	3.21
9	25.03	0.0858	2616505	21.4	**85.7**	1.1	**86.8**	3.15
12	24.96	0.0846	2521874	21.2	**84.8**	0.0	**85.9**	3.12

II. Quercetin Dihydrate Content: 2a Samples (Qu:C 1:0.1 in buffer pH3)

Day #	Average Weight (mg)	Average Quercetin Conc (mg/mL)	Average Peak Area	Average Quercetin (mg/sample)	Average Recovery (%)	Average Recovery SD	**Avg corrected Recovery (%)**	pH
0	25.00	0.0974	3142824	24.3	**97.4**	1.9	**100.0**	3.21
1	24.89	0.0936	2965597	23.4	**94.0**	0.5	**96.6**	3.22
3	25.01	0.0920	2853443	23.0	**91.9**	0.1	**94.4**	3.22
6	25.07	0.0880	2726785	22.0	**87.8**	0.5	**90.1**	3.20
9	25.17	0.0854	2603574	21.3	**84.8**	1.2	**87.1**	3.10
12	24.95	0.0826	2458048	20.6	**82.7**	2.3	**85.0**	3.08

III. Quercetin Dihydrate Content: 3a Samples (Qu:C 1:0.5 in buffer pH3)

Day #	Average Weight	Average Quercetin Conc	Average Peak Area	Average Quercetin	Average Recovery	Average Recovery	**Avg corrected Recovery**	pH

	(mg)	(mg/mL)		(mg/sample)	(%)	SD	(%)	
0	24.91	0.0990	3196222	24.7	**99.4**	1.1	**100.0**	3.20
1	24.98	0.0950	3010379	23.8	**95.1**	0.3	**95.7**	3.20
3	25.11	0.0944	2930302	23.6	**94.0**	0.9	**94.6**	3.21
6	24.88	0.0889	2756049	22.2	**89.4**	0.2	**89.9**	3.20
9	25.13	0.0864	2636598	21.6	**86.0**	1.1	**86.5**	3.09
12	25.02	0.0854	2545495	21.4	**85.4**	1.5	**85.9**	3.08

IV. Quercetin Dihydrate Content: 4a Samples (Qu:C 1:1 in buffer pH3)

Day #	Average Weight (mg)	Average Quercetin Conc (mg/mL)	Average Peak Area	Average Quercetin (mg/sample)	Average Recovery (%)	Average Recovery SD	**Avg corrected Recovery (%)**	pH
0	25.25	0.0992	3203423	24.8	**98.2**	1.7	**100.0**	3.20
1	25.02	0.0962	3050053	24.1	**96.1**	1.0	**97.9**	3.17
3	25.00	0.0959	2978796	24.0	**95.9**	0.5	**97.7**	3.18
6	25.14	0.0940	2919298	23.5	**93.5**	1.8	**95.2**	3.19
9	25.08	0.0889	2714688	22.2*	**89.9**	0.3	**91.6**	3.08
12	25.09	0.0906	2703858	22.6	**90.2**	1.6	**91.9**	3.09

* n = 2

V. Quercetin Dihydrate Content: 1b Samples (Qu in buffer pH6)

Day #	Average Weight (mg)	Average Quercetin Conc (mg/mL)	Average Peak Area	Average Quercetin (mg/sample)	Average Recovery (%)	Average Recovery SD	**Avg corrected Recovery (%)**	pH
0	25.16	0.0965	3114068	24.1	**95.9**	0.4	**100.0**	6.18
1	25.02	0.0647	2017793	16.2	**64.6**	0.7	**67.4**	6.12
3	25.03	0.0553	1687734	13.8	**55.3**	0.3	**57.6**	6.09
6	24.97	0.0419	1245598	10.5	**42.0**	1.2	**43.8**	6.07
9	24.97	0.0339	989485	8.5	**33.9**	3.8	**35.4**	5.94
12	24.97	0.0283	786676	7.1	**28.3**	1.0	**29.5**	5.89

VI. Quercetin Dihydrate Content: 2b Samples (Qu:C 1:0.1 in buffer pH6)

Day #	Average Weight (mg)	Average Quercetin Conc (mg/mL)	Average Peak Area	Average Quercetin (mg/sample)	Average Recovery (%)	Average Recovery SD	**Avg corrected Recovery (%)**	pH
0	25.04	0.0975	3145860	24.4	**97.3**	0.8	**100.0**	6.15
1	25.09	0.0666	2080157	16.6	**66.4**	1.5	**68.2**	6.08
3	24.87	0.0547	1667470	13.7	**55.0**	3.2	**56.5**	6.03

6	25.19	0.0433	1289481	10.8	**43.0**	2.6	**44.2**	6.03
9	24.94	0.0300	866059	7.5	**30.0**	7.4	**30.9**	5.90
12	25.03	0.0214	575243	5.3	**21.4**	6.0	**22.0**	5.84

VII. Quercetin Dihydrate Content: 3b Samples (Qu:C 1:0.5 in buffer pH6)

Day #	Average Weight (mg)	Average Quercetin Conc (mg/mL)	Average Peak Area	Average Quercetin (mg/sample)	Average Recovery (%)	Average Recovery SD	**Avg corrected Recovery (%)**	pH
0	25.09	0.0974	3144656	24.4	**97.1**	1.1	**100.0**	6.04
1	24.83	0.0859	2712144	21.5	**86.5**	1.2	**89.1**	5.96
3	24.95	0.0750	2315133	18.8	**75.2**	0.7	**77.4**	5.91
6	25.08	0.0590	1795296	14.8	**58.8**	0.6	**60.6**	5.86
9	25.00	0.0485	1447965	12.1	**48.5**	8.2	**50.0**	5.80
12	25.04	0.0393	1125837	9.8	**39.1**	9.9	**40.3**	5.69

VIII. Quercetin Dihydrate Content: 4b Samples (Qu:C 1:1 in buffer pH6)

Day #	Average Weight (mg)	Average Quercetin Conc (mg/mL)	Average Peak Area	Average Quercetin (mg/sample)	Average Recovery (%)	Average Recovery SD	**Avg corrected Recovery (%)**	pH
0	24.99	0.0974	3144613	24.4	**97.5**	1.9	**100.0**	5.98
1	25.02	0.0939	2914993	23.5	**93.8**	1.1	**96.3**	5.87
3	24.91	0.0829	2578234	20.7	**83.2**	2.7	**85.3**	5.81
6	24.81	0.0796	2455170	19.9	**80.2**	1.7	**82.3**	5.83
9	24.98	0.0697	2112684	17.4*	**71.3**	3.2	**73.2**	5.77
12	25.11	0.0767	2278706	19.2	**76.4**	4.1	**78.3**	5.70

*n = 2

Interim Report of Quercetin Stability Study -3-

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October 13, 2008

1. Objective

This study was intended to compare the effects of different antioxidants (EGCG and propyl gallate) on the stability of quercetin under different pH conditions (pH = 3 and 6). The accelerated condition (75°C for 12 days) was selected from a typical extrapolation table for 1 year (365 days) at room temperature.

Table 1: Extrapolation table of temp vs. time for 1 year storage at room temperature based on the Arrhenius equation

Temp (°C)	Time (Day)
20-25 (average room temp)	365
30-35	182-183
40-45	90-91
50-55	45-46
60-65	22-23
70-75	11-12

2. Protocol

Eight different quercetin compositions (n = 3 per group) were prepared as shown below. Each sample (0.05% of quercetin; 25mg of quercetin in 50 mL of water) in a glass bottle with a lid was placed in a dark thermostatic oven at 75°C. The samples were analyzed on days 1, 3, 6, 9, and 12 by HPLC.

1a: Quercetin in buffer pH = 3 (control)

2a: Quercetin + Ascorbic acid (1:0.5) in buffer pH = 3

3a: Quercetin + EGCG (1:0.5) in buffer pH = 3

4a: Quercetin + propyl gallate (1:0.5) in buffer pH = 3

1b: Quercetin in buffer pH = 6 (control)

2b: Quercetin + Ascorbic acid (1:0.5) in buffer pH = 6

3b: Quercetin + EGCG (1:0.5) in buffer pH = 6

4b: Quercetin + propyl gallate (1:0.5) in buffer pH 6

3. Results and discussion

The study results are summarized in Table 1. Quercetin in combination with different antioxidants such as ascorbic acids (Sample 2a), EGCG (Sample 3a) and propyl gallate (Sample 4a)

with 1: 0.5 ratio at pH = 3 showed similar degradation patterns under these conditions. The quercetin degradation rates in all the samples 1a-4a were significantly retarded at day 9 and the degradation almost reached a plateau on day 12. This tendency is the same as that observed in our previous study reported on Aug 29. Quercetin's content was reduced about 18%, 24% and 22% in sample 2a, 3a and 4a, respectively, when heated for 12 days at 75°C. It is noted that propyl gallate and EGCG did not show any protective effect on quercetin degradation in comparison with quercetin alone (sample 1a). This result is in good accordance with our hypothesis that only the antioxidant dissolved in water with a redox potential comparable to that of quercetin can protect against quercetin's oxidative degradation. On the other hand, in the higher pH = 6, quercetin's content was reduced about 48%, 64% and 58% in sample 2b, 3b and 4b, respectively, when heated for 12 days at 75°C. However, Both EGCG and propyl gallate showed some protective effect on quercetin degradation in comparison with quercetin alone. It can be explained that as the pH becomes higher, the water-solubility of EGCG and propyl gallate go up, increasing the active concentrations dissolved in water. According to HPLC profiles, the degradation products identified were the same products as when quercetin was treated by flow-through aeration over the course of 3.5 hrs in a literature. These results indicate that the quercetin decomposition is initiated by oxygen under the accelerated condition, thus the decomposition ratio depends only on the concentration of oxygen in the air of the reaction media.

4. Conclusion

The protective effect on quercetin degradation is thought to depend on the active antioxidant concentration dissolved in the water. Water-soluble ascorbic acid among three antioxidants showed the best protecting effect on quercetin oxidative degradation at pH=3. EGCG and propyl gallate, practically water insoluble ones, showed no protecting effect at pH =3. On the other hand, at the higher pH 6, some protective effects were observed in both EGCG and propyl gallate, although the quercetin degradation ratio at the higher pH=6 is significantly increased in all the samples (1b-4b). It can be explained by the increase of the water-solubility of EGCG and propyl gallate at the higher pH 6.

Table 1:

I. Quercetin Dihydrate Content: 1a Samples (Qu in buffer pH3)

Day #	Average Weight (mg)	Average Quercetin Conc (mg/mL)	Average Peak Area	Average Quercetin (mg/sample)	**Average Recovery (%)**	Average Recovery SD	**Avg corrected Recovery (%)**	pH
0	25.18	0.0980	3155975	24.5	**97.3**	0.7	**100.0**	**3.13**
1	25.01	0.0911	2947679.667	22.8	**91.0**	1.6	**93.5**	**3.1**
2.98	25.12	0.0886	2748312.333	22.1	**88.1**	0.4	**90.6**	**3.12**
6	25.00	0.0811	2509833	20.3	**81.1**	1.7	**83.4**	**3.06**
9	25.07	0.0775	2268842	19.4	**77.3**	3.5	**79.4**	**3.05**
12	25.05	0.0773	2266012	19.3	**77.1**	2.1	**79.3**	**3.01**

II. Quercetin Dihydrate Content: 2a Samples (Qu:C 1:0.5 in buffer pH 3)

Day #	Average Weight (mg)	Average Quercetin Conc (mg/mL)	Average Peak Area	Average Quercetin (mg/sample)	**Average Recovery (%)**	Average Recovery SD	**Avg corrected Recovery (%)**	pH
0	24.84	0.0976	3182123	24.4	**98.2**	1.1	**100.0**	**3.13**
1	24.93	0.0938	3037276	23.5	**94.1**	0.2	**95.8**	**3.08**
3	24.96	0.0927	2878202	23.2	**92.9**	1.3	**94.6**	**3.08**
6	24.88	0.0877	2716854	21.9	**88.1**	1.1	**89.8**	**3.03**
9	24.99	0.0844	2474257	21.1	**84.4**	1.8	**85.9**	**3.04**
12	24.95	0.0815	2393223	20.4	**81.7**	3.0	**83.2**	**2.96**

III. Quercetin Dihydrate Content: 3a Samples (Qu:EGCG 1:0.5 in buffer pH 3)

Day #	Average Weight (mg)	Average Quercetin Conc (mg/mL)	Average Peak Area	Average Quercetin (mg/sample)	**Average Recovery (%)**	Average Recovery SD	**Avg corrected Recovery (%)**	pH
0	25.12	0.0994	3244431	24.9	**99.0**	0.4	**100.0**	**3.13**
1	24.86	0.0931	3012674	23.3	**93.6**	0.5	**94.5**	**3.10**
3	25.02	0.0868	2693464	21.7	**86.8**	1.6	**87.6**	**3.12**
6	24.94	0.0817	2528828	20.4	**81.9**	1.8	**82.8**	**3.06**
9	24.95	0.0776	2270417	19.4	**77.7**	1.4	**78.5**	**3.07**
12	24.94	0.0753	2204657	18.8	**75.5**	1.3	**76.2**	**3.01**

IV. Quercetin Dihydrate Content: 4a Samples (Qu:propyl gallate 1:0.5 in buffer pH 3)

Day #	Average Weight (mg)	Average Quercetin Conc (mg/mL)	Average Peak Area	Average Quercetin (mg/sample)	**Average Recovery (%)**	Average Recovery SD	**Avg corrected Recovery (%)**	pH
0	25.02	0.0996	3249148	24.9	**99.5**	1.0	**100.0**	**3.13**
1	24.97	0.0928	3003189	23.2	**92.9**	0.9	**93.3**	**3.12**
3	25.05	0.0888	2673393	22.2	**88.6**	1.8	**89.1**	**3.13**
6	24.91	0.0860	2662541	21.5	**86.3**	2.1	**86.7**	**3.07**
9	25.06	0.0802	2350262	20.1	**80.0**	2.3	**80.4**	**3.08**
12	25.11	0.0783	2297243	19.6	**78.0**	1.5	**78.4**	**3.03**

V. Quercetin Dihydrate Content: 1b Samples (Qu in buffer pH6)

Day #	Average Weight (mg)	Average Quercetin Conc (mg/mL)	Average Peak Area	Average Quercetin (mg/sample)	**Average Recovery (%)**	Average Recovery SD	**Avg corrected Recovery (%)**	pH
0	25.10	0.0965	3106659	24.1	**96.1**	1.1	**100.0**	**6.20**
1	24.92	0.0687	2218192	17.2	**68.9**	2.0	**71.7**	**6.08**
3	25.18	0.0512	1590856	12.8	**50.8**	2.0	**52.9**	**5.90**
6	24.98	0.0332	998930	8.3	**33.3**	4.9	**34.6**	**5.85**
9	25.05	0.0225	619775	5.6	**22.5**	3.0	**23.4**	**5.84**
12	24.84	0.0188	502102	4.7	**18.9**	2.2	**19.6**	**5.85**

VI. Quercetin Dihydrate Content: 2b Samples (Qu:C 1:0.5 in buffer pH 6)

Day #	Average Weight (mg)	Average Quercetin Conc (mg/mL)	Average Peak Area	Average Quercetin (mg/sample)	**Average Recovery (%)**	Average Recovery SD	**Avg corrected Recovery (%)**	pH
0	24.88	0.0970	3122808	24.3	**97.5**	0.1	**100.0**	**6.08**
1	24.84	0.0865	2799576	21.6	**87.1**	1.3	**89.3**	**5.89**
3	24.90	0.0745	2336107	18.6	**74.8**	3.5	**76.7**	**5.74**
6	25.18	0.0629	1892219	15.7	**62.4**	7.5	**64.0**	**5.69**
9	25.14	0.0563	1634491	14.1	**56.0**	3.0	**57.5**	**5.73**
12	24.78	0.0513	1483789	12.8	**51.8**	6.0	**53.1**	**5.70**

VII. Quercetin Dihydrate Content: 3b Samples (Qu:EGCG 1:0.5 in buffer pH 6)

Day #	Average Weight (mg)	Average Quercetin Conc (mg/mL)	Average Peak Area	Average Quercetin (mg/sample)	**Average Recovery (%)**	Average Recovery SD	**Avg corrected Recovery (%)**	pH
0	25.02	0.0955	3074900	23.9	**95.5**	0.9	**100.0**	**6.19**
1	25.08	0.0819	2539524	20.5	**81.7**	1.5	**85.5**	**6.03**
3	25.11	0.0618	1931448	15.5	**61.6**	2.8	**64.5**	**5.86**
6	25.06	0.0462	1391001	11.6	**46.1**	3.1	**48.3**	**5.82**
9	25.15	0.0370	1076325	9.3	**36.8**	10.3	**38.5**	**5.88**
12	24.96	0.0363	1029411	9.1	**36.4**	11.0	**38.1**	**5.83**

VIII. Quercetin Dihydrate Content: 4b Samples (Qu:propyl gallate 1:0.5 in buffer pH6)

Day #	Average Weight (mg)	Average Quercetin Conc (mg/mL)	Average Peak Area	Average Quercetin (mg/sample)	**Average Recovery (%)**	Average Recovery SD	**Avg corrected Recovery (%)**	pH
0	25.13	0.0978	3147838	24.4	**97.3**	0.9	**100.0**	**6.19**
1	24.81	0.0831	2577835	20.8	**83.8**	0.7	**86.1**	**6.05**
3	24.97	0.0640	2000293	16.0	**64.1**	0.3	**65.8**	**5.88**
6	25.00	0.0538	1619091	13.4	**53.8**	1.4	**55.3**	**5.84**
9	24.91	0.0411	1199414	10.3	**41.2**	7.4	**42.4**	**5.85**
12	24.93	0.0418	1195430	10.4	**41.9**	1.7	**43.1**	**5.82**

Certificate of Analysis

Emission Date : 12.11.2007

Material:	BR1410096.9020
Material Description:	QU995 FOOD GRADE QP FA QUERCETIN
Batch:	MM 02-7080
Inspection Plan:	F400024 – 1 / 1
Inspection Method:	PF400024 / 1
Manufacturing Date:	07/2007
Expiration Date:	07/2012

Test	Specification	Results
Description	Corresponds	Corresponds
Identity (HPLC)	Corresponds	Corresponds
Quercetin Assay (HPLC)	not less than 99,5 %	99,7 %
Aspect in Ethanol 2% (W/V)	not more than 15 NTU	5 NTU
Aspect in DMF 1% (W/V)	Corresponds	Corresponds
Arsenic (As)	not more than 1 ppm	<1 ppm
Cadmium (Cd)	not more than 1 ppm	<1 ppm
Cobalt (Co)	not more than 1 ppm	<1 ppm
Mercury (Hg)	not more than 1 ppm	<1 ppm
Nickel (Ni)	not more than 1 ppm	<1 ppm
Lead (Pb)	not more than 1 ppm	<1 ppm
Related Substances (TLC)	not more than 1 %	<1 %
Sulfated Ash	not more than 0,15 %	0,03 %
Water Assay (Karl Fischer)	not more than 4,0 %	1,3 %
Sulphate	not more than 1000 ppm	<1000 ppm
Chloride	not more than 1000 ppm	<1000 ppm
Apparent Density	not less than 0,25 g/ml	0,34 g/ml
Residual Ethanol	not more than 3000 ppm	382 ppm
Loss on Drying	not more than 4,0 %	0,9 %

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Certificate of Analysis

Emission Date : 12.11.2007

Material:	BR1410096.9020
Material Description:	QU995 FOOD GRADE QP FA QUERCETIN
Batch:	MM 02-7080
Inspection Plan:	F400024 – 1 / 1
Inspection Method:	PF400024 / 1
Manufacturing Date:	07/2007
Expiration Date:	07/2012

Test	Specification	Results
Total aerobic microbal count	not more than 5000 CFU/g	125 CFU/g
Total yeasts and molds count	not more than 500 CFU/g	60 CFU/g
Salmonella sp	Absent/10 g	Absent/10 g
Escherichia coli	Absent/g	Absent/g
Staphylococcus aureus	Absent/g	Absent/g
Pseudomonas aeruginosa	Absent/g	Absent/g

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Certificate of Analysis

Emission Date : 12.11.2007

Material:	BR1410096.9020
Material Description:	QU995 FOOD GRADE QP FA QUERCETIN
Batch:	MM 02-7080
Inspection Plan:	F400024 – 1 / 1
Inspection Method:	PF400024 / 1
Manufacturing Date:	07/2007
Expiration Date:	07/2012

Test	Specification	Results

Obs.:

Cas: (117-39-5)
Storage conditions:
Max. Temperature: 40°C / max. Humidity: 75%
Additional information:
Preserve closed and protected from light
Non-irradiated
Batch size: 960 kg

Moises E. de Carvalho Junior
Quality Assurance

Nivaldo M. Pereira
Responsible Pharmacist

This document has been produced electronically and is valid without a signature
Produced by Merck S.A. Brazil exclusively for Quercegen Pharma LLC, Newton, MA, USA
QU995 is Quercetin with a purity listed above measured by dry weight.
Maximum water content does not exceed 4,0%

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METHODS MANUAL

DETERMINATION OF QUERCETIN BY HPLC

TM200.131 – Excerpts from the method. *Proprietary information removed.*

Principle and Application

High Performance Liquid Chromatography (HPLC) is used to separate quercetin in beverage matrices, ingredients, and solid samples. The analyte is detected by Ultraviolet (UV) absorption and quantified against known standards. The amount of organic solvent (methanol) and acidic aqueous solution have been optimized so that the analyte is resolved and separated from matrix components in a relatively short analysis time.

Scope

This method measures quercetin in beverages, ingredients, and water dispersible solid materials. The method is validated for beverages.

Accuracy and Precision

Analyte	Concentration (mg/100 g)	Accuracy (%Recovery)	Single-Laboratory Data	
			Sigma	Precision (95% CI)
Quercetin (Beverages)	52	99.8	1.033	4.0
	104	100.5	1.318	2.5
	146	99.2	1.831	2.5

Accuracy and precision were determined by a single laboratory validation. Precision at the 95% confidence interval is the %RSD calculated using two standard deviations (2 sigma).

Equipment

- High Performance Liquid Chromatograph (HPLC) system comprising:
  - Autosampler
  - On-line degasser
  - Column thermostat
  - Single wavelength detection, UV-visible or Diode Array Detector
  - Software for data acquisition and processing
- Column: Waters Symmetry Shield, RP18, 250 x 4.6 mm 5 µm, Cat #186000112
- Guard Column: Phenomenex C18 4 mm x 3.0 mm with Holder, Cat #AJO-4287
- 13 mm x 0.45 µm PVDF membrane syringe filters, Whatman Cat #6779-1304
- Analytical balance ±0.0001 g
- Stir plate and magnetic stir bars
- Vacuum apparatus consisting of glass chamber, stopcocks, and collection rack, VWR Cat #BJ9400 or equivalent - used to degas mobile phase
- Laboratory Grade Sonicator, Branson or equivalent
- Digital timer
- 0.45 µm filter discs for filtration of mobile phase – Whatman 47 mm Cat #7404-004
- Handheld Variable Speed Shearer – "Tissue Tearor" Biospec Products Model #985370
- Volumetric flasks: clear: 25 mL, 500 mL, and 2 L. Amber colored 100 mL.
- Pipettors: 1 mL adjustable (must be calibrated); 5 mL adjustable (must be calibrated)
- Pipette tips: 1 mL, 5 mL

METHODS MANUAL

DETERMINATION OF QUERCETIN BY HPLC

- Disposable syringes with luer tip: 5 mL
- Disposable Transfer Pipettes
- Amber colored HPLC vials and caps
- Vials with closed top caps, VWR Cat #EP139-20ACT

Reagents / Chemicals

1. Quercetin Dihydrate, ≥98% purity, CAS #6151-25-3, Indofine Chemical, Cat #P-102
2. Methanol, HPLC grade, VWR Cat #MX0488-1
3. o-Phosphoric acid, minimum 85%, VWR Cat #MK278814
4. Deionized (DI) Water
5. Mobile phase preparation (isocratic) - 75% Methanol:25% 0.5% o-phosphoric acid

Equipment Preparation

1. **HPLC Parameters**
   - Column: Waters Symmetry Shield RP18 5µm, 250 x 4.6 mm analytical column. A Phenomenex C18 guard cartridge is required to extend column lifetime.
   - Column temperature: 45°C
   - Mobile phase: Isocratic - 75% Methanol:25% 0.5% o-phosphoric acid solution
   - Flow rate: 1 mL/min
   - Injection volume: 10 µL
   - Total run time: 10 min
   - UV-visible or Diode Array Detector: set at 260 nm wavelength.
   - Typical pressure: Approximately 120-180 bar, pressure changes with column usage.
   - Typical retention time: quercetin - 4.9 min

   Always equilibrate HPLC system by running mobile phase through at a flow rate of 1 mL/min for at least 20 min prior to use.

Sample Preparation

1. A 1.00 ml sample is pipetted to a tared 50 ml volumetric flask on a balance and the weight of the sample is recorded to the nearest 0.0001 g.
2. The sample is treated with an methanol solution to dissolve the Quercetin.
3. The sample is filtered through a syringe filter into a HPLC vial and injected for analysis.

References

1. Hasler and Sticher. "Identification and Determination of the Flavonoids from Ginkgo Biloba by High-Performance Liquid Chromatography." Journal of Chromatography, 605 (1992) 41-48.
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3. Antonella Saijaa, Antonio Tomainoa, et al. "In Vitro Antioxidant and Photo Protective Properties and Interaction with Model Membranes of Three New Quercetin Esters. European Journal of Pharmaceutics and Biopharmaceutics. Vol 56 (2003) 167–174.
4. [redacted] Good Lab Practice (GLP) Procedures 30000.1000, 30000.1100, 30000.1200, 30000.1300 and 30000.1400.

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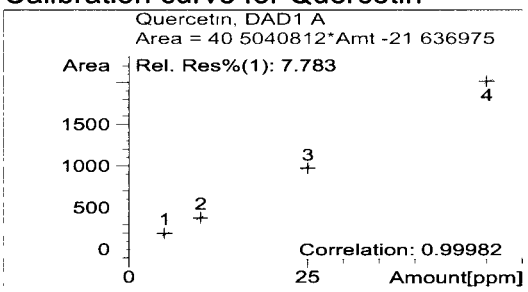
METHODS MANUAL

DETERMINATION OF QUERCETIN BY HPLC

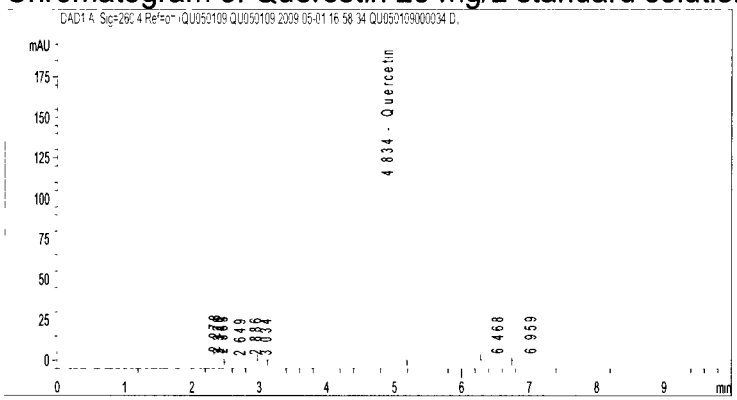
Appendix A: Calibration Curve

Calibration curve for Quercetin

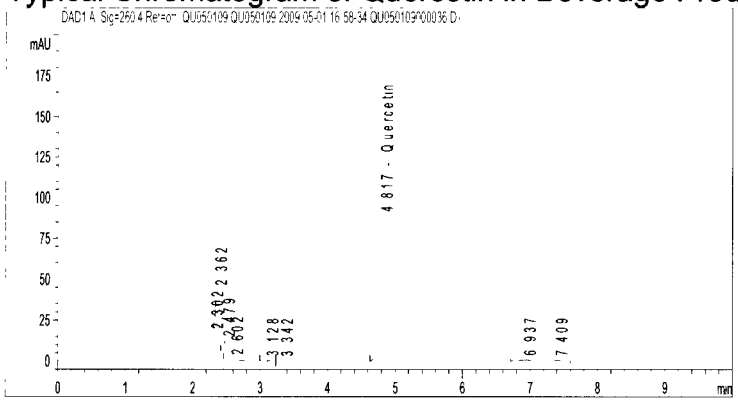


Appendix B: Standard and Sample Chromatograms

Chromatogram of Quercetin 25 mg/L standard solution



Typical Chromatogram of Quercetin in Beverage Product Matrix and Ingredient



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APPENDIX B

ESTIMATED CONSUMPTION OF QUERCETIN FROM PROPOSED FOOD USES

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Estimated Daily Intake of Quercetin by the U.S. Population from Proposed Food-Uses

Prepared by:

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April 5th, 2010

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Estimated Daily Intake of Quercetin by the U.S. Population from Proposed Food-Uses

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Table B-6 Estimated Daily per Kilogram Body Weight Intake of Quercetin from Individual Proposed Food-Uses by Male Adults (Aged 20 Years and Over) Within the United States (2003-2004, 2005-2006 NHANES Data)..... B-6

Table B-7 Estimated Daily per Kilogram Body Weight Intake of Quercetin from Individual Proposed Food-Uses by the Total Population (All Ages) Within the United States (2003-2004, 2005-2006 NHANES Data)..... B-7

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Estimated Daily Intake of Quercetin by the U.S. Population from Proposed Food-Uses

1.0 INTRODUCTION

Cantox Health Sciences International has completed an assessment of the consumption of quercetin by the United States (U.S.) population as proposed for use in beverages and beverage bases, grain-based products and pastas, processed fruits and fruit juices, and soft candies.

Estimates for 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, 2007, 2008). 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. Calculations for the mean and 90th percentile all-person and all-user intakes, and percent consuming were performed for each of the individual proposed food-uses of quercetin. Similar calculations were used to determine the estimated total intake of quercetin resulting from all proposed food-uses of quercetin combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

infants, ages 0 to 2;
children, ages 3 to 11;
female teenagers, ages 12 to 19;
male teenagers, ages 12 to 19;
female adults, ages 20 and up;
male adults, ages 20 and up; and
total population (all age and gender groups combined).

2.0 FOOD CONSUMPTION SURVEY DATA

2.1 Survey Description

National Health and Nutrition Examination Surveys (NHANES) for the years 2003-2004 and 2005-2006 are available for public use. NHANES are conducted as a continuous, annual survey, and are released in 2-year cycles. Each year about 7,000 people from 15 different locations across the U.S. are interviewed, and approximately 5,000 complete the health examination component of the survey. Any combination of consecutive years of data collection is a nationally representative sample of the U.S. population. It is well established that the length of a dietary survey affects the estimated consumption of individual users and that short-term

surveys, such as the typical 1-day dietary survey, overestimate consumption over longer time periods (Anderson, 1988). Because two 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) are available from the NHANES 2003-2004, 2005-2006 survey, these data were used to generate estimates for the current intake analysis.

NHANES 2003-2004, 2005-2006 survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person, and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S, of which 15 PSUs are visited per year. Small counties were combined to attain a minimum population size. These PSUs were segmented and households were chosen within each segment. One or more participants within a household were interviewed. For NHANES 2003-2004 12,761 individuals were selected for the sample, 10,122 were interviewed (79.3%), and 9,643 were sampled (75.6%). For NHANES 2005-2006 12,862 individuals were selected for the sample, 10,348 were interviewed (80.4%), and 9,950 were sampled (77.4%).

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2003-2004 and 2005-2006 collected socioeconomic, physiological and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. Sample weights were incorporated with NHANES 2003-2004 and 2005-2006 data to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (CDC, 2006; USDA, 2009a,b).

2.2 Statistical Methods

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of quercetin by the U.S. population. Estimates for the daily intake of quercetin represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2003-2004, 2005-2006 data; these average amounts comprised the distribution from which mean and percentile intake estimates were produced. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. All-person intake refers to the estimated intake of quercetin averaged over all individuals surveyed, regardless of whether they potentially consumed food products containing quercetin, and therefore includes "zero" consumers (those who reported no intake of food products containing

quercetin during the 2 survey days). All-user intake refers to the estimated intake of quercetin by those individuals potentially consuming food products containing quercetin, hence the "all-user" designation. Individuals were considered users if they consumed 1 or more food products containing quercetin on either Day 1 or Day 2 of the survey.

2.3 Statistical Reliability

Mean or percentile intake estimates based on small sample sizes or with high variability relative to the mean [assessed using the coefficient of variation (CV)] may be less statistically reliable than estimates based on adequate sample sizes or low variability relative to the mean (LSRO, 1995). Data presented herein for the estimated daily intake of quercetin follow the guidelines proposed by the Human Nutrition Information Service/National Center for Health Statistics Analytic Working Group for evaluating the reliability of statistical estimates adopted in the "Third Report on Nutrition Monitoring in the United States", whereby an estimated mean may be unreliable if the CV is equal to or greater than 30% (LSRO, 1995). The CV is the ratio of the estimated standard error of the mean to the estimated mean, expressed as a percentage (LSRO, 1995). Therefore, for the estimated intakes of quercetin presented herein, values were considered statistically unreliable if the CV was equal to or greater than 30% or the sample size is less than 30 respondents. These values were not considered when assessing the relative contribution of specific food-uses to total quercetin consumption and are marked with an asterisk.

3.0 FOOD USAGE DATA

The individual proposed food-uses and use-levels for quercetin employed in the current intake analysis are summarized in Table 3-1. Food codes representative of each proposed food-use were chosen from the NHANES 2003-2004, 2005-2006 24-hour dietary recall surveys (CDC, 2006; USDA, 2009a,b). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (CFR, 2008a). Product-specific adjustment factors were developed based on data provided in the standard recipe file for the CSFII 1994-1996, 1998 survey (USDA, 2000). All food codes included in the current intake assessment are listed in Appendix C.

Table 3-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Quercetin in the U.S.

Food Category	Proposed Food-Uses	Quercetin Level (mg/serving)	Serving Size (g or mL)	Use-Levels (%)
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 Candy	Dietetic or Low-Calorie Soft Candies	250 to 500	6	4.2 to 8.3

4.0 FOOD SURVEY RESULTS

Estimates for the total daily intakes of quercetin from all proposed food-uses are provided in Tables 4.1-1 and 4.1-2. Estimates for the daily intake of quercetin from individual proposed food-uses in the U.S. are summarized in Tables A-1 to A-7 and B-1 to B-7 of Appendices A and B, respectively. Tables A-1 to A-7 provide estimates for the daily intake of quercetin per person (mg/day), whereas Tables B-1 to B-7 provide estimates for the daily intake of quercetin on a per kilogram body weight basis (mg/kg body weight/day).

4.1 Estimated Daily Intake of Quercetin from All Proposed Food-Uses

The estimated total intake of quercetin from all proposed food-uses in the U.S. by population group is summarized in Table 4.1-1. Table 4.1-2 presents these data on a per kilogram body weight basis. These intakes were calculated using the both proposed use levels for soft candies (*i.e.* 250 and 500 mg/serving); however, the differences between these results were miniscule (less than 1 mg/person/day for the total population) and therefore, only the results for the higher use level are presented in Tables 4.1-1 and 4.1-2.

Approximately 37.4% of the total U.S. population was identified as potential consumers of quercetin from the proposed food-uses (6,252 actual users identified). Consumption of these types of foods by the total U.S. population resulted in estimated mean all-person and all-user intakes of quercetin of 67 mg/person/day (1 mg/kg body weight/day) and 205 mg/person/day (4 mg/kg body weight/day), respectively. The 90th percentile all-person and all-user intakes of quercetin from all proposed food-uses by the total population were 182 mg/person/day (4 mg/kg body weight/day) and 461 mg/person/day (9 mg/kg body weight/day), respectively.

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Table 4.1-1 Summary of the Estimated Daily Intake of Quercetin from All Proposed Food-Uses in the U.S. by Population Group (2003-2004, 2005-2006 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
				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

Within the individual population groups, the largest percentage of potential consumers of quercetin was identified in children where 55.9% of the population group (1,528 actual users) reported consuming foods in which quercetin is proposed for use. The lowest percentage of potential consumers was observed to occur in female adults with 27.3% of the survey population (566 actual users) reported consuming the same foods. On an individual population basis, the greatest mean all-person and all-user intakes of quercetin were determined to occur in male teenagers at 144 and 298 mg/person/day, respectively. The estimates for the mean all-person and all-user intakes of quercetin on an absolute basis were observed to be lowest in female adults at 39 and 152 mg/person/day, respectively. On a body weight basis, the estimates for the mean all-person and all-user intakes of quercetin were highest in infants, with intakes of 7 and 22 mg/kg body weight/day, respectively. The lowest all-person and all-user mean intakes on a per kilogram body weight basis was observed to occur in female adults, with values of 1 and 2 mg/kg body weight/day, respectively.

Table 4.1-2 Summary of the Estimated Daily per Kilogram Body Weight Intake of Quercetin from All Proposed Food-Uses in the U.S. by Population Group (2003-2004, 2005-2006 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
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

When heavy consumers (90th percentile) were assessed, the estimates for the all-person and all-user intakes of quercetin from all proposed food-uses was observed to be largest in male teenagers at 450 and 668 mg/person/day, respectively. The lowest estimates for the 90th percentile all-person and all-user intakes of quercetin were observed to occur in female adults with values of 105 and 288 mg/person/day, respectively. On a body weight basis, the estimates for the all-person and all-user 90th percentile intakes of quercetin were largest in children and infants, respectively, with values of 12 and 53 mg/kg body weight/day, respectively. The lowest all-person and all-user 90th percentile estimates for the intake of quercetin were observed to be 1 and 4 mg/kg body weight/day, respectively, occurring in female adults and teenagers, respectively.

4.2 Estimated Daily Intake of Quercetin From All Individual Food-Uses

In addition to examining the intake of quercetin resulting from concurrent consumption of all proposed food uses, the consumption of each individual proposed food uses also were assessed. Estimates for the mean and 90th percentile daily intakes of quercetin from each individual proposed food-use are summarized in Tables A-1 to A-7 and B-1 to B-7 on a mg/day and mg/kg body weight/day basis, respectively. Tables A-7 and B-7 summarize the estimates for the mean all-person intakes of quercetin by the total population (all ages) from each of the individual proposed food-uses on a mg/person/day and mg/kg body weight/day basis, respectively.

For all proposed food uses, the estimated intakes of quercetin were considered statistically unreliable if the CV was equal to or greater than 30% or the sample size was less than 30 individuals. Based on these criteria, the all-person and all-user intake estimates found the intake for soft candies and fruit smoothies were observed to be statistically unreliable in infants,

male and female teenagers, and male adults and as such these were not included in the discussion of the results.

4.2.1 All-Person Intakes from Individual Food Types

The total U.S. population was observed to be significant consumers of fruit-flavored juice-based drinks with 25.9% of the population identified as potential consumers of this proposed food use. Sports and energy drinks and cereal and energy bars were both observed to be consumed by approximately 7% of the total population while all other food uses were consumed by less than 2% of the total U.S. population. Consumption of sports and energy drinks provided the largest mean all-person intake of quercetin within the total U.S. population at 27 mg/person/day, while the highest 90th percentile intake was observed to result from the consumption of fruit-flavored juice-based drinks at 88 mg/person/day. On a body weight basis, the mean all-person intake of sports and energy drinks was equivalent to 0.5 mg/kg body weight/day and the 90th percentile intake of fruit-flavored juice-based drinks was equivalent to 1.6 mg/kg body weight/day.

Within the individual population groups, the consumption of fruit-flavored juice-based drinks provided the highest estimate for the mean all-person consumption of quercetin for infants, children, and female teenagers and adults. In male teenagers and adults the consumption of sports and energy drinks was estimated to provide the highest mean all-person intakes of quercetin. For the 90th percentile intake estimates, the highest intake was observed to result from the consumption of fruit-flavored juice-based drinks in all population groups with the exception of male teenagers, for whom the consumption of sports and energy drinks continued to be the largest source of quercetin. The highest mean and 90th percentile all-person intakes of quercetin resulting from any single proposed food use was observed to occur in male teenagers consuming energy, sport and isotonic drinks at 97 and 328 mg/person/day, respectively. On a body weight basis, the consumption of fruit-flavored juice-based drinks by infants resulted in the largest estimates for the mean and 90th percentile all-person intakes of quercetin at 1.4 and 5.2 mg/kg body weight/day, respectively.

4.2.2 All-User Intakes from Individual Food Types

Tables A-7 and B-7 also summarize the estimates for the mean all-user intakes of quercetin by the total population (all ages) from each of the individual food-uses on a mg/person/day and mg/kg body weight/day basis, respectively. For all-user intakes, the contribution of each food-use to the overall intake is a function of both the estimated intake of quercetin resulting from the consumption of the food, as well as the percentage of users identified as consumers of the food. For example, for the total population the consumption of sports and energy drinks resulted in an estimated mean all-user quercetin intake of 401 mg/person/day; however, only 1,164 users (7.0% of the total population) were identified as potential consumers of sports and energy drinks. As a result the mean all-user intake of quercetin resulting from its proposed use in fruit-flavored juice-based drinks estimated to be 125 mg/person/day based on 4,320 identified users

(25.9% of the total population), made a more significant contribution to the overall intake of quercetin.

Similar to the results observed for the mean all-user intake, the consumption of fruit-flavored juice-based drinks by the total U.S. population also made the most significant contribution to the 90th percentile estimate for the intake of quercetin at 245 mg/person/day. Within the individual population groups, the consumption of fruit-flavored juice-based drinks made the most significant contribution to the mean and 90th percentile intake estimates for the consumption of quercetin by infants, children, and female teenagers and adults. For male teenagers and adults the most significant contribution to the mean and 90th percentile estimates for the intake of quercetin resulted from the consumption of sports and energy drinks. The consumption of sports and energy drinks by male teenagers made the most significant overall contribution to the estimates for the mean and 90th percentile all-user intake of quercetin with intakes of 507 and 1,000 mg/person/day, respectively.

5.0 CONCLUSIONS

Consumption data and information pertaining to the individual proposed food-uses of quercetin were used to estimate the all-person and all-user intakes of quercetin for specific demographic groups and for the total U.S. population. This type of intake methodology 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 consumed relatively infrequently.

In summary, 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.

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APPENDIX A

Estimated Daily Intake of Quercetin from Individual Proposed Food-Uses by Different Population Groups Within the United States

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Table A-1 Estimated Daily Intake of Quercetin from Individual Proposed Food-Uses by Infants (Aged 0 to 2 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90th Percentile	Mean	90th Percentile
Beverages and Beverage Bases						
Sports and Energy Drinks	5.4	103	16	na	274	635
Grain Products and Pastas						
Cereal and Energy Bars	4.0	76	2	na	27	58
Grain-Based Beverages	1.6	30	<1	na	14	29
Processed Fruits and Fruit Juices						
Fruit-Flavored Juice-Based Drinks (non-carbonated and carbonated)	20.0	382	21	65	109	206
Fruit Smoothie Drinks	0.9	19	1*	na	69*	197*
Soft Candies						
Dietetic or Low-Calorie Soft Candies (8.3)	1.4	26	45*	na	1,924*	3,333*
Dietetic or Low-Calorie Soft Candies (4.2)	1.4	26	22*	na	962*	1,667*

na = not available
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

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Table A-2 Estimated Daily Intake of Quercetin from Individual Proposed Food-Uses by Children (Aged 3 to 11 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90th Percentile	Mean	90th Percentile
Beverages and Beverage Bases						
Sports and Energy Drinks	8.2	224	28	47	279	523
Grain Products and Pastas						
Cereal and Energy Bars	9.5	259	5	18	40	68
Grain-Based Beverages	1.4	40	0	na	9	18
Processed Fruits and Fruit Juices						
Fruit-Flavored Juice-Based Drinks (non-carbonated and carbonated)	45.0	1230	40	128	103	203
Fruit Smoothie Drinks	1.0	28	1	na	63	105
Soft Candies						
Dietetic or Low-Calorie Soft Candies (8.3)	1.9	51	22	na	1,004	1,625
Dietetic or Low-Calorie Soft Candies (4.2)	1.9	51	11	na	502	813

na = not available

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Table A-3 Estimated Daily Intake of Quercetin from Individual Proposed Food-Uses by Female Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90th Percentile	Mean	90th Percentile
Beverages and Beverage Bases						
Sports and Energy Drinks	7.4	148	22	na	284	508
Grain Products and Pastas						
Cereal and Energy Bars	10.3	205	7	23	45	98
Grain-Based Beverages	0.7	14	0	na	6	11
Processed Fruits and Fruit Juices						
Fruit-Flavored Juice-Based Drinks (non-carbonated and carbonated)	33.7	669	34	119	132	272
Fruit Smoothie Drinks	1.7	34	2	na	100	190
Soft Candies						
Dietetic or Low-Calorie Soft Candies (8.3)	0.9	17	5*	na	1,118*	2,166*
Dietetic or Low-Calorie Soft Candies (4.2)	0.9	17	3*	na	559*	1,083*

na = not available
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

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Table A-4 Estimated Daily Intake of Quercetin from Individual Proposed Food-Uses by Male Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90th Percentile	Mean	90th Percentile
Beverages and Beverage Bases						
Sports and Energy Drinks	14.6	282	97	328	507	1,000
Grain Products and Pastas						
Cereal and Energy Bars	9.0	174	7	27	57	101
Grain-Based Beverages	0.6	11	0	na	11	13
Processed Fruits and Fruit Juices						
Fruit-Flavored Juice-Based Drinks (non-carbonated and carbonated)	32.6	633	37	141	161	304
Fruit Smoothie Drinks	1.1	22	2	na	123	184
Soft Candies						
Dietetic or Low-Calorie Soft Candies (8.3)	0.4	8	2*	na	824*	542*
Dietetic or Low-Calorie Soft Candies (4.2)	0.4	8	1*	na	412*	896*

na = not available
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

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Table A-5 Estimated Daily Intake of Quercetin from Individual Proposed Food-Uses by Female Adults (Aged 20 Years and Over) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90th Percentile	Mean	90th Percentile
Beverages and Beverage Bases						
Sports and Energy Drinks	2.4	104	7	na	341	937
Grain Products and Pastas						
Cereal and Energy Bars	5.8	250	3	na	37	68
Grain-Based Beverages	1.4	61	0	na	8	11
Processed Fruits and Fruit Juices						
Fruit-Flavored Juice-Based Drinks (non-carbonated and carbonated)	18.0	769	17	63	112	233
Fruit Smoothie Drinks	1.9	79	2	na	102	197
Soft Candies						
Dietetic or Low-Calorie Soft Candies (8.3)	0.9	37	10	na	953	2,375
Dietetic or Low-Calorie Soft Candies (4.2)	0.9	37	5	na	511	1,139

na = not available

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Table A-6 Estimated Daily Intake of Quercetin from Individual Proposed Food-Uses by Male Adults (Aged 20 Years and Over) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90th Percentile	Mean	90th Percentile
Beverages and Beverage Bases						
Sports and Energy Drinks	7.9	303	39	na	458	937
Grain Products and Pastas						
Cereal and Energy Bars	4.9	187	3	na	39	71
Grain-Based Beverages	1.1	41	0	na	10	18
Processed Fruits and Fruit Juices						
Fruit-Flavored Juice-Based Drinks (non-carbonated and carbonated)	16.7	637	21	66	153	312
Fruit Smoothie Drinks	1.4	54	2	na	149	353
Soft Candies						
Dietetic or Low-Calorie Soft Candies (8.3)	0.4	16	6*	na	1,378*	3,500*
Dietetic or Low-Calorie Soft Candies (4.2)	0.4	16	3*	na	689*	1,750*

na = not available
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

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Table A-7 Estimated Daily Intake of Quercetin from Individual Proposed Food-Uses by the Total Population (All Ages) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90th Percentile	Mean	90th Percentile
Beverages and Beverage Bases						
Sports and Energy Drinks	7.0	1,164	27	na	401	781
Grain Products and Pastas						
Cereal and Energy Bars	6.9	1151	3	na	40	68
Grain-Based Beverages	1.2	197	<1	na	9	18
Processed Fruits and Fruit Juices						
Fruit-Flavored Juice-Based Drinks (non-carbonated and carbonated)	25.9	4320	23	88	125	245
Fruit Smoothie Drinks	1.4	236	2	na	114	198
Soft Candies						
Dietetic or Low-Calorie Soft Candies (8.3)	0.9	155	11	na	1,138	3,250
Dietetic or Low-Calorie Soft Candies (4.2)	0.9	155	5	na	569	1,625

na = not available

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APPENDIX B

Estimated Daily per Kilogram Body Weight Intake of Quercetin from Individual Proposed Food-Uses by Different Population Groups Within the United States

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Table B-1 Estimated Daily per Kilogram Body Weight Intake of Quercetin from Individual Proposed Food-Uses by Infants (Aged 0 to 2 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg)		All-User Consumption (mg/kg)	
			Mean	90th Percentile	Mean	90th Percentile
Beverages and Beverage Bases						
Sports and Energy Drinks	5.4	103	1.4	na	23.9	53.4
Grain Products and Pastas						
Cereal and Energy Bars	4.0	76	0.1	na	2.3	4.6
Grain-Based Beverages	1.6	30	<0.1	na	1.1	2.2
Processed Fruits and Fruit Juices						
Fruit-Flavored Juice-Based Drinks (non-carbonated and carbonated)	20.0	382	1.7	5.4	8.4	16.6
Fruit Smoothie Drinks	0.9	19	0.1	na	5.7	17.9
Soft Candies						
Dietetic or Low-Calorie Soft Candies (8.3)	1.4	26	3.7*	na	158.4*	361.1*
Dietetic or Low-Calorie Soft Candies (4.2)	1.4	26	1.9*	na	79.5*	180.6*

na = not available
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

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Table B-2 Estimated Daily per Kilogram Body Weight Intake of Quercetin from Individual Proposed Food-Uses by Children (Aged 3 to 11 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg)		All-User Consumption (mg/kg)	
			Mean	90th Percentile	Mean	90th Percentile
Beverages and Beverage Bases						
Sports and Energy Drinks	8.2	224	0.9	1.6	8.9	17.5
Grain Products and Pastas						
Cereal and Energy Bars	9.5	259	0.2	0.7	1.5	2.7
Grain-Based Beverages	1.4	40	<0.1	na	0.5	0.8
Processed Fruits and Fruit Juices						
Fruit-Flavored Juice-Based Drinks (non-carbonated and carbonated)	45.0	1230	1.6	4.9	4.0	8.1
Fruit Smoothie Drinks	1.0	28	<0.1	na	2.4	3.8
Soft Candies						
Dietetic or Low-Calorie Soft Candies (8.3)	1.9	51	1.0	na	45.7	84.2
Dietetic or Low-Calorie Soft Candies (4.2)	1.9	51	0.5	na	22.8	42.1

na = not available

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Table B-3 Estimated Daily per Kilogram Body Weight Intake of Quercetin from Individual Proposed Food-Uses by Female Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg)		All-User Consumption (mg/kg)	
			Mean	90th Percentile	Mean	90th Percentile
Beverages and Beverage Bases						
Sports and Energy Drinks	7.4	148	0.4	na	5.1	9.4
Grain Products and Pastas						
Cereal and Energy Bars	10.3	205	0.1	0.4	0.8	1.7
Grain-Based Beverages	0.7	14	<0.1	na	0.1	0.2
Processed Fruits and Fruit Juices						
Fruit-Flavored Juice-Based Drinks (non-carbonated and carbonated)	33.7	669	0.6	2.0	2.2	4.7
Fruit Smoothie Drinks	1.7	34	<0.1	na	1.7	2.6
Soft Candies						
Dietetic or Low-Calorie Soft Candies (8.3)	0.9	17	0.1*	na	20.7*	34.5*
Dietetic or Low-Calorie Soft Candies (4.2)	0.9	17	0.1*	na	10.4*	17.2*

na = not available
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

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Table B-4 Estimated Daily per Kilogram Body Weight Intake of Quercetin from Individual Proposed Food-Uses by Male Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg)		All-User Consumption (mg/kg)	
			Mean	90th Percentile	Mean	90th Percentile
Beverages and Beverage Bases						
Sports and Energy Drinks	14.6	282	1.4	5.2	7.5	15.8
Grain Products and Pastas						
Cereal and Energy Bars	9.0	174	0.1	0.5	1.0	1.6
Grain-Based Beverages	0.6	11	<0.1	na	0.2	0.2
Processed Fruits and Fruit Juices						
Fruit-Flavored Juice-Based Drinks (non-carbonated and carbonated)	32.6	633	0.6	2.0	2.6	5.4
Fruit Smoothie Drinks	1.1	22	<0.1	na	2.0	3.8
Soft Candies						
Dietetic or Low-Calorie Soft Candies (8.3)	0.4	8	<0.1*	na	13.0*	17.7*
Dietetic or Low-Calorie Soft Candies (4.2)	0.4	8	<0.1*	na	6.5*	8.9*

na = not available
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

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Table B-5 Estimated Daily per Kilogram Body Weight Intake of Quercetin from Individual Proposed Food-Uses by Female Adults (Aged 20 Years and Over) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg)		All-User Consumption (mg/kg)	
			Mean	90th Percentile	Mean	90th Percentile
Beverages and Beverage Bases						
Sports and Energy Drinks	2.4	104	0.1	na	5.1	11.4
Grain Products and Pastas						
Cereal and Energy Bars	5.8	250	<0.1	na	0.5	1.0
Grain-Based Beverages	1.4	61	<0.1	na	0.1	0.2
Processed Fruits and Fruit Juices						
Fruit-Flavored Juice-Based Drinks (non-carbonated and carbonated)	18.0	769	0.2	0.8	1.6	3.2
Fruit Smoothie Drinks	1.9	79	<0.1	na	1.4	2.8
Soft Candies						
Dietetic or Low-Calorie Soft Candies (8.3)	0.9	37	0.1	na	13.1	32.4
Dietetic or Low-Calorie Soft Candies (4.2)	0.9	37	0.1	na	6.5	16.2

na = not available

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Table B-6 Estimated Daily per Kilogram Body Weight Intake of Quercetin from Individual Proposed Food-Uses by Male Adults (Aged 20 Years and Over) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg)		All-User Consumption (mg/kg)	
			Mean	90th Percentile	Mean	90th Percentile
Beverages and Beverage Bases						
Sports and Energy Drinks	7.9	303	0.5	na	5.4	10.7
Grain Products and Pastas						
Cereal and Energy Bars	4.9	187	<0.1	na	0.5	0.9
Grain-Based Beverages	1.1	41	<0.1	na	0.1	0.3
Processed Fruits and Fruit Juices						
Fruit-Flavored Juice-Based Drinks (non-carbonated and carbonated)	16.7	637	0.2	0.8	1.8	3.7
Fruit Smoothie Drinks	1.4	54	<0.1	na	1.9	4.2
Soft Candies						
Dietetic or Low-Calorie Soft Candies (8.3)	0.4	16	0.1*	na	14.4*	32.6*
Dietetic or Low-Calorie Soft Candies (4.2)	0.4	16	<0.1*	na	7.2*	16.3*

na = not available
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

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Table B-7 Estimated Daily per Kilogram Body Weight Intake of Quercetin from Individual Proposed Food-Uses by the Total Population (All Ages) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg)		All-User Consumption (mg/kg)	
			Mean	90th Percentile	Mean	90th Percentile
Beverages and Beverage Bases						
Sports and Energy Drinks	7.0	1164	0.5	na	7.0	14.0
Grain Products and Pastas						
Cereal and Energy Bars	6.9	1151	0.1	na	0.8	1.7
Grain-Based Beverages	1.2	197	<0.1	na	0.3	0.6
Processed Fruits and Fruit Juices						
Fruit-Flavored Juice-Based Drinks (non-carbonated and carbonated)	25.9	4320	0.5	1.6	2.7	5.5
Fruit Smoothie Drinks	1.4	236	<0.1	na	1.8	3.6
Soft Candies						
Dietetic or Low-Calorie Soft Candies (8.3)	0.9	155	0.4	na	38.5	73.7
Dietetic or Low-Calorie Soft Candies (4.2)	0.9	155	0.2	na	19.3	36.8

na = not available

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APPENDIX C

Representative NHANES 2003-2004, 2005-2006 Food Codes for All Proposed Food-Uses of Quercetin in the United States

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Representative NHANES 2003-2004, 2005-2006 Food Codes for All Proposed Food-Uses of Quercetin in the United States

Beverages and Beverage Bases

Sports and Energy Drinks

(Quercetin concentration of 250 mg/RACC, RACC of 240 mL)
[Quercetin] = 1.04×10^{-1}%

92553000	Fruit-flavored thirst quencher beverage, low calorie
92560000	Fruit-flavored thirst quencher beverage
92560100	Gatorade Thirst Quencher sports drink
92560200	Powerade sports drink
92570100	Fluid replacement, electrolyte solution
92570500	Fluid replacement, 5% glucose in water
92650000	Red Bull Energy Drink
92651000	Energy drink

Powdered sports and isotonic drinks
(Adjusted for reconstitution assuming 16 g powder employed to create a 240 mL beverage)
[Quercetin] = 1.56%

92900300	Fruit-flavored thirst quencher beverage, dry concentrate, not reconstituted

Grain Products and Pastas

Cereal and Energy Bars

(Quercetin concentration of 50 mg/RACC, RACC of 40 g)
[Quercetin] = 1.3×10^{-1}%

53530000	Breakfast tart
53530010	Breakfast tart, lowfat
53540000	Breakfast bar, NFS
53540200	Breakfast bar, cereal crust with fruit filling, lowfat
53540250	Breakfast bar, cereal crust with fruit filling, fat free
53540500	Breakfast bar, date, with yogurt coating
53540600	Milk 'n Cereal bar
53542100	Granola bar, oats, sugar, raisins, coconut
53542200	Granola bar, oats, fruit and nuts, lowfat
53542210	Granola bar, nonfat
53543100	Granola bar, peanuts, oats, sugar, wheat germ
53544450	PowerBar (fortified high energy bar)
53541200	Meal replacement bar
53544200	Granola bar, chocolate-coated
53544210	Granola bar, with coconut, chocolate-coated
53544220	Granola bar with nuts, chocolate-coated
53544250	Granola bar, coated with non-chocolate coating
53544300	Granola bar, high fiber, coated with non-chocolate yogurt coating
53544400	Granola bar, with rice cereal
91780010	Snickers Marathon Energy bar
91781010	Snickers Marathon Protein bar

Grain-Based Beverages

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(Quercetin concentration of 10 mg/RACC, RACC of 240 mL)
[Quercetin] = 4.2×10^{-3}%

92201010	Postum
92202010	Chicory
92203000	Cereal beverage
92203110	Cereal beverage with beet roots, from powdered instant
92204000	Mate, sweetened beverage made from dried green leaves
92205000	Rice beverage
92306100	Corn beverage
92610010	Horchata, Puerto Rican
92610110	Coconut beverage, Puerto Rican
92611010	Oatmeal beverage, Puerto Rican
92611100	Oatmeal beverage with milk (Atole de avena)
92611510	Rice beverage, Mexican, (Horchata)
92612010	Sugar cane beverage, Puerto Rican
92613010	Atole (corn meal beverage)
92613510	Corn beverage with chocolate and milk (Champurrado, Atole de Chocolate)

Powdered Grain-Based Beverages
(Adjusted for not being reconstituted, assuming 15 g of powder to 240 mL of water)
[Quercetin] = 6.7×10^{-2}%

92203200	Cereal beverage with beet roots, powdered instant, dry
92291300	Postum, dry powder

Processed Fruit and Fruit Juices

Fruit-Flavored Drinks (Non-Carbonated and Carbonated)
(Quercetin concentration of 125 mg/RACC, RACC of 240 mL)
[Quercetin] = 5.2×10^{-2}%

Fruit-Flavored Drinks, Non-carbonated

92510150	Apple juice drink
92510170	Apple-cranberry-grape juice drink
92510200	Apple-orange-pineapple juice drink
92510220	Apricot-pineapple juice drink
92510610	Fruit juice drink
92510630	Fruit juice drink, NFS
92510820	Grape juice drink
92510910	Grapefruit juice drink
92510950	Guava juice drink
92511200	Orange-mango juice drink
92511230	Orange-apricot juice drink
92511250	Citrus fruit juice drink
92511260	Orange-cranberry juice drink
92511270	Orange-peach juice drink
92511280	Orange-grape-banana juice drink
92511290	Papaya juice drink
92511310	Pineapple-grapefruit juice drink
92511340	Pineapple-orange juice drink
92511350	Orange-raspberry juice drink
92530410	Citrus drink with vitamin C added
92530510	Cranberry juice drink with vitamin C added

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92530520	Cranberry-apple juice drink with vitamin C added
92530610	Fruit juice drink, with high vitamin C
92530810	Grapefruit juice drink with vitamin C added
92530840	Guava juice drink with vitamin C added
92530950	Vegetable and fruit juice drink, with vitamin C added
92531020	Orange breakfast drink, made from frozen concentrate
92531030	Fruit juice drink, with thiamin (vitamin B1) and vitamin C
92531110	Pineapple-grapefruit juice drink with vitamin C added
92531120	Pineapple-orange juice drink with vitamin C added
92531150	Pineapple-orange-grapefruit juice drink with vitamin C added
92550030	Fruit juice drink, low calorie
92550050	Apple-white grape juice drink, low calorie, with vitamin C added
92550110	Cranberry juice drink, low calorie, with vitamin C added
92550210	Cranberry-apple juice drink, low calorie, with vitamin C added
92550300	Grapefruit juice drink, low calorie, with vitamin C added
92551700	Juice drink, low calorie
92552020	Fruit juice drink, reduced sugar, with thiamin (vitamin B1) and vitamin C
92552100	Orange-cranberry juice drink, low calorie, with vitamin C added
92582100	Citrus juice drink, calcium fortified
92582110	Fruit juice drink, with thiamin (vitamin B1) and vitamin C plus calcium
92510120	Apple-cherry drink
92510310	Banana-orange drink
92510410	Black cherry drink
92510720	Fruit punch, made with fruit juice and soda
92510730	Fruit punch, made with soda, fruit juice, and sherbet or ice cream
92510810	Grapeade and grape drink
92511220	Orange drink
92511240	Orange-lemon drink
92530310	Cherry drink with vitamin C added
92550350	Light orange juice beverage, 40-50% juice, lower sugar and calories, with artificial sweetener

Fruit-Flavored Drinks, Carbonated

92431000	Carbonated juice drink, NS as to type of juice
92432000	Carbonated citrus juice drink
92433000	Carbonated noncitrus juice drink

Fruit Smoothie Drinks
(Quercetin concentration of 125 mg/RACC, RACC of 240 mL)
[Quercetin] = 5.2×10^{-2}%

11553000	Fruit smoothie drink, made with fruit or fruit juice and dairy products
11553100	Fruit smoothie drink, NFS
64134000	Fruit smoothie drink, made with fruit or fruit juice only (no dairy products)

Soft Candies

Dietetic or Low-Calorie Soft Candies
(Quercetin concentration of 250 to 500 mg/RACC, RACC of 6 g)
[Quercetin] = 4.2 to 8.3%

91770000	Dietetic or low calorie candy, NFS
91770010	Dietetic or low calorie gumdrops

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91770030	Dietetic or low calorie candy, chocolate covered
91703080	Caramel, all flavors, sugar free
91708100	Fruit snack candy, with added vitamin C

Submission End

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