A critical review of the data related to the safety of quercetin and lack of evidence of *in vivo* toxicity, including lack of genotoxic/carcinogenic properties

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Abstract

Quercetin is a naturally-occurring flavonol (a member of the flavonoid family of compounds) that has a long history of consumption as part of the normal human diet. Because a number of biological properties of quercetin may be beneficial to human health, interest in the addition of this flavonol to various traditional food products has been increasing. Prior to the use of quercetin in food applications that would increase intake beyond that from naturally-occurring levels of the flavonol in the typical Western diet, its safety needs to be established or confirmed. This review provides a critical examination of the scientific literature associated with the safety of quercetin. Results of numerous genotoxicity and mutagenicity, short- and long-term animal, and human studies are reviewed in the context of quercetin exposure *in vivo*. To reconcile results of *in vitro* studies, which consistently demonstrated quercetin-related mutagenicity to the absence of carcinogenicity *in vivo*, the mechanisms that lead to the apparent *in vitro* mutagenicity, and those that ensure absence of quercetin toxicity *in vivo* are discussed. The weight of the available evidence supports the safety of quercetin for addition to food.
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1. Introduction

Quercetin [3,3′,4′,5,7-pentahydroxyflavone, CAS no. 117-39-5] is one of several naturally-occurring dietary fla- vonol compounds belonging to a broad group of polyphe- nolic flavonoid substances (see Fig. 1). Flavonoids are characterized by a phenyl benzo(γ)pyrone-derived structure consisting of two benzene rings (A and B in Fig. 1) linked
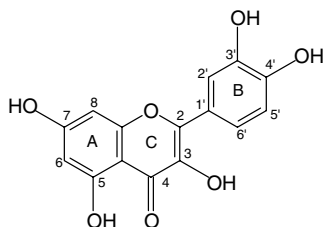
Fig. 1. Chemical structure of quercetin.

by a heterocyclic pyran or pyrone ring (C in Fig. 1) (Kühnau, 1976; Morand et al., 1998). In plants, the flavonol aglycone is most commonly present conjugated at the 3-position of the unsaturated C-ring with a sugar moiety, forming *O*-β-glycosides such as quercitrin or rutin (Merck, 2001). Quercetin can be obtained from plants via extraction of the quercetin glycosides followed by hydrolysis to release the aglycone and subsequent purification. Flavonols exhibit numerous biological and pharmacological effects, including anti-oxidant, chelation, anti-carcinogenic, cardioprotective, bacteriostatic, and secretory properties (Gross et al., 1996; Middleton et al., 2000; PDRNS, 2001). In plants, these compounds are involved in energy production (Theoharides et al., 2001) and exhibit strong anti-oxidant properties, possibly protecting plants against harmful ultraviolet rays (Wiczkowski et al., 2003).

The Joint FAO/WHO Expert Committee on Food Additives evaluated quercetin for use in food in 1977 (JECFA, 1977), but the limited amount of toxicity data available at the time of the evaluation precluded establishing an acceptable daily intake (ADI). Subsequently, in 1998 the International Agency for Research on Cancer evaluated quercetin for its potential carcinogenic risk to humans, assigning an overall Group 3 classification (i.e., *not classifiable as to its carcinogenicity to humans*) (IARC, 1999). Until recently, quercetin has been marketed in the United States primarily as a dietary supplement (Theoharides and Bielory, 2004), with recommended daily doses of supplemental quercetin of 200–1200 mg (PDRNS, 2001). In 2004, food-grade quercetin of high purity (i.e., 98.5% and higher, and marketed as QU 985, QU 995, QU 998, and QU 1000) was self-affirmed as generally recognized as safe for use in a variety of foods at use-levels in the range of 0.008–0.5% or 10–125 mg/serving (Quercegen Pharma, LLC (personal communication), 2004). Based on the specified use-levels of quercetin in foods such as breakfast cereals, chewing gum, fats and oils, frozen dairy desserts and mixes, grain products and pastas, hard and soft candies, milk and plant protein products, beverages and beverage bases, and processed fruits and fruit juices, it was calculated that under a worst-case scenario of estimating intake, a heavy-end consumer of quercetin (90th percentile) would not be exposed to more than 4.70 mg quercetin/kg body weight/day (226 mg quercetin/person/day) from the intended addition of quercetin to foods. In Japan, quercetin is permitted as a food additive under the *List of Existing Food Additives* (MHLW, 1996).

Because of the prevalence of quercetin in the diet and its potential clinical and food applications, the safety of quercetin has been evaluated extensively in a variety of genotoxicity assays and a full range of acute, subchronic, chronic, and reproductive toxicity studies. In an attempt to reconcile the differences observed between *in vitro* results demonstrating quercetin-related mutagenic activity and the absence of carcinogenicity *in vivo*, several reviews of some of the data available for quercetin were conducted. Most recently, Okamoto (2005) provided an extensive overview of the safety data available for quercetin. Presently, the bioavailability and anti-oxidant properties of quercetin appear to be two areas of intense research. Specifically, validation of quercetin as a potent anti-oxidant *in vivo*, but also realizing its potential for pro-oxidant activity following oxidation, are of prime interest in an effort to determine its clinical applicability and acceptability for use in food. Moreover, the pro-oxidant state of quercetin, as a consequence of its potent anti-oxidant activity, may provide insight into its apparent *in vitro* mutagenicity. Regardless, the available data suggest that *in vivo* protective mechanisms adequately limit any potential for adverse effects related to quercetin pro-oxidant activity.

The present review is a critical evaluation of the safety of quercetin, which expands on some of the points presented by Okamoto (2005) and considers additional data to further support the absence of dietary quercetin-related carcinogenicity *in vivo*. Searches of several scientific literature databases (e.g., PubMed, MEDLINE®, EMBASE®, and BIOSIS Previews®) were conducted through January 2007 and only papers related to mechanism of action, metabolic fate, genotoxicity and mutagenicity, potential short- and long-term animal toxicity and carcinogenicity, and human safety were selected for inclusion in this review. The implications of the *in vitro* results and their usefulness in determining the potential for quercetin toxicity *in vivo*, in light of the largely negative results obtained in animal studies, are assessed, with special emphasis placed on the pro-oxidant properties of quercetin as a potential mechanism for its *in vitro* mutagenicity.

2. Natural dietary occurrence of quercetin

Flavonols occur ubiquitously in the human diet as glycosides, with wide distribution in the edible portions of food plants, including berries, citrus, and various other fruits, leafy vegetables, roots, tubers and bulbs, herbs and spices, legumes, and cereal grains, as well as in tea and cocoa (Brown, 1980). Fruits and vegetables, particularly apples, cranberries, blueberries, and onions, are the primary sources of naturally-occurring dietary quercetin of the typical Western diet and contain the flavonol at levels as high as ~350 ppm (expressed as the aglycone) (Day and Williamson, 1999; Harnly et al., 2006). Black tea, as well as red wine and various fruit juices, also were identified as rich dietary sources of quercetin (Hertog et al., 1993; Sampson et al., 2002).

In the United States, the average daily intake of all flavonoids (flavanones, flavones, flavonols, anthocyanins, catechins, and biflavans) from a normal mixed diet is estimated at ~1 g/day [expressed as quercitrin equivalents, where one biflavan molecule is equal to 2 molecules of quercitrin], of which, depending on seasonal variations, 160–175 mg/day is accounted for by flavanones, flavones, and flavonols (Kühnau, 1976; Brown, 1980). It is estimated that flavonol glycosides, expressed as quercetin equivalents, are consumed at levels of up to ~100 mg/day (Brown, 1980; Jones and Hughes, 1982; NTP, 1992; Rimm et al., 1996; USDA, 2000; Sampson et al., 2002). National dietary record-based cohort assessments (e.g., from Australia, the Netherlands, Finland, Italy, Croatia, Japan, and the United States) of the intake of quercetin from the habitual diet indicated mean consumption levels in the range of less than 5 mg to ~40 mg quercetin/day (Hertog et al., 1995; Rimm et al., 1996; Knekt et al., 1997; Kimira et al., 1998; Johannot and Somerset, 2006; Lin et al., 2006); however, daily levels of quercetin as high as 200–500 mg may be attained by high-end consumers of fruits and vegetables, especially in cases where the individuals consume the peel portion of quercetin-rich fruits and vegetables, such as tomatoes, apples, and onions (Jones and Hughes, 1982; USDA, 2000).

3. Biological properties of quercetin

As cited by Middleton et al. (2000) and Erlund (2004), anti-oxidant, anti-carcinogenic, anti-inflammatory, and cardioprotective properties are several key biological functions ascribed to quercetin. Middleton et al. (2000) stressed the anti-carcinogenic properties of quercetin and other flavonoids. Galati and O'Brien (2004) also reviewed the ability of certain flavonoids to prevent tumor development and also raised the possibility of flavonoid–drug interactions. It remains to be determined whether these properties of quercetin are effected independently or share a common mechanism of action. For example, several authors have related the anti-carcinogenic and anti-inflammatory effects to the anti-oxidant and free radical scavenging properties of quercetin (Stavric, 1994; Formica and Regelson, 1995). The anti-oxidant properties are largely a function of the chemical structure of quercetin, particularly the presence and location of the hydroxyl (–OH) substitutions and the catechol-type B-ring (Rice-Evans et al., 1996; Wang et al., 2006). Bors et al. (1990) elucidated the structural features of an effective anti-oxidant, which consisted of the presence of (i) an *ortho*-dihydroxy or catechol group in the B-ring, (ii) a 2,3-double bond, and (iii) hydroxyl substitution at positions 3 and 5. Several authors have pointed out that quercetin, which is characterized by a hydroxylation pattern of 3, 5, 7, 3′, and 4′ and a catechol B-ring, possesses all the structural elements characteristic of an anti-oxidant (Silva et al., 2002; Rietjens et al., 2005); however, by exerting its anti-oxidant activity, quercetin may be converted into reactive products (Metodiewa et al., 1999; Boots et al., 2003).

In vitro, the oxidative degradation of quercetin has been reported to result in the formation of a free radical *ortho*-semiquinone intermediate, which may subsequently be converted to the parent compound or alternatively to an *ortho*-quinone, accompanied by the production of reactive oxygen species (ROS), such as superoxide and hydrogen peroxide (H_2O_2) (Metodiewa et al., 1999; Boots et al., 2003). The *ortho*-quinone is subject to tautomerisation to produce one of three possible quinone methides. Oxidation of quercetin by H_2O_2 in the presence of horseradish peroxidase or tyrosinase to the reactive intermediates, *ortho*-quinone/quinone methides, was demonstrated *in vitro* (Boersma et al., 2000; Awada et al., 2002; Kaldas et al., 2005). Consequently, the possible pro-oxidant activity of quercetin, particularly at high-dose levels, must be addressed (Rietjens et al., 2005). The pro-oxidant mechanism may be responsible for the *in vitro* mutagenic activity of quercetin. For example, under aerobic conditions, quercetin was reported to produce dose-dependent DNA damage and lipid peroxidation in isolated rat liver nuclei (Sahu and Washington, 1991) and damage in calf thymus DNA (Rahman et al., 1992), effects that were increased in the presence of copper and iron ions and which the authors concluded were the result of oxygen radicals produced by auto-oxidation of the flavonol.

Formation of the *ortho*-quinone/quinone methide intermediates, which are capable of covalently binding to DNA, is very likely a major contributor in quercetin-related mutagenicity (Rietjens et al., 2005). Quercetin binding to cellular DNA and protein has been observed *in vitro* following oxidation of quercetin presumably by H_2O_2/peroxidase (Walle et al., 2003). Such oxidative conditions (i.e., H_2O_2 and catalytic amounts of horseradish peroxidase) also were shown to significantly increase the binding capacity of quercetin (25 μM) to human serum albumin (13.8 μM); however, the addition of glutathione (GSH) at normally occurring cellular concentrations (5 mM) was observed to completely suppress the binding (Kaldas et al., 2005). Although binding of the quercetin aglycone to DNA also was observed in intact cultured human cell lines (i.e., intestinal Caco-2 and hepatic HepG2 cells) (Walle et al., 2003; van der Woude et al., 2006), which do not contain any detectable levels of intracellular oxidative enzymes, methylation of quercetin at the 3′- and 4′-hydroxyl moiety decreased the binding capacity by more than 50% (van der Woude et al., 2006).

A number of *in vitro* studies were performed with quercetin under several experimental conditions to monitor various indices of anti-oxidant/pro-oxidant activity. In an effort to resolve the role of quercetin as an anti-oxidant versus a pro-oxidant, Lee et al. (2003) incubated mouse thymocytes with quercetin (50 μM) and observed that, while quercetin alone did not induce any cytotoxicity, it did exhibit anti-oxidant activity by protecting cells against oxidative stress-mediated apoptosis. Conversely, in human cells

tested at concentrations of up to 300 μM, quercetin was moderately cytotoxic in lung embryonic fibroblasts, but did induce significant levels of cytotoxicity in umbilical vein endothelial cells [i.e., 50% lethal concentrations (LC_{50}) of 303 and 61 μM, respectively] (Matsuo et al., 2005). Furthermore, when added to cultured lung embryonic fibroblasts, quercetin increased ROS levels, but not in a dose-dependent fashion. In human red blood cells, quercetin was shown to oxidize some erythrocyte oxyhemoglobin, producing methemoglobin, with an increase in activity observed in the presence of extracellular horseradish peroxidase and H_2O_2, but did not markedly increase the incidence of hemolysis (Galati et al., 2002). Following an investigation of the anti-oxidant/pro-oxidant effects of 10 μM quercetin on nuclear factor κB (NF-κB) binding activity, which is recognized to increase in the presence of ROS, and DNA integrity in HepG2 cells, Musonda and Chipman (1998) concluded that the anti-oxidant potential of quercetin surpasses its oxidative potential in a cellular environment, as quercetin did not induce DNA strand breaks, but did inhibit H_2O_2-mediated DNA strand breakage and NF-κB activity.

Evaluated *in vitro* in rat hepatocytes with added H_2O_2 and peroxidase, quercetin was demonstrated to rapidly co-oxidize ascorbate, which was likely mediated by the semiquinone radical; however, in contrast to its phenol B-ring-containing counterpart, kaempferol, quercetin did not co-oxidize reduced β-nicotinamide adenine dinucleotide (NADH) or GSH (Galati et al., 2002). The absence of NADH oxidation, which requires *ortho*-quinone formation, supports the fact that the semiquinone quercetin intermediate was likely responsible for ascorbate oxidation. Although quercetin is oxidized to an *ortho*-quinone intermediate, the *ortho*-quinone isomerizes to quinone methides, which are not conducive to either NADH or ascorbate oxidation. The quercetin-induced depletion of GSH levels that accompanied the oxidative reaction in the isolated hepatocytes was determined to be due to GSH conjugation of the quinone methide reactive products, rather than GSH oxidation. Thus, it does not appear that the quercetin oxidative products oxidize GSH to form reactive thiyl radicals (GS). Conjugation of the quercetin intermediates with GSH resulting in the formation of mono-GSH and bis-GSH conjugates was confirmed in an earlier study (Galati et al., 2001).

Using levels of GSH, enzymatic activity, and markers of lipid peroxidation as indicators of anti-oxidant/pro-oxidant activity, several studies were conducted in rats and mice to evaluate these potential quercetin-related effects *in vivo*. In comparison to a control group, significant increases in the levels of hepatic and pulmonary glutathione-*S*-transferase (GST) and GSH levels were noted in an 8-week mouse study in which the test animals received up to 2.6 mg quercetin/kg body weight/day via the drinking water (Gandhi and Khanduja, 1993). In contrast, Duarte et al. (2001) reported a decrease in levels of liver GSH in quercetin-treated (10 mg quercetin/

kg body weight/day by gavage) normotensive Wistar Kyoto rats compared to untreated controls, which was not accompanied by any changes in glutathione peroxidase (GP_x) activity or glutathione reductase (GR) levels. Levels of liver and plasma malondialdehyde (MDA), a lipid peroxidation product, in quercetin-treated rats were comparable to controls or slightly reduced (not statistically significant), respectively. In another study, increases in GST and GR activities were observed in segments of the colon mucosa of Sprague–Dawley rats maintained on quercetin-supplemented diets (up to 1% of the diet or ∼500 mg/kg body weight/day); however, no changes in the activity of liver enzymes were reported (Fischer et al., 2002).

Choi et al. (2003, 2005) assessed potential quercetin-related pro-oxidant activity in male Sprague–Dawley rats following 4- to 6-week oral treatments with up to 20 mg quercetin/rat/day (approximately 78 mg/kg body weight/day). Quercetin was observed to significantly decrease hepatic levels of GSH, while concomitantly increasing, albeit only slightly and not at levels of statistical significance, the activity of GP_x and decreasing GR activity compared to controls. Moreover, quercetin increased serum and liver vitamin E levels and reduced hepatic MDA levels, particularly in vitamin E-deprived rats. Interestingly, in combination with daidzein (20 mg/day), a flavonoid present in soy, the reduction in hepatic GSH levels and GR activity was prevented. Conversely, Rangan et al. (2002) observed elevated levels of renal MDA production in male Wistar rats fed quercetin in the diet for a period of 21 days at dose levels of 285 or 1133 mg/kg body weight/day. In another study in which quercetin was administered to rats for a period of 5 weeks at dose levels of 10 mg/kg body weight/day (2 mg daily) by gavage, plasma levels of thiobarbituric acid (TBARS) and nitrates plus nitrites (NO_x), markers of oxidative liver stress, were comparable to controls (García-Saura et al., 2005). With the exception of a slight reduction in liver GSH levels in quercetin-treated rats, no significant changes were observed in liver GST, GP_x, or GR activity between test and control rats. Moreover, gavage administration of quercetin to rats at a dose level of 135 mg/kg body weight/day for 3 consecutive days did not significantly enhance the activity of oxidative scavengers, superoxide dismutase (SOD) and catalase (Cierniak et al., 2004). Although variations in levels of GSH and enzymatic activity relevant to anti-oxidative processes indicate that quercetin is active *in vivo*, the long-term oral animal toxicity studies (as discussed below) demonstrate the safety of dietary quercetin.

4. Metabolic fate of quercetin

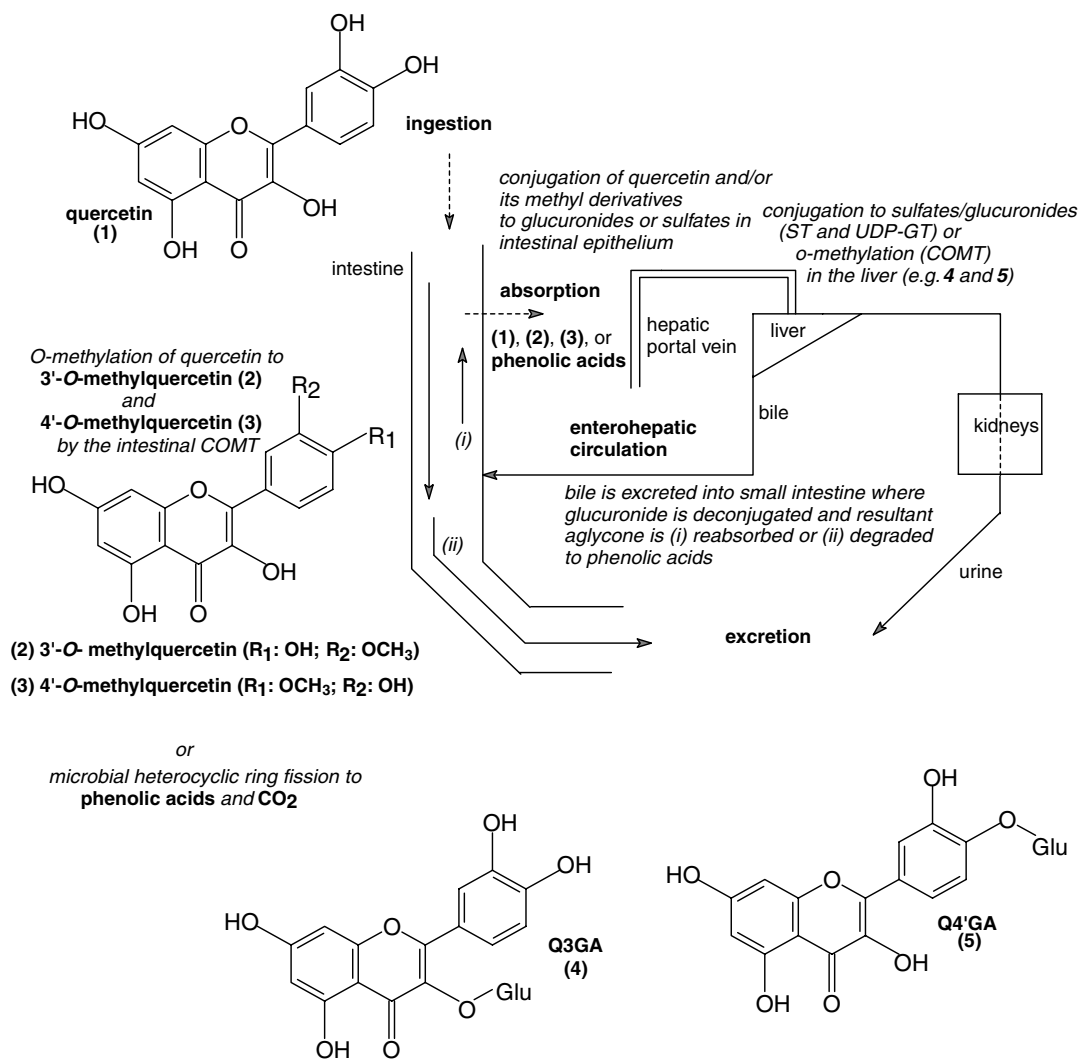### 4.1. Absorption and metabolism

Since the potential toxicity of quercetin, as well as any of its putative beneficial pharmacological effects are largely dependent on its bioavailability following oral administra-

tion, the absorption, distribution, metabolism, and excretion of quercetin have been extensively studied in laboratory animals and humans. As depicted in Fig. 2, quercetin may be *O*-methylated, primarily resulting in the formation of 3′-*O*-methylquercetin (isorhamnetin) and to a smaller extent, 4′-*O*-methylquercetin (tamaraxetin), sulfated, or glucuronidated at one of the hydroxyl groups in the absorptive cells of the intestinal epithelium following ingestion of the aglycone (Crespy et al., 1999; Murota et al., 2000; Rechner et al., 2002; Murota and Terao, 2005; Graf et al., 2006). Subsequently, the resulting quercetin derivatives and any remaining unmetabolized quercetin are released into the circulation via the hepatic portal vein. Alternatively, Murota and Terao (2005) recently demonstrated that quercetin may be absorbed systemically from the gastrointestinal tract via the lymph. In the liver, quercetin and its derivatives are further subjected to conjuga-

tion, resulting in the formation of sulfate and/or glucuronide derivatives (Shali et al., 1991; Morand et al., 1998; Oliveira and Watson, 2000; Boersma et al., 2002). Additionally, the catechol-*O*-methyltransferase (COMT) enzymes of the liver and kidneys also may participate in further methylation of quercetin or its derivatives (Onishi et al., 1982; Zhu et al., 1994; De Santi et al., 2002; O'Leary et al., 2003; Graf et al., 2006).

Alternatively, quercetin may be degraded to one of several different phenolic acids (e.g., 3,4-dihydroxyphenylacetic acid) and carbon dioxide (CO_2) by the colonic microflora (heterocyclic ring fission) (Murray et al., 1954; Booth et al., 1956; DeEds, 1968; Krishnamurty et al., 1970; Stelzig and Ribeiro, 1972; Ueno et al., 1983; Gross et al., 1996; Pietta et al., 1997; Braune et al., 2001; Justesen and Arrigoni, 2001; Rechner et al., 2002; Olthof et al., 2003; Weldin et al., 2003). Chen et al. (2005) specifically evaluated the

Adapted from Day and Williamson (1999)

Fig. 2. Schematic representation of the absorption, metabolism, and excretion of quercetin in mammals.

relative contribution of the gut and liver in the metabolism of quercetin in rats. More than 90% of an orally administered dose of quercetin was metabolized in the gut prior to absorption, while metabolism in the liver accounted for a further 3%.

Following administration of an oral dose of radiolabeled quercetin to male ACI rats, only ∼20% of the radiolabel was estimated to be absorbed (Ueno et al., 1983). More recently, Chen et al. (2005) demonstrated absorption of ∼60% of total quercetin (i.e., free and conjugated quercetin and its metabolites) and 5% of unchanged quercetin following oral administration of a single dose of 10 mg/kg body weight of quercetin to male Sprague–Dawley rats. Evaluated in ileostomy patients, 24% of total quercetin was absorbed following ingestion of 100 mg of the quercetin aglycone (Hollman et al., 1995, 1997), while in healthy subjects provided 100 mg of radiolabeled quercetin, up to 53% of the total administered radioactivity was absorbed (Walle et al., 2001). In plasma samples collected from laboratory animals and humans following quercetin administration or consumption of quercetin-rich foods or herbal extracts, sulfate and glucuronide conjugates of quercetin and its *O*-methylated derivatives were identified (Zhu et al., 1994; Manach et al., 1995, 1997, 1998, 1999; Morand et al., 1998, 2000a,b; Ader et al., 2000; Morrice et al., 2000; Oliveira and Watson, 2000; Day et al., 2001; Moon et al., 2001). One hour following single intragastric administration of quercetin (50 mg/kg body weight) to male Sprague–Dawley rats, 93% of quercetin was metabolized, with quercetin glucuronides, sulfoglucuronides, and sulfates, as well as isorhamnetic conjugates identified as the major metabolites (Justino et al., 2004).

4.2. Bioavailability

Following single or repeat oral administration of 75–1000 mg quercetin (∼300–4000 mg/kg body weight), total plasma quercetin concentrations (i.e., free and conjugated quercetin and its metabolites) between 12.2 and up to 100 nmol/mL were detected in Wistar and Lister rats (Manach et al., 1997, 1999; Nakamura et al., 2000; Carbonaro and Grant, 2005). Although free quercetin was not detected in the plasma with single dosing (1 g/kg body weight), unconjugated quercetin was present at levels of 0.56 nmol/mL (0.17 μg/mL) in male Wistar rats following repeat-dose administration (10 days at 1 g/kg body weight) (Nakamura et al., 2000). In a more recent study, free quercetin was found in the plasma, albeit only at a concentration of 0.9 μmol/L (0.27 μg/mL), following single intragastric treatment of male Sprague–Dawley rats with 50 mg quercetin/kg body weight (Justino et al., 2004). Conversely, in weanling rats administered quercetin in the diet at 0.45% for a period of 6 weeks (equivalent to doses of up to 58.5 mg/day during the last week of treatment), plasma samples did not contain any detectable levels of the aglycone (Graf et al., 2006). While low concentrations of free quercetin were identified in liver and kidney tissues (i.e.,

less than 8% of total quercetin identified in these tissues), the authors suggested that this may have occurred as a result of *ex vivo* hydrolysis of the quercetin metabolites.

In humans, total plasma quercetin levels (i.e., quercetin, quercetin glycosides, glucuronides, and sulfates) between 29 and 248 ng/mL were attained following ingestion of single meals consisting of quercetin-rich foods (∼50 mg quercetin) (Hollman et al., 1996; de Vries et al., 1998; McAnlis et al., 1999); however, daily ingestion of 114 mg quercetin from onions for 7 continuous days resulted in total quercetin plasma levels of 453 ng/mL (Janssen et al., 1998). While in earlier human trials the unconjugated quercetin aglycone was not identified in plasma samples following oral administration of the flavonol (Manach et al., 1998; Erlund et al., 2000; Walle et al., 2000; Graefe et al., 2001), more recent trials utilizing improved detection methods have demonstrated trace quantities of the aglycone in the plasma of one subject ingesting fried onions (Mullen et al., 2004) and mean plasma levels of up to 8 ng/mL in another study in which participants were provided 500 mg quercetin three times daily (t.i.d.) for a period of 5 days (120–350 ng/mL of total quercetin) (Wang and Morris, 2005). Following single-dose administration of 10 mg quercetin per 70 kg of body weight dissolved in different beverages (i.e., vegetable juice, white wine, or grape juice), mean plasma aglycone levels of up to 25 ng/mL were identified, with the highest plasma levels observed when quercetin was provided in wine (Goldberg et al., 2003). Some variability in plasma concentrations of the quercetin aglycone, as observed between the Wang and Morris (2005) and Goldberg et al. (2003) studies, is expected as a result of differences in methods of analysis. Also, the food matrix in which quercetin is administered appears to play a significant role in the bioavailability of the free aglycone following ingestion (Goldberg et al., 2003).

While both animal and human studies have demonstrated that following oral consumption of quercetin as much as 60% of the dose is absorbed (as total quercetin), extensive metabolism as a result of the first-pass effect ensures that the free unconjugated quercetin aglycone circulates in plasma at extremely low concentrations.

4.3. Distribution

Several authors reported on the distribution of quercetin following oral administration to determine possible target organs of the anti-oxidant activity of quercetin. Although acute treatment of Wistar rats with quercetin resulted in equal distribution of systemically-absorbed quercetin across all major tissues (Abrahamse et al., 2005), in rats fed quercetin in the diet for a period of 11 weeks, quercetin was observed to concentrate in several organs (i.e., lung, testes, kidney, thymus, heart, liver), with the highest concentrations of quercetin and its methylated derivatives, particularly isorhamnetin, detected in the pulmonary tissue (de Boer et al., 2005). Quercetin was primarily identified in its conjugated form in the tissue samples; however, free

quercetin was extracted from some organs (i.e., lungs, liver, kidney, and testes) at concentrations up to 40% of total extracted quercetin. As noted by the authors, although tissue β-glucuronidase deconjugation of quercetin may occur *in vivo*, conversion of conjugated quercetin to the aglycone upon extraction also could not be fully discounted.

4.4. Excretion

Systemically-absorbed quercetin may be eliminated in the urine (Gugler et al., 1975; Onishi et al., 1982; Ueno et al., 1983; de Vries et al., 1998; Young et al., 1999; Nakamura et al., 2000; Wang et al., 2003a,b) or alternatively, may be secreted into the bile and excreted in the feces (Ueno et al., 1983). Also, as previously indicated, quercetin can undergo microbial degradation in the colon to phenolic acids and CO_2, which is exhaled in the breath (Ueno et al., 1983; Walle et al., 2001; Abrahamse et al., 2005). Unabsorbed quercetin and the phenolic acid microfloral degradation products are eliminated in the feces (Gugler et al., 1975; Ueno et al., 1983; Nakamura et al., 2000). In experimental rat models, absorbed quercetin (∼20% of the administered dose) was excreted as expired CO_2 (35%), or via the feces (45%) and urine (10%) as glucuronide or sulfate conjugates following oral administration (Ueno et al., 1983). Excretion of quercetin in the urine and feces in human subjects accounted for only 3.3–5.7% and 0.21–4.6% of an orally administered dose (100 mg), with the majority of the radioactivity recovered as CO_2 (i.e., 41.8–63.9%) (Walle et al., 2001). Given its extensive metabolism, quercetin is mostly recovered in the form of various metabolic products; however, Wang and Morris (2005) demonstrated that small quantities of unchanged quercetin (15.5–74.9 μg) were excreted in the urine within an 8 h period by human subjects ingesting quercetin supplements (500 mg × t.i.d.).

The biliary secretion of quercetin in rats is suggestive of a potential for enterohepatic circulation following deconjugation of the quercetin conjugates back to the aglycone in the lower intestine (Ueno et al., 1983). Although the potential for biliary recirculation could theoretically contribute to an extended bioavailability (Erlund, 2004; Abrahamse et al., 2005), it is dependent on the colonic deconjugation of the quercetin derivatives to the aglycone. Furthermore, subsequent degradation of the quercetin aglycone to phenolic acids may reduce the amount of quercetin re-entering the circulation (Crespy et al., 1999). Following ingestion of quercetin (100 mg), half-lives in the range of 31–50 h were observed in humans, with peak plasma levels observed at 30 min and again at 8 h post-treatment (Walle et al., 2001). Although such an extended half-life and biphasic elimination (Gugler et al., 1975; Hollman et al., 1996; Ader et al., 2000; Skibola and Smith, 2000; Walle et al., 2001; Khaled et al., 2003) are supportive of enterohepatic recirculation, it also might be indicative of the binding of quercetin to plasma proteins presumably following initial enzymatic conversion (Walle et al., 2001; Carbonaro and Grant, 2005). In both rats and humans, quercetin and its derivatives were shown to possess strong affinity for serum albumin (Manach et al., 1995; McAnlis et al., 1999; Sengupta and Sengupta, 2003). Furthermore, although recent studies conducted in rats confirm excretion of quercetin via the bile in the form of glucuronide/sulfate and methylate conjugates, enterohepatic recirculation was considered unlikely due to a similar metabolic profile of quercetin in rats with and without bile duct cannulation (Chen et al., 2005).

5. Toxicological studies of quercetin

5.1. In vitro genotoxicity studies

In vitro, quercetin consistently tested positive for mutagenic activity in most standard strains of *Salmonella typhimurium* (Bjeldanes and Chang, 1977; Hardigree and Epler, 1978; Seino et al., 1978; Brown and Dietrich, 1979; Ochiai et al., 1984; Stoewsand et al., 1984; Ueno et al., 1984; Hatcher and Bryan, 1985; Busch et al., 1986; Rueff et al., 1986, 1992; Crebelli et al., 1987; Schimmer et al., 1988; Nguyen et al., 1989; Vrijsen et al., 1990; NTP, 1992; Czeczot, 1994; Cross et al., 1996) and induced SOS activity (Rueff et al., 1986; Dayan et al., 1987; Llagostera et al., 1987; Czeczot and Kusztelak, 1993; Czeczot et al., 1993), reverse mutations, and DNA single strand breaks in *Escherichia coli* (Hardigree and Epler, 1978; Czeczot et al., 1993; Czeczot, 1994) irrespective of metabolic activation. Quercetin did not, however, exhibit any mutagenicity in a forward mutation assay alone or with a bioactivation system in two *Bacillus subtilis* strains (MacGregor, 1979). The mutagenicity observed in bacterial test systems was confirmed in eukaryotic cells, including yeast cells, at relatively high concentrations (up to 10 mg/incubation mixture) (Hardigree and Epler, 1978). Additionally, in hamster and mouse cells and human lymphocytes, quercetin exposure induced chromosomal aberrations, DNA single strand breaks, and micronucleus formation (Yoshida et al., 1980; Meltz and MacGregor, 1981; Carver et al., 1983; Rueff et al., 1986; Kubiak and Rudek, 1990; Popp and Schimmer, 1991; NTP, 1992; Gasper et al., 1994; Caria et al., 1995). Mutations also were detected at the *tk* locus, but not at other loci (*hprt, hgprt, aprt,* ATPase), in mouse and hamster cells (Meltz and MacGregor, 1981; Carver et al., 1983; van der Hoeven et al., 1984). Positive results were obtained in cell transformation assays in mouse (Meltz and MacGregor, 1981) and hamster cells (Umezawa et al., 1977). On the other hand, in rat hepatocytes, quercetin tested negative for induction of DNA repair (Kato et al., 1985; Cross et al., 1996).

In the Ames assay, methylation of quercetin at the different hydroxyl groups (e.g., 3′-OH, 4′-OH, 3-OH, 4-OH, 5-OH, 7-OH) significantly attenuated or entirely abolished its mutagenic activity (MacGregor and Jurd, 1978; Brown and Dietrich, 1979; Hatcher et al., 1981; Onishi et al., 1982;

Czeczot et al., 1990). Urine and fecal samples collected from rats administered quercetin orally demonstrated detectable levels of mutagenicity in some (MacGregor, 1979; Stoewsand et al., 1984; Crebelli et al., 1987), but not all (Hatcher et al., 1981) *Salmonella* assays. Conversely, plasma samples obtained from rats administered quercetin by gavage or via the diet exhibited no mutagenic activity in *S. typhimurium* (Crebelli et al., 1987), thus clearly indicating that absorbed quercetin is rapidly and efficiently metabolized to non-mutagenic derivatives.

5.2. In vivo genotoxicity studies

The results of quercetin-related mutagenicity/genotoxicity observed *in vitro* have not been confirmed by *in vivo* experiments. With oral administration to mice and rats, quercetin consistently did not induce any significant changes in several mutagenicity/genotoxicity endpoints (i.e., micronuclei, chromosomal aberrations, sister chromatid exchange, unscheduled DNA synthesis, and alkali-labile DNA damage) in somatic cells in comparison to untreated controls (MacGregor, 1979; Aeschbacher et al., 1982; Ishikawa et al., 1985; Ngomuo and Jones, 1996; Taj and Nagarajan, 1996; Cierniak et al., 2004). Likewise, no significant increases in the number of revertants or micronuclei were observed in a host-mediated assay (*S. typhimurium* TA98) in male mice treated orally with quercetin (Aeschbacher et al., 1982). While positive results were obtained in some but not all of the *in vivo* mutagenicity/genotoxicity assays following intraperitoneal treatment with quercetin (MacGregor, 1979; Sahu et al., 1981; Aravindakshan et al., 1985; Caria et al., 1995; Ngomuo and Jones, 1996; da Silva et al., 2002), in assessing the safety of dietary quercetin, the relevance of such findings is questionable.

5.3. Acute toxicity studies

Although an early study indicated a relatively low-oral LD_{50} value for quercetin (i.e., in the range of 160 mg quercetin/kg body weight) (Sullivan et al., 1951), the purity of the quercetin product administered was not specified, and subsequent longer-term animal toxicity studies demonstrated that quercetin is tolerated at oral dose levels exceeding this LD_{50} value by several fold (see below). Moreover, no symptoms of toxicity were reported in rabbits receiving single intravenous injections of quercetin dose levels of 100–150 mg/kg body weight or two injections of up to 136 mg quercetin/kg body weight (Ambrose et al., 1952). Urine samples collected following quercetin administration were highly pigmented.

5.4. Subchronic and chronic toxicity/carcinogenicity studies

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 weights, food and water consumption, clinical chemistry, and organ weights in male and female Swiss mice administered quercetin in the diet at doses of 30, 300, or 3000 mg/kg body weight/day for a period of 28 days in comparison to a control group (Ruiz et al., 2006). The variations in the hematological parameters were within historical limits for this mouse strain. Study-end histopathological examination of various organs was reported to be unremarkable. In another 28-day feeding study, dietary administration of ~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) produced no significant effects on body or organ weight gains, biochemical parameters, or intestinal histopathology of Swiss mice in comparison to a control group (Barrenetxe et al., 2006). Although variations were observed in the small intestinal enzymatic activity, the physiological implications of this finding were stated by the authors to be unclear.

In a 5-week study evaluating the potential effects of quercetin treatment on experimental renovascular hypertension in rats, quercetin administered to a group of normotensive male Wistar rats at daily dose levels of 10 mg/ kg body weight by gavage did not induce any body or relative kidney or heart weight variations in comparison to untreated controls (García-Saura et al., 2005). Moreover, excretion of protein in the urine of quercetin-treated rats also was comparable to that of controls. Administration of quercetin to hypertensive rats reduced systolic blood pressure, cardiac hypertrophy, and proteinuria. Enhanced proteinuria was, however, observed in a 21-day feeding study in high-dose (1133 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). In this study, no variations in final body weight, body weight gain, kidney weights, or food intake were reported in the quercetin-treated groups compared to a basal diet control group. With the exception of variations in the levels of fecal bile acid excretion, no significant effects related to food intake, body weight, or relative and absolute liver weight were reported by Nakamura et al. (2000) in a group of male Wistar rats administered quercetin in the diet for 22 consecutive days at dose levels of up to 1000 mg/kg - body weight/day. In 15-week-old male Long-Evans Cinnamon (LEC) rats, an inbred mutant strain that accumulates copper in the liver, administration of quercetin in the diet at a concentration of 1% (~500 mg/kg body weight/day) for a period of 6 weeks was associated with a statistically significant increase in the incidence of tubular necrosis in comparison to basal diet controls (Kitamura et al., 2005); however, no variations were observed in body weights, relative liver and kidney weights, or clinical biochemistry results between the quercetin-treated group and controls.

Quercetin has been studied for its potential carcinogenicity in numerous long-term experimental animal studies, the majority of which indicated no evidence of significantly increased incidences of neoplasm formation related to its

oral administration (Ambrose et al., 1952; 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); however, two other studies, a 58-week study (Pamukcu et al., 1980) and a 2-year carcinogenicity bioassay conducted by the National Toxicology Program (NTP) (Dunnick and Hailey, 1992; NTP, 1992), indicated positive findings for quercetin-related carcinogenicity in rats fed high-amounts of the compound in the diet.

Specifically, the 2-year rat feeding study conducted by the NTP demonstrated 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 F344/N rats exposed to 40 000 ppm dietary quercetin (∼2000 mg quercetin/kg body weight/day) (Dunnick and Hailey, 1992; NTP, 1992), an amount that would correspond to the ingestion of 140 g of quercetin by a 70 kg individual. In comparison to a single occurrence of adenoma in the control group, 9 out of 50 high-dose male rats (40 000 ppm) exhibited renal tubule adenomas or adenocarcinomas, but only following step-section analysis. The appropriateness of using step-section results, which revealed additional incidences of hyperplasia and adenomas not observed following single-section analysis, in statistical tests, and comparing the incidence of post-treatment lesions with the spontaneous incidence of lesions as reported following standard examination has been considered questionable by several authors (Hirono, 1992; Ito, 1992). At lower levels of 1000 ppm (∼50 mg/kg body weight/day) and 10 000 ppm (∼500 mg/kg body weight/day), no statistically significant adverse effects (at $p \leqslant 0.01$) were reported (Dunnick and Hailey, 1992; NTP, 1992). Only yellow–brown, granular staining of the epithelial cells in the proximal segments of the gastrointestinal tract, including the glandular stomach and to a smaller degree the distal portions (i.e., duodenum and colon), which increased in intensity with increased exposure to quercetin, was observed in rats receiving up to 10 000 ppm (500 mg/kg body weight/day) of quercetin in the diet. It was concluded by the study authors that, under the conditions of the study, there was some evidence of carcinogenic activity of quercetin in male F344/N rats based on an increased incidence of renal tubule cell adenomas, and no evidence of carcinogenic activity of quercetin in female F344/N rats (NTP, 1992). A summary of the results of the NTP study is presented in Table 1.

As summarized in Table 2, in contrast to the NTP study, no significant renal histopathological effects were observed in other toxicity studies conducted prior to the NTP study with treatment periods greater than one year (Ambrose et al., 1952; Stoewsand et al., 1984), including another 2-year feeding study in which F344/DuCrj rats were maintained on diets containing 1.25 or 5% quercetin (providing 427 or 1926, and 497 or 2372 mg/kg body weight/day for males and females, respectively) followed by an 8-week recovery period (Ito et al., 1989). An increase in relative kidney weights in high-dose males was reported to be

Table 1
Incidence of renal tubule lesions in F344/N rats in the 2-year feed study of quercetin conducted by the National Toxicology Program (NTP)

	0 ppm (control)	1000 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[a]	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 (Single and Step Sections)[b]				
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[c]	0/49 (0%)	0/49 (0%)	1/50 (2%)	0/50 (0%)
Evaluation of step sections				
Hyperplasia	1/49 (2%)	–[d]	–	3/50 (6%)
Adenoma	1/49 (2%)	–	–	0/50 (0%)
Combined				
Hyperplasia	2/49 (4%)	–	–	4/50 (8%)
Adenoma	1/49 (2%)	–	–	0/50 (0%)

Table adapted from NTP (1992).
[*] $p \leqslant 0.01$ pair-wise comparison between control and dose group.
[a] Historical incidence for 2-year NTP feed studies with untreated control groups 4/499 (0.8% ± 1.1%), range 0–4%.
[b] Where the combined incidence of lesions identified in the single and step-section evaluations are less than the addition of both, it is because the same lesion was identified in both examinations.
[c] Historical incidence for 2-year NTP feed studies with untreated control groups 1/499 (0.2% ± 0.6%), range 0–2%.
[d] Step sections not conducted at the 1000 and 10 000 ppm dose levels.

within the normal range and was considered by Ito et al. (1989) to be the 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 recorded for the laboratory. An increase in cecal hyperplastic polyp formation was observed in both sexes at the high-dose levels; however, statistical significance was attained only in males and the lesions were deemed to be non-neoplastic. Additionally, the study also included assessments of hematology and urinalysis parameters, as well as non-renal organ weights, which apart from some variability considered to be consistent with reduced growth of the test animals, did not demonstrate any quercetin-related toxicity.

In a 64-week study in which quercetin was provided to F344 rats at dietary concentrations of 0.1 or 0.2% (providing ∼41 and 76 mg quercetin/kg body weight/day and 29

Table 2
Summary of long-term toxicological studies with quercetin conducted prior to the National Toxicology Program (NTP, 1992) 2-year rat feed study

Species (strain, sex)	Duration (days)	Dietary quercetin concentration (dose)	Toxicological endpoints evaluated	Results[*]	References
Rat (strain not specified; M&F)	410	0 (control), 0.25, 0.5, or 1% (~125, 250, and 500 mg/kg body weight/day, respectively)	Survival, food consumption, body weight gain, hematology, absolute organ weights[a], and histopathology[b]	No toxicologically significant adverse effects	Ambrose et al. (1952)
Rat (F344; M&F)	448	0 (control), 0.1, or 0.2% [~40.7/29 and 75.9/57.5 (M/F) mg/kg body weight/day, respectively]	Survival, food consumption, body weight gain, hemoglobin, packed red blood cell volume, clinical chemistry,[c] and histopathology	M (0.2%): ↓ AST and BUN; No other statistically significant variations[d]	Stoewsand et al. (1984)
Rat (F344/DuCrj; M&F)	728	0 (control), 1.25, or 5% (~427/497 and 1926/2372 (M/F) mg/kg body weight/day, respectively)	Survival, overt symptoms of toxicity, food and water consumption, body weight gain, urinalysis, hematology, clinical chemistry, and gross and histopathological examination of a wide-range of major organs	M/F (5%): ↓ body weights and serum glucose (M only); ↑ relative brain weights and kidney weights (M only)[e] *Histopathology* (statistically significant variations in comparison to controls)- M (5%): ↑ hyperplastic cecal polyps (11/50)	Ito et al. (1988)

M = male; F = female; AST = aspartate aminotransferase; BUN = blood urea nitrogen.

[*] Values in parentheses indicated the dietary quercetin level at which the effects were reported to be observed.

[a] Adrenals, kidneys, spleen, liver, heart, and testes.

[b] Adrenals, kidneys, spleen, liver, heart, testes, thyroid, lung, pancreas, stomach, small intestine, bladder, and female reproductive organs.

[c] Blood urea nitrogen, aspartate aminotransferase, alanine aminotransferase, and α-fetoprotein (as a marker for hepatocarcinogenesis).

[d] All lesions observed were determined to be related to normal rat ageing, including a sarcoma of the uterus, the only malignant tumor observed in the study.

[e] Weight variations within normal limits of variation; attributed to reduced weight.

and 58 mg quercetin/kg body weight/day, in males and females, respectively), all non-neoplastic and neoplastic lesions of the skin (papilloma), spleen (congestion combined with a moderate lymphoid increase that could be due to early mononuclear-cell leukemia), thyroid (mild follicular hyperplasia), uterus (endometrial fibrosis, glandular hyperplasia, and stromal sarcoma), and adipose tissue (granulomatous steatitis) were typical of aging rats and were not attributed to quercetin exposure *per se* (Stoewsand et al., 1984). No adverse effects, including an absence of pathobiological abnormalities as determined by microscopic evaluation of a wide range of selected tissues (kidneys, liver, and intestine among others), were observed in a 410-day feeding study in which groups of male and female rats (strain not specified) were fed quercetin at up to 1% of the diet (providing ~500 mg/kg body weight/day) (Ambrose et al., 1952).

In addition to these traditional toxicological studies, several other studies have been performed that specifically assessed the potential carcinogenicity of quercetin. The results of these studies are presented in Table 3. With the exception of the study conducted by Pamukcu et al. (1980) in the Norwegian rat in which urinary bladder and intestinal tumors were reported following exposure to dietary quercetin, other studies consistently produced negative results for enhanced induction of any organ tumors in a variety of laboratory animals including ACI and F344 rats, ddY and A/JJms mice, and golden hamsters at concentrations of up to 10% dietary quercetin (providing up to 12 g/kg body weight/day) (Saito et al., 1980; Hirono et al., 1981; Hosaka and Hirono, 1981; Morino et al., 1982; Takanashi et al., 1983).

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 its potential carcinogenic activity. In their study, 29% (2/7) and 17% (3/18) of male and female Norwegian rats, respectively, receiving quercetin as 0.1% of their diet (providing ~52 and 47 mg/kg body weight/day in males and females, respectively) for a period of 58 weeks developed urinary bladder tumors. Intestinal tumors were observed in 6/7 and 14/18 quercetin-treated male and female rats, respectively.

Two additional dietary rat studies, the details of which were identified in abstract form only, indicated pathological changes of the colon and ileum following quercetin administration at a concentration of 5% of the diet (providing ~2500 mg/kg body weight/day) for a period of 6 months (Bokkenheuser and Winter, 1990) and an increased incidence of liver and bile duct tumors in female F344 rats administered quercetin at up to 2% of the diet (~1000 mg/kg body weight/day) (Ertürk et al., 1983). Although a control group was included in the latter study [a control group was not discussed in Bokkenheuser and Winter (1990) study], the statistical significance of the results was not

Table 3
Summary of carcinogenicity study results with quercetin administered via the diet

Species	Duration	Dietary concentration (corresponding dose[*])	Results	References
Mouse (A/JJms; M&F)	23 weeks (161 days)	0 or 5% Qu (7500 mg/kg bw/d)	Qu (5%): No difference in lung tumor incidence and multiplicity between Qu-test and control groups	Hosaka and Hirono (1981)
Mouse (ddY; M&F)	~842 days (Life-long exposure)	0 or 2% Qu (3000 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)
Rat (strain not specified)	6-months (~180 days)	0 or 5% Qu (2500 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 only)
Rat (Norwegian; M&F)	58 weeks (406 days)	0 or 0.1% Qu [52 (M) and 47 (F) mg/kg bw/d] 0 or 33% BF [11 (M) and 10 (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[a] [2/7 (M); 3/18 (F)] BF (33%): Reduced growth rate; intestinal [7/8 (M), 10/11 (F)] and urinary bladder tumors [6/8 (M); 8/11 (F)]	Pamukcu et al. (1980)
Rat (F344; 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)
Rat (F344; F)	Life-long exposure (~750 days)	0, 1, or 2% Qu (500, and 1000 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 only)
Rat (ACI; M&F)	540 days 850 days	0, 1, or 5% Qu (500 and 2500 mg/kg bw/d) 0 or 10% Qu (5000 mg/kg bw/d)	Qu (5%): Body weights (M only) significantly reduced; cecal adenoma [1/8 (M)] Qu (10%): Cecal adenoma [1/19 (M)] and adenocarcinoma [2/19 (M)]	Hirono et al. (1981)
Hamster (Syrian gold; M&F)	733 days	0 or 10% Qu (12000 mg/kg bw/d)	Qu (10%): No difference in the incidence of forestomach papillomas and adrenal cortical adenomas between test and control animals; ileal adenocarcinoma [1/20 (F)]	Morino et al. (1982)

M = male; F = female; Qu = quercetin; BF = Bracken fern.
[*] Unless specifically reported by the study authors, the corresponding doses were calculated using US FDA (1993).
[a] Frequency of bladder tumors significantly reduced compared to bracken fern test group.

discussed. In light of the absence of full study reports, the usefulness of these two studies in a safety assessment of quercetin is limited.

In all other carcinogenicity studies, none of the tumors identified occurred with statistically greater incidence compared to concurrently maintained control groups. The incidence of tumor formation in F344 rats receiving quercetin as 0.1% of the diet (providing ~50 mg/kg body weight/day) for a period of 540 days did not differ significantly from that observed in the control group (Takanashi et al., 1983). With the exception of the occurrence of one lung adenoma and one jejunal adenocarcinoma in male quercetin-treated rats, all other tumors identified in the test group also were present in control animals. Quercetin administered in the diet at 5% (~7500 mg/kg body weight/day) for a period of 23 weeks to A/JJms mice did not induce a

significant difference in the incidence and multiplicity of lung tumors (Hosaka and Hirono, 1981).

In a 733-day golden Syrian hamster study, histopathology revealed papillomas of the forestomach and cortical adenomas of the adrenal gland with statistically equal frequency in the control and quercetin-test group (provided as ~12000 mg/kg body weight/day via the diet) (Morino et al., 1982). Only a single case of adenocarcinoma of the ileum was detected in the test group, without parallel occurrence in the control group. Lesions of the cecum, colon, adrenal cortex, and testis observed by Hirono et al. (1981) in ACI rats at dietary quercetin concentrations of 5% (providing ~2500 mg/kg body weight/day) and higher, administered for at least 540 days, also were observed in the control group. Likewise, with the exception of a single incidence of a heart sarcoma, the development

Table 4
Summary of anti-carcinogenicity study results of quercetin administered in conjunction with known carcinogens

Species (strain; sex)	Initiator	Test compound (dose; route; treatment duration)		Conclusion	References
		Initiation	Promotion		
Unspecified target organ					
Mouse (C57BL/6; female)	MCA	Qu (20 mg; single i.m.) (420-day observation period)		Absence of tumors without MCA initiation	Ishikawa et al. (1985)
		Qu (0.5–20 mg) + MCA (0.1 or 1.0 mg; single i.m.)		Enhanced MCA-induced carcinogenesis with quercetin	
		MCA (0.1 mg; single i.m.)	Qu (50 or 500 mg/kg bw/d; diet; life-span)		
Hamster (Syrian; male and female)	Croton oil	Qu (4500 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 (1300 mg/kg bw/d; diet; 351 days)	Basal diet (350 days)		
		Qu (1300 mg/kg bw/d; diet; 351 days)	Croton oil (1%; 350 days)		
		Croton oil (1%; diet; 351 days)	Basal diet (350 days)		
Urinary bladder					
Rat (F344; male)	BHBN	Water (4 weeks)	Qu (2500 mg/kg bw/d; diet; 25 weeks)	Absence of quercetin-related promoting activity and lack of quercetin-associated carcinogenicity in the bladder	Hirose et al. (1983)
		Qu (2500 mg/kg bw/d; diet; 4 weeks)	Water or BHBN (0.01%) (29 weeks)	No effects in liver and kidneys	
		Basal diet (4 weeks)	BHBN (0.001%; 29 weeks)		
		BHBN (0.01%; 4 weeks)	Qu (2500 mg/kg bw/d; diet) or basal diet (25 weeks)		
Rat (F344; male)	BBN	Water (4 weeks)	Qu (2000 mg/kg bw/d; diet; 34 weeks)	No changes in the urinary bladder	Fukushima et al. (1983)
		BBN (water, 4 weeks)	Qu (2000 mg/kg bw/d; diet; 34 weeks)	↓ bladder PN hyperplasia and papilloma; Incidences of simple hyperplasia	
Kidneys					
Hamster (Syrian; male)	Estradiol	Qu (0, 360, or 3600 mg/kg bw/d; diet; 5.5 months)		Absence of tumor formation	Zhu and Liehr (1994)
		Qu (0, or 3600 mg/kg bw/d; diet; 6.3 months)			
		Estradiol (2 × 25 mg; s.c.)[a]	Qu (0, 360, or 3600 mg/kg bw/d; diet; 2 weeks + 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 3600 mg/kg bw/d; diet; 2 weeks + 6.3 months)		
Gastrointestinal tract					
Mouse (CF1; male and female)	AOM	Saline[b]	Qu (0 or ~3000 mg/kg bw/day; diet; 8 weeks)	Qu-induced non s.s. ↑ in FAH	Yang et al. (2000)
		AOM[b] (↑'ing doses of 5 to 10 mg/kg bw/week; s.c.; 6 weeks)		Qu-induced ↓ in AOM-induced FAH	
Mouse (CF1; female)	AOM (azoxy-methanol)	Qu (3000 mg/kg bw/d; diet; 9 weeks)		No effects	Deschner et al. (1991)
		Qu (0 or 3000 mg/kg bw/d; diet) + AOM (weekly injections) (9 weeks)		Qu-induced ↓ in no. of cells in S-phase and in no. of FAH in colonic tissue.	
		AOM[c] (weekly injections; 3×)	Qu (0, 150, 750, or 3000 mg/kg bw/d; diet; 48 weeks)	Qu-induced ↓ (4-fold) in colonic tumors	
Mouse (C57BL/6; male)	ENNG	Qu (3000 mg/kg bw/day; diet; 16 weeks)		No duodenal tumors detected	Matsukawa et al. (2002)
		ENNG (water; 4 weeks)	Qu (0, 300, or 3000 mg/kg bw/d; diet;16 weeks)	↑ in size and average number of duodenal tumors	

Rat (F344; male)	AOM		Qu (1680 mg/kg bw/d; diet; 46 weeks)	Adenocarcinoma (small intestine) (1/12)	Pereira et al. (1996)
		AOM (30 mg/kg bw; s.c.)[d]	Qu (840 or 1680 mg/kg bw/d; diet; 46 weeks)	↑ incidence and no. of colonadenocarcinomas, but not adenomas; no effect observed on the small intestine	
Rat (F344; male)	AOM		Qu (0 or 25 mg/kg bw/d; diet; 5 weeks)	No adverse effect (no histopathology in liver, kidneys, lungs and heart, and no ACF)	Tanaka et al. (1999)
		AOM (s.c.; 1/week; 3 weeks)[e]	Basal diet (5 weeks) Qu (25 mg/kg bw/d; diet; 5 weeks)[f]	Qu-induced ↓ in ACF	
Rat (F344; male)	AOM		Qu (0 or ~5, 50, or 500 mg/kg bw/d; diet; 41 weeks)	No colorectal tumors	Dihal et al. (2006)
		AOM (s.c.) (2× at 1 week interval)[b]	Basal diet (38 weeks) Qu (5, 50, or 500 mg/kg bw/d; diet; 38 weeks)	No differences in ACF numbers and multiplicity between Qu + AOM-treated and control rats at 8 weeks following last AOM injection Qu-induced dose-dependent ↓ in colorectal tumor number, multiplicity, and diameter	
Rat (Sprague–Dawley; female)	AOM		Qu (100 mg/kg bw; diet; 5×/week; 7 weeks)	No difference in multiplicity and incidence of ACFs in Qu + AOM compared to AOM-only rats	Exon et al. (1998)
		AOM (s.c.; 2×; on days 2 and 9 of 7-week feeding period)	Qu (100 mg/kg bw; diet; 5×/week; 7 weeks)		
Rat (Wistar; male)	ENNG		Qu (500 mg/kg bw/d; diet; 16 weeks)	Absence of duodenal tumor formation	Werner et al. (1985)
			Qu (500 mg/kg bw/d) + ENNG (120 mg/L; water) (16 weeks)	↑incidence of duodenal tumors (not s.s.)	
Rat (Sprague–Dawley; male and female)	MAM	Saline (i.p.; 1/week for 3 weeks)	Basal diet (459 days) Qu (500 mg/kg bw/d; diet; 459 days)	Absence of tumor formation	Kato et al. (1984)
		MAM (i.p.; 1/week for 3 weeks)	Basal diet (459 days) Qu (500 mg/kg bw/d; diet; 459 days)	↓ colonic adenomas in MAM-Qu compared to MAM-control	
Rat (F344; male)	DMBDD[g]		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 weeks)	Qu fully inhibited small intestinal tumors (promotional phase)	
		DMBDD (4 weeks)	Qu (500 mg/kg bw/d; diet; 31 weeks)	↓ incidence and number of total small intestinal tumors	
Liver and pancreas Rat (ACI; male)	PB		Qu (50 or 500 mg/kg bw/d; i.p.; 6 days)	No hepatic lesions	Kato et al. (1985)
		Qu (50 or 500 mg/kg bw/d; i.p.; 6 days)	PB (diet; 467 days)		
Rat (Wistar; male and female)	NMU	Saline (single i.p. at 3 days of age)	Basal diet Qu (500 mg/kg bw/d; initiated at 30 days of age for 20 weeks)	↑ pancreas wts (relative) Qu-sal (F) compared to sal-control group ↓ bw Qu-NMU (M & F) compared to sal-control and NMU-control	Barotto et al. (1998)
		NMU (single i.p. at 3 days of age)	Basal diet Qu (500 mg/kg bw/d; diet; initiated at 30 days of age for 20 weeks)	↑ pancreas and liver wts (relative) 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 3/14(M) and 1/14(M) microcarcinoma Qu-NMU rats	
Rat (Wistar; male and female)	NMU	Saline (single i.p. at 3 days of age)	Basal diet ± PB (500 ppm)	↑ DYF (pancreas) of Qu-NMU (total, M&F) compared to NMU-control, but ↓ DYF of Qu-NMU-PB (total & M) compared to NMU-PB	Valentich et al. (2006)
		NMU (single i.p. at 3 days of age)	Basal diet ± PB (500 ppm) Qu (500 mg/kg bw/d; diet) ± PB (500 ppm) (initiated at 30 days of age for 20 weeks)[h]	Carcinoma *in situ* (pancreas) observed in 3/15 Qu-NMU males and 1/15 NMU-PB males, but not in any NMU- or Qu-NMU-PB animals of either sex	

Table 4 (continued)

Species (strain; sex)	Initiator	Test compound (dose; route; treatment duration)		Conclusion	References
		Initiation	Promotion		
Rat (F344; male)	DEN	Saline DEN[i] (200 mg/kg bw; i.p.; single)	Qu (1250 mg/kg bw/d; diet; 6 weeks) Qu (1250 mg/kg bw/d; diet; 6 weeks)	No liver tumors No change in GST-P$^+$ foci (liver) compared to DEN-only group	Ito et al. (1988)
Mammary glands					
Rat (Sprague–Dawley; female)	7,12-DMBA; NMU	Qu (1,000 mg/kg bw/d; diet; 20 weeks) 7,12-DMBA[d] (gavage) or NMU[d] (i.v.; single)	Qu [1000 (7,12-DMBA only) or 2500 mg/kg bw/d; diet; 20 weeks]	No palatable tumors identified Qu + 7,12-DMBA ↓ (dose-dependent) incidence and multiplicity of tumors Qu + NMU ↓ induction of tumors; less effective than w/7,12-DMBA	Verma et al. (1988)
Rat (Sprague–Dawley; female)	7,12-DMBA	7,12-DMBA[d] (12 mg; gavage)	Qu (500 or 1000 mg/kg bw/d; diet; 107 days)	Qu ↓ incidence and multiplicity of mammary tumors (not s.s.) & ↑ tumor latency	Pereira et al. (1996)
Rat (Wistar; female)	9,10-DMBA	9,10-DMBA	Qu (0, 15, 25, 35, or 45 mg/kg bw; weekly injections on days 1, 7, 14, 21, and 28)	Qu-induced dose-dependent ↓ in tumor volume	Devipriya et al. (2006)
Lungs					
Mouse (ICR; male and female)	B[a]P	Basal diet (90 days) B[a]P (1 × 100 mg/kg bw; i.p.)[d]	Basal diet (83 days) Qu (0.5, 1, or 2 g/kg bw/day; gavage; 83 days)	↑ Number of animals with normal bronchi epithelium and ↓ number of animals with atypical hyperplasia in Qu + B[a]P group compared to B[a]P group	Jin et al. (2006)
Skin carcinogenesis model					
Mouse (CD-1; female)	TPA	Qu (100 mg/animal; topical application; 2/week; 5 weeks) Qu (100 mg/animal; topically; 2/week; 5 weeks)	Acetone (topically; 2/week; 47 weeks) TPA (topically; 2/week; 47 weeks)	Absence of tumor-initiating activity	Sato et al. (1987)

ACF = Aberrant crypt foci; AOM = Azoxymethane; B[a]P = Benzo[a]pyrene; BBN = N-Nitroso-n-butyl-(4-hydroxybutyl)amine; BHBN = N-Butyl-N-(-4-hydroxybutyl)nitrosamine; DEN = Diethylnitrosamine; DMBA = Dimethylbenz[a]anthracene; DYF = Dysplastic foci; ENNG = N-ethyl-N′-nitro-N-nitrosoguanidine; F = Female; FAH = 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; ± = with or without.

[a] Quercetin treatment was initiated 2 weeks before estradiol administration and continued thereafter for 5.5 months. Estradiol administration repeated at 3 months following 1st injection.
[b] Quercetin treatment started 2 weeks prior to carcinogen treatment.
[c] Quercetin treatment started after 1st injection.
[d] Quercetin treatment started 1 week prior to carcinogen treatment.
[e] AOM injections administered for the 3 weeks of the total 5-week treatment period.
[f] Quercetin treatment initiated 1 week prior to 3-week AOM treatment.
[g] "DMBDD treatment": i.p. injections of diethylnitrosamine (100 mg/kg body weight × 1) and N-methylnitrosourea (20 mg/kg body weight × 4), s.c. injections of 1,2-dimethylhydrazine (40 mg/kg body weight × 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.
[h] Dams of quercetin-treated pups also had been treated with quercetin during pregnancy and lactation.
[i] Quercetin treatment started 2 weeks following DEN initiation.

of leukemia, as well as lung, forestomach, and mammary gland tumors in the test animals (receiving ∼3000 mg quercetin/kg body weight/day via the diet) did not differ significantly from controls (Saito et al., 1980).

5.5. Two-stage carcinogenicity studies

Anticarcinogenic activity is one of several biological properties ascribed to quercetin (Stavric, 1994; Formica and Regelson, 1995; Middleton et al., 2000; Skibola and Smith, 2000; PDRNS, 2001; Erlund, 2004). Accordingly, as summarized in Table 4, numerous 2-stage (initiation–promotion) carcinogenicity studies have been performed to determine the potential effect of quercetin on chemical-induced carcinogenesis.

The evidence related to the promotional activity of quercetin on chemical-induced organ-specific carcinogenesis is somewhat inconsistent. 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 any quercetin-related promotion of carcinogenesis or were supportive of a chemoprotective effect (Morino et al., 1982; Fukushima et al., 1983; Hirose et al., 1983; Kato et al., 1984, 1985; Sato et al., 1987; Ito et al., 1988; Verma et al., 1988; Deschner et al., 1991; Akagi et al., 1995; Exon et al., 1998; Tanaka et al., 1999; Yang et al., 2000; Devipriya et al., 2006; Dihal et al., 2006; Jin et al., 2006). While quercetin was shown to enhance the formation of azoxymethane (AOM)-induced gastrointestinal tumors in the study conducted by Pereira et al. (1996), as well as following *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (ENNG) initiation (Werner et al., 1985; Matsukawa et al., 2002), in other studies, quercetin demonstrated inhibitory effects on colon tumorigenesis, not only following AOM induction (Deschner et al., 1991; Exon et al., 1998; Tanaka et al., 1999; Dihal et al., 2006), but also when gastrointestinal carcinogenesis was induced with methylazoxymethanol acetate (MAM) and DMBDD[1] (Kato et al., 1984; Akagi et al., 1995). Zhu and Liehr (1994) reported quercetin-related promotion of tumor formation in the renal tissues of hamsters administered subcutaneous estradiol implants. Increased incidences of pancreatic dysplastic foci were noted in rats receiving a single intraperitoneal administration of *N*-nitrosomethylurea (NMU) at 3 days of age, 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 9, 10- or 7,12-dimethylbenz[*a*]anthracene (DMBA)-initiated mammary gland tumors (Verma et al., 1988; Pereira et al., 1996; Devipriya et al., 2006), *N*-nitroso-*n*-butyl-(4-hydroxybutyl)amine (BBN)-induced urinary bladder neoplasms (Fukushima et al., 1983), and benzo[*a*]pyrene-induced blood lymphocyte damage and pulmonary precancerous pathologic changes (Jin et al., 2006). In several other studies, quercetin did not act to promote (Morino et al., 1982; Hirose et al., 1983; Ito et al., 1988) or initiate (Kato et al., 1985; Sato et al., 1987) chemical-induced carcinogenesis in laboratory animals.

Additionally, these studies also further support 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 un-initiated quercetin-only-treated groups included in these studies. In light of the Pamukcu et al. (1980) findings of increased incidence of urinary tumors following quercetin administration, studies evaluating the urinary bladder as a potential target organ of quercetin-mediated tumorigenicity are of particular interest. Urinary bladder tumors were not observed in two separate 2-stage carcinogenesis studies in which quercetin-only-treated male F344 rats received up to 5% of the flavonol in the diet (providing ∼2000–2500 mg/kg body weight/day) for up to 25 weeks (Fukushima et al., 1983; Hirose et al., 1983). In other similar studies, no kidney lesions were observed following administration of quercetin at dietary concentrations of up to 3% (providing ∼3600 mg/kg body weight/day) to male hamsters for ∼6 months (Zhu and Liehr, 1994).

5.6. Reproductive and developmental studies

In light of its *in vitro* mutagenic activity, the potential effect of quercetin on germ cells also has been the subject of several evaluations. In mammalian cells, including human germ cells, some variable results were reported with respect to sperm motility and viability following quercetin exposure *in vitro* (Nass-Arden and Breitbart, 1990; Swisher et al., 1994; Trejo et al., 1995; Khanduja et al., 2001). Likewise, significant sperm abnormalities were observed in male (C57 × BALB/B)F1 mice following intraperitoneal administration of quercetin (32 mg/kg body weight/day) for a period of 5 days (Rastogi and Levin, 1987). Quercetin administered orally at dose levels of up to 150 mg/kg body weight/day to male Swiss albino mice did not induce any significant increases in the frequency of abnormal sperm (Nandan and Rao, 1983).

In a one-generation reproductive toxicity study, no toxicologically significant adverse effects related to reproduction or fetal development, and no teratological abnormalities were observed in Sprague–Dawley rats receiving gavage administrations of quercetin at dose levels ranging between 2 and 2,000 mg/kg body weight/day on a single occasion (gestation day 9) or on gestation days 6 through 15 (Willhite, 1982). No signs of reproductive or developmental toxicities were reported in another study

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

conducted with F344 rats in which the females and males of the parental generation were provided dietary quercetin at concentrations of up to 0.2%, with corresponding doses of up to ~58 and 76 mg/kg body weight/day, respectively, for a period of 64 weeks (Stoewsand et al., 1984).

6. Clinical and epidemiological studies related to the safety of quercetin

Although limited, clinical data relating to the safety of quercetin were available from a number of randomized, double blind clinical tolerance and efficacy studies (Ferry et al., 1996; Shoskes et al., 1999; Kiesewtter et al., 2000; Lozoya et al., 2002). In studies in which quercetin or plant extracts containing quercetin glycosides were provided to study subjects for oral ingestion for periods of up to 12 weeks at dose levels ranging between 3 and 1000 mg quercetin/day, no compound-related adverse effects were reported, including the absence of any variations in hematology, clinical chemistry, and urinalysis parameters (Shoskes et al., 1999; Kiesewtter et al., 2000; Lozoya et al., 2002). Likewise, no adverse effects were reported in male and female subjects following ingestion of single oral doses of quercetin (8.5 mg/kg body weight/day or ≈600 mg for an average 70 kg individual) in a study demonstrating a quercetin-related increase in adenosine levels (Blardi et al., 1999). Currently in the recruitment phase, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH) has developed a protocol for a double blind, placebo-controlled trial investigating the potential effects of 1 or 2 g of supplemental quercetin on glucose absorption (NLM, 2007).

The safety of quercetin, administered via intravenous infusion in escalating doses (60–2000 mg/m^2) (~1.5–51.3 mg/kg body weight for a 70 kg individual; 3–8 patients and 1–11 courses per dose level) at 3-week intervals or weekly at dose levels of 945 or 1400 mg/m^2 or ~24.3 and 35.9 mg/kg body weight, respectively (6–8 patients and 8–18 courses per dose level), was assessed in an extensive phase I clinical trial designed to investigate potential quercetin-related anti-proliferative properties in patients not responsive to typical cancer treatments (Ferry et al., 1996). At dose levels of up to 420 mg/m^2 or ~10.8 mg/kg body weight (756 mg quercetin), no adverse effects were noted. At the higher dose levels of up to 51.3 mg/kg body weight (~3591 mg quercetin), more pronounced and longer-lasting pain at the site of injection, dyspnea, emesis, and nephrotoxicity (grades 1–4; determined by serum creatinine levels) were reported in test subjects; however, the clinical symptoms lasted only a short period of time after every injection of the test substance, and of the patients reported to experience renal toxicity, as indicated by increased serum creatinine levels, none were reported to exhibit any evidence of nephritis, infection, or obstructive uropathy following ultrasound.

In addition to the human clinical trials, a series of epidemiological studies consistently demonstrated an absence of any correlation between flavonoid intakes (up to 68 mg total flavonoids/day) from the habitual diet, represented in large proportion by a high-quercetin content, and incidences of cancer (all types of cancer combined, and lung, breast, gastrointestinal tract, or respiratory tract cancer specifically) (Hertog et al., 1994, 1995; Goldbohm et al., 1995; Lin et al., 2006). Knekt et al. (1997, 2002) demonstrated an inverse association between total flavonoid intake (up to ~40 mg/day), 95% of which was determined to be quercetin, and overall cancer incidence, as well as the incidence of lung cancer specifically.

7. Discussion

Interest in the use of quercetin in various food applications has been increasing due to its potential anti-oxidant and other beneficial health properties; however, the toxicity profile for quercetin is complicated by consistent findings of positive mutagenic effects *in vitro* and reports of positive findings in two carcinogenicity studies (Pamukcu et al., 1980; NTP, 1992). Numerous additional animal studies have consistently failed to demonstrate any relationship between quercetin administration and increased tumor incidence. As such, the potential carcinogenicity of quercetin has long been considered a contentious issue (NAS, 1982a,b). Several previously published reviews have indicated that quercetin, although displaying mutagenic activity *in vitro*, is not carcinogenic *in vivo* (Das et al., 1994; Stavric, 1994; Formica and Regelson, 1995; Lamson and Brignall, 2000; Erlund, 2004). Although an acceptable dose level was not specified, in the most recent review of quercetin safety, Okamoto (2005) also concluded that orally administered quercetin is unlikely to cause any adverse effects. The present critical review of all relevant toxicity studies was therefore undertaken to bring better perspective and understanding to the implications of these positive findings.

Quercetin is one of the most abundant polyphenolic flavonols occurring naturally as an *O*-β-glycoside in the diet (Day and Williamson, 1999). Several biological and pharmacological functions have been ascribed to quercetin, including strong anti-oxidant and anti-inflammatory properties (Middleton et al., 2000). Although quercetin has been clearly demonstrated to act as a free radical scavenger *in vitro*, the chemical structure of quercetin and features of its mechanism of action (i.e., plateauing of the anti-mutagenic response, lack of linear dose–response, and a decrease in the response with dose levels beyond a certain concentration) suggest that quercetin may, under certain conditions, also exhibit pro-oxidant effects (Geetha et al., 2005). The pro-oxidant activity of quercetin is likely attributable to its ability to auto-oxidize or be converted to *ortho*-semiquinone and *ortho*-quinone/quinone methide intermediates via enzymatic oxidation, which may contribute to the generation of ROS and GSH depletion (Metodiewa et al., 1999; Boots et al., 2003); however, the significance of this mechanism *in vivo* has not been fully resolved.

The results of a considerable number of *in vitro* mutagenicity and genotoxicity assays indicated consistent quercetin-related mutagenic effects. While the anti-mutagenic activity of quercetin at high concentrations is likely attributable to its role as a free radical scavenger (Geetha et al., 2005), its *in vitro* mutagenic properties may be due to the formation of the quinone methides, which are active alkylating DNA-reactive intermediates (Rietjens et al., 2005). Thus, at high concentrations, the quercetin-related mutagenicity that is observed *in vitro* in the presence of oxygen appears to be mediated via a pro-oxidant mechanism, not unlike the mechanism underlying the *in vitro* mutagenic and genotoxic properties, including clastogenicity, characteristic of other strong anti-oxidants, such as ascorbic acid (vitamin C) and *tert*-butylhydroquinone (TBHQ) at unrealistically high concentrations (JECFA., 1998, 2005).

In evaluating the structural features that may contribute to the apparent genotoxic activity of quercetin and other plant flavonoids, MacGregor and Jurd (1978) listed the presence of a 2,3-double bond, a 4-keto group, 3- and 5-hydroxyl substitution, and B-ring substitution (OH- at positions 3′ and 4′ in the case of quercetin) as crucial determinants of mutagenicity. Specifically, the quercetin hydroxyl groups at the 3′ and 4′ positions may be oxidized to form quinone intermediates. This is supported by observations of a reduced or absent mutagenic response associated with methylated quercetin derivatives, especially 3′-O-, 4′-O-, and 3′,4′-di-O-methylquercetin in the Ames assay (MacGregor and Jurd, 1978; Brown and Dietrich, 1979; Onishi et al., 1982; Czeczot et al., 1990). It is therefore not surprising that, there is an overlap in the structural features consistent with the anti-oxidant properties of quercetin (i.e., a catechol B-ring, a 2,3-double bond, and 3- and 5-OH groups) (Bors et al., 1990) and those facilitating the mutagenicity *in vitro*.

Interestingly, while dietary quercetin is almost completely metabolized as a result of the first-pass effect in the gut and liver, the conjugated derivatives have been observed to retain some of the anti-oxidant properties (Manach et al., 1998). For example, although substitutions at the 3′-OH and 4′-OH positions (isorhamnetin and tamaraxetin, respectively) resulted in a decrease in anti-oxidant activity (Yamamoto et al., 1999), conjugation with glucuronide and/or sulfate, which most likely occurs at the 5-OH and 7-OH groups of the A-ring (Galati et al., 2001; Justino et al., 2004), but also may occur at other positions including 3, 3′, and 4′ (Moon et al., 2001), did not significantly modify the anti-oxidant activity of quercetin (Justino et al., 2004). It has been shown that the presence of the ortho-catechol group in the B-ring (3′,4′-OH) is most conducive to the anti-oxidant properties of quercetin. Retention of anti-oxidant activity of metabolized quercetin was confirmed in blood samples withdrawn from rats administered quercetin orally (Justino et al., 2004).

Although quercetin has consistently tested positive for mutagenicity and genotoxicity *in vitro*, the implications of these findings in an assessment of human safety have not been established. Among relevant considerations, differences in the mechanism of action responsible for the quercetin-related mutagenicity between test systems have been identified, which suggest that the results obtained *in vitro*, particularly in the Ames assay, may not be appropriate to evaluate potential quercetin toxicity following oral exposure. In the *Salmonella* mutagenicity assay, quercetin acts as a direct mutagen (Caria et al., 1995). As such, the positive results obtained in the absence of exogenous enzymatic activation are enhanced in the presence of metabolic activation, as oxidative scavengers such as SOD and catalase present in the S9 system effectively prevented the auto-oxidative degradation of quercetin (Ueno et al., 1984). The decreased mutagenic potential of degraded quercetin was demonstrated in a study in which xanthine and xanthine oxidase were added to the test system as generators of superoxide radicals, which enhanced the degradation of quercetin (Ueno et al., 1984). Evaluated at 2 different pH levels, quercetin-induced SOS activity in *E.coli* cells was increased at pH 6.0 in comparison to pH 7.4, as the more basic pH supported the oxidative degradation of quercetin and consequently reduced quercetin-related mutagenicity (Rueff et al., 1992). Also, at the more basic pH, an increase in the mutagenic response was noted following addition of the oxidative scavenger, SOD. In contrast, in eukaryotic test systems in which ROS generation is expected to mediate the positive mutagenic response (i.e., pro-oxidant mechanism), quercetin-related mutagenicity was increased under basic conditions (pH 8.0) (Gasper et al., 1994) at which spontaneous auto-oxidization is expected to occur, and either not enhanced (Gasper et al., 1994; Caria et al., 1995) or reduced in the presence of scavenging enzymes added as part of the S9 system (Meltz and MacGregor, 1981; Rueff et al., 1986; NTP, 1992; Caria et al., 1995).

Of importance, the positive mutagenicity/genotoxicity results observed *in vitro* have not been confirmed *in vivo* following oral administration of quercetin. Although quercetin is a strong anti-oxidant, which may under certain conditions have pro-oxidant properties as a result of auto-oxidation or enzymatic conversion to *ortho*-semiquinone and *ortho*-quinone/quinone methides, *in vivo*, several protective mechanisms act in concert (e.g., GSH conjugation, other anti-oxidants, and pre- and post-absorptive biodegradation) to limit its potential to act as a pro-oxidant/genotoxicant. For example, while the anti-oxidant properties of quercetin *in vitro* may be due at least in part to an ability to chelate transition metal ions such as iron and copper (Sestili et al., 1998), *in vivo* transition metals are complexed by proteins and are unlikely to catalyze the auto-oxidation of polyphenols significantly (Galati et al., 2002). Nevertheless, 1% quercetin in the diet exacerbated renal tubular necrosis in the LEC strain of rats prone to accumulate copper (Kitamura et al., 2005). In theory, formation of oxidized quinone metabolites *in vivo* could lead to binding with various cell constituents, including DNA, resulting in genotoxic effects (Metodiewa et al., 1999); however, in attempting to determine the mechanism of quercetin mutagenicity

in relation to its lack of consistent carcinogenicity *in vivo*, Rietjens et al. (2005) suggested that although the *ortho*-quinone/quinone methide oxidative metabolites may be involved in DNA-adduct formation, as supported by results of *in vitro* studies with quercetin, the DNA-adduct formation may be transient. In fact, incubation of purified 6- or 8-glutathionyl quercetin conjugates in 25 mM potassium phosphate in D_2O resulted in an isomeric mixture of both compounds with time (Boersma et al., 2000). Additional evidence to support the transient nature of quercetin-DNA-adduct formation is derived from *in vitro* studies conducted by Walle et al. (2003) in which it was demonstrated that incubation of quercetin with some cell types (Hep G2 cells) produced a time-dependent decrease in the binding of quercetin to DNA or protein, with higher levels of adduct formation observed at 10 min than at 2 h. Furthermore, methylated quercetin metabolites (3'- and 4'-*O*-methylquercetin) were associated with substantially lower levels of DNA-adduct formation in comparison to the aglycone (van der Woude et al., 2006). Although it remains to be elucidated whether the reversible nature of the DNA adducts results in the regeneration of the parent DNA base and, thus, detoxification or, alternatively, depurination of the DNA base, it has been speculated that if the former is true, the transient nature of the quercetin-DNA-adducts may help to explain the absence of quercetin-related carcinogenic effects *in vivo*, despite the apparent genotoxicity observed *in vitro* (van der Woude et al., 2005).

Moreover, quercetin has demonstrated high-selectivity for albumin such that, in comparison to other proteins, binding to human serum albumin was at least ~10-fold greater (Kaldas et al., 2005). Although not entirely consistent among different cell lines, quercetin binding to DNA and intracellular protein was markedly reduced in human intestinal Caco-2 cells in cell culture medium than when the assay was performed in buffer solution (Walle et al., 2003). It was noted by the study investigators that the high-affinity binding of quercetin to serum protein in the culture medium (10% fetal bovine serum) may have prevented the distribution of quercetin into the cell. While some authors have indicated that formation of albumin–quercetin complexes following absorption of the aglycone may prolong its elimination and consequently increase the possibility for the flavonol to dissociate and interact with cellular proteins and DNA (Skibola and Smith, 2000), others have suggested that the binding of quercetin and, specifically, the reactive ortho-quinone/quinone methide metabolites of oxidized quercetin, to albumin attenuates the possibility of ensuing oxidative damage and therefore serves as a protective and detoxification mechanism *in vivo* (Kaldas et al., 2005).

Although oxidation of quercetin was shown to significantly increase binding of quercetin to human serum albumin, the addition of GSH at normally occurring cellular concentrations (5 mM) was observed to completely suppress the binding (Kaldas et al., 2005). Based on unexpected results demonstrating that only slightly increased levels of quercetin DNA/protein-adducts were formed *in vitro* in cells expressing tyrosinase or peroxidase-type oxidative enzymes in comparison to cells in which such activity was not detected, it was suggested that oxidative quercetin quinone metabolites may bind preferentially to GSH, thus limiting the potential for DNA-binding (van der Woude et al., 2005). Furthermore, the authors noted that in the presence of the average cellular concentration of GSH (5 mM), depletion was not likely to occur as a result of GSH-scavenging of quinone-type metabolites under the conditions of the assay. Effective scavenging of the reactive quercetin oxidation products by GSH and formation of 1 of 2 possible adducts (i.e., 6-GS-quercetin and 8-GS-quercetin) also has been confirmed by others (Metodiewa et al., 1999; Boersma et al., 2000; Boots et al., 2003). Additionally, consumed as part of a normal diet, quercetin does not act in isolation, but rather becomes a component of an integral network of other anti-oxidants including ascorbate, which act together to regenerate the parent compound from the oxidized derivatives (Stavric, 1994; Boots et al., 2003). Thus, once quercetin has exerted its anti-oxidant function and is presumably converted into a reactive pro-oxidant, it may be recycled by other anti-oxidants to the parent compound or react with GSH to form GS-quercetin conjugates; however, when both GSH and ascorbate are present, quercetin reacts preferentially with GSH (Boots et al., 2003).

Most importantly, as indicated by the metabolism data obtained *in vivo*, the potential for oxidative stress-mediated genotoxicity is minimized by the preferential microbial degradation of quercetin in the intestine, as well as the *O*-methylation and glucuronidation/sulfation of the flavonol including at the site of absorption resulting in a limited bioavailability of the aglycone *in vivo* and subsequent rapid excretion in the urine. As previously discussed, quercetin is extensively metabolized to non-mutagenic compounds such as 3'-*O*-methylquercetin and 4'-*O*-methylquercetin (MacGregor and Jurd, 1978; Hatcher et al., 1981; Onishi et al., 1982; Czeczot et al., 1990; Zhu and Liehr, 1993; Zhu et al., 1994), which likely contributes significantly to the absence of quercetin-induced mutagenicity *in vivo* and the absence of carcinogenic activity (Zhu and Liehr, 1993; Morand et al., 1998; van der Woude et al., 2006). The low bioavailability of the free aglycone as a result of biodegradation is further compounded by possible binding of quercetin to the small intestinal epithelium, preventing systemic uptake (Carbonaro and Grant, 2005). Thus, although several defense mechanisms exist to protect against potential quercetin-induced toxicity, it should be emphasized that following oral administration, the aglycone demonstrates a very low order of bioavailability, which in itself is a crucial control point limiting any adverse effects. Using quercetin as an example to evaluate whether *in vitro* mutagenicity is an appropriate indicator of carcinogenicity, Rietjens et al. (2005) also argued that the positive mutagenic activity observed with quercetin *in vitro* may not necessarily adequately predict a real risk for carcinogenesis

in vivo, particularly at low dose levels. Although positive results are observed in *in vitro* test systems of quercetin, the limited absorption of quercetin and almost complete metabolism in the intestinal tract should be considered when extrapolating these results in an assessment of potential quercetin-induced genotoxicity and overall safety of quercetin in humans (Gross et al., 1996). Short- and long-term animal studies using oral routes of exposure are a more reliable and appropriate model for the assessment of the actual carcinogenic potential of quercetin associated with its presence in food.

When tested *in vivo*, except by intraperitoneal administration (Sahu et al., 1981; da Silva et al., 2002), quercetin consistently yielded negative results for mutagenic activity in somatic cells (MacGregor, 1979; Aeschbacher et al., 1982; Caria et al., 1995; Ngomuo and Jones, 1996; Taj and Nagarajan, 1996; Cierniak et al., 2004). Consistent with the *in vivo* mutagenicity/genotoxicity data, which highlight the importance of quercetin degradation in the limitation of its activity, the data pertaining to the potential reproductive and developmental effects also support the importance of the first-pass effect in eliminating quercetin-related toxicity following oral administration. The importance of the first-pass effect is further emphasized by studies demonstrating sperm abnormalities in mice following intraperitoneal (Rastogi and Levin, 1987), but not oral administration (Nandan and Rao, 1983). As suggested by Rastogi and Levin (1987), the significant elevation in the percent of abnormal sperm cells following intraperitoneal, but not oral treatment, may be a result of quercetin bypassing intestinal degradation prior to contact with the germ cells when provided via non-oral routes of administration.

Two findings of concern emerge from the long-term animal toxicity and carcinogenicity studies: renal tumors in male rats and bladder and intestinal tumors in male and female rats. Several considerations support a conclusion that the renal effects of quercetin in the rats are not relevant to human safety. Although the results of the 2-year NTP rat feeding study indicated a statistically significant increase in the incidence of renal proliferative lesions, which occurred only in the presence of nephropathy in high-dose males, no toxicologically significant adverse effects were reported at quercetin dose levels at or below 500 mg/kg body weight/day or in females at 2000 mg/kg body weight/day. In evaluating the relevance of these male kidney lesions in an assessment of quercetin safety for human consumption, several factors should be considered. In addition to the already significant age-related background incidence of kidney diseases in the male F344 rat, compound-related kidney neoplasms also may occur with greater frequency in males of this particular strain of rat (NTP, 1992). For example, comparable effects related to chronic nephropathy only with greater severity also were observed in male F344/N rats administered hydroquinone (also a strong anti-oxidant) in another 2-year NTP study (NTP, 1989), which has been attributed to enhancement of the severity of chronic progressive nephropathy with stimulation of tubular proliferation (Hard et al., 1997).

As initially discussed by Ito (1992) and recently reiterated in a review by Rietjens et al. (2005), the possible role of $\alpha_{2\mu}$-globulin-related nephropathy, which occurs only in male rats, may have been a contributing factor in the formation of the renal lesions in males following quercetin exposure in the NTP study. Consistent with the criteria established by the US Environmental Protection Agency (US 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 indicative of $\alpha_{2\mu}$-globulin nephropathy. High-incidences of protein casts also were observed in a 2-stage carcinogenicity study (Fukushima et al., 1983) and enhanced proteinuria was observed at the highest dose level tested in a 21-day rat feeding study (Rangan et al., 2002). Also, quercetin exacerbated nephrotoxicity in male LEC rats (Kitamura et al., 2005).

Additionally, it is of interest to note that the severity of the lesions observed in the NTP rat study also may have been further enhanced as a result of the high protein content of the NIH-07 basal diet used in this study. More specifically, several short- and long-term studies were conducted by the NTP in F344 rats to assess the possible 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 ($\sim$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 in the high-dose male test rats may have 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.

As pointed out by Okamoto (2005), the occurrence of tumors only following long-term administration at high-dose levels of quercetin is characteristically associated with non-mutagenic carcinogens. Furthermore, not only were kidney lesions not observed prior to the 24-month evaluation, but as already stated, the tumors were observed only at nephrotoxic dose levels, which is further supportive of a potential secondary response rather than a direct carcinogenic effect (Hirono, 1992). 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 semi-quantitative 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 tumors of spontaneous origin. Furthermore, the re-evaluation did not reveal any cellular alterations that would indicate 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.

Using male and female Norwegian rats, urinary bladder and intestinal tumors were observed by Pamukcu et al. (1980) at much lower dose levels (~50 mg quercetin/kg body weight/day) and following a shorter period of time (58 weeks). These results were not confirmed in any of the longer term studies performed with several-fold higher dose levels of quercetin, including the 2-year NTP study. 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). Other authors have suggested that the discrepancy observed in the carcinogenic response between the Pamukcu et al. (1980) rat study and other long-term studies may be attributable to strain- or species-specific differences in tumor susceptibility, or alternatively, variations in experimental conditions (Boorman and Hollander, 1974; NTP, 1992). For example, several authors postulated that the differential findings of the Pamukcu et al. (1980) study may be due, at least in part, to compositional differences in the basal diets used as vehicles for the administration of quercetin (Morino et al., 1982; Lamson and Brignall, 2000; Okamoto, 2005). We consider it likely, however, 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, 1982a) were reported to develop the same types of tumors, albeit at a higher level of incidence than those observed following treatment with quercetin. It is now well accepted that BF contains the carcinogen, ptaquiloside, which induces ileal and bladder tumors in rats (Hirono et al., 1987). Therefore, although the study specifies a greater than 99% level of purity for the quercetin test material, the potential of cross-contamination needs to be considered as a possible explanation for the unique appearance of these tumors limited to this single study, particularly since other studies of quercetin conducted for longer periods of time and at higher dose levels failed to reproduce the intestinal and urinary bladder tumors. Given the lack of reproducibility of these study results and the possibility of contamination by BF, the Pamukcu et al. (1980) study should not be considered in the evaluation of quercetin safety.

Other long-term toxicity studies conducted not only in rats, but also in hamsters and mice, demonstrated the absence of significant histopathological effects, including in the urinary bladder and kidneys, at comparable and in some cases higher dose levels of quercetin (Ambrose et al., 1952; Saito et al., 1980; Hirono et al., 1981; Hosaka and Hirono, 1981; Morino et al., 1982; Ito et al., 1989). Likewise, the absence of renal tissue neoplasms was confirmed by a 2-stage carcinogenicity study that included a quercetin-only control group (Zhu and Liehr, 1994). Several additional activation–promotion studies (Fukushima et al., 1983; Hirose et al., 1983) also support that the urinary bladder tumors observed exclusively in Pamukcu et al. (1980) rat study were not directly related to treatment with quercetin.

Furthermore, in the 2-stage carcinogenicity studies, quercetin consistently did not demonstrate any initiation activity related to oral administration, and although a few equivocal results were reported with respect to some enhancing activity of quercetin, the majority of studies failed to demonstrate any quercetin-related promotion of carcinogenicity, or conversely, demonstrated chemoprotective effects. In cases where questionable results related to quercetin-promoting properties were observed, these can be largely attributed to poor study design. For example, the study conducted by Pereira et al. (1996) cannot be considered a true 2-stage carcinogenicity study as AOM was administered subcutaneously 8 days following commencement 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 should be considered to be of limited relevance to the assessment of quercetin safety. Also, in the study evaluating the effect of quercetin on ENNG-induced duodenal tumorigenicity, in which quercetin was report-

edly associated with an increase in the multiplicity and size of tumors, but not in tumor incidence, all 19 animals in the ENNG-control group exhibited duodenal tumors (Matsukawa et al., 2002). It is therefore apparent that the dose of the initiating agent administered, resulting in a 100% tumor incidence, was too high in this study to properly evaluate the promotional potential of quercetin, and hence, this study is inappropriate for inclusion in the evaluation of the tumorigenic potential of quercetin. The weight of evidence from long-term animal studies and initiation–promotion studies indicates that quercetin is neither a carcinogen nor a promoter of tumor development in the kidneys or any other organs when administered in the diet of laboratory animals at reasonable multiples of human exposure.

There were no reported significant adverse health effects following oral administration of quercetin to humans at doses up to 1000 mg/day for up to 12 weeks. Although nephrotoxicity was reported in a phase I intravenous injection trial, the parenteral route of administration is inappropriate for assessing the safety of orally administered quercetin, because bioavailability of the dietary quercetin aglycone is low or negligible as a result of extensive metabolism. Based on the most recent study demonstrating human plasma aglycone levels of 8 ng/mL following oral administration of 1500 mg quercetin/day (Wang and Morris, 2005), an unrealistically high oral dose (i.e., ~28 kg of quercetin) would be required in order to attain a plasma quercetin level comparable to that following intravenous administration of 722 mg quercetin (~150 mg/L), the dose level above which nephrotoxicity occurred in some study subjects. It should be noted that the study group consisted of subjects with compromised health (i.e., cancer patients non-responsive to traditional therapy), and not unlikely a somewhat incapacitated detoxification system. In addition to the controlled clinical trials, a number of epidemiological reports were reviewed which unequivocally support the absence of any relationship between flavonoid consumption from the diet and an increased risk of cancer. The results of the human clinical trials, corroborated by the epidemiological data, together with a safe history of use as a dietary supplement, support findings from oral toxicity studies in animals that the consumption of quercetin at levels proposed for use in foods would not be expected to cause any adverse health effects.

In summary, a number of points needs to be considered to understand why quercetin is mutagenic *in vitro*, but not mutagenic/carcinogenic *in vivo*. Firstly, the metabolic fate of quercetin *in vivo*, particularly the first-pass effect, significantly reduces the potential for quercetin-mediated adverse effects following oral consumption. For example, *O*-methylation results in the elimination of the essential structural features that underlie quercetin-related mutagenicity. Secondly, several protective mechanisms exist *in vivo* to limit the pro-oxidant activity of the small quantities of systemically available unmetabolized quercetin, and combined with the low bioavailability of the quercetin aglycone act in unison to limit the possibility for the occur-

rence of any of the adverse effects that are observed *in vitro*. Moreover, given the very low-order bioavailability of the quercetin aglycone, these protective mechanisms are not expected to be saturated even at higher levels of quercetin exposure. Therefore, while enzymatic or chemical oxidation of quercetin resulting in the formation of *ortho*-quinone/quinone methides warrants concern over possible pro-oxidant and genotoxic activity, which is in fact confirmed by *in vitro* study results, the first-pass effect, which ensures the metabolism of a considerable amount of an orally administered dose (>90% in rats), as well as several other protective mechanisms, which prevent the occurrence of any adverse effects as a result of the low levels of systemically available aglycone, provide a mechanistic understanding for the absence of any carcinogenic effects in the long-term animal studies. The dose levels in the long-term animal studies at which no toxicologically significant adverse effects were reported support the addition of food-grade quercetin to foods at levels resulting in exposure estimates approximating intakes of naturally-occurring quercetin from the diet by consumers with a high fruit and vegetable intake (i.e., 200–500 mg/day).

8. Conclusion

From a critical evaluation of the available literature on the biological effects of quercetin, including data related to safety, it may be concluded that quercetin, at estimated dietary intake levels, would not produce adverse health effects.

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