Putative Cancer Chemopreventive Agents of Dietary Origin – How Safe Are They?

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Abbreviations: DIM, 3,3’-diindolylmethane; I3C, indole-3-carbinol; NOEL, no effect level; ip, intraperitoneal, iv, intravenous; sc, subcutaneous; NSAID, non steroidal anti-inflammatory drugs; FMO1, flavin monooxygenase 1
Abstract: As cancer chemopreventive agents are intended for use by healthy individuals as prophylactics to prevent or retard the development of cancer, they must be amenable to ingestion over prolonged periods without toxicity. Therefore putative chemopreventive agents need to undergo stringent testing to ensure their safety with regard to chronic exposure in humans. The diet is thought to be a source of chemopreventive agents, and dietary compounds are generally considered to be of low hazard, albeit this notion has not often been put to the test. Here the safety information available for 5 dietary putative chemopreventive compounds, indol-3-carbinol, curcumin, quercetin, epigallocatechin gallate (EGCG) and capsaicin, is reviewed. For these agents normal dietary intake, doses used in clinical trials, efficacious doses in rodents and, where available, toxic doses are compared. For curcumin, quercetin and capsaicin, toxicological data is only available from studies in rodents. Information on long-term effects in animals beyond 28 or 90 days is lacking for EGCG. Capsaicin and quercetin are suspected carcinogens. Indole-3-carbinol and quercetin can modulate the absorption of other drugs given concomitantly. Without further investigation of their toxicology, it is difficult to recommend any of these agents for long-term use in the healthy population.

Introduction

Many studies of cancer epidemiology over the last 25 years suggest a relationship between diet and cancer incidence. In 1981, Doll and Peto (1) first suggested that 35% of cancer deaths in the United States were related to diet and therefore potentially avoidable. This notion is supported by more recent additional evidence (2). The relationship between cancer and diet is strongly supported by epidemiological studies which show that cancer incidence can change with migration,
by cohort studies relating cancer incidence to diet, and by investigation of special exposure groups in populations that consume unusual diets (1,2). A diet rich in fruit and vegetables has been proposed to reduce the incidence of a number of malignancies (2 – 6), although a systematic recent review of nutritional interventions in cancer patients suggests that evidence for an improvement of survival or disease prognosis of cancer patients by dietary modification is lacking (7).

Phytochemicals have for centuries been used in the prevention and treatment of diseases, and some are thought to inhibit tumorigenesis. The anticarcinogenic properties of phytochemicals have been scientifically tested in vivo using carcinogen-induced and transgenic rodent models (3). The utilisation of agents in a healthy population to inhibit the initiation or development of cancer is termed chemoprevention (8). In contrast, chemotherapy aims at the eradication of established cancer. In general, drugs used in chemotherapy are developed by the pharmaceutical industry and undergo extensive testing not only of efficacy, but also toxicity, mutagenicity and carcinogenicity. A governmentally appointed regulatory authority reviews this data extensively prior to registration for use in oncology. In contrast, dietary phytochemicals suspected of possessing cancer chemopreventive properties are often assumed to be safe and can, therefore, bypass some of the detailed toxicological investigation required for chemotherapeutic drugs. However, because their intended use is for long-term consumption by healthy humans, it is crucial that their toxicology is fully understood. Chemopreventive agents inhibit the development of cancer by altering cellular biology at the molecular level (8). They must be amenable to prolonged ingestion often at levels well in excess of normal dietary intake without inducing adverse effects. Recently concern has been raised as to the safety of non-steroidal anti-inflammatory drugs (NSAIDs) and selective
cyclooxygenase-2 inhibitors, when administered with cancer chemopreventive intent over prolonged periods of time (9,10). Under these conditions NSAIDs such as sulindac or piroxicam can exert severe gastrointestinal side effects, and cyclooxygenase-2 inhibitors, eg. celecoxib, seem to increase the risk of adverse cardiovascular events (9,10). In the light of these safety concerns diet-derived phytochemicals might be considered safe alternatives to synthetic drugs as putative cancer chemopreventive agents. However, the expectation that they possess an unblemished safety record can be fallacious, as borne out for example by the detrimental outcome of the clinical intervention study of beta-carotene as a lung cancer chemopreventive agent (11). In the light of the paucity of information on the safety of putatively chemopreventive phytochemicals, in this paper preclinical and early clinical safety information is reviewed for five representative dietary agents. Tables 1 to 5 summarize the acute and chronic toxicities of these agents, which are indole-3-carbinol (I3C, Table 1), curcumin (Table 2), quercetin (Table 3), capsaicin (Table 4) and epigallocatechin gallate (Table 5). Table 6, which presents normal dietary intake juxtaposed with recommended health food dose, chemopreventive dose in rodents, clinical trial dose and no effect level (NOEL) from toxicity testing, should help with the assessment of the safety of these substances. The ultimate aim of the review is to increase the awareness of chemopreventive phytochemical safety evaluation. In the following, specific toxicities and biochemical properties predisposing for potential drug interactions are presented for each of these five agents.

**Indole-3-carbinol**

I3C (for structure see Fig. 1) is a component of cruciferous vegetables with chemopreventive properties in several rodent models (12-17). In humans it has been
studied for the treatment of cervical intraepithelial neoplasia, breast cancer prevention and recurrent respiratory papillomatosis (18 – 21).

Specific toxicities. The preclinical toxicity of I3C has been reviewed (22). In a clinical trial tremor and unsteadiness were observed in three individuals on I3C at 800mg/day (~11.4mg/kg/day, po) or more (18). I3C was not mutagenic with or without metabolic activation in the Ames test and did not induce mutations in a shuttle vector system in human cells (23), nor did it cause chromosome breaks in mouse bone marrow cells at oral doses of up to 1g/kg (24). I3C has been shown to act as co-carcinogen. At doses of >250ppm it enhanced the hepatocarcinogenicity of aflatoxin B1 in the rainbow trout (25). Similarly, the incidence of dimethylhydrazine-induced colon cancer was increased in rats which received I3C 0.1% with the diet (26). It is not clear whether co-carcinogenesis was mediated by parent I3C or its in vivo decomposition products, the major one of which is di-indolyl methane (DIM).

Potential for drug interactions. I3C modulated the activity of both phase 1 and phase 2 drug metabolism when administered with the diet or by gavage (27,28,29,30-33). These experiments indicate that I3C can induce CYP1A1, CYP1A2, CYP2B1 and CYP3A2 in liver and CYP1A1 in colon, whilst at the same time inhibiting flavin-containing monooxygenase 1 (FMO1) (28,31,33). Activities of liver UDP glucuronosyl transferase, glutathione S-transferase and quinone reductase were increased in mice which received I3C 50mg/kg for 10 days (27). In rats fed I3C at 0.5% for 14 days, CYP1A2 and CYP2B1, aflatoxin B1 aldehyde reductase, glutathione S-transferase Yc2 and quinone reductase were induced (32). Similar changes to the metabolism profile of the rat were observed after feeding I3C or DIM at either 1000 or 2500ppm for 4 weeks (33). Both compounds reduced FMO1 protein expression significantly. I3C induced hepatic CYP levels, so that these rats metabolized
tamoxifen and nicotine faster than controls. Guinea pigs, mice and rabbits which received I3C at 2000ppm for 4 weeks showed induction of hepatic CYP1A1/2, whilst FMO1 levels were either unaffected or only slightly reduced (34).

**Curcumin**

Curcumin (diferuloylmethane, for structure see Fig. 1) is a component of turmeric, the powdered rhizome of the plant *Curcuma longa* long used as a spice in Asian cuisine. Curcumin has been shown to have both anti-inflammatory and chemopreventive properties particularly against chemically induced colon cancer in rats and in the *Apc*<sup>Min</sup> mouse (35-37). Curcumin has also been shown to reduce tumor number and volume in a variety of carcinogen-induced or genetic rodent models of carcinogenesis (38, 39). Recently, curcumin has been given orally to patients with advanced colorectal cancer and in patients with high-risk or pre-malignant lesions in phase I trials (40, 41).

*Specific toxicities.* When given to patients with cancer at 3600mg/day for 4 months (41) or 800mg/day for 3 months (40) curcumin caused mild diarrhoea, increased serum alkaline phosphatase and increased serum lactate dehydrogenase in a few individuals (40). Curcumin was not mutagenic in a number of Salmonella typhimurium tests as summarised by the World Health Organisation in 1996 (42). A slight but significant dose-related increase in sister chromatid exchange in bone marrow cells was observed in mice which received curcumin ip at doses ranging from 25–200mg/kg (43). However, chromosome aberrations were not recorded. Chronic feeding in rats of diets containing 100-1000 ppm curcumin for 3 or 6 months did not cause an increase in chromosome aberrations, whilst feeding 500 or 1000ppm for a more extended time period (9 months) increased chromosome aberrations slightly but significantly (43). Curcumin at 15µM and more damaged DNA as indicated by the
Comet assay *in vitro* in human gastric mucosal cells and peripheral blood lymphocytes (44). In mice, dietary curcumin (0.015%) failed to increase the incidence of micronucleated polychromatic erythrocytes or cause structural or numerical aberrations in bone marrow chromosomes, pregnancy rate, number of live or dead embryos or total implants (45).

**Potential for drug interactions.** Curcumin at 4-20µM inhibited the activity of CYP1A1, 1A2 and 2B1 as measured by resorufin substrate assays in liver microsomes from rats pre-exposed to phenobarbitone or benzo(a)pyrene (46). When fed to male ddY mice at 2% for 30 days, curcumin significantly induced the activities of glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase and catalase in liver and kidney (47). A similar induction in liver and kidney was observed for glutathione S-transferase and quinone reductase (47). Curcumin (40-60µM) inhibited the activity of endogenous cellular NAD(P)H:quinone oxidoreductase in cultured human colon carcinoma and myeloid leukemia cells and induced p53 degradation resulting in inhibition of p53-mediated apoptosis (48). Curcumin (50µM) impaired the function of the p53 tumor suppressor gene in RKO colon cancer cells (49).

**Quercetin**

Quercetin (3,3’,4’,5,7-pentahydroxyflavone, Fig. 1), the aglycone of quercetrin and rutin, is an integral component of the human diet and occurs in onions, apples and red wine. It has shown chemopreventive properties in rodents (50-53) and has been subjected to a phase 1 clinical trial in cancer patients (54).

**Specific toxicities.** In this clinical trial quercetin at 1400mg/m^2^ (~36mg/kg, iv) and above caused kidney toxicity (54). A review in 1983 by IARC concluded that there
was limited evidence for the carcinogenicity of quercetin in experimental animals (55), and further carcinogenicity studies did not change this conclusion (56). The effect of quercetin on rodent carcinogenesis was studied in azoxymethane-induced colon cancer and aberrant crypt formation in rats (57, 58), estradiol tumorigenesis in hamster kidney (59) and 7,12- dimethylbenz[a]anthracene-induced mammary carcinogenesis (58). At dietary concentrations of 0.3-3% quercetin increased tumor incidence. A review of more than 30 publications on the genetic effects of quercetin in *in vitro* and *in vivo* test systems concluded that whilst not mutagenic *in vivo*, it produced cytogenetic damage in both human and rodent cells (56). Mice that received two ip doses of quercetin at 625, 1250 or 2500mg/kg 24h apart, were assessed for genotoxicity using the micronucleus and the comet assay (60). Results for the micronucleus assay were all negative apart from those in which mice received 1250mg/kg. In the comet assay, quercetin caused a small but significant increase in bone marrow damage index and frequency (60). There has been one publication on the teratogenicity of quercetin (61). Rats received quercetin as a single oral dose of up to 2g/kg on day 9 of pregnancy or daily on days 6-15 of pregnancy with examination on day 20. Apart from a reduction in foetus weight in the offspring of mice on 2g/kg, there was no other indication of teratogenicity (61). A dominant lethal assay was carried out in both rats and mice after two ip doses of 200, 300 or 400mg/kg/quercetin given to males 24h apart. Each male was paired with successive pairs of females weekly over a 6 week period. There was no significant difference between control and experimental groups with regard to dead implantations in either species. A significant loss of fertility in male mice was observed at 300 and 400mg/kg, but this effect was not observed in rats (62). Sperm abnormalities were induced in mice 5 weeks after a total dose of 80mg/kg quercetin ip split into 5 consecutive daily doses.
The percentage of abnormal sperm increased from a control level of 1.0 to 14.8%, and a 32% reduction in testicular weight was associated with a 28% reduction in sperm count (63).

*Potential for drug interactions.* The effect of quercetin on drug metabolizing enzymes was assessed by measuring the ability of liver microsomal enzymes from rats fed 0.3% quercetin for 2 weeks in the diet (~192 mg/kg/day) to metabolise ‘specific’ substrates (64). There was no change in activity of phase1 or 2 enzymes including NADPH-cytochrome P450, NADPH-cytochrome b5 reductase, CYP1A1, CYP1A, CYP2B1, CYP2B, CYP2E1, CYP3A and CYP4A, glutathione transferase and UDP-glucuronosyltransferase (64). In contrast, hepatic activities of CYP1A and CYP2B were induced in rats given 3 x 80mg/kg quercetin ip over 3 days (65). Quercetin has also been shown to modulate the absorption of other drugs. When dosed orally to rats at 2, 10 or 20mg/kg, 20 min prior to an oral dose of paclitaxel, it increased the bioavailability of paclitaxel by between 1.8-3.0 fold (66). Oral co-administration of quercetin (40 or 50mg/kg) also increased the total bioavailability of digoxin (0.02mg/kg) in pigs by 170% and the digoxin peak plasma level by 413% with lethal consequences (67). In contrast, quercetin given orally at 50mg/kg to pigs or rats significantly decreased, by approximately 40%, the bioavailability of an oral dose of 10mg/kg of cyclosporine (68). *The ability of quercetin to modulate the absorption of other drugs may have been mediated by changes in the concentration of gut wall p-glycoproteins, proteins pumps which can expel xenobiotic molecules* (67, 68).

**Capsaicin**

Capsaicin (8-methyl-N-vanillyl-6-nonenamide, Fig. 1) is found in chilli peppers at concentrations of between 0.1-1.0 %. It is responsible for the burning
sensation experienced after ingestion of chilli. It is consumed worldwide as an ingredient of spicy foods, particularly in Latin America and Asia.

Capsaicin has shown cancer chemopreventive properties in a number of rodent studies (69-72). It has also been shown to inhibit angiogenesis, a chemopreventive and chemotherapeutic mechanism (73). Capsaicin has been used topically in cancer patients in the management of long-term neuropathic pain resulting from surgery (74).

Specific toxicities. Signs of poisoning after iv injection to dogs or cats included bradycardia, a fall in blood pressure and apnea (75). Dogs were more sensitive to this effect than cats. Capsaicin also affects peripheral and central nervous systems (75). These effects seem related to thermoregulation and desensitisation of the primary afferent neurons within the peripheral sensory system and account for its use in pain management.

There have been a number of investigations of the genotoxicity of capsaicin with conflicting results (76). Human peripheral lymphocytes treated with capsaicin with and without metabolic activation were examined for genotoxicity using micronucleus and sister chromatid exchange assays (77). Both tests gave positive results. However other investigators using purer material in tests for chromosomal aberration, bacterial mutation and the rodent micronucleus test did not confirm the genotoxicity of capsaicin. Genotoxicity was not established when material of 98.7% purity was used (78), whilst in another study 99% pure capsaicin afforded only weak positive results (79) leading to the suggestion that impurities in the capsaicin material under test may have been a critical factor in the earlier experiments, in which it was found to be genotoxic (77). Rodent studies (80) and epidemiology tentatively suggest that capsaicin may be carcinogenic (81, 82-84).

Potential for drug interactions. Capsaicin is metabolized predominantly by liver
cytochrome P450 and carboxylesterases to an array of O-demethylated, hydroxylated, and N-dehydrogenated products (76, 85). It is not known whether any of these metabolites share the chemopreventive properties of the parent molecule. Capsaicin is thought to generate potentially toxic reactive intermediates including epoxides, quinones and phenoxy radicals (85). It has been shown to act as a suicide inhibitor of CYP2E1 (76). This action is thought to be responsible for its ability to attenuate the mutagenicity and carcinogenicity of vinyl carbamate and N-nitrosodimethylamine (70). Capsaicin may well modulate the activity of other CYP isozymes particularly those in the CYP1A and CYP2B families, and it has been shown to induce metabolism by glutathione S-transferase and quinone reductase in mice at a dietary dose of 500ppm (70, 76, 85).

**Epigallocatechin-3-gallate**

The flavanol epigallocatechin-3-gallate (EGCG, Fig 1) is the most abundant catechin in green tea, the unfermented extract of the leaves of *Camellia sinensis*. Green tea contains up to 25% catechins (% dry weight) of which EGCG constitutes 9-13%, while epicatechingallate, epigallocatechin and epicatechin make up the remainder (86). EGCG has demonstrated chemopreventive properties in a number of rodent studies (87-92). Epidemiological studies have linked tea consumption with carcinogenesis both positively and negatively (86, 93-95).

**Specific toxicities.** Daily oral doses for 4 weeks of EGCG (800 mg/day) in 40 volunteers caused minor adverse effects (96). Similarly green tea extract at 5.05g/m² (~129 mg/kg) containing 13.2% EGCG (~17mg/kg) caused mild toxicity (97). In the latter case these symptoms were probably related to the caffeine content of the tea. Interestingly, of the catechins tested, EGCG was the one responsible for asthmatic and nasal symptoms elicited in three patients exposed to green tea dust (98). In a 90 day
study of polyphenon E, a formulation of green tea polyphenols containing 53.4% EGCG and 30.5% other catechins, the oral NOEL values were 600mg/kg/day for the dog and 90mg/kg/day for the rat (99). Histological examination of rats surviving to the end of the experiment revealed pancreatic necrosis and hepatic acinar necrosis. Although toxicity was similar to that seen with pure EGCG, polyphenon E was considered to be more toxic to rats than would have been predicted on the basis of its EGCG content (99). In a rat model of transient focal cerebral ischemia, EGCG was given intraperitoneally to rats at 50 mg/kg/day on the day of ischemia induction and daily for 72h thereafter (100). Although EGCG was associated with a decrease in the volume of the brain infarcts in this model, it also resulted in a 50% increase in the incidence of intracerebral hemorrhage.

The dermal toxicity of EGCG was assessed in shaved and depilated BALB/c and in hairless SKH1 mice. EGCG contained in a hydrophilic ointment at 1, 3 or 10% was applied to the skin daily for 30 days. No toxicity was observed in shaved BALB/c or hairless SKH1 mice (101). However, in depilated BALB/c mice treated with the 10% EGCG formulation, erythema and a papular rash were observed by day 5 associated with a 7% loss of bodyweight at day 15 (101).

Tea polyphenols are thought to be antimutagenic (102). The genotoxicity of polyphenon E was examined in a battery of in vitro and in vivo assays, none of which showed convincing evidence of mutagenicity (103).

The developmental toxicity of polyphenon E was assessed in Sprague-Dawley rats (104). Time-mated female rats were dosed daily by gavage from day 6 to day 15 of gestation with 125, 250, 500 or 1000mg/kg polyphenon E. No signs of maternal toxicity were observed. Resorption rates, litter size, sex ratio and fetal body weights
were similar in all dosage groups. The NOEL for polyphenon E in this experiment was considered to be >1000mg/kg (104).

Tea catechin-induced estrogenic responses on either uterine weight or uterine peroxidase activity were not observed in immature mice dosed ip with 30 or 50mg/kg/day EGCG for 3 days. However, when mice were co-treated with $17\beta$-estrodiol and EGCG, uterine peroxidase activity was induced 2.3-fold above that elicited by $17\beta$-estrodiol alone (105). EGCG alone at the higher of the two doses caused a marked increase in plasma alanine aminotransferase activity and mild liver damage (105).

**Potential for drug interactions.** Green tea polyphenol formulations and EGCG have been shown to affect drug metabolizing enzymes. In rats, 4 week treatment with a 2% aqueous solution of green tea extract resulted in a doubling of liver CYP1A1 and CYP1A2 activities (106). In SKH-1 hairless mice given 0.2% green tea polyphenols in the drinking water for 30 days, glutathione peroxidase, glutathione S-transferase, quinone reductase and catalase activity were induced 1.5 – 2 fold in small intestine, liver and lung, but not in skin (107). EGCG was administered to mice ip for 7 days at 12.5, 25 or 50 mg/kg/ day (108). At 50 mg/kg EGCG caused severe hepatic necrosis coincident with markedly increased plasma alkaline phosphatase. Ovarian aromatase was inhibited by 56% at 12.5 and 25mg/kg EGCG, and hepatic CYP3A and CYP2E1 activity and protein levels were slightly increased at 25mg/kg (108). The livers of rats given EGCG by portal vain perfusion (0.2-10mg/kg) were examined for glutathione S-transferase activity (109). There was a dose- and time-related increase in glutathione S-transferase protein expression (109). This increase was particularly associated with the GSTM2 subunit (109).
Discussion

The information presented above on five putative dietary cancer chemopreventive agents provides mosaic pieces which, when properly assembled and completed, will ultimately allow assessment of their safety. All five agents have undergone clinical evaluation, four of them in trials with a rationale related to cancer prevention or treatment (18-21,40,41,54,96,97), the fifth, capsaicin, in experimental treatments of neuropathic pain (74) and dyspepsia (110). If these agents are really to be considered as cancer chemopreventive interventions, humans will be exposed to them for prolonged periods of time, a scenario which requires a clear understanding of any potential hazard they might pose. To probe whether the available information is sufficient to make definitive judgements as to the safety of these agents, the data is interpreted in the following from three angles: i) adverse effects in humans and safe dose levels, ii) potential for drug interaction and iii) gaps in knowledge and future studies required.

Adverse effects in humans and safe dose levels

In general the published adverse effects of phytochemicals of the type investigated here tend to be of a minor nature, exemplified by results for curcumin and green tea. In contrast, the information gathered for capsaicin, I3C and quercetin suggest that they may cause more severe toxicity. Table 6 suggests that the rodent NOEL values for curcumin, I3C and EGCG are close to, or overlap with, recommended health food doses and doses used in clinical trials. Undoubtedly a comparison of doses in animals with those in humans needs to be interpreted with extreme caution. Nevertheless this dose proximity is noteworthy, as it may explain the adverse effects published for I3C in the clinical trial (18) alluded to above. There is no
published NOEL value for quercetin. In rats quercetin at a dose as low as 2mg/kg was efficacious in terms of enhancement of paclitaxel bioavailability (66). The kidney toxicity seen in the clinical trial of iv quercetin mentioned above (54) was seen at 32.6 mg/kg, approximately twice the recommended oral health food dose (111, 112). Comparisons between species of toxicities of a specific compound administered by different routes can be misleading and need to be read with great care, nonetheless it seems worth bearing in mind that this iv dose was administered on a weekly basis, whilst consumption of nutraceuticals and health food products tends to be daily [54, 111,112].

Potential drug interactions

Changes in metabolism and absorption caused by nutraceuticals might markedly alter the pharmacokinetics of medications given concurrently with the phytochemical with potential unwanted corollaries. Quercetin, I3C, capsaicin and EGCG are capable of altering phase 1 and phase 2 metabolism (27,29,31,64, 65, 85,108). The ability of quercetin to enhance intestinal absorption of drugs given concurrently has been considered responsible for severe toxicity, e.g. in pigs coadministration of quercetin with digoxin was lethal (67). In rodents I3C (128mg/kg/day) crossed the placenta and induced CYP enzymes in the foetus (29). CYP1A2 activity reflected by caffeine metabolism was elevated in 94% of women who received I3C at 400mg/day for 4 weeks followed by 800mg/day for a further 4 weeks, with a 4.1-fold mean increase in metabolism (19). These examples cast doubt on the suitability of unregulated availability of phytochemicals as nutraceuticals or in health foods at recommended doses well in excess of normal dietary intake.
Gaps in safety information and desirable future studies

Considerable uncertainty and gaps of knowledge exist for these compounds with respect to three issues germane to safety: i) discrepancies between study results, ii) potential carcinogenicity/teratogenicity, and iii) ability to affect drug metabolizing enzymes. These gaps require more experimental work to help complement the portfolio of safety data necessary to support further clinical development. Frequently results from different studies on the same agent are incompatible with each other. For example the acute toxicity of quercetin in an early investigation (113) is not commensurate with that seen in later experiments, in which far larger doses given on a daily basis were without ill effect. The discrepancy may stem from toxic impurities in early quercetin formulations. Consistent with this suspicion rats exposed to a technical quercetin formulation presented with cataracts, which were caused by an impurity as discovered when the formulation was purified and retested (114). So it seems pertinent to re-examine the acute toxicity of pure quercetin. Whilst the toxicities of curcumin, I3C and EGCG have been investigated in dogs (115, 116,117), quercetin and capsaicin have only been evaluated in rodents. Some findings should be experimentally confirmed, for example the negative effect of quercetin on rodent testes weight and its ability to induce sperm abnormalities when given ip at cumulative doses (16-160 mg/kg) (63).

As to the risk of carcinogenicity of these agents, life-time carcinogenicity studies have as yet not been performed on EGCG beyond 90 days and on I3C beyond 12 months. For curcumin, the ability to impair p53 tumour suppressor activity in cultured cells (48,49) requires confirmation in animals in vivo. With respect to teratogenicity, capsaicin has thus far not been tested. More research is also required to understand potential effects of the agents on the absorption, distribution, metabolism
and excretion of co-administered drugs, particularly to assess whether the effects described above may be applicable to humans. Furthermore it seems worth exploring which of these agents can cross the placenta. **All of these gaps suggest strongly that additional toxicity studies of these agents are highly opportune to clarify the potential safety of these agents.**

In conclusion, there seems to be a strong case for more extensive toxicological testing of agents of dietary origin. This is especially true if they are sold as health foods at relatively high doses without medical supervision. A somewhat similar conclusion was reached by Galati and O’Brien (118), who addressed molecular aspects of the toxicity of chemopreventive agents. It is tantalizing to consider that, if any of these agents was to be marketed by the pharmaceutical industry, extensive extra testing would be required before they could be made available on a long-term basis to healthy individuals.

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Figure legend

**Figure 1.** Chemical structures of the five chemoprevention agents discussed in this paper. 1. Indole-3-carbinol, 2. Curcumin, 3. Quercetin, 4. Capsaicin, 5. Epigallocatechin gallate.