Chlorogenic Acid
[327-97-9]

and

Caffeic Acid
[331-39-5]

Review of Toxicological Literature

Prepared for

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Contract No. N01-ES-65402

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July 1998

EXECUTIVE SUMMARY

The nomination by Drs. Gold, Ames, and Slone, University of California, Berkeley, of chlorogenic acid and caffeic acid is based on their occurrence in high concentrations in food and the apparent lack of carcinogenicity data.

Both chlorogenic and caffeic acids are constituents of numerous plant species from the families Umbelliferae, Cruciferae, Cucurbitaceae, Polygonaceae, Compositae, Labiatae, Solanaceae, Leguminosae, Saxifragaceae, Caprifoliaceae, Thaceae, and Valerianaceae. Thus, they occur in many common fruits, vegetables, spices, medicinal plants, and beverages.

Information on the commercial availability of chlorogenic acid was not found, but caffeic acid is available in small quantities from a number of U. S. producers.

No information was found on uses of chlorogenic acid. The use of caffeic acid for treating asthma and allergies has been investigated in drug development studies. Plants containing chlorogenic acid and/or caffeic acid have been used as herbal remedies and possess some of the following pharmacological properties: antiarthritic, antidiarrheal, antiinflammatory, antirheumatic, antitumor, antiviral, astringent, cardiotonic, carminative, chloretic, coronary vasodilatory, diaphoretic, diuretic, gastric sedative, hypotensive, intestinal antiseptic, purgative, and spasmolytic effects. Medicinal plants containing chlorogenic and/or caffeic acid have also been used as remedies for the common cold, hematemesis, hematuria, hemorrhoids, lumbago, neuralgia, tinnitus, and toothache.

Exposure to chlorogenic and/or caffeic acid occurs primarily via the oral route from the ingestion of foods, beverages, and herbal remedies. Inhalation exposure occurs from tobacco smoking.

Chlorogenic and caffeic acids may be expected to be found in the wastes generated by industries making coffee and processed potatoes. No information on the regulatory status of chlorogenic and caffeic acids was found.

In humans, the carcinogenic potency of caffeic acid has been estimated based on an average human intake of 10^6 ng/kg body wt/day (1 mg/kg body wt/day). The estimated number of cancer cases was less than 1000 per 1 million individuals.

In human immunological studies, both positive and negative results were found when individuals allergic to green coffee were tested for allergic reactions to chlorogenic acid by subcutaneous (s.c.) injection or skin scratch tests. Caffeic acid did not produce allergic reactions when administered s.c. to individuals allergic to green coffee, nor did it induce sensitization when applied by dermal patch to a woman allergic to beeswax.

No information on the chemical disposition, metabolism, or toxicokinetics of chlorogenic acid in humans was found. Ingestion of caffeic acid, however, produces a

number of metabolites, including glucuronides of *m*-coumaric acid and *m*-hydroxyhippuric acid. Oral administration of caffeic acid to volunteers resulted in rapid (no precise time specified) urinary excretion of *O*-methylated derivatives (ferulic, dihydroferulic, and vanillic acids), while *m*-hydroxyphenyl derivatives were excreted later (time not provided).

In one study using rats, chlorogenic acid was hydrolyzed in the stomach and intestine to caffeic and quinic acids. In isolated rat livers that were perfused with caffeic acid, 93% of the caffeic acid appeared unchanged after one liver passage; oxidation, methylation, and cyclization products were found in the perfusion medium, and glucuronides and sulfates of caffeic acid were identified in the bile. Following intravenous (i.v.) administration to rabbits, most of the dose was excreted unchanged in the urine within 2 hours. For caffeic acid, the elimination kinetics fit a two-compartment model when administered orally to rats or i.v. to rabbits.

For both chlorogenic and caffeic acids, the oral LD_{50} for redwing blackbirds was greater than 100 mg/kg (0.282 and 0.555 mmol/kg, respectively).

Few toxic effects resulting from acute exposure to chlorogenic or caffeic acid were noted in the reviewed studies. In rats dosed intraperitoneally (i.p.), chlorogenic acid at 4000 mg/kg (11.29 mmol/kg) induced death in 4 of 6 animals, and caffeic acid at 1500 mg/kg (8.326 mmol/kg) induced death in 5 of 8 animals, but doses of chlorogenic acid and caffeic acid lower than 2437 mg/kg (6.878 mmol/kg) and 1250 mg/kg (6.938 mmol/kg), respectively, were non-lethal.

Subchronic exposure of mice to chlorogenic or caffeic acid in the diet reduced aryl hydrocarbon hydroxylase (AHH) and glutathione-*S*-transferase (GST) levels in the intestine, but did not induce clinical symptoms of toxicity. In rats, the effects of feeding chlorogenic or caffeic acid in the diet include reduced kidney and adrenal weights (chlorogenic acid), hyperplasia of the forestomach (chlorogenic and caffeic acids), and increased antioxidant capacity (caffeic acid).

No information on chronic exposure to chlorogenic acid was found, but chronic exposure to caffeic acid in the diet induced hyperplasia of the forestomach (mice, rats, and hamsters), hyperplasia of the kidney (mice and rats), and increased liver and kidney weights (rats).

Chlorogenic acid in the diet inhibited benzo[*a*]pyrene (B*a*P)-induced increases in liver aryl hydrocarbon hydroxylase and liver glucuronosyl transferase in mice. In rats chlorogenic acid inhibited paraquat-induced increases in liver catalase, liver glutathione peroxidase, and liver glutathione reductase and peroxidized corn oil-induced increases in serum total cholesterol, serum triglycerides, serum alanine aminotransferase, serum aspartate aminotransferase, and serum and liver lipid peroxides.

Based on one rat study, the adverse reproductive effects of i.p. treatment with chlorogenic or caffeic acid consisted only of fetal rib defects.

In mice, 2% (20,000 ppm) chlorogenic acid in the diet for 96 weeks induced papillomas and carcinomas of the forestomach, alveolar type II-cell tumors of the lung, and renal cell adenomas, while in rats 1 or 2% (10,000 or 20,000 ppm) caffeic acid in the diet for 51 weeks to 2 years induced papillomas of the forestomach and renal adenomas. One study in which rats were exposed to 2% (20,000 ppm) caffeic acid in the diet for two years showed treatment-induced carcinomas of the forestomach, whereas two studies with shorter exposure durations showed no such effect.

Initiation/promotion carcinogenicity studies for chlorogenic acid were not found, but in studies using rats, caffeic acid was shown to exert strong promotion activity for forestomach carcinogenesis when administered in the diet for 51 weeks after a single dose of a carcinogen (e.g., 7,12-dimethylbenz[*a*]anthracene [DMBA], *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine [MNNG]); caffeic acid treatment for shorter durations (i.e., 35 weeks) did not promote the induction of tumors.

Chlorogenic acid inhibited the number of DMBA-initiated/12-*O*-tetradecanoylphorbol-13-acetate (TPA)-promoted skin tumors when administered topically to mice concomitantly with the DMBA and TPA, but not when administered prior to the initiator and promoter. Caffeic acid also inhibited formation of DMBA-initiated/TPA-promoted skin tumors, but to a lesser extent than chlorogenic acid. Chlorogenic acid, when administered i.p. to mice, reduced the number of B*a*P-induced lung tumors, and, when administered in the diet of hamsters, reduced the number of methylazoxymethanol acetate (MAM acetate)-induced colon tumors, colon adenocarcinomas, and hepatocellular foci. In mice, caffeic acid in the diet reduced the number of B*a*P-induced tumors of the forestomach, while i.p. administration of caffeic acid for ten days after a single s.c. injection of sarcoma-180 cells inhibited sarcoma-180 tumor growth. In rats, caffeic acid in the diet reduced the incidence of 4-nitroquinoline-1-oxide (4-NQO)-induced tongue neoplasms. Dietary treatment of rats with caffeic acid inhibited the formation of neoplasms and preneoplasms of the forestomach induced by treatment with diethylnitrosamine (DEN), *N*-methyl-*N*-nitrosourea (MNU), *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN), 1,2-dimethylhydrazine (DMH), and 2,2'-dihydroxydi-*n*-propylnitrosamine (DHPN).

Both chlorogenic acid and caffeic acid induced strand breaks in DNA in acellular test systems that favored formation of oxygen radicals, particularly in the presence of transition metals. These chemicals were not mutagenic in standard bacterial mutagenicity assays. However, caffeic acid, in the presence of Mn^{2+} and in the absence of S9 activation induced mutations in *Salmonella typhimurium* strains TA98 and TA100; removal of the transition metal ions or addition of liver S9 eliminated the mutagenic response. Both chlorogenic acid and caffeic acid induced mitotic gene conversion in *Saccharomyces cerevisiae* strain D7 under conditions of alkaline pH and in the absence of S9. Caffeic acid was also able to induce gene conversion at normal

pH, without S9, although the response was weaker. As with the other genotoxicity assays, addition of transition metal ions enhanced the recombinogenic response in *S. cerevisiae*, but S9 eliminated all activity, even in the presence of metal ions. Neither chlorogenic acid nor caffeic acid induced 8-azaguanine resistance in Chinese hamster V79 cells, but both compounds were clastogenic in mammalian cells *in vitro*. Induction of chromosomal aberrations was seen in Chinese hamster ovary (CHO) cells treated with chlorogenic acid or caffeic acid in the absence of S9; addition of S9 eliminated the clastogenicity. Addition of Mn^{2+} enhanced the response seen with caffeic acid. Both chemicals induced forward mutations at the *tk* locus in mouse lymphoma L5178Y cells, but chlorogenic acid required S9 for a positive response and caffeic acid was only positive in the absence of S9. Chlorogenic acid or caffeic acid did not induce chromosomal damage in mice or rats *in vivo*.

Co-mutagenicity data was limited to a single study of the clastogenicity of chlorogenic acid with and without arecoline, in CHO cells; there was a significant enhancement of clastogenic activity when both chemicals were administered in combination, compared to the responses elicited by the individual chemicals. The addition of Mn^{2+} further enhanced the clastogenic response.

Caffeic acid inhibited the induction of DNA single strand breaks in phage ØX174 DNA by H_2O_2 and cytochrome c. Both positive and negative results were observed in tests of the antigenotoxicity of chlorogenic acid and caffeic acid in *S. typhimurium strains* TA98 and TA100. In *S. typhimurium* strain TA1535, however, both compounds were reported to inhibit the mutagenicity of MNNG, and chlorogenic acid also inhibited the mutagenicity of nitrosation products of nitrosoproline in the absence of S9. Neither chlorogenic acid nor caffeic acid inhibited the mutagenicity of ultraviolet (UV) radiation in *E. coli*. In Chinese hamster V79 cells, both chlorogenic acid and caffeic acid inhibited the mutagenicity of B[*a*]P 7,8-diol-9,10-epoxide-2 in the absence of S9. In *in vivo* studies, oral administration of chlorogenic acid to gamma-irradiated mice significantly reduced the incidence of micronuclei in bone marrow erythrocytes.

In *in vitro* immunotoxicity tests using rat mast cells, both chlorogenic and caffeic acid inhibited histamine release induced by compound 48/80 or by concanavalin A plus phosphatidylserine, although caffeic acid appeared to be more effective than chlorogenic acid. Chlorogenic acid reduced serum complement activity in normal human serum and caffeic acid reduced guinea pig serum complement activity. Caffeic acid also inhibited leukotriene production in mouse peritoneal macrophages.

Chlorogenic acid, administered by i.v. injection, did not induce allergic reactions in monkeys that were first sensitized by topical applications of sera from humans who were allergic to green coffee. In mice, topical application of chlorogenic acid, but not caffeic acid, inhibited TPA-induced edema of the ear. Similarly, i.p. injection of caffeic acid to rats inhibited edema induced by carrageenan or formalin.

Other data reviewed on chlorogenic and caffeic acid included antibacterial activity, cytotoxicity, effect on cell proliferation, effect on enzymes *in vitro*, hepatoprotective activity *in vitro*, inhibition of the nitrosation reaction *in vitro*, inhibition of oxidation *in vitro*, and miscellaneous effects identified in human studies. Caffeic acid was more potent than chlorogenic acid in its ability to inhibit nitrosamine formation and reduce nitrite levels *in vitro*. In contrast to caffeic acid, treatment with the *O*-methylated metabolite ferulic acid in the diet did not induce rat forestomach carcinogenesis, but ferulic acid, like caffeic acid, was found to be a potent antioxidant *in vitro*. Caffeic acid phenethyl ester was a more potent inhibitor of leukotriene production than caffeic acid in calcium ionophore A23187-stimulated murine peritoneal macrophages.

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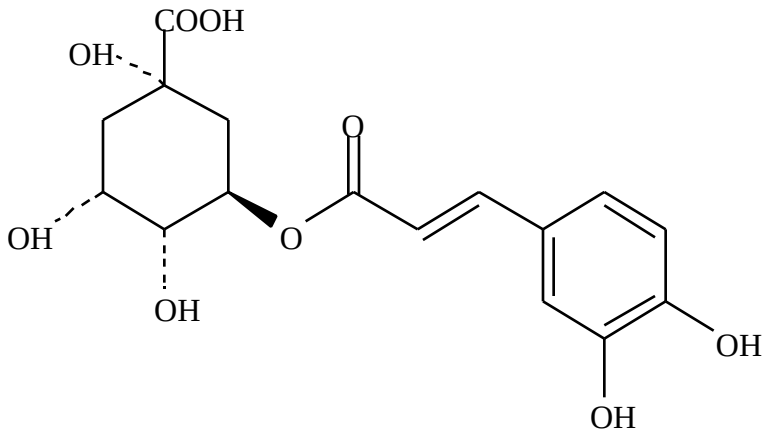
1.0 BASIS FOR NOMINATION

The nomination by Drs. Gold, Ames, and Slone, University of California, Berkeley, of chlorogenic and caffeic acid for testing is based on their occurrence in high concentrations in food and the apparent lack of carcinogenicity data.

2.0 INTRODUCTION

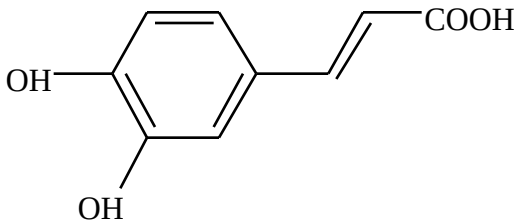
Chlorogenic Acid

[327-97-9]



Caffeic Acid

[331-39-5]



2.1 Chemical Identification

Chlorogenic acid ($C_{16}H_{18}O_9$; mol. wt. = 354.31), an ester of caffeic acid and quinic acid, is also called:

Cyclohexanecarboxylic acid, 3-[[3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-1,4,5-trihydroxy-, [1*S*-(1α,3*ß*,4α,5α)- (9CI)
3-Caffeoylquinic acid
3-*O*-Caffeoylquinic acid
3-(3,4-Dihydroxycinnamoyl)quinic acid
3-*O*-(3,4-Dihydroxycinnamoyl)-*D*-quinic acid
[1*S*-(1α,3*ß*,4α,5α)]-3-[[3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-1,4,5-trihydroxycyclohexanecarboxylic acid
NSC 407296
1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid 3-(3,4-dihydroxycinnamate)
trans-Chlorogenic acid

The term "chlorogenic acids" typically includes at least five groups of isomers including caffeoylquinic acids, dicaffeoylquinic acids, and feruloylquinic acids (Clifford and Wright, 1976; cited by Trugo and Macrae, 1984).

Caffeic acid ($C_9H_8O_4$; mol. wt. = 180.16) is also called:

3-(3,4-Dihyroxyphenyl)-2-propenoic acid (9CI)
5(4)-(2-Carboxyethenyl)-1,2-dihydroxybenzene
4-(2'-Carboxyvinyl)-1,2-dihydroxybenzene
3,4-Dihydroxybenzeneacrylic acid
3,4-Dihydroxycinnamic acid
3-(3,4-Dihydroxyphenyl)propenoic acid
3-(3,4-Dihydroxyphenyl)-2-propenoic acid

2.2 Physical-Chemical Properties

2.2.1 Chlorogenic Acid

Property	Information	Reference
Physical State	forms needles from water	Budavari (1996)
Melting Point (^oC)	208	Budavari (1996)
Boiling Point (^oC)	not found	
Density	not found	
Soluble in:	hot water, alcohol, acetone	Budavari (1996)
Slightly Soluble in:	water at 25^oC, ethyl acetate	Budavari (1996)

Heating chlorogenic acid with dilute hydrochloric acid yields caffeic acid and quinic acid (Budavari, 1996). When recrystallized from water, the hemihydrate is formed.

The enzymatic oxidation of chlorogenic acid produces mainly polymers (Oszmianski and Lee, 1990). The enzymatic oxidation products of a catechin-chlorogenic acid mixture were mainly copolymers with higher polarity than either catechin or chlorogenic acid, and were less brown in color.

2.2.2 Caffeic Acid

Property	Information	Reference
Physical State	yellow prisms from water	Lide (1991)
Melting Point (^{o}C)	225, with decomposition	Lide (1991)
Boiling Point (^{o}C)	not found	
Density	not found	
Soluble in:	hot water, cold alcohol	Budavari (1996)
Slightly Soluble in:	cold water	Budavari (1996)

Caffeic acid exists in *cis* and *trans* forms; the *trans* form is the predominant form in nature (Janssen Chimica, 1991; cited by IARC, 1993). When solutions of caffeic acid and chlorogenic acid, a derivative of caffeic acid, are exposed to sunlight or ultraviolet light, the *trans* form of caffeic acid is partially converted to the *cis* form, which may be converted to the lactone, aesculetin (Grodzinska-Zachwieja et al., 1973; Hartley and Jones, 1975; Borges and Pinto, 1989; all cited by IARC, 1993).

Enzymatic oxidation products of caffeic acid include caffeic acid *o*-quinone, which is generated enzymatically within the first minutes of incubation with polyphenol oxidase, and a number of secondary products (Cheynier and Moutounet, 1992). Caffeic acid *o*-quinone is highly unstable and is rapidly replaced by various condensation products, the first of which are formed by reaction of caffeic acid and its *o*-quinone. These products are also enzymatically oxidizable. Following initial oxidation steps, the reaction proceeds further by nonoxidative mechanisms, yielding a series of relatively polar compounds.

2.3 Commercial Availability

No information on the commercial availability of chlorogenic acid was found.

Caffeic acid is not known to be a significant commercial product (IARC, 1993). It is available as the *trans* isomer in small quantities from Aldrich Chemical Co., Fluka Chemie AG, Janssen Chimica, Lancaster Synthesis, Riedel-de Haen, and ICI America (IARC, 1993).

3.0 PRODUCTION PROCESSES AND ANALYSES

Both chlorogenic acid and caffeic acid can be isolated from green coffee beans (Budavari, 1996).

4.0 PRODUCTION AND IMPORT VOLUMES

No information was found on the production and import volumes of chlorogenic or caffeic acid.

In 1990, the United States imported 1387 tons (1258 Mg) of green coffee beans (International Coffee Organization, 1990; cited by Wasserman et al., 1993).

5.0 USES

No information was found on uses of chlorogenic acid, but the use of caffeic acid for treating asthma and allergies has been investigated (Koshihara et al., 1984; Murota and Koshihara, 1985; both cited by IARC, 1993).

Plants containing chlorogenic acid are used for medicinal purposes. *Betulae folium*, *Orthosiphonis folium,* and *Solidaginis herba* are herbal remedies used in Germany for their diuretic effects (Schneider-Leukel et al., 1992).

Some plants containing both chlorogenic and caffeic acid are used as herbal remedies. Hawthorn (*Crataegus oxyacantha*) is used as a cardiotonic and coronary vasodilator (Budavari, 1996). Angelica (*Angelica archangelica*) is used as a carminative, diaphoretic, and diuretic (Budavari, 1996). Dandelions (*Taraxacum officinale*) are used for their chloretic, antirheumatic, and diuretic properties (Williams et al., 1996). Lemon balm (*Melissa officinalis*) inhibits viral development and tumor cell division (Chlabicz and Galasinski, 1986; cited by IARC, 1993), and burdock (*Arctium lappa*) is used as a diuretic (Leung, 1980; cited by IARC, 1993).

Many medicinal plants contain caffeic acid. In China, Du Huo (*Angelica pubescens*) is used in traditional medicine as a remedy for arthritis, lumbago, edema, and the common cold (Chen et al., 1995). The fern species *Davallia mariesii* is used in Korean folk medicine to treat the common cold, neuralgia, stomach cancer, and in Chinese traditional medicine to treat lumbago, rheumatism, toothache, and tinnitus (Cui et al., 1990; cited by IARC, 1993). In India, roots of the thorny evergreen shrub, *Carissa spinarum*, are used as a purgative and for the treatment of rheumatism (Raina et al., 1971; cited by IARC, 1993). *Ixora jamanica* flowers are used as an antitumor agent, gastric sedative, intestinal antiseptic, and astringent (Nair and Pannikkar, 1990; cited by IARC, 1993). In China, Korea, and Japan, the dried leaves of wormwood (*Artemisia* sp.) have been used in traditional medicine to treat inflammation, hematemesis, hematuria, hemorrhoids, and diarrhea (Kimura et al., 1985a). Elephant creeper (*Argyreia speciosa*) seeds exhibit hypotensive and spasmolytic medicinal properties (Agarwal and Rastogi, 1974; cited by IARC, 1993). Coltsfoot (*Tussilago farfara*) flowers have antispasmodic properties (Didry et al., 1980; cited by IARC, 1993)

6.0 ENVIRONMENTAL OCCURRENCE AND PERSISTENCE

Chlorogenic and caffeic acid are constituents of fruits, leaves, and other tissues of numerous dicotyledenous plant species from the families Caprifoliaceae, Compositae, Cruciferae, Cucurbitaceae, Labiatae, Leguminosae, Polygonaceae, Saxifragaceae, Solanaceae, Theaceae, Umbelliferae, and Valerianaceae (Herrmann, 1956; Litvinenko et al., 1975; both cited by IARC, 1993).

6.1 Medicinal Plants Containing Chlorogenic and Caffeic Acid

Medicinal plants containing chlorogenic acid include *B. folium, O. folium,* and *S. herba* (Schneider-Leukel et al., 1992).

Medicinal plants containing chlorogenic and caffeic acid include hawthorn (*C. oxyacantha*) berries, flowers, and leaves and Angelica roots (*A. archangelica*) (Budavari, 1996), lemon balm (*M. officinalis*) (Chlabicz and Galasinski, 1986; cited by IARC, 1993), timothy grass (*Phleum*

pratense) (Mino and Harada, 1974; cited by IARC, 1993), burdock (*A. lappa*) roots (Leung, 1980; cited by IARC, 1993), and dandelions (*T. officinale*) (Williams et al., 1996).

Medicinal plants containing caffeic acid include Du Huo (*A. pubescens*) (Chen et al., 1995), a fern (*D. mariesii*) (Cui et al., 1990; cited by IARC, 1993), roots of a thorny evergreen shrub (*C. spinarum*) (Raina et al., 1971; cited by IARC, 1993), *I. jamanica* (Nair and Pannikkar, 1990; cited by IARC, 1993), *Centaurium umbellatum* (Hatjimanoli and Debelmas, 1977; cited by IARC, 1993), wormwood (*Artemisia* sp.) (Kimura et al., 1985a); coltsfoot (*T. farfara*) (Didry et al., 1980; cited by IARC, 1993), essential oil of Scotch broom (*Cytisus scoparius*) flowers (Kurihara and Kikuchi, 1980; cited by IARC, 1993), elephant creeper (*A. speciosa*) seeds (Agarwal and Rastogi, 1974; cited by IARC, 1993), essential oil of fennel (*Foeniculum vulgare*) (Trenkle, 1971; cited by IARC, 1993), and germander speedwell (*Veronica chamaedrys*) (Swiatek et al., 1971; cited by IARC, 1993).

6.2 Plants Used to Make Beverages that Contain Chlorogenic and Caffeic Acid

Chlorogenic and caffeic acid constitute about 6% of the chemical composition of green coffee beans (*Coffea arabica*) (Budavari, 1996). Another source stated that chlorogenic acid constitutes about 9% of the chemical composition of green *C. robusta* beans and about 7% of green *C. arabica* beans (Wasserman et al., 1993). The coffee plant is a native of tropical Africa but has been cultivated in many other tropical countries, including Java, West Indies, and Brazil (Budavari, 1996).

Chlorogenic acid is present in tea (*Camillia sinensis*) (concentration not provided), originally native to Southeast Asia, which is now cultivated in over 30 countries (Graham, 1992). Chlorogenic acid is a constituent of chicory (*Cichorium intybus*) roots (Clifford et al., 1987) and maté (the leaves of *Ilex paraguensis*), which is grown in some South American countries, including Brazil, Uruguay, Argentina, and Paraguay (Budavari, 1996).

6.3 Fruits, Vegetables, and Spices Containing Chlorogenic and Caffeic Acid

Vegetables that contain chlorogenic acid and/or caffeic acid include broad bush beans; red and sugar beetroots; Chinese, red, savoy, and white cabbages; carrots; cauliflower; celery; kale; kohlrabi; eggplant; lettuce; onions; peas; sweet peppers; potatoes; regular and black radishes; rhubarb; rutabagas; tomatoes; and zucchinis (Schmidtlein and Herrmann, 1975a,b,c; Stöhr and Herrmann, 1975a,b,c,d; all cited by IARC, 1993). Other vegetables containing chlorogenic acid and/or caffeic acid include artichokes (Leung, 1980; Hinou et al., 1989; both cited by IARC, 1993), red peppers (Kusnawidjaja et al., 1969; cited by IARC, 1993), sweet potatoes (Hayase and Kato, 1984; cited by IARC, 1993), soy beans (Pratt and Birac, 1979; cited by IARC, 1993), and spinach (Kusnawidjaja et al., 1969; cited by IARC, 1993).

The presence of chlorogenic acid in many species of the potato (Solanaceae) family has been extensively investigated. Chlorogenic acid constitutes up to 90% of the total phenolic content of potato tubers (Friedman, 1997) and its main function is presumably as a defense against phytopathogens (Deshpande et al., 1984; cited by Friedman, 1997). The average chlorogenic acid concentration per 100 g of potato sprout, leaf, or root material was 754 mg, 224 mg, and 26 mg, respectively (Friedman, 1997). The average chlorogenic acid concentration found in tubers was 17 mg chlorogenic acid/100 g tuber. About 50% of the phenolic compounds in potato tubers were located in the peel and adjoining tissues (Hasegawa et al., 1966; cited by Friedman, 1997). Temperature and organic content of the soil affect the chlorogenic acid content of potatoes (Kaldy and Lynch, 1983; cited by Friedman, 1997). Both chlorogenic and caffeic acid contents of potatoes increase from potato wounding or exposure to pathogens and viral infections (Dinkle, 1964; cited by Friedman, 1997). Chlorogenic acid content in potato tubers is greater when potatoes are stored for prolonged periods at low temperatures than when stored at higher temperatures (Mondy et al., 1966; cited by Friedman, 1997). Potatoes that have turned green due to exposure to light after harvest contain higher levels of chlorogenic acid (Dao and Friedman, 1994; Friedman, 1997). No correlation was found between the chlorogenic acid content of potatoes and the rate of browning during growth and after harvest (Mapson et al., 1963; cited by Friedman, 1997). However, chlorogenic acid may cause bluish-gray discoloration

of boiled or steamed potatoes following exposure to air, known as “after-cooking blackening or darkening” (Friedman, 1997).

Fruits containing chlorogenic acid and/or caffeic acid include blueberries; black, red, and white currants; green, yellow, and red gooseberries; grapefruits; lemons; oranges; strawberries; sweet melons; and watermelons (Schmidtlein and Herrmann, 1975a,b,c; Stöhr and Herrmann, 1975a,b,c,d; all cited by IARC, 1993). Others include apples (Iwahashi et al., 1990; cited by IARC, 1993), apricots (Kusnawidjaja et al., 1969; cited by IARC, 1993), coconuts (Kusnawidjaja et al., 1969; cited by IARC, 1993), and grapes (Shahrzad and Bitsch, 1996).

Herbs and spices containing chlorogenic and/or caffeic acid include aniseed, caraway, cloves, coriander, star anise (Dirks and Herrmann, 1984), chives, fennel, garlic, horseradish, and parsley (Schmidtlein and Herrmann, 1975a,b,c; Stöhr and Herrmann, 1975a,b,c,d; all cited by IARC, 1993).

6.4 Other Agricultural Products Containing Chlorogenic and Caffeic Acid

Other agricultural products containing chlorogenic and/or caffeic acid include rolled oats (Kusnawidjaja et al., 1969; cited by IARC, 1993), sunflower seeds and meal (Pomenta and Burns, 1971; Felice et al., 1976; both cited by IARC, 1993), tobacco leaves (Anderson and Vaughn, 1970; cited by IARC, 1993), and castor beans (Freedman et al., 1962).

6.5 Presence of Chlorogenic Acid in Wastes

Chlorogenic acid and caffeic acid may be expected in the wastes generated by industries making coffee and processed potatoes. Anaerobic treatment has been found to reduce the chlorogenic acid content of waste from the instant coffee-making process to 60% of its original level (Azhar and Stuckey, 1994).

7.0 HUMAN EXPOSURE

Human exposure occurs from the ingestion of medicinal or dietary plants containing chlorogenic or caffeic acid, some of which are listed in **Section 6** of this report.

Both acids are present in a wide variety of common dietary vegetables, fruits, and spices. While uncooked potatoes contain an average of 17 mg chlorogenic acid/100 g (170 ppm) tuber, oven-baked potatoes, French fried potatoes, mashed potato flakes, and prepared potato skins were found to contain no chlorogenic acid (Lyon and Barker, 1984; Dao and Friedman, 1996; both cited by Friedman, 1997). Boiling and microwaving reduced the amount of chlorogenic acid to 35% and 55% of the original level, respectively. Stewed potatoes contained the same quantity of chlorogenic acid as raw potatoes (Friedman, 1997). Potato flour was found to contain 341 ppm chlorogenic acid (34.1 mg chlorogenic acid/100 g flour) and 59 ppm caffeic acid (5.9 mg caffeic acid/100 g flour) (Sosulski et al., 1982).

Additionally, beverages are prepared from some chlorogenic acid- and/or caffeic acid-containing plants. For coffee, 15 to 325 mg of chlorogenic acids are consumed from one cup prepared using 10 g of ground coffee beans (Viani, 1988; cited by IARC, 1993). In the United States, the average cup of brewed coffee contains 190 mg total chlorogenic acids (Clinton, 1985; cited by IARC, 1993). The per capita consumption of coffee in 1993 was 26 gallons (SRI Int., 1998). Thus, the average person ingests 79.04 g chlorogenic acids/year from drinking coffee. Brewed coffee has a higher chlorogenic acid content than instant coffee (Wasserman et al., 1993). Regular (Graham, 1992), chicory, and dandelion tea (Clifford et al., 1987), fruit juices, and wine are other sources of oral exposure (IARC, 1993). Cherry juice was found to contain 85 mg chlorogenic acid/L and 3.7 mg caffeic acid/L (Shahrzad and Bitsch, 1996). The amount of caffeic acid in black and green grape juice was 1.05 and 0.37 mg/L, respectively. In South America, exposure to chlorogenic and caffeic acid occurs from drinking maté (a type of tea).

Caffeic acid has been identified in the smoke condensates used to smoke fish and meat (Ohshima et al., 1989), and may be ingested from eating these products.

Heat processing of dehulled, defatted sunflower flour, which is done to remove or inactivate toxic compounds (e.g., trypsin inhibitors, hemagglutinins), also reduces the content of chlorogenic acid and, to a lesser extent, caffeic acid (Sastry and Subramanian, 1985).

Inhalation exposure to chlorogenic and caffeic acids occurs from tobacco smoking.

8.0 REGULATORY STATUS

A search of CFR titles 7, 21, 29, 40, and 49 was performed, but no regulations pertaining to chlorogenic acid or caffeic acid were found.

9.0 TOXICOLOGICAL DATA

9.1 General Toxicology

9.1.1 Human Data

9.1.1.1 Cancer Studies

Caffeic acid is considered a risk factor in the development of diet-related cancer (Lutz and Schlatter, 1992). A daily dose of about 1 mg/kg/day (5.55 μmol/ kg/day) is estimated to result in less than 1000 cancer cases per 1 million individuals. The authors stated, however, that the risk of cancer from human exposure levels may be much lower or even nonexistent since caffeic acid also has anticarcinogenic properties when combined with genotoxic carcinogens.

9.1.1.2 Anti-cancer Studies

In a study analyzing the effects of tea and tea components as modifiers of *in vivo* nitrosation reactions in humans, volunteers ingested 300 mg sodium nitrite followed in 30 minutes by ingestion of 300 mg sodium nitrite, 300 mg proline, and 300 mg (1.67 mmol) caffeic acid (Stich, 1982). The same nitrite, proline, and caffeic acid doses were ingested again 10 and 20 minutes later. Controls ingested 300 mg sodium nitrite followed in 30 minutes by ingestion of 300 mg proline. From an analysis of urine samples collected over the following 24-hour period, caffeic acid strongly inhibited the formation of nitrosoproline compared to the levels found in the control group. Nitroso compounds are known mutagens and carcinogens.

9.1.1.3 Immunological Studies

One study investigated the allergic response to chlorogenic and caffeic acids in ten coffee industry workers known to be allergic to green coffee (Freedman et al., 1962). When chlorogenic acid and caffeic acid (doses not provided) were injected subcutaneously (s.c.), only chlorogenic acid produced allergic reactions. When chlorogenic acid was analyzed against serum from an individual allergic to green coffee using the passive transfer method, doses as low as 20 µM (7.1 µg/mL) induced a positive reaction.

However, Layton et al. (1968) disputed published reports on the allergenic activity of chlorogenic acid and stated, based on experiments in humans and monkeys, that chlorogenic acid is not one of the allergens responsible for atopic allergy to green coffee. In one of their experiments, pure chlorogenic acid (dose not provided) did not cause skin reactions in 29 coffee-allergic patients or 400 castor bean-allergic patients evaluated by direct skin scratch tests (Layton et al., 1965a). Similarly, 200 patients who were allergic to castor bean protein did not show a significant skin reaction to a 1% (10,000 ppm) solution of chlorogenic acid (Layton et al., 1965b). In a third study, a 1% (10,000 ppm) synthetic chlorogenic acid solution induced no reaction when applied intradermally to 30 patients with a severe allergy to green coffee and/or castor beans (Layton et al., 1966). In another experiment, six healthy young nonatopic male volunteers were sensitized with reaginic sera from 16 patients allergic to green coffee (Layton et al., 1968). Twenty-four hours later, the volunteers received challenge treatments of a 1% (10,000 ppm) solution of either purified natural or synthetic chlorogenic acid by the skin-scratch test. No reactions were observed in the sites challenged with the purified natural chlorogenic acid, but four of the serum-sensitized sites reacted strongly when challenged with commercial chlorogenic acid.

In a subsequent study, Karr et al. (1978) determined that eight coffee workers with occupational allergic disease demonstrated serum IgE antibodies specific for etiologic green coffee bean and castor bean antigens, but that these antigens were distinct and unrelated to chlorogenic acid.

One 32-year-old housewife who experienced allergic reactions to beeswax was not sensitized by 5% (50,000 ppm) caffeic acid in patch tests (Garcia et al., 1995).

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

9.1.2.1 Humans

No information on the chemical disposition, metabolism, or toxicokinetics of chlorogenic acid in humans was found. However, a number of metabolites of caffeic acid have been identified, and the proposed metabolic pathways are shown in **Figure 1** (Arnaud, 1988; cited by IARC, 1993). The main metabolites of caffeic acid are presumably glucuronides of *m*-coumaric acid and *m*-hydroxyhippuric acid. When caffeic acid was administered orally to human volunteers, *O*-

Figure 1. Proposed Metabolic Pathways of Caffeic Acid.

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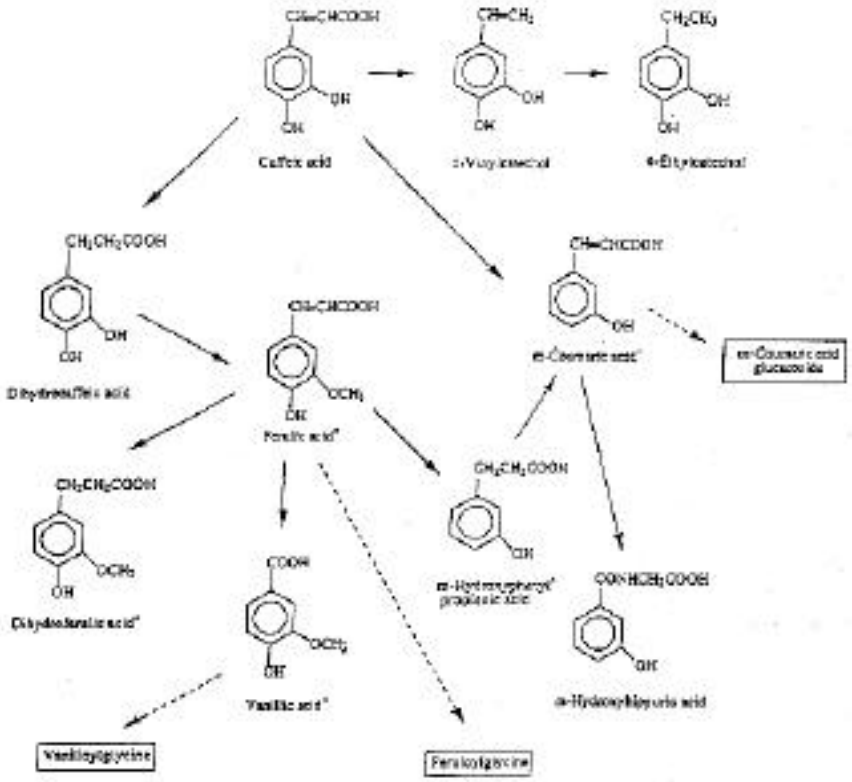
Figure 1. Proposed Metabolic Pathways of Caffeic Acid.



[Excerpt from IARC, 1993; Original Source: Arnaud, 1988]
*known metabolites

[Excerpt from IARC, 1993; Original Source: Arnaud, 1988]

***known metabolites**

methylated derivatives (ferulic, dihydroferulic, and vanillic acids) were excreted rapidly in the urine, while the *m*-hydroxyphenyl derivatives were excreted later. The dehydroxylation reactions were attributed to the action of intestinal bacteria.

9.1.2.2 Rats

In rats, chlorogenic acid is hydrolyzed in the stomach and intestine to caffeic and quinic acids (Czok et al., 1974).

In a pharmacokinetic study, female Sprague-Dawley rats were administered caffeic acid intravenously (i.v.) at 40 mg/kg (0.22 mmol/kg) or *per os* (p.o.) at 120 mg/kg (0.666 mmol/kg) (Camarosa et al., 1988). Intravenous administration resulted in a distribution half-life of 4.6 minutes and an elimination half-life of 1.7 hours. Following p.o. administration, the absorption half-life was 4.7 minutes, the distribution half-life was 8.7 minutes, and the elimination half-life was 3.1 hours; with p.o. administration, caffeic acid kinetics fit a two-compartment model.

When isolated Wistar rat livers were perfused with caffeic acid, 93.3% of the caffeic acid appeared unchanged after one liver passage (Gumbinger et al., 1993). Oxidation products (i.e., cyclolignan derivatives), methylation products (i.e., ferulic and isoferulic acid), and a cyclization product (i.e., esculetin) were also found in the perfusion medium. Glucuronides and sulfates of caffeic acid were identified in the bile.

9.1.2.3 Rabbits

Following p.o. administration of caffeic acid (10 mg/kg; 0.056 mmol/kg) to male New Zealand white rabbits, caffeic acid levels peaked in plasma within 30 minutes and gradually decreased over time (Uang et al., 1995). Traces were still present at the last sampling time (6 hours post-treatment). Following i.v. administration of the same dose, levels of caffeic acid decreased rapidly in the plasma, with only trace amounts present 80 minutes after treatment.

When caffeic acid was administered i.v. at 5, 10, or 25 mg/kg (0.028, 0.056, or 0.14 mmol/kg) to male New Zealand white rabbits, the concentration-time profiles fit a two-compartment model for each dose (Uang and Hsu, 1997). Total-body clearance and the

elimination rate constant from the central compartment was greater at the low dose than at the two higher doses. Further, the terminal elimination and mean residence times were less at the low dose than at the two higher doses. The percentage of caffeic acid excreted in the urine was 63.4, 60.0, and 55.4 % at the low, mid, and high doses, respectively; these percentages were not statistically different. Most of the unchanged caffeic acid was excreted within 2 hours.

9.1.3 Acute Exposure

LD_{50} values for chlorogenic acid and caffeic acid are presented in **Tables 1** and **2**, respectively. The details of studies discussed in this section are presented in **Table 3**.

Table 1. LD_{50} Values for Chlorogenic Acid

Route	Species (sex and strain)	LD_{50}	Reference
oral	redwing blackbird (sex and strain n.p.)	> 100 mg/kg (0.282 mmol/kg)	Schafer et al. (1983)

Abbreviations: n.p. = not provided

Table 2. LD_{50} Values for Caffeic Acid

Route	Species (sex and strain)	LD_{50}	Reference
oral	redwing blackbird (sex and strain n.p.)	> 100 mg/kg (0.555 mmol/kg)	Schafer et al. (1983)

Abbreviations: n.p. = not provided

In mice, s.c. administration of chlorogenic acid (3.5-270 mg/kg; 9.9-762 µmol/kg) did not increase motility or oxygen consumption, and intraperitoneal (i.p.) administration (135 mg/kg; 381 µmol/kg) did not affect hexobarbital-induced sleeping time (Hach and Heim, 1971). In rats, i.p. administrations of 4000 mg chlorogenic acid/kg (11.29 mmol/kg) induced death in 4 of 6 treated animals, while doses lower than 2497 mg/kg (6.878 mmol/kg) were nontoxic (Chaube and Swinyard, 1976).

In mice, an i.p. injection of caffeic acid (10 mg/kg; 0.056 mmol/kg) 30 minutes prior to an injection of formalin markedly inhibited the formalin-induced pain response (Chen et al., 1995). A 1500 mg/kg (8.326 mmol/kg) i.p. dose of caffeic acid induced death in 5 of 8 rats, but doses lower than 1250 mg/kg (6.938 mmol/kg) were nontoxic (Chaube and Swinyard, 1976). Additionally, oral administration of caffeic acid (50-500 mg/kg; 0.28-2.78 mmol/kg) did not induce clinical symptoms of toxicity in rats, but did inhibit glutathione-*S*-transferase (GST)

activity in the liver (Ploemen et al., 1993).

Table 3. Acute Exposure to Chlorogenic Acid and Caffeic Acid

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
mice (white NMRI, age n.p.)	15 M	chlorogenic acid, purity n.p.	3.5-270 mg/kg (9.9-762 µmol/kg), s.c.	single exposure; observation period n.p.	Treatment did not increase motility or oxygen consumption.	Hach and Heim (1971)
mice (white NMRI, age n.p.)	M (number n.p.)	chlorogenic acid, purity n.p.	hexobarbital i.p. and 135 mg/kg (381 µmol/kg) chlorogenic acid, i.p.	single exposure; observation period n.p.	Chlorogenic acid treatment did not affect hexobarbital-induced sleeping time.	Hach and Heim (1971)
rats (Wistar, 9-wk-old)	22 F	chlorogenic acid, purity n.p.	400-4000 mg/kg (1.13-11.29 mmol/kg) i.p.	single exposure; observed for 11 days	The HD induced 4/6 deaths. Lower doses were nontoxic.	Chaube and Swinyard (1976)
mice (ICR, age n.p.)	M (number n.p.)	caffeic acid and formalin, purities n.p.	10 mg caffeic acid/kg (0.056 mmol/kg) i.p. 30 min. prior to injection of 10, 50, or 100 mg formalin/kg (0.33, 1.7, or 3.33 mmol/kg) into the paw	single exposure; observed for 30 minutes after formalin injection	Caffeic acid markedly inhibited both the early and late phases of pain response (i.e., licking of injected paw) induced by formalin (i.e., acted as an analgesic).	Chen et al. (1995)
rats (Wistar, 8 to 9-wk-old)	8 M per dose	caffeic acid, purity n.p.	50, 100, 250, or 500 mg/kg (0.28, 0.555, 1.39, or 2.78 mmol/kg) p.o.	single exposure; sacrificed after 18 hours	No clinical symptoms were observed at any dose. A marginal, significant relationship between the dose level and the irreversible inhibition of GST activity was observed in the liver. At the HD, inhibition was 14%. No effects were observed in the kidneys or intestinal mucosa.	Ploemen et al. (1993)
rats (Wistar, 9-wk-old)	24 F	caffeic acid, purity n.p.	400-1500 mg/kg (2.22-8.326 mmol/kg) i.p.	single exposure; observed for 11 days	The HD induced 5/8 deaths. Lower doses were nontoxic.	Chaube and Swinyard (1976)

Abbreviations: s.c. = subcutaneous; F = female; GST = glutathione *S*-transferase; HD = high dose; i.p. = intraperitoneal injection; M = male; n.p. = not provided; p.o. = *per os* = by mouth

9.1.4 Short-Term and Subchronic Exposure

The details of these studies are presented in **Table 4**.

9.1.4.1 Mice

Exposure of mice for 10 weeks to chlorogenic acid or caffeic acid in the diet (0.2%, 2000 ppm) did not induce clinical symptoms of toxicity. However, these mice experienced reduced aryl hydrocarbon hydroxylase (AHH) and UDP-glucuronosyl transferase (UDPGT) levels in the intestine, but not in the liver (Kitts and Wijewickreme, 1994). Chlorogenic acid also reduced GST levels in the intestine. Cytochrome b5 and P-450 activities in the liver were not affected by either treatment.

9.1.4.2 Rats

Among the short-term and subchronic effects of chlorogenic and caffeic acid in rats were reduced kidney and adrenal weights, hyperplasia of the forestomach, and increased plasma antioxidant capacity. Rats exposed to 1% (10,000 ppm) chlorogenic acid in the diet for 3 weeks had reduced kidney and adrenal weights (Eklund, 1975), while chlorogenic or caffeic acid administered at 2% (20,000 ppm) in the diet for 4 weeks induced forestomach hyperplasia in 17% and 100% of the animals, respectively (Hirose et al., 1987). Another study found that 2% (20,000 ppm) caffeic acid in the diet of rats increased cell proliferation (measured as an increase in DNA synthesis) in the forestomach epithelium within 12 hours, and induced forestomach hyperplasia three days after the start of treatment (Ito et al., 1993). At the end of 24 weeks, moderate forestomach hyperplasia was induced in 100% of the rats. In a study with the same dose and 24-wk exposure time, the animals had severe papillary hyperplasia of the forestomach (Kagawa et al., 1993). The antioxidant effect of caffeic acid was noted when 0.2 (2000 ppm) or 0.8% (8000 ppm) caffeic acid in the diet increased levels of α-tocopherol in plasma and lipoprotein (Nardini et al., 1997). Daily i.p. injections up to 500 mg chlorogenic acid/kg/day (1.41 mmol/kg/day) or 187.5 mg caffeic acid/kg/day (1.041 mmol/kg/day) for 8 days were not lethal (Chaube and Swinyard, 1976).

9.1.5 Chronic Exposure

The details of studies with caffeic acid are presented in **Table 5**. No information on chronic exposure to chlorogenic acid was found.

Chronic exposure of mice, rats, and hamsters to caffeic acid in the diet at 2% (20,000 ppm) for 96 to 104 weeks, 1%-2% (10,000-20,000 ppm) for 24, 48, or 104 weeks, and 1% (10,000 ppm) for 20 weeks, respectively, induced hyperplasia of the forestomach (Hirose et al., 1986; Hagiwara et al., 1991; cited by IARC, 1993; Hirose et al., 1992; Kagawa et al., 1993).

Table 4. Short-Term and Subchronic Exposure to Chlorogenic Acid and Caffeic Acid

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
9.1.4.1 Mice						
mice (Balb/c; age n.p.)	9-12 F	chlorogenic acid, purity n.p.	0.2% (2000 ppm) in the diet	10-week exposure; sacrificed at the end of treatment	Treatment did not affect body, liver, or intestine weights. Chlorogenic acid significantly reduced AHH, UDPGT, and GST levels in the intestine, but not in the liver. Cytochrome b_5 and P-450 activities in the liver were not affected.	Kitts and Wijewickreme (1994)
mice (Balb/c; age n.p.)	9-12 F	caffeic acid, purity n.p.	0.2% (2000 ppm) in the diet	10-week exposure; sacrificed at the end of treatment	Treatment did not affect body, liver, or intestine weights. Caffeic acid significantly reduced AHH and UDPGT levels in the intestine, but not in the liver. GST activity in the intestine and liver and cytochrome b_5 and P-450 activities in the liver were not affected.	Kitts and Wijewickreme (1994)
9.1.4.2 Rats						
rats (Sprague-Dawley, 3-wk-old)	5 M	chlorogenic acid, 'pure' as determined by TLC	1% (10,000 ppm) in a casein diet	3-week exposure; sacrificed at the end of treatment	Treatment did not change growth, protein uptake, protein efficiency ratio, biological value, digestibility, nitrogen balance, or hematological values. The weights of the kidneys and adrenals were significantly reduced, but other organ weights were not affected.	Eklund (1975)
rats (Fischer 344 (F344), 6-wk-old)	6 M	chlorogenic acid, >98% pure	2% (20,000 ppm) in the diet	4-week exposure; sacrificed at the end of treatment	Body and liver weights were not affected by treatment. Hyperplasia of the forestomach was induced in one animal.	Hirose et al. (1987)
rats (Wistar, 9-wk-old)	4 F at the LD, 5 F at the MD, and 6 F at the HD	chlorogenic acid, purity n.p.	60, 100, or 500 mg/kg/day (0.17, 0.282, or 1.41 mmol/kg/day) i.p.	8-day exposure; sacrificed 9 days post-treatment	Treatment did not induce lethality.	Chaube and Swinyard (1976)

Abbreviations: AHH = aryl hydrocarbon hydroxylase; F = female; GST = glutathione-S-transferase; HD = high dose; i.p. = intraperitoneal injection; LD = low dose; M = male; MD = mid-dose; n.p. = not provided; TLC = thin-layer chromatography; UDPGT = UDP-glucuronosyl transferase

Table 4. Short-Term and Subchronic Exposure to Chlorogenic Acid and Caffeic Acid (continued)

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
rats (F344, 6-wk-old)	5 M	caffeic acid, >98% pure	2% (20,000 ppm) in the diet	4-week exposure; sacrificed at the end of treatment	Body and liver weights were not affected by treatment. Treatment induced epithelial hyperplasia of the forestomach in 5/5 rats, compared with none in the control group.	Hirose et al. (1987)
rats (F344, 6-wk-old)	25 M	caffeic acid, purity n.p.	2% (20,000 ppm) in the diet	exposed for 12 h or 1, 3, or 7 days; sacrificed at the end of treatment	Increased DNA synthesis was seen in forestomach epithelium at all time points; hyperplasia of the forestomach epithelium was noted at 3-7 days at an incidence of 80-100%. Toxic effects (ulceration, erosion) were seen in 60% of the rats on day 7.	Ito et al. (1993)
	10 M			24-wk exposure; sacrificed at the end of treatment	Moderate forestomach hyperplasia was noted at 24 wk in 100% of rats.	
rats (F344, 6-wk-old)	9 M	caffeic acid, >98% pure	2% (20,000 ppm) in the diet	24-week exposure; sacrificed at the end of treatment	All treated animals had severe papillary hyperplasia of the forestomach. 78% (7/9) of the animals had mild basal cell hyperplasia and 11% (1/9) had moderate basal cell hyperplasia of the forestomach.	Kagawa et al. (1993)
rats (albino Sprague-Dawley CD, age n.p.)	10 M per dose	caffeic acid, purity n.p.	0.2 or 0.8% (2000 or 8000 ppm) in the diet Estimated caffeic acid doses: 40 or 60 mg/kg/day (0.22 or 0.33 mmol/kg/day)	6-week exposure; sacrificed at the end of treatment	Treatment did not affect food intake, weight gain, final weight, relative liver weight, or plasma fatty acids composition. Treatment at both doses increased plasma and lipoprotein concentrations of α-tocopherol. After eating, caffeic acid was present in the plasma, which doubled plasma total antioxidant capacity. The results demonstrated the antioxidant effect of caffeic acid by direct contribution to the antioxidant defense system and a sparing effect on α-tocopherol.	Nardini et al. (1997)

Abbreviations: AHH = aryl hydrocarbon hydroxylase; F = female; GST = glutathione-S-transferase; HD = high dose; i.p. = intraperitoneal injection; LD = low dose; M = male; MD = mid-dose; n.p. = not provided; TLC = thin-layer chromatography; UDPGT = UDP-glucuronosyl transferase

Table 4. Short-Term and Subchronic Exposure to Chlorogenic Acid and Caffeic Acid (continued)

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
rats (Wistar, 9-wk-old)	9 F at the 2 lower doses (combined) and 6 F each at the 2 higher doses	caffeic acid, purity n.p.	20, 40, 100, or 187.5 mg/kg/day (0.11, 0.22, 0.555, or 1.041 mmol/kg/day) i.p.	8-day exposure; sacrificed 9 days post-treatment	Treatment did not induce lethality.	Chaube and Swinyard (1976)

Abbreviations: AHH = aryl hydrocarbon hydroxylase; F = female; GST = glutathione-S-transferase; HD = high dose; i.p. = intraperitoneal injection; LD = low dose; M = male; MD = mid-dose; n.p. = not provided; TLC = thin-layer chromatography; UDPGT = UDP-glucuronosyl transferase

Table 5. Chronic Exposure to Caffeic Acid

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
mice ($B6C3F_1$, 6-wk-old)	30 F, 30 M	caffeic acid, ≥ 98% pure	2% (20,000 ppm) in the diet Average intakes: 3126 mg/kg/d (17.35 mmol/kg/d) for F; 2120 mg/kg/d (11.77 mmol/kg/d) for M	96-wk exposure; sacrificed at the end of treatment	Incidences of epithelial hyperplasia of the forestomach and renal tubular-cell hyperplasia were statistically increased in both sexes.	Hagiwara et al. (1991; cited by IARC, 1993)
rats (F344, 6-wk-old)	30 F; 30 M	caffeic acid, ≥ 98% pure	2% (20,000 ppm) in the diet Average intakes: 814 mg/kg/d (4.52 mmol/kg/d) for F; 678 mg/kg/d (3.76 mg/kg/d) for M	2-yr exposure; sacrificed at the end of treatment	Incidences of forestomach hyperplasia and renal tubular-cell hyperplasia were significantly increased in both sexes.	Hagiwara et al. (1991; cited by IARC, 1993)
rats (F344, 6-wk-old)	15 M	caffeic acid, >98% pure	1% (10,000 ppm) in the diet Estimated caffeic acid dose based on average food consumption of 16 g/ rat/day: 160 mg/rat/day (0.890 mmol/rat/day)	51-wk exposure; sacrificed at the end of treatment	Treatment did not affect body weight, but significantly increased liver and kidney weights. Hyperplasia of the forestomach was induced in all treated animals.	Hirose et al. (1992)
rats (F344, 6-wk-old)	20 M	caffeic acid, >98% pure	150 mg MNNG/kg in dimethyl sulfoxide by gavage, followed 1 week later by 1% (10,000 ppm) caffeic acid in the diet Estimated caffeic acid dose based on average food consumption of 16 g/ rat/day: 160 mg/rat/day (0.890 mmol/rat/day)	51-wk exposure; sacrificed at the end of treatment	Treatment did not affect body weights, but significantly increased liver and kidney weights compared to rats administered MNNG only. Hyperplasia of the forestomach was induced in all treated animals.	Hirose et al. (1992)

Table 5. Chronic Exposure to Caffeic Acid (continued)

Abbreviations: F = female; i.p. = intraperitoneal injection; LD = low dose; M = male; MNNG = *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; n.p. = not provided

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
rats (F344, 6-wk-old)	10 M	caffeic acid, >98% pure	2% (20,000 ppm) in the diet	48-week exposure; sacrificed at the end of treatment	At the end of the observation period, 20% (2/10) of the animals had moderate papillary hyperplasia of the forestomach and 60% (6/10) had mild basal cell hyperplasia. The authors concluded that papillary hyperplasia induced by caffeic acid regresses after cessation of insult, but basal cell hyperplasia does not.	Kagawa et al. (1993)
rats (F344, 6-wk-old)	10 M	caffeic acid, purity n.p.	2% (20,000 ppm) in the diet	48-wk exposure; sacrificed at the end of treatment	The incidences of moderate forestomach hyperplasia decreased from 100% at 24 wk to 20% at 48 wk. Thus, the induced hyperplasia was reversible when exposure to caffeic acid ceased.	Ito et al. (1993)
rats (F344, 6-wk-old)	30 M	caffeic acid, >98% pure	2% (20,000 ppm) in the diet	2-year exposure; sacrificed at the end of treatment	Treatment significantly induced hyperplasia of the kidney (21/30).	Hirose et al. (1993)
rats (F344/DuCrj, 6-wk-old)	18 M	caffeic acid, purity n.p.	2% (20,000 ppm) in the diet	2-year exposure; sacrificed at the end of treatment	Treatment significantly reduced body weight and elevated relative liver weights.	Hagiwara et al. (1996)
hamsters (Syrian golden, 7-wks-old)	15 M	caffeic acid, >98% pure	1% (10,000 ppm) in the diet	20-week exposure; sacrificed at the end of treatment	The dose level chosen was the LD_{50} in rats. Treatment significantly increased incidences of mild (4/15) and moderate (10/15) hyperplasia the forestomach.	Hirose et al. (1986)

Abbreviations: F = female; i.p. = intraperitoneal injection; LD = low dose; M = male; MNNG = *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; n.p. = not provided

Additionally, at these dose levels, hyperplasia of the kidney was induced in mice and rats (Hagiwara et al., 1991; cited by IARC, 1993). Ito et al. (1993) found that treating rats with 2% (20,000 ppm) caffeic acid for 48 weeks induced reversible forestomach hyperplasia, as was demonstrated by a decrease in the incidence of forestomach hyperplasia 24 weeks after the last treatment. Furthermore, in the rat studies, caffeic acid in the diet increased liver (Hirose et al., 1992; Hagiwara et al., 1996) and kidney weights (Hirose et al., 1992), while body weight was reduced in one study (Hagiwara et al., 1996) but not in another (Hirose et al., 1992).

9.1.6 Modulation of Xenobiotic-Induced Metabolic Changes

The details of these studies are presented in **Table 6**.

9.1.6.1 Mice

In mice, administration of 0.2% (2000 ppm) chlorogenic or caffeic acid in the diet for 10 weeks significantly inhibited benzo[*a*]pyrene (B*a*P)-induced increases in AHH and GST activity in the intestine but not in the liver (Kitts and Wijewickreme, 1994).

9.1.6.2 Rats

In rats, chlorogenic acid in the diet exerted a protective effect on paraquat-induced oxidative stress by suppressing the paraquat-induced decreases in food intake, body weights, and levels of liver phospholipids (Tsuchiya et al., 1996). Chlorogenic acid also inhibited the paraquat-induced increases in levels of liver catalase, liver glutathione peroxidase, and liver glutathione reductase. Chlorogenic acid (0.2% or 2000 ppm) and paraquat (0.02% or 200 ppm) were fed simultaneously in the diet for 10 days.

One study in rats investigated the effects of chlorogenic and caffeic acid on changes in lipid metabolism induced by oral administration of peroxidized corn oil, and showed that both substances inhibited corn oil-induced elevations of serum total cholesterol, serum triglycerides, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), and serum and liver lipid peroxides (Kimura et al., 1985b). Peroxidized corn oil was given orally two times a

day for a week. During the second week, chlorogenic acid at 12.5 or 25 mg/kg (0.0353 or 0.071 mmol/kg, respectively) or caffeic acid at 25 or 50 mg/kg (0.14 or 0.28 mmol/kg, respectively) was administered together with the corn oil.

Table 6. Modulation of Xenobiotic-Induced Metabolic Changes

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
9.1.6.1 Mice						
mice (Balb/c, age n.p.)	9-12 F	chlorogenic acid and B*a*P, purities n.p.	2% (20,000 ppm) chlorogenic acid in the diet for 10 weeks plus a single dose of 50 mg B*a*P/kg by gavage	see dose for exposure; sacrificed 18 hours after B*a*P treatment	Chlorogenic acid significantly inhibited B*a*P-induced increases in AHH and UDPGT activities in the intestine, but not in the liver. The authors stated that the presence of chlorogenic acid in the diet may have an integral role in modulating the carcinogenic potential of reactive xenobiotics such as B*a*P.	Kitts and Wijewickreme (1994)
mice (Balb/c, age n.p.)	9-12 F	caffeic acid and B*a*P, purities n.p.	2% (20,000 ppm) caffeic acid in the diet for 10 weeks, plus a single dose of 50 mg B*a*P/kg by gavage	see dose for exposure; sacrificed 18 hours after B*a*P treatment	Caffeic acid significantly inhibited B*a*P-induced increases in AHH and UDPGT activities in the intestine, but not in the liver. The authors stated that the presence of caffeic acid in the diet may have an integral role in modulating the carcinogenic potential of reactive xenobiotics such as B*a*P.	Kitts and Wijewickreme (1994)
9.1.6.2 Rats						
rats (Wistar-King, 6-wk-old)	6 M per dose	chlorogenic acid, purity n.p.	10 mL/kg peroxidized corn oil p.o. twice per day for 1 week, followed by 12.5 or 25 mg/kg (0.0353 or 0.071 mmol/kg) chlorogenic acid p.o. twice per day together with peroxidized corn oil for an additional week	see dose for exposure period; sacrificed at the end of treatment	Both chlorogenic acid doses reduced levels of serum total cholesterol, lipid peroxides and triglycerides, and liver lipid peroxides compared to the rats fed peroxidized corn oil only. Both doses also inhibited corn oil-induced increases in serum ALT and AST. The high dose of chlorogenic acid slightly inhibited the peroxidized corn oil-induced reduction in body weight, but did not affect the liver weight.	Kimura et al. (1985b)

Abbreviations: AHH = aryl hydrocarbon hydroxylase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; B*a*P = benzo[*a*]pyrene; F = female; GSH-Px = glutathione peroxidase; GSSG-R = glutathione reductase; M = male; n.p. = not provided; p.o. = *per os* = by mouth; UDPGT = glucuronosyl transferase

Table 6. Modulation of Xenobiotic-Induced Metabolic Changes (continued)

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
Rats (Wistar, 5-wk-old)	5 M	chlorogenic acid and paraquat, purities n.p.	0.2% (2000 ppm) chlorogenic acid in the diet and 0.02% (200 ppm) paraquat in the diet	10-day exposure; sacrificed at the end of treatment	Chlorogenic acid suppressed the decrease in food intake and body weight observed in rats fed only paraquat. The authors stated that chlorogenic acid may prevent paraquat-induced oxidative damage to the lungs, liver, kidneys, and heart. Chlorogenic acid inhibited the increased levels of liver catalase, liver GSH-Px, and liver GSSG-R induced by paraquat; the levels were reduced to those of the controls fed basal diet alone. However, chlorogenic acid had no effect on the paraquat-induced increase in GSH-Px activity in erythrocytes. Chlorogenic acid had no effect on the decrease of liver levels of triacylglycerol and total lipids induced by paraquat, but did inhibit the paraquat-induced decrease in liver phospholipids.	Tsuchiya et al. (1996)
rats (Wistar-King, 6-wk-old)	6 M per dose	caffeic acid, purity n.p.	10 mL/kg peroxidized corn oil p.o. twice per day for 1 week, followed by 25 or 50 mg/kg (0.14 or 0.28 mmol/kg) caffeic acid p.o. twice per day, together with peroxidized corn oil for an additional week	see dose for exposure period; sacrificed at the end of treatment	The low dose of caffeic acid slightly inhibited the peroxidized corn oil-induced reduction in body weight, but did not affect the liver weight. The low dose also reduced levels of serum total cholesterol, lipid peroxides and triglycerides, and liver lipid peroxides compared to the rats fed peroxidized corn oil only, whereas the high dose reduced only serum triglyceride and liver lipid peroxide levels. Both doses inhibited corn oil-induced increases in serum ALT and AST.	Kimura et al. (1985b)

Abbreviations: AHH = aryl hydrocarbon hydroxylase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; B*a*P = benzo[*a*]pyrene; F = female; GSH-Px = glutathione peroxidase; GSSG-R = glutathione reductase; M = male; n.p. = not provided; p.o. = *per os* = by mouth; UDPGT = glucuronosyl transferase

9.2 Reproductive and Teratological Effects

The details of these studies are presented in **Table 7**.

In the only reported rat study, chlorogenic acid (5-500 mg/kg/day; 0.01-1.41 mmol/kg/day) and caffeic acid (40-187.5 mg/kg/day; 0.22-1.041 mmol/kg/day) i.p. on days 5 through 12 of gestation induced rib defects in 10% and 4% of the fetuses, respectively (Chaube and Swinyard, 1976). Fetal central nervous system defects and maternal and fetal mortality were not induced at any dose.

9.3 Carcinogenicity

The details of these studies are presented in **Table 8**.

9.3.1 Mice

In mice, chlorogenic acid, when surgically implanted in the urinary bladder in purified cholesterol pellets (dose not provided), did not induce bladder carcinomas (Wang et al., 1976). However, feeding 2% (20,000 ppm) chlorogenic acid in the diet for 96 weeks induced squamous-cell papillomas and carcinomas of the forestomach (predominantly in males), alveolar type II-cell tumors of the lung (only in males), and renal-cell adenomas (predominantly in females) (Hagiwara et al., 1991; cited by IARC, 1993).

9.3.2 Rats

In rats, maintenance on a diet containing caffeic acid at 1 or 2% (10,000 or 20,000 ppm) for 51 to 104 weeks resulted in a significantly increased frequency of papillomas of the forestomach (Hagiwara et al., 1991; cited by IARC, 1993; Hirose et al., 1992, 1993). However, such effects were not observed in rats maintained on a diet containing 2% (20,000 ppm) caffeic acid for 24 or 48 weeks (Kagawa et al., 1993). Similar exposure-duration results were also found for the induction of carcinomas of the forestomach. One study in which rats were exposed to 2% (20,000 ppm) caffeic acid for 2 years (Hagiwara et al., 1991; cited by IARC, 1993) showed treatment-induced carcinomas of the forestomach, while studies with shorter exposure periods

(24-51 weeks) were negative (Hirose et al., 1992; Kagawa et al., 1993). One 2-year study showed that 2% (20,000 ppm) caffeic acid in the diet increased the incidence of renal adenomas (Hagiwara et al., 1991; cited by IARC, 1993). A 7-week study showed that 500 ppm caffeic acid in the diet did not induce neoplasms and preneoplastic lesions of the tongue (Tanaka et al., 1993), and 2% (20,000 ppm) caffeic acid in the diet for two years did not induce hepatocellular adenomas or carcinomas (Hagiwara et al., 1996), glandular stomach adenomas or carcinomas, or kidney adenomas but did produce significant hyperplasia (Hirose et al., 1993).

Table 7. Reproductive Effects of Chlorogenic Acid and Caffeic Acid

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
rats (Wistar, 9-wk-old)	8 pregnant F in the 5-40 mg/kg/d group and 6 pregnant F each at higher doses	chlorogenic acid, purity n.p.	5-40, 60, 100, or 500 mg/kg/d (0.01-0.11, 0.17, 0.282, or 1.41 mmol/kg/d) i.p.	exposed on days 5-12 of gestation; sacrificed on day 21 of gestation	Treatment did not induce maternal or fetal mortality. No central nervous system defects were observed. 10% of the fetuses (30/289) had rib defects and one failed to develop the mandible, whereas the control group had no such effects (0/356).	Chaube and Swinyard (1976)
rats (Wistar, 9-wk-old)	5 pregnant F at two lower doses and 6 pregnant F at two higher doses	caffeic acid, purity n.p.	40, 60, 100, or 187.5 mg/kg/d (0.22, 0.33, 0.555, or 1.041 mmol/kg/d) i.p.	exposed on days 5-12 of gestation; sacrificed on day 21 of gestation	Treatment did not induce maternal or fetal mortality. No central nervous system defects were observed. 4% of the fetuses (12/274) had rib defects and the growth of one fetus was severely retarded, whereas the control group had no such effects (0/356).	Chaube and Swinyard (1976)

Abbreviations: F = female; i.p. = intraperitoneal injection; n.p. = not provided

Table 8. Carcinogenicity of Chlorogenic Acid and Caffeic Acid

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
9.3.1 Mice						
mice (Swiss albino, age n.p.)	49 (sex n.p.)	chlorogenic acid, 'pure'	chlorogenic acid (dose n.p.) in purified cholesterol pellets, surgically implanted into the urinary bladder	single implantation; observed for 1 yr	Treatment did not induce bladder carcinomas.	Wang et al. (1976)
mice ($B6C3F_1$, 6-wk-old)	30 F, 30 M	caffeic acid, ≥ 98% pure	2% (20,000 ppm) in the diet Average intakes: 3126 mg/kg/d (17.35 mmol/kg/d) for F; 2120 mg/kg/d (11.77 mmol/kg/d) for M	96-wk exposure; sacrificed at the end of treatment	Treatment induced squamous-cell papillomas (0/29 F; 4/30 M) and carcinomas (1/29 F; 3/30 M) in the forestomach. Renal-cell adenomas were observed in 8/29 F and 1/30 M. Alveolar type II-cell tumors (adenomas plus carcinomas) of the lung were induced in 27% of the M (8/30), which is higher than the M spontaneous rate (i.e., 2.2-13.9%). (Original data were not provided.)	Hagiwara et al. (1991; cited by IARC, 1993)
9.3.2 Rats						
rats (F344, 6-wk-old)	30 F; 30 M	caffeic acid, ≥ 98% pure	2% (20,000 ppm) in the diet Average intakes: 814 mg/kg/d (4.52 mmol/kg/d) for F; 678 mg/kg/d (3.76 mg/kg/d) for M	2-yr exposure; sacrificed at the end of treatment	Treatment induced squamous-cell papillomas (24/30 F; 23/30 M) and carcinomas (15/30 F; 17/30 M) in the forestomach. Renal tubular-cell adenomas were induced in 4/30 M. The control was an untreated group of 30 males and females; control data not provided but increase stated to be significantly different at $p<0.01$.	Hagiwara et al. (1991; cited by IARC, 1993)
rats (F344, 6-wk-old)	15 M	caffeic acid, >98% pure	1% (10,000 ppm) in the diet Estimated caffeic acid dose based on average food consumption of 16 g/rat/d: 160 mg/rat/d (0.89 mmol/rat/d)	51-wk exposure; sacrificed at the end of treatment	Treatment induced papillomas of the forestomach (4/15) but did not induce carcinomas or sarcomas.	Hirose et al. (1992)

Abbreviations: GST-P^+ = glutathione *S*-transferase-positive; F = female; M = male; n.p. = not provided; TGFα = tumor growth factor α

Table 8. Carcinogenicity of Chlorogenic Acid and Caffeic Acid (continued)

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
rats (F344, 6-wk-old)	9 M	caffeic acid, >98% pure	2% (20,000 ppm) in the diet	24-wk exposure; sacrificed at the end of treatment	The incidence of forestomach papillomas (1/9) was not significantly increased. No carcinomas were induced.	Kagawa et al. (1993)
	10 M			24-wk exposure; observed for an additional 24 weeks	No papillomas or carcinomas were observed at the end of the observation period. The purpose of this testing group was to determine the reversibility of induced forestomach tumors. However, the study failed due to the lack of forestomach tumors in rats treated for 24 weeks.	
	10 M			48-wk exposure; sacrificed at the end of treatment	The incidence of forestomach papillomas (3/10) was not significantly increased. No carcinomas were induced.	
rats (F344, 6-wk-old)	8 M	caffeic acid, >97% pure	500 ppm in the diet	7-wk exposure; sacrificed 32 weeks after the start of the experiment	Tongue neoplasms or preneoplastic lesions were not induced (only site evaluated). Also, the treatment did not affect mean body or liver weights.	Tanaka et al. (1993)
rats (F344, 6-wk-old)	30 M	caffeic acid, >98% pure	2% (20,000 ppm) in the diet	2-yr exposure; sacrificed at the end of treatment	The incidence of forestomach papillomas (23/30) and carcinomas (17/30) was significantly increased compared to control group (0/30 for both tests). Incidences of glandular stomach and kidney adenomas were not significantly increased. No glandular stomach carcinomas were observed.	Hirose et al. (1993)
rats (F344, 6-wk-old)	30 M	caffeic acid, purity n.p.	2% (20,000 ppm) in the diet	2-yr exposure; sacrificed at the end of treatment	No hepatocellular adenomas or carcinomas were observed. From an analysis of 6 M, treatment decreased GST-P^+ foci and TGFα in the liver to 58 and 57% of those of the control, respectively. The authors stated that this decrease was indicative of an inhibitory activity toward hepatocarcinogenicity.	Hagiwara et al. (1996)
9.3.3 Hamsters						

Abbreviations: GST-P^+ = glutathione *S*-transferase-positive; F = female; M = male; n.p. = not provided; TGFα = tumor growth factor α

Table 8. Carcinogenicity of Chlorogenic Acid and Caffeic Acid (continued)

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
hamsters (Syrian golden, 8-wk-old)	10 M, 10 F	chlorogenic acid, purity n.p.	0.025% (250 ppm) in the diet	24-week exposure; sacrificed at the end of treatment	Treatment did not induce liver or large intestine tumors.	Mori et al. (1986)
hamsters (Syrian golden, 7-wk-old)	15 M	caffeic acid, >98% pure	1% (10,000 ppm) in the diet	20-wk exposure; sacrificed at the end of treatment	The dose level chosen was the LD_{50} in rats. No papillomas of the forestomach were induced.	Hirose et al. (1986)

Abbreviations: GST-P^+ = glutathione *S*-transferase-positive; F = female; M = male; n.p. = not provided; TGFα = tumor growth factor α

9.3.3 Hamsters

Feeding hamsters 0.025% (250 ppm) chlorogenic acid in the diet for 24 weeks did not induce liver or colon tumors (Mori et al., 1986), and 1% (10,000 ppm) caffeic acid in the diet for 20 weeks did not induce papillomas of the forestomach (Hirose et al., 1986).

9.4 Initiation/Promotion Carcinogenicity Studies

The details of these caffeic acid studies are presented in **Table 9**. Initiation/promotion carcinogenicity studies were not located for chlorogenic acid.

In studies using rats, caffeic acid was shown to exert strong promotion activity for forestomach carcinogenesis when administered in the diet for 51 weeks after a single initiating dose of a carcinogen; caffeic acid treatment for shorter durations (35 weeks) did not promote. In the 51-week studies, tumors were initiated with 7,12-dimethylbenz[*a*]anthracene (DMBA) by gavage and promoted with 0.5% (5000 ppm) caffeic acid in the diet (Hirose et al., 1988; cited by IARC, 1993), or were initiated with MNNG by gavage and promoted with 1% (10,000 ppm) caffeic acid in the diet (Hirose et al., 1992; 1993). In the 35-week study, rats were treated with a single MNNG dose by gavage, followed by 0.5% (5000 ppm) caffeic acid in the diet (Hirose et al., 1991; cited by IARC, 1993).

9.5 Anticarcinogenicity

The details of these studies are presented in **Table 10**.

9.5.1 Mice

Topical chlorogenic acid treatment prior to treatment with the initiator DMBA and the promoter TPA did not effectively inhibit DMBA plus TPA-induced neoplasia of the skin (Lesca, 1983). However, topical application of chlorogenic or caffeic acid with the promoter did reduce the number of skin tumors induced by DMBA plus TPA, although caffeic acid was somewhat less active than chlorogenic acid (Huang et al., 1988). Similarly, chlorogenic acid, administered i.p., reduced the number of B*a*P-induced lung tumors (Lesca, 1983), and caffeic acid,

administered in the diet, reduced the number of B*a*P-induced forestomach tumors (Wattenberg et al., 1980; cited by IARC, 1993). When caffeic acid was administered to mice by i.p. injection 10 days after a single s.c. injection of sarcoma-180 cells, tumor incidence was reduced by 21.4% (Inayama et al., 1984), but a single i.p. injection of caffeic acid 4 days prior to i.p. injection of sarcoma-180 cells did not have antitumor activity, as measured by an increased lifespan in treated mice (Miyamoto et al., 1992).

Table 9. Initiation/Promotion Studies of Caffeic Acid

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
rats (Sprague-Dawley, 50-d-old)	20 F	caffeic acid, >99% pure, and DMBA, purity n.p.	25 mg/kg DMBA by gavage in sesame oil, followed 1 wk later by 0.5% (5000 ppm) caffeic acid administered in the diet for 51 wk	see dose for exposure; sacrificed at the end of treatment	The incidence of forestomach papillomas (6/19) was significantly increased compared to the controls administered DMBA alone (0/19). No induced tumors of the mammary glands, ear ducts, liver, or kidneys were found.	Hirose et al. (1988; cited by IARC, 1993)
rats (F344, 6-wk-old)	15 M per dose	caffeic acid, >98% pure, and MNNG, purity n.p.	150 mg/kg MNNG in dimethyl sulfoxide by gavage, followed 1 wk later by 0.5% (5000 ppm) caffeic acid in the diet for 35 wk	see dose for exposure; sacrificed at the end of treatment	MNNG plus caffeic acid treatment did not significantly increase the incidence of squamous cell carcinomas of the forestomach compared to treatment with MNNG alone. No induced tumors of the esophagus, intestines, liver, and kidneys were found.	Hirose et al. (1991; cited by IARC, 1993)
rats (F344, 6-wk-old)	20 M	caffeic acid, >98% pure, and MNNG, purity n.p.	150 mg MNNG/kg in dimethyl sulfoxide by gavage, followed 1 wk later by 1% (10,000 ppm) caffeic acid in the diet for 51 wk Estimated caffeic acid dose based on average food consumption of 16 g/rat/d: 160 mg/rat/d (0.89 mmol/rat/d)	see dose for exposure period; sacrificed at the end of treatment	The incidences of forestomach papillomas (19/20) and squamous cell carcinomas (20/20) were significantly increased compared to treatment with MNNG alone (7/20 and 2/20, respectively), but the increase of forestomach sarcomas was not statistically significant. Treatment with caffeic acid alone induced papillomas in 27% (4/15) of the animals but no carcinomas or sarcomas, while a basal diet with no treatment induced none of the lesions. The authors concluded that caffeic acid exerted strong promotion activity for rat forestomach carcinogenesis.	Hirose et al. (1992)
rats (F344, 6-wk-old)	15-20 M	caffeic acid, >98% pure, and MNNG, purity n.p.	150 mg MNNG/kg i.g., followed 1 wk later by 1% (10,000 ppm) caffeic acid in the diet for 51 wk.	see dose for exposure period; sacrificed at the end of treatment	MNNG plus caffeic acid significantly increased the incidence of forestomach squamous cell carcinomas (100%) compared to the controls administered MNNG alone (26%). The incidence of glandular stomach carcinomas was not significantly increased compared to the controls.	Hirose et al. (1993)

Abbreviations: DMBA = 7,12-dimethylbenz[*a*]anthracene; F = female; i.g. = intragastric injection; M = male; MNNG = *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; n.p. = not provided

Table 10. Anticarcinogenicity of Chlorogenic Acid and Caffeic Acid

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
9.5.1 Mice						
mice (NMRI Swiss, 28-d-old)	20 M	chlorogenic acid, DMBA, and TPA, purities n.p.	200 µg/d (0.564 µmol/d) chlorogenic acid applied to the shaven backs for 6 d. 30 min after chlorogenic acid treatment on the 3rd day, 25 µg DMBA was topically applied. 1 wk after DMBA treatment, 10 µg TPA was applied twice per wk for 15 wk	see dose for exposure period; sacrificed at the end of treatment	Chlorogenic acid did not effectively inhibit neoplasia of the skin initiated with DMBA and promoted with TPA.	Lesca (1983)
mice (CD-1, 8-wk-old)	30 F per dose	chlorogenic acid, DMBA, and TPA, purities n.p.	0.200 µmol DMBA applied topically in acetone, followed 1 wk later by a topical application of 0.005 nmol TPA plus 10 or 20 µmol (3.5 or 7.1 mg) chlorogenic acid twice per wk for 19 wk	see dose for exposure period; sacrificed at the end of treatment	The HD significantly reduced the number of DMBA+TPA-induced skin tumors per mouse.	Huang et al. (1988)
mice (A/J, 28-d-old)	20 M	chlorogenic acid and B*a*P, purities n.p.	100 mg B*a*P/kg i.p. 5 i.p. injections of 100 mg chlorogenic acid/kg (282 µmol/kg) administered 1 hour before and 6, 24, 48, and 72 hours after B*a*P treatment	see dose for exposure period; sacrificed 6 mo after the B*a*P treatment	Chlorogenic acid reduced the number of B*a*P-induced lung tumors per mouse.	Lesca (1983)

Abbreviations: AOM = azoxymethane; B*a*P = benzo[*a*]pyrene; BBN = *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine; DEN = diethylnitrosamine; DHPN = 2,2'-dihydroxy-di-*n*-propylnitrosamine; DMBA = 7,12-dimethyl[*a*]anthracene; DMH = 1,2-dimethylhydrazine; HD = high dose; MAM = methylazoxymethanol; MNU = *N*-methyl-*N*-nitrosourea; MTD = maximum tolerated dose, defined as the highest dose which does not cause $\geq$ 10% reduction in weight gain or final weight after 6 wk of administration; n.p. = not provided; 4-NQO = 4-nitroquinoline-1-oxide; TPA = 12-*O*-tetradecanoylphorbol-13-acetate

Table 10. Anticarcinogenicity of Chlorogenic Acid and Caffeic Acid (continued)

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
mice (ICR/Ha, 9-wk-old)	17 F	caffeic acid, 99% pure, and B*a*P, purity n.p.	10 g caffeic acid/kg of diet (10,000 ppm). Starting on day 8, mice were also given 1 mg B*a*P by gavage twice per wk for 4 wk. The diet containing caffeic acid was removed 3 d after the last B*a*P treatment	see dose for exposure period; sacrificed at 211 d of age	Caffeic acid significantly reduced the number of forestomach tumors/mouse (3.1 tumors/mouse in treated mice vs. 5.0 in control mice administered B*a*P alone).	Wattenberg et al. (1980; cited by IARC, 1993)
mice (CD-1, 8-wk-old)	30 F per dose	caffeic acid, DMBA, and TPA, purities n.p.	200 nmol DMBA applied topically in acetone, followed 1 wk later by a topical application of 5 nmol TPA plus 10 or 20 µmol (1800 or 3600 µg) caffeic acid twice per wk for 19 wk	see dose for exposure period; sacrificed at the end of treatment	The HD significantly reduced the number of DMBA+TPA-induced tumors per mouse, although caffeic acid was somewhat less active than chlorogenic acid.	Huang et al. (1988)
mice (ICR, age n.p.)	10 M	caffeic acid, purity n.p.	Sarcoma 180 cells administered s.c. into the left groin on day zero. 10 mg/kg/d (0.056 mmol/kg/d) caffeic acid i.p. on days 1 through 10	see dose for exposure period; sacrificed on day 21	Caffeic acid reduced sarcoma 180 tumor activity by 21.4%.	Inayama et al. (1984)
mice (ddY, age n.p.)	F (number n.p.)	caffeic acid, purity n.p.	caffeic acid (dose n.p.) i.p., followed 4 d later by i.p. injection of sarcoma-180 cells.	single exposure; observed for 60 d	Caffeic acid treatment showed negligible activity in terms of increasing lifespan.	Miyamoto et al. (1992)
9.5.2 Rats						

Abbreviations: AOM = azoxymethane; B*a*P = benzo[*a*]pyrene; BBN = *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine; DEN = diethylnitrosamine; DHPN = 2,2'-dihydroxy-di-*n*-propylnitrosamine; DMBA = 7,12-dimethyl[*a*]anthracene; DMH = 1,2-dimethylhydrazine; HD = high dose; MAM = methylazoxymethanol; MNU = *N*-methyl-*N*-nitrosourea; MTD = maximum tolerated dose, defined as the highest dose which does not cause $\geq$ 10% reduction in weight gain or final weight after 6 wk of administration; n.p. = not provided; 4-NQO = 4-nitroquinoline-1-oxide; TPA = 12-*O*-tetradecanoylphorbol-13-acetate

Table 10. Anticarcinogenicity of Chlorogenic Acid and Caffeic Acid (continued)

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
rats (F344, 7-wk-old)	M (number n.p.)	chlorogenic acid and AOM, purities n.p.	0.4 or 0.8 MTD or up to 2% (20,000 ppm) chlorogenic acid in the diet. After 1 wk, either 2 injections of 15 mg AOM/kg 1 wk apart or 1 injection of 30 mg AOM/kg was given	5-wk exposure; sacrificed at the end of treatment	Chlorogenic acid did not significantly reduce the number of AOM-induced aberrant crypt foci (i.e., precancerous lesions), which are considered as intermediate biomarkers for colon cancer in rodents.	Steele et al. (1994)
rats (Sprague-Dawley, 50- or 120-d-old)	F (number n.p.)	chlorogenic acid and MNU, purities n.p.	0.4 or 0.8 MTD or up to 2% (20,000 ppm) chlorogenic acid in the diet. After 1 wk, a single i.v. injection of 50 mg MNU/kg was given	>180-d exposure; sacrificed at the end of treatment	Chlorogenic acid did not significantly reduce the number of MNU-induced mammary tumors, which are invasive and predominantly adenocarcinomas.	Steele et al. (1994)
rats (F344, 6-wk-old)	15 M	caffeic acid, >97% pure and 4-NQO, >98% pure	500 ppm caffeic acid in the diet for 7 wk. At the beginning of the second wk, rats were also administered 20 ppm 4-NQO in the drinking water for 5 wk	see dose for exposure period; sacrificed 32 wk after the start of the experiment	Caffeic acid significantly reduced the incidence of 4-NQO-induced tongue neoplasms (0/15 in treatment group v. 9/15 in control group administered 4-NQO alone). Treatment also significantly reduced mean body weights compared to control group administered 4-NQO alone.	Tanaka et al. (1993)
rats (F344, 7-wk-old)	M (number n.p.)	caffeic acid and AOM, purities n.p.	0.4 or 0.8 MTD or up to 2% (20,000 ppm) caffeic acid in the diet. After 1 wk, either 2 injections of 15 mg AOM/kg 1 wk apart or 1 injection of 30 mg AOM/kg was given	5-wk exposure; sacrificed at the end of treatment	Caffeic acid did not significantly reduce the number of AOM-induced aberrant crypt foci (i.e., precancerous lesions), which are considered as intermediate biomarkers for colon cancer in rodents.	Steele et al. (1994)

Abbreviations: AOM = azoxymethane; B*a*P = benzo[*a*]pyrene; BBN = *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine; DEN = diethylnitrosamine; DHPN = 2,2'-dihydroxy-di-*n*-propylnitrosamine; DMBA = 7,12-dimethyl[*a*]anthracene; DMH = 1,2-dimethylhydrazine; HD = high dose; MAM = methylazoxymethanol; MNU = *N*-methyl-*N*-nitrosourea; MTD = maximum tolerated dose, defined as the highest dose which does not cause $\geq$ 10% reduction in weight gain or final weight after 6 wk of administration; n.p. = not provided; 4-NQO = 4-nitroquinoline-1-oxide; TPA = 12-*O*-tetradecanoylphorbol-13-acetate

Table 10. Anticarcinogenicity of Chlorogenic Acid and Caffeic Acid (continued)

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
rats (F344, age n.p.)	M (number n.p.)	caffeic acid, DEN, MNU, BBN, DMH, and DHPN, purities n.p.	100 mg DEN/kg i.p. at day 0 plus 20 mg MNU/kg i.p. 4 times during wk 1-2 plus 0.05% BBN in the drinking water during weeks 1-2 plus 40 mg DMH/kg s.c. 4 times during weeks 3-4 plus 0.1% DHPN in the drinking water during weeks 3-4 plus 1% caffeic acid in the diet (10,000 ppm; 56 mmol/kg) during weeks 4 through 28 or 36	see dose for exposure period; sacrificed at the end of treatment (either at 28 or 36 wk)	Caffeic acid significantly inhibited carcinogen-induced neoplasms and preneoplastic lesions of the forestomach.	Ito et al. (1996)
9.5.3 Hamsters						
hamsters (Syrian golden, 8-wk-old)	12 M, 12 F	chlorogenic acid and MAM acetate, purities n.p.	single i.v. injection of 20 mg/kg MAM acetate plus 0.025% (250 ppm) chlorogenic acid in the diet	24-wk exposure; sacrificed at the end of treatment	Chlorogenic acid significantly reduced the total incidence of large intestine tumors and adenocarcinomas compared to control group administered MAM acetate alone. Treatment also significantly reduced the number of hepatocellular foci compared to the controls administered MAM acetate alone.	Mori et al. (1986)

Abbreviations: AOM = azoxymethane; B*a*P = benzo[*a*]pyrene; BBN = *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine; DEN = diethylnitrosamine; DHPN = 2,2'-dihydroxy-di-*n*-propylnitrosamine; DMBA = 7,12-dimethyl[*a*]anthracene; DMH = 1,2-dimethylhydrazine; HD = high dose; MAM = methylazoxymethanol; MNU = *N*-methyl-*N*-nitrosourea; MTD = maximum tolerated dose, defined as the highest dose which does not cause $\geq$ 10% reduction in weight gain or final weight after 6 wk of administration; n.p. = not provided; 4-NQO = 4-nitroquinoline-1-oxide; TPA = 12-*O*-tetradecanoylphorbol-13-acetate

9.5.2 Rats

Neither chlorogenic nor caffeic acid significantly reduced the number of azoxymethane (AOM)-induced aberrant crypt foci (i.e., precancerous lesions) when administered in the diet for five weeks; aberrant crypt foci are considered as intermediate biomarkers for colon cancer in rodents (Steele et al., 1994). Chlorogenic acid also did not significantly reduce the number of *N*-methyl-*N*-nitrosourea (MNU)-induced mammary tumors when administered in the diet for more than 25 weeks (Steele et al., 1994). However, caffeic acid in the diet for seven weeks significantly reduced the incidence of 4-nitroquinoline-1-oxide (4-NQO)-induced tongue neoplasms (Tanaka et al., 1993). Dietary treatment with caffeic acid for 24 or 32 weeks significantly inhibited neoplasms and preneoplastic lesions of the forestomach induced by diethylnitrosoamine (DEN), MNU, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN), 1,2-dimethylhydrazine (DMH), and 2,2'-dihydroxy-di-*n*-propylnitrosamine (DHPN) (Ito et al., 1996).

9.5.3 Hamsters

Chlorogenic acid in the diet for 24 weeks significantly reduced the number of methylazoxymethanol acetate (MAM acetate)-induced large intestinal tumors and adenocarcinomas, and MAM acetate-induced hepatocellular foci (Mori et al., 1986).

9.6 Genotoxicity

The details of the these studies are presented in **Table 11**.

In general, the results indicate that both chlorogenic acid and caffeic acid are capable of inducing DNA strand breaks *in vitro* in isolated or plasmid DNA, particularly in the presence of transition metals that enhance the formation of H_2O_2 and oxygen radicals. The chemicals, by themselves, tended to be nonmutagenic in bacterial assays. Mitotic gene conversion in yeast and clastogenicity in mammalian cells were observed *in vitro* in the absence of S9 metabolic activation; these effects were eliminated in the presence of S9. Both chemicals were mutagenic in the mouse lymphoma assay. They were not clastogenic *in vivo*.

9.6.1 Acellular Assays

Both chlorogenic acid and caffeic acid induce DNA strand breaks in acellular test systems that favor the formation of oxygen radicals, particularly in the presence of transition metals that promote the generation of H_2O_2. Chlorogenic acid (250 µM; 88.6 µg/mL) induced double strand breaks in isolated γDNA (Yamada et al., 1985). DNA damage was also induced in phage ØX174 RF I DNA treated with chlorogenic acid (doses not provided) in the presence of cupric ions (Cu^{2+})

Table 11. Genotoxicity of Chlorogenic Acid and Caffeic Acid

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
9.6.1 Acellular Assays							
isolated λDNA	DNA double strand breaks	-	chlorogenic acid, purity n.p.	250 µM (88.6 µg/mL)	weakly positive	DNA was treated for 6 h. The response was weaker than that seen with caffeic acid.	Yamada et al. (1985)
isolated plasmid pBR322 DNA	DNA strand breaks in the presence and absence of NO-releasing compounds	-	chlorogenic acid, purity n.p.	100 µM (35 µg/mL) in presence and absence of 100 µM DEA-NO, spermine-NO, or SNP	positive in the presence of DEA-NO and spermine-NO negative alone and in the presence of SNP	A synergistic interaction between chlorogenic acid and NO-releasing compounds, which may occur in human dietary exposure situations, was observed.	Yoshie and Ohshima (1997)
phage øX174 RF I DNA	DNA strand breaks in the presence of Cu^{2+}	-	chlorogenic acid, purity n.p.	n.p.	weakly positive	Copper redox cycle and H_2O_2 generation were two major factors involved in the observed DNA damage.	Li and Trush (1994)
isolated λDNA	DNA double strand breaks	-	caffeic acid, purity n.p.	250 µM (45.0 µg/mL)	positive	DNA was treated for 6 h.	Yamada et al. (1985)
isolated DNA from plasmid pbcNI carrying human c-Ha-*ras*-1	DNA strand breaks in the presence and absence of transition metal ions	-	caffeic acid, purity n.p.	n.p.	positive in the presence of Cu^{2+} only		Inoue et al. (1992)

Abbreviations: CHO = Chinese hamster ovary; DEA-NO = diethanolamine-nitric oxide; F = female; i.p. = intraperitoneal; NA = not applicable; NCE = normochromatic erythrocytes**;** NO = nitric oxide; n.p. = not provided; PCE = polychromatic erythrocytes; p.o. = *per os* = by mouth; SNP = sodium nitroprusside; +/- = presence/absence

Table 11. Genotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
phage øX174 DNA	DNA single strand breaks in the presence or absence of Fe^{3+}	-	caffeic acid, purity n.p.	100, 200, 300, 400, or 500 µM (18, 36, 54, 72, or 90 µg/mL)	positive in the presence of Fe^{3+} only	DNA breakage induced in the presence of Fe^{3+} was dose-dependent.	Nakayama et al. (1993)
phage øX174 RF I DNA	DNA strand breaks in the presence of Cu^{2+}	-	caffeic acid, purity n.p.	n.p.	positive	Copper redox cycle and H_2O_2 generation were two major factors involved in the observed DNA damage.	Li and Trush (1994)
9.6.2 Prokaryotic Systems							
Salmonella typhimurium strain TA98	*his* gene mutations	+/-	chlorogenic acid, purity n.p.	0.166 or 1.66 µmol/plate (58.8 or 588 µg/plate)	negative		MacGregor and Jurd (1978)
S. typhimurium strains TA98 and TA100	*his* gene mutations in the presence and absence of transition metal ions	+/-	chlorogenic acid, purity n.p.	19 or 28 mg/plate (53 or 79 µmol/plate)	positive in both strains in the presence of Mn^{2+} negative in both strains in the presence and absence of S9 and in the presence of Cu^{2+} only	Assays using transition metals were conducted in the absence of S9.	Stich et al. (1981a)
S. typhimurium strain TA98	*his* gene mutations	+	chlorogenic acid, purity n.p.	1, 3, 6, or 9 mg/mL (3, 9, 20 or 30 mM)	negative	A suspension test was used.	San and Chan (1987)

Abbreviations: CHO = Chinese hamster ovary; DEA-NO = diethanolamine-nitric oxide; F = female; i.p. = intraperitoneal; NA = not applicable; NCE = normochromatic erythrocytes**;** NO = nitric oxide; n.p. = not provided; PCE = polychromatic erythrocytes; p.o. = *per os* = by mouth; SNP = sodium nitroprusside; +/- = presence/absence

Table 11. Genotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
S. typhimurium strains TA98, TA100, TA1535, TA1537, TA1538	*his* gene mutations	+/-	chlorogenic acid, purity n.p.	0.333-10 mg/plate (0.940-28 µmol/plate)	negative		Fung et al. (1988)
S. typhimurium strain BA13	*L*-arabinose resistance, forward mutations	-	chlorogenic acid, purity n.p.	0.3-28 µmol/plate (0.1-9.9 mg/plate)	weakly positive	The lowest effective dose was 23 µmol.	Ariza et al. (1988)
S. typhimurium strain TA98	*his* gene mutations	+/-	caffeic acid, purity n.p.	150 µg/plate (0.832 µmol/plate)	negative		MacGregor and Jurd (1978)
S. typhimurium strains TA98 and TA100	*his* gene mutations with and without transition metal ions	+/-	caffeic acid, purity n.p.	6 or 10 mg/plate (30 or 55 µmol/plate)	positive in both strains in the presence of Mn^{2+} negative in both strains in the presence and absence of S9 and in the presence of Cu^{2+} only	Assays using transition metal ions were conducted in the absence of S9.	Stich et al. (1981b; cited by IARC, 1993)
S. typhimurium strain TA98	*his* gene mutations	+	caffeic acid, purity n.p.	1, 2, 3, 6, or 9 mg/mL (6, 11, 17, 33, or 50 mM)	negative		San and Chan (1987)
S. typhimurium strains TA98, TA100, TA1535, TA1537, TA1538	*his* gene mutations	+/-	caffeic acid, purity n.p.	0.333-10 mg/plate (1.85-55 µmol/plate)	negative		Fung et al. (1988)
S. typhimurium strain BA13	*L*-arabinose resistance, forward	-	caffeic acid, purity n.p.	0.3-10 µmol/plate	positive	The lowest effective dose was 4 µmol.	Ariza et al. (1988)

Abbreviations: CHO = Chinese hamster ovary; DEA-NO = diethanolamine-nitric oxide; F = female; i.p. = intraperitoneal; NA = not applicable; NCE = normochromatic erythrocytes; NO = nitric oxide; n.p. = not provided; PCE = polychromatic erythrocytes; p.o. = *per os* = by mouth; SNP = sodium nitroprusside; +/- = presence/absence

Table 11. Genotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
	mutations			(0.05-1.8 mg/plate)			
9.6.3 Lower Eukaryotic Systems							
Saccharomyces cerevisiae strain D7	gene conversion at the *trp* locus in the presence of Mn^{2+} or Cu^{2+}	+/-	chlorogenic acid, purity n.p.	20, 40, or 80 mg/mL (56, 110 or 230 mM)	positive in the absence of S9 and in the presence of Cu^{2+} or Mn^{2+} negative in the presence of S9	Assays using transition metal ions were conducted in the absence of S9. Mutagenic response in the presence of Mn^{2+} was very strong.	Stich et al. (1981a)
S. cerevisiae strain D7	mitotic gene conversion	-	chlorogenic acid, purity n.p.	1 mg/mL (3 mM)	positive	Tested at pH 10.	Rosin (1984)
S. cerevisiae strain D7	gene conversion at the *trp* locus in the presence of Mn^{2+} or Cu^{2+}	+/-	caffeic acid, purity n.p.	20, 30, or 40 mg/mL (110, 170, or 220 mM)	positive in the absence of S9 and in the presence of Cu^{2+} or Mn^{2+} negative in the presence of S9	Assays using transition metals were conducted in the absence of S9. Response with Mn^{2+} was very strong.	Stich et al. (1981a)
S. cerevisiae strain D7	mitotic gene conversion	-	caffeic acid, purity n.p.	300 µg/mL (1.66 mM)	positive	Tested at pH 10.	Rosin (1984)
9.6.4 *In Vitro* Mammalian Systems							
Chinese hamster V79-6 cells	formation of 8-azaguanine resistant colonies	-	chlorogenic acid, 'pure'	500 nmol/mL (177 µg/mL)	negative	Dose was not cytotoxic.	Wood et al. (1982)
mouse lymphoma L5178Y cells	forward mutations, *tk* locus	+/-	chlorogenic acid, purity n.p.	6.545-10 mg/mL (18.47-28 mM)	+S9: positive -S9: negative		Fung et al. (1988)

Abbreviations: CHO = Chinese hamster ovary; DEA-NO = diethanolamine-nitric oxide; F = female; i.p. = intraperitoneal; NA = not applicable; NCE = normochromatic erythrocytes**;** NO = nitric oxide; n.p. = not provided; PCE = polychromatic erythrocytes; p.o. = *per os* = by mouth; SNP = sodium nitroprusside; +/- = presence/absence

Table 11. Genotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
mouse lymphoma L5178Y cells	forward mutations, *tk* locus	+/-	chlorogenic acid, purity n.p.	0.50-2.43 mg/mL (1.4-6.86 mM)	+S9: positive -S9: negative		Fung et al. (1988)
CHO cells	chromosomal aberrations in the presence of transition metal ions	+/-	chlorogenic acid, purity n.p.	10-40 µg/mL (28-110 µM)	-S9: positive +S9: negative	The positive response was enhanced in the presence of Mn^{2+} and Cu^{2+}.	Stich et al. (1981a)
CHO cells	chromosomal aberrations in the presence and absence of mouse intestinal cells	+/-	chlorogenic acid, purity n.p.	125, 150, or 250 µg/mL (353, 420, or 706 µM)	positive in the absence of mouse cells or S9 negative in the presence of either cells or S9	Cultured mouse intestinal cells were used as an activation enzyme source.	Whitehead et al. (1983)
Chinese hamster V79-6 cells	formation of 8-azaguanine resistant colonies	-	caffeic acid, 'pure'	500 nmol/mL (90.1 µg/mL)	negative	Dose was not cytotoxic.	Wood et al. (1982)
mouse lymphoma L5178Y cells	forward mutations, *tk* locus	+/-	caffeic acid, purity n.p.	307 µg/mL (1.7 mM)	-S9: positive +S9: negative		Fung et al. (1988)
CHO cells	chromosomal aberrations in the presence and absence of Mn^{2+}	+/-	caffeic acid, purity n.p.	200 µg/mL (1.1 mM)	-S9: positive +S9: negative	The positive response was enhanced in the presence of Mn^{2+}.	Stich et al. (1981b; cited by IARC, 1993)
CHO cells	chromosomal aberrations in the presence and absence of mouse	+/-	caffeic acid, purity n.p.	240, 260, 280, or 350 µg/mL (1.3, 1.4, 1.6, or	positive in the absence of mouse intestinal cells or S9 negative in the presence of	Cultured mouse intestinal cells were used as an activation enzyme source.	Whitehead et al. (1983)

Abbreviations: CHO = Chinese hamster ovary; DEA-NO = diethanolamine-nitric oxide; F = female; i.p. = intraperitoneal; NA = not applicable; NCE = normochromatic erythrocytes; NO = nitric oxide; n.p. = not provided; PCE = polychromatic erythrocytes; p.o. = *per os* = by mouth; SNP = sodium nitroprusside; +/- = presence/absence

Table 11. Genotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
	intestinal cells			1.9 mM)	mouse intestinal cells or S9		
CHO cells	chromosomal aberrations	-	caffeic acid, purity n.p.	250 µg/mL (1.4 mM)	positive		Hanham et al. (1983; cited by IARC, 1993)
human Raji cells	DNA strand breaks in the presence and absence of transition metal ions	-	caffeic acid, purity n.p.	n.p.	positive in the presence of Mn^{2+} only		Inoue et al. (1992)
9.6.5 *In Vivo* Mammalian Systems							
rats (Sprague-Dawley [NIH/HAN;SPF]; 5 to 7-wk-old)	induction of micronucleated erythrocytes in the bone marrow	NA	chlorogenic acid, chromato-graphically pure	150 mg/kg (420 µmol/kg) p.o. twice, administered 24 h apart	negative in NCE and PCE	The animals were killed 6 h after the 2nd dosing. The experiment was performed with irradiated and nonirradiated chemical.	Hossain et al. (1976)
mice ($B6C3F_1$; age n.p.)	induction of micronucleated erythrocytes in the bone marrow	NA	caffeic acid, purity n.p.	2400 mg/kg (13.3 mmol/kg) in the diet,	negative		Raj et al. (1983; cited by IARC, 1993)

Abbreviations: CHO = Chinese hamster ovary; DEA-NO = diethanolamine-nitric oxide; F = female; i.p. = intraperitoneal; NA = not applicable; NCE = normochromatic erythrocytes; NO = nitric oxide; n.p. = not provided; PCE = polychromatic erythrocytes; p.o. = *per os* = by mouth; SNP = sodium nitroprusside; +/- = presence/absence

Table 11. Genotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
				duration n.p.			
mice (C56B1/6J, age n.p.)	induction of micronuclei in intestinal epithelial cells	NA	caffeic acid, purity n.p.	800 mg/kg (4.44 mmol/kg) in the diet, duration n.p.	negative		Wargovich et al. (1983; cited by IARC, 1993)
mice (C56B1/6J, age n.p.)	induction of micronuclei in intestinal epithelial cells	NA	caffeic acid, purity n.p.	4800 mg/kg (26.6 mmol/kg) in the diet, duration n.p.	negative		Wargovich et al. (1985; cited by IARC, 1993)

Abbreviations: CHO = Chinese hamster ovary; DEA-NO = diethanolamine-nitric oxide; F = female; i.p. = intraperitoneal; NA = not applicable; NCE = normochromatic erythrocytes**;** NO = nitric oxide; n.p. = not provided; PCE = polychromatic erythrocytes; p.o. = *per os* = by mouth; SNP = sodium nitroprusside; +/- = presence/absence

(Li and Trush, 1994). Yoshie and Ohshima (1997) reported the induction of strand breaks in plasmid pBR322 DNA treated with chlorogenic acid (100 µM) in the presence of 100 µM of the nitric oxide (NO)-releasing compounds diethanolamine-NO or spermine-NO, but no breakage occurred when treatment was with chlorogenic acid alone or with chlorogenic acid in combination with sodium nitroprusside ($Na_2[Fe(CN)_5NO]$). The authors proposed that a similar type of synergistic action with chlorogenic acid and NO-releasing compounds might occur in normal human dietary exposure situations.

Caffeic acid (100-500 µM; 18.0-90.1 µg/mL) in the presence of ferric ions (Fe^{3+}) induced single strand breaks in ØX174 phage DNA in a dose-dependent manner (Nakayama et al., 1993). In the absence of Fe^{3+}, no induction of DNA damage was observed. Similar results were also seen with caffeic acid (doses not provided) in the presence of Cu^{2+} with isolated DNA from plasmid pbcNI, which carries the human c-Ha-*ras*-1 oncogene (Inoue et al., 1992). Yamada et al. (1985) reported the induction of double strand breaks in isolated gamma-DNA after treatment with 250 µM caffeic acid for 6 hours; the effect was stronger than that seen with the same concentration of chlorogenic acid. Li and Trush (1994) reported that caffeic acid induced strand breaks in phage ØX174 RF I DNA in the presence of Cu^{2+}; the authors suggested that the copper redox cycle and generation of H_2O_2 were two major factors involved in the observed DNA damage induced by either caffeic acid or chlorogenic acid.

9.6.2 Prokaryotic systems

In contrast to the induction of DNA damage in acellular assays with chlorogenic acid and caffeic acid, the chemicals were not mutagenic in bacterial mutagenicity assays. No induction of *his* gene mutations was observed in *S. typhimurium* strain TA98 treated with chlorogenic acid (the highest dose reported was 10 mg/plate [28 µmol/plate]) with or without S9 metabolic activation (MacGregor and Jurd, 1978; San and Chan, 1987; Fung et al., 1988). Caffeic acid (up to 9 mg/mL; 50 mM) did not induce mutations in *S. typhimurium* strain TA98, with or without S9 (San and Chan, 1987; Fung et al., 1988). Furthermore, both compounds were nonmutagenic in

S. typhimurium tester strains TA100, TA1535, TA1537, and TA1538, with or without S9 (Fung et al., 1988).

However, caffeic acid, at concentrations of 6 or 10 mg/plate (30 or 60 µmol/plate) in the absence of S9 and with Mn^{2+} induced gene mutations in *S. typhimurium* strains TA98 and TA100; mutants were not induced in either strains without Mn^{2+}, or with both Mn^{2+} and S9 (Stich et al., 1981a). Thus, transition metals, as in the DNA damage tests described above, are also important in the induction of genetic damage by these phenolics in *S. typhimurium.*

There was a report of mutation induction by caffeic acid (up to 10 µmol/plate; 1.8 mg/plate) and by chlorogenic acid (up to 28 µmol/plate; 9.9 mg/plate), in the absence of S9, in *S. typhimurium* strain BA13, which detects induction of forward mutations resulting in L-arabinose resistance (Ariza et al., 1988). In this assay, caffeic acid gave a stronger response than chlorogenic acid. The authors, who tested 6 compounds found in coffee solutions, concluded that, although they observed mutation induction by these two compounds, caffeic acid and chlorogenic acid mutagenicity could not account for the mutagenic activity they detected in coffee because the lowest effective doses for these two compounds in this *Salmonella* Ara test were much higher than corresponding concentrations measured in the coffee samples assayed.

9.6.3 Lower Eukaryotic Systems

Both chlorogenic acid (1 mg/mL; 3 mM) and caffeic acid (300 µg/mL; 1.66 mM) induced mitotic gene conversion in *S. cerevisiae* strain D7, when treatment was carried out in an alkaline environment (pH 10) in the absence of S9 metabolic activation (Rosin, 1984). Caffeic acid, 40 mg/mL (220 mM), also induced gene conversion in *S. cerevisiae* D7 at pH 7.4 in the absence of S9, although this response was weak (Stich et al., 1981a). A stronger response was obtained in this assay with caffeic acid in the presence of Mn^{2+}, but no activity was seen with caffeic acid in the presence of S9, with or without Mn^{2+} (Stich et al., 1981a). Thus, Mn^{2+} enhanced the mutagenic response seen with caffeic acid in *S. cerevisiae* D7, but S9 enzymes eliminated caffeic acid's recombinogenic activity, regardless of the presence of transition metal ions.

9.6.4 *In Vitro* Mammalian Systems

Neither chlorogenic acid nor caffeic acid (500 µM; 177 and 90.1 µg/mL, respectively) induced 8-azaguanine resistance in Chinese hamster V79-6 cells; the test was conducted in the absence of S9 activation (Wood et al., 1982). However, both compounds were mutagenic at the tk locus in mouse lymphoma L5178Y cells (Fung et al., 1988). Chlorogenic acid was active in the presence of S9 but not in the absence of S9, whereas caffeic acid was active in the absence of S9 but not in the presence of S9. Chlorogenic acid induced an 8-fold increase in mutation frequency over the control level, while caffeic acid induced a 5-fold increase.

Both chlorogenic and caffeic acids are clastogenic in mammalian cells *in vitro*. Induction of chromosomal aberrations was observed in Chinese hamster ovary (CHO) cells after treatment with chlorogenic acid (Whitehead et al., 1983; Stich et al., 1981a) or caffeic acid (Whitehead et al., 1983; Stich et al., 1981a; Hanham et al., 1983; cited by IARC, 1993) in the absence of S9; addition of S9 activation enzymes eliminated the clastogenic activity of both compounds. As in the assays discussed above, addition of transition metal ions enhanced the positive responses seen with chlorogenic and caffeic acids in the absence of S9 (Stich et al., 1981a,b).

Caffeic acid (dose not provided) in the presence of Mn^{+2} with human Raji cells induced DNA strand breaks (Inoue et al., 1992).

9.6.5 *In Vivo* Mammalian Systems

In contrast to the clastogenicity of chlorogenic and caffeic acids *in vitro*, they did not induce chromosomal damage *in vivo*. Male Sprague-Dawley rats administered two p.o. chlorogenic acid doses of 150 mg/kg (420 µmol/kg) 24 hours apart showed no increases in the frequencies of micronucleated polychromatic erythrocytes (PCE) or normochromatic erythrocytes in bone marrow (Hossain et al., 1976). No induction of micronucleated PCE was noted in bone marrow cells of female $B6C3F_1$ mice treated with caffeic acid in the diet (doses up to 2400 mg/kg; 13.3 mmol/kg) (Raj et al., 1983; cited by IARC, 1993) or in intestinal epithelial cells of mice after dietary exposure to caffeic acid doses up to 4800 mg/kg (26.6 mmol/kg) (Wargovich et al., 1983).

9.7 Cogenotoxicity

The details of this study are presented in **Table 12**.

Only one paper was identified that described cogenotoxicity data, and that publication contained information on chlorogenic acid only.

Chlorogenic acid (25, 50, 100, and 200 µg/mL; 71, 140, 282, or 564 µM) was tested for clastogenicity in CHO cells, with and without arecoline and Mn^{2+} (Stich et al., 1981c). Chlorogenic acid, alone, was not clastogenic at these doses. However, a dose-related, synergistic increase in chromosomal damage was detected in cultures treated with chlorogenic acid and either arecoline or Mn^{2+}. The enhancement was greatest with Mn^{2+}.

9.8 Antigenotoxicity

The details of these studies are presented in **Table 13**.

9.8.1 Acellular Systems

Chlorogenic acid and caffeic acid (doses not provided) were effective inhibitors of aflatoxin B_1 (AFB)-DNA adduct formation in a reconstituted microsomal monooxygenase system (Firozi and Bhattacharya, 1995). Additionally, pretreatment of purified cytochrome P450 with chlorogenic acid or caffeic acid rendered the cytochrome partially inactive in catalyzing AFB-DNA adduct formation in the reconstituted system. The authors concluded that natural polyphenols may have the ability to modulate chemical carcinogenesis by modulating cytochrome P450 function.

Caffeic acid (50-500mM; 9-90 mg/mL) inhibited the induction of DNA single strand breaks by H2O2 and cytochrome c in intact supercoiled phage ØX174 DNA (I) (Nakayama et al., 1993).

Table 12. Cogenotoxicity of Chlorogenic Acid

Test System	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
CHO cells	clastogenic activity in presence or absence of arecoline or Mn^{2+}	-	chlorogenic acid and arecoline, purities n.p.	25, 50, 100, or 200 µg/mL (71, 140, 282, or 564 µM) chlorogenic acid and 50, 100, 200, and 400 µg/mL arecoline	enhancement of chromosomal damage	No clastogenic activity when tested alone. Dose-dependent, synergistic enhancement of clastogenic activity when tested together. More enhancement with Mn^{2+}.	Stich et al. (1981c)

Abbreviations: CHO = Chinese hamster ovary; n.p. = not provided

Table 13. Antigenotoxicity of Chlorogenic Acid and Caffeic Acid

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
9.8.1 Acellular Systems							
reconstituted microsomal monooxygenase system	inhibition of aflatoxin B_1-DNA adduct formation	-	chlorogenic acid, purity n.p.	n.p.	inhibition seen	The reconstituted system contained purified cytochrome P450 and NADPH-cytochrome P450 reductase. Pretreatment of purified cytochrome P450 with chlorogenic acid rendered cytochrome P450 partially inactive in catalyzing aflatoxin B1-DNA adduct formation. The authors concluded that natural polyphenols may have the ability to modulate chemical carcinogenesis by modulating cytochrome P450 function.	Firozi and Bhattacharya (1995)
phage ØX174 DNA	inhibition of DNA single strand breaks induced by H_2O_2 and cytochrome *c*	-	caffeic acid, purity n.p.	50, 100, 200, 300, 400, or 500 µM (9, 18, 36, 54, 72, or 90 µg/mL)	inhibition seen	Inhibition was dose-dependent.	Nakayama et al. (1993)

Abbreviations: AFB_1 = aflatoxin B_1; AF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; B*a*P = benzo[*a*]pyrene; B*a*P 7,8-diol-9,10-epoxide-2 = (±)-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; ENU = ethyl nitrosourea; Glu-p-2 = 2-aminopyrido[1,2-*a*:3',2'-*d*]imidazole; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ = 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; $MeIQ_x$ = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; MMC = mitomycin C; MNNG = *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; MNU = methylnitrosourea; MX = 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone; NA = not applicable; n.p. = not provided; 4-NQO = 4-nitroquinoline 1-oxide; PCE = polychromatic erythrocytes; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; p.o. = *per os* = by mouth; Trp-p-1 = 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole; Trp-p-2 = 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; +/- = presence/absence

Table 13. Antigenotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
reconstituted microsomal monooxygenase system	inhibition of aflatoxin B_1-DNA adduct formation	-	caffeic acid, purity n.p.	n.p.	inhibition seen	The reconstituted system contained purified cytochrome P450 and NADPH-cytochrome P450 reductase. Pretreatment of purified cytochrome P450 with chlorogenic acid rendered cytochrome P450 partially inactive in catalyzing aflatoxin B1-DNA adduct formation. The authors concluded that natural polyphenols may have the ability to modulate chemical carcinogenesis by modulating cytochrome P450 function.	Firozi and Bhattacharya (1995)
9.8.2 Prokaryotic Systems							
S. typhimurium strain TA98	inhibition of *his* gene mutations induced by the pyrolyzate of albumin	+	chlorogenic acid, purity n.p.	200 µg/plate (0.564 µmol/plate)	inhibition seen when the chlorogenic acid/ albumin mixture was heated at 250°C or 550°C	Chlorogenic acid was added to 1 g of albumin and the mixture was heated. Treatment was not toxic.	Fukuhara et al. (1981)
S. typhimurium strain TA100	inhibition of *his* gene mutations induced by B*a*P 7,8-diol-9,10-	-	chlorogenic acid, 'pure'	up to 300 nmol/mL (106 µg/mL)	inhibition seen		Wood et al. (1982)

Abbreviations: AFB_1 = aflatoxin B_1; AF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; B*a*P = benzo[*a*]pyrene; B*a*P 7,8-diol-9,10-epoxide-2 = (±)-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; ENU = ethyl nitrosourea; Glu-p-2 = 2-aminopyrido[1,2-*a*:3',2'-*d*]imidazole; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ = 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; $MeIQ_x$ = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; MMC = mitomycin C; MNNG = *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; MNU = methylnitrosourea; MX = 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone; NA = not applicable; n.p. = not provided; 4-NQO = 4-nitroquinoline 1-oxide; PCE = polychromatic erythrocytes; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; p.o. = *per os* = by mouth; Trp-p-1 = 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole; Trp-p-2 = 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; +/- = presence/absence

Table 13. Antigenotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
	epoxide-2						
S. typhimurium strain TA1535	inhibition of *his* gene mutations induced by a model nitrosation system	-	chlorogenic acid, purity n.p.	up to 10 mg/mL (28 mM)	inhibition seen	The nitrosation reaction mixture consisted of methylurea and sodium nitrite in standard buffer, adjusted to pH 3.6. The mixture was incubated at room temperature for 35 min before being neutralized to pH 7.4. Chlorogenic acid was added to the methylurea just prior to the addition of sodium nitrite.	Stich et al. (1982a)
S. typhimurium strain TA98	inhibition of *his* gene mutations induced by B*a*P or cigarette-smoke condensate	+	chlorogenic acid, purity n.p.	250 or 500 µg/plate (0.706 or 1.41 µmol/plate)	no inhibition		Terwel and van der Hoeven (1985)
S. typhimurium strain TA100	inhibition of *his* gene mutations induced by B*a*P 7,8-diol-9,10-epoxide-2	-	chlorogenic acid, purity n.p.	0.25 µmol (88.6 µg)	inhibition seen	86% inhibition was observed. Treatment was not toxic.	Huang et al. (1985)
S. typhimurium	inhibition of *his* gene mutations	+	chlorogenic acid, purity	up to 0.05	no inhibition		Alldrick et

Abbreviations: AFB_1 = aflatoxin B_1; AF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; B*a*P = benzo[*a*]pyrene; B*a*P 7,8-diol-9,10-epoxide-2 = (±)-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; ENU = ethyl nitrosourea; Glu-p-2 = 2-aminopyrido[1,2-*a*:3',2'-*d*]imidazole; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ = 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; $MeIQ_x$ = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; MMC = mitomycin C; MNNG = *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; MNU = methylnitrosourea; MX = 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone; NA = not applicable; n.p. = not provided; 4-NQO = 4-nitroquinoline 1-oxide; PCE = polychromatic erythrocytes; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; p.o. = *per os* = by mouth; Trp-p-1 = 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole; Trp-p-2 = 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; +/- = presence/absence

Table 13. Antigenotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
strain TA98	induced by IQ, MeIQ, $MeIQ_x$, Trp-p-1, or Trp-p-2		n.p.	µmol (18 µg)			al. (1986)
S. typhimurium strain TA1535	inhibition of *his* gene mutations induced by MNNG	-	chlorogenic acid, purity n.p.	up to 10 mg/mL (28 mM)	inhibition seen	Inhibition observed only with concurrent administration of chlorogenic acid and MNNG. Doses were not toxic.	Chan et al. (1986)
S. typhimurium strain TA98	inhibition of *his* gene mutations induced by Trp-p-2	-	heat-treated chlorogenic acid, purity n.p.	10 mg (28 µmol) (see comments)	no inhibition	Chlorogenic acid plus buffer and celite was heated to 190°C for 30 min. Water was added, the contents centrifuged, and the supernatant tested.	Yamaguchi and Iki (1986)
			heat-treated chlorogenic	10 mg chlorogenic	inhibition seen	Chlorogenic acid, heat-treated to simulate the coffee	

Abbreviations: AFB_1 = aflatoxin B_1; AF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; B*a*P = benzo[*a*]pyrene; B*a*P 7,8-diol-9,10-epoxide-2 = (±)-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; ENU = ethyl nitrosourea; Glu-p-2 = 2-aminopyrido[1,2-*a*:3',2'-*d*]imidazole; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ = 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; $MeIQ_x$ = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; MMC = mitomycin C; MNNG = *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; MNU = methylnitrosourea; MX = 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone; NA = not applicable; n.p. = not provided; 4-NQO = 4-nitroquinoline 1-oxide; PCE = polychromatic erythrocytes; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; p.o. = *per os* = by mouth; Trp-p-1 = 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole; Trp-p-2 = 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; +/- = presence/absence

Table 13. Antigenotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
			acid plus sucrose, purities n.p.	acid plus 11.4 mg sucrose		roasting process, induced 99% inhibition when sucrose was added.	
S. typhimurium strain TA98	inhibition of *his* gene mutations induced by AFB_1	+	chlorogenic acid, purity n.p.	1, 3, 6, or 9 mg/mL (3, 9, 20, or 30 mM)	inhibition seen	Inhibitory effect was observed when chlorogenic acid and AFB_1 were administered concurrently, but not when exposure to AFB_1 was followed by chlorogenic acid. Doses were not cytotoxic.	San and Chan (1987)
S. typhimurium strain TA100	inhibition of *his* gene mutations induced by AFB_1 or MNNG	+	chlorogenic acid, purity n.p.	0.1 or 0.5 µmol (40 or 200 µg)	inhibition of AFB_1-induced mutations no inhibition of MNNG-induced mutations	Doses were not toxic to cells.	Francis et al. (1989)
S. typhimurium strain TA98	inhibition of *his* gene mutations induced by IQ	+	chlorogenic acid, purity n.p.	0.1, 1, 10, or 100 µM (0.04, 0.4, 3.5, or 35.4 µg/mL)	no inhibition		Ayrton et al. (1992)
S. typhimurium strain TA1535	inhibition of *his* gene mutations induced by a	+/-	chlorogenic acid, purity	up to 5 mg/mL (14 mM)	inhibition seen	The nitrosation reaction mixture, consisting of an aqueous fraction of a	Stich (1992)

Abbreviations: AFB_1 = aflatoxin B_1; AF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; B*a*P = benzo[*a*]pyrene; B*a*P 7,8-diol-9,10-epoxide-2 = (±)-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; ENU = ethyl nitrosourea; Glu-p-2 = 2-aminopyrido[1,2-*a*:3',2'-*d*]imidazole; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ = 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; $MeIQ_x$ = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; MMC = mitomycin C; MNNG = *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; MNU = methylnitrosourea; MX = 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone; NA = not applicable; n.p. = not provided; 4-NQO = 4-nitroquinoline 1-oxide; PCE = polychromatic erythrocytes; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; p.o. = *per os* = by mouth; Trp-p-1 = 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole; Trp-p-2 = 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; +/- = presence/absence

Table 13. Antigenotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
	nitrosation reaction mixture		n.p.			common Chinese salt-preserved fish and sodium nitrite, was not toxic to cells. The inhibition of nitrosation reaction-induced mutagenicity was dose-dependent.	
S. typhimurium strains TA98 and TA100	inhibition of *his* gene mutations induced by cigarette smoke condensate or extract of oral Swedish moist snuff	+	chlorogenic acid, purity n.p.	up to 7 mg/plate (20 µmol/plate)	smoke condensate: inhibition of mutagenicity in TA98 and TA100 snuff extract: inhibition of mutagenicity in TA98, no inhibition in TA100	Authors stated that because mutant survival and slight cytotoxicity cannot be accurately determined in this test, the anti-mutagenicity of chlorogenic acid requires confirmation in another test system.	Romert et al. (1994)
S. typhimurium strain TA98	inhibition of *his* gene mutations induced by Trp-p-1 or Glu-p-2	+	chlorogenic acid, purity n.p.	1, 2.5, or 5 mg/plate (3, 7.1, or 10 µmol/plate)	inhibition seen	Inhibition was dose-dependent.	Yamada and Tomita (1996)
S. typhimurium strain TA98	inhibition of *his* gene mutations induced by 4-	-	chlorogenic acid, purity n.p.	1, 2.5, or 5 mg/plate (3, 7.1, or 10	no inhibition		Yamada and Tomita (1996)

Abbreviations: AFB_1 = aflatoxin B_1; AF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; B*a*P = benzo[*a*]pyrene; B*a*P 7,8-diol-9,10-epoxide-2 = (±)-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; ENU = ethyl nitrosourea; Glu-p-2 = 2-aminopyrido[1,2-*a*:3',2'-*d*]imidazole; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ = 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; $MeIQ_x$ = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; MMC = mitomycin C; MNNG = *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; MNU = methylnitrosourea; MX = 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone; NA = not applicable; n.p. = not provided; 4-NQO = 4-nitroquinoline 1-oxide; PCE = polychromatic erythrocytes; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; p.o. = *per os* = by mouth; Trp-p-1 = 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole; Trp-p-2 = 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; +/- = presence/absence

Table 13. Antigenotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
	NQO or AF-2			µmol/plate)			
E. coli strain B/r WP2	inhibition of mutations induced by UV	-	chlorogenic acid, purity n.p.	2 or 4 mg/plate (6 or 11 µmol/plate)	no inhibition	No cytotoxicity was observed.	Shimoi et al. (1986)
S. typhimurium strain TA100	inhibition of *his* gene mutations induced by B*a*P 7,8-diol-9,10-epoxide-2	-	caffeic acid, 'pure'	up to 500 nmol/mL (90.1 µg/mL)	inhibition seen	Maximum inhibition observed at 300 nmol/plate.	Wood et al. (1982)
S. typhimurium strain TA100	inhibition of *his* gene mutations induced by B*a*P 7,8-diol-9,10-epoxide-2	-	caffeic acid, purity n.p.	0.25 µmol (45.0 µg)	inhibition seen	Inhibited B*a*P 7,8-diol-9,10-epoxide-2-induced mutagenicity by 68% Treatment was not cytotoxic.	Huang et al. (1985)
S. typhimurium strain TA98	inhibition of *his* gene mutations induced by IQ, MeIQ, $MeIQ_x$, Trp-p-1, or Trp-p-2	+	caffeic acid, purity n.p.	up to 0.05 µmol (9.0 µg)	no inhibition		Alldrick et al. (1986)
S. typhimurium strain TA1535	inhibition of *his* gene mutations induced by MNNG	-	caffeic acid, purity n.p.	10, 20, 30, or 40 mg/mL (56, 110, 170, or 220 mM)	inhibition seen	Inhibitory effect was observed only when caffeic acid and MNNG were administered concurrently. Doses were not cytotoxic.	Chan et al. (1986)

Abbreviations: AFB_1 = aflatoxin B_1; AF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; B*a*P = benzo[*a*]pyrene; B*a*P 7,8-diol-9,10-epoxide-2 = (±)-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; ENU = ethyl nitrosourea; Glu-p-2 = 2-aminopyrido[1,2-*a*:3',2'-*d*]imidazole; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ = 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; $MeIQ_x$ = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; MMC = mitomycin C; MNNG = *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; MNU = methylnitrosourea; MX = 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone; NA = not applicable; n.p. = not provided; 4-NQO = 4-nitroquinoline 1-oxide; PCE = polychromatic erythrocytes; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; p.o. = *per os* = by mouth; Trp-p-1 = 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole; Trp-p-2 = 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; +/- = presence/absence

Table 13. Antigenotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
S. typhimurium strain TA98	inhibition of *his* gene mutations induced by Trp-p-2	-	heat-treated caffeic acid, purity n.p.	4.6 mg (26 µmol) (see comments)	no inhibition	Caffeic acid plus buffer and celite was heated to 190°C for 30 min. Water was added, the contents centrifuged, and the supernatant tested.	Yamaguchi and Iki (1986)
			heat-treated caffeic acid and sucrose, purities n.p.	4.6 mg caffeic acid plus 11.4 mg sucrose	inhibition seen	Caffeic acid, heat-treated to simulate the coffee roasting process, induced 96% inhibition when sucrose was added.	
S. typhimurium strain TA98	inhibition of *his* gene mutations induced by AFB_1	+	caffeic acid, purity n.p.	1, 2, 3, 6, or 9 mg/mL (6, 11, 17, 33, or 50 mM)	inhibition seen	Inhibitory effect was observed when caffeic acid and AFB_1 were administered concurrently, but not when exposure to AFB_1 was followed by caffeic acid. Treatment was not	San and Chan (1987)

Abbreviations: AFB_1 = aflatoxin B_1; AF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; B*a*P = benzo[*a*]pyrene; B*a*P 7,8-diol-9,10-epoxide-2 = (±)-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; ENU = ethyl nitrosourea; Glu-p-2 = 2-aminopyrido[1,2-*a*:3',2'-*d*]imidazole; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ = 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; $MeIQ_x$ = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; MMC = mitomycin C; MNNG = *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; MNU = methylnitrosourea; MX = 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone; NA = not applicable; n.p. = not provided; 4-NQO = 4-nitroquinoline 1-oxide; PCE = polychromatic erythrocytes; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; p.o. = *per os* = by mouth; Trp-p-1 = 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole; Trp-p-2 = 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; +/- = presence/absence

Table 13. Antigenotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
						cytotoxic.	
S. typhimurium strain TA100	inhibition of *his* gene mutations induced by AFB_1 or MNNG	+	caffeic acid, purity n.p.	0.1 or 0.5 µmol (20 or 90 µg)	inhibition seen	Doses were not cytotoxic.	Francis et al. (1989)
S. typhimurium strain TA100	inhibition of *his* gene mutations induced by 4-NQO	-	caffeic acid, purity n.p.	0.33, 1.0, 3.3, or 10 µmol/ plate (59, 180, 590, or 1800 µg/plate)	inhibition seen		Camoirano et al. (1994)
S. typhimurium strain TA98	inhibition of *his* gene mutations induced by unfractionated mainstream cigarette smoke	+	caffeic acid, purity n.p.	0.33, 1.0, 3.3, or 10 µmol/ plate (59, 180, 590, or 1800 µg/plate)	inhibition seen	The dose inhibiting 50% of mutagenicity (MID_{50}) was 3.4 µmol/plate (610 µg/plate).	Camoirano et al. (1994)
S. typhimurium strain TA98	inhibition of *his* gene mutations induced by $MeIQ_x$ or PhIP	+	caffeic acid, purity n.p.	0.2, 0.6, 2, or 6 µg (1, 3, 11, or 33 nmol)	no inhibition		Malaveille et al. (1996)
S. typhimurium strain TA98	inhibition of *his* gene mutations	+	caffeic acid, purity n.p.	1, 2.5, or 5 mg/plate (6,	inhibition seen	Inhibition was dose-dependent.	Yamada and Tomita

Abbreviations: AFB_1 = aflatoxin B_1; AF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; B*a*P = benzo[*a*]pyrene; B*a*P 7,8-diol-9,10-epoxide-2 = (±)-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; ENU = ethyl nitrosourea; Glu-p-2 = 2-aminopyrido[1,2-*a*:3',2'-*d*]imidazole; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ = 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; $MeIQ_x$ = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; MMC = mitomycin C; MNNG = *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; MNU = methylnitrosourea; MX = 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone; NA = not applicable; n.p. = not provided; 4-NQO = 4-nitroquinoline 1-oxide; PCE = polychromatic erythrocytes; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; p.o. = *per os* = by mouth; Trp-p-1 = 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole; Trp-p-2 = 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; +/- = presence/absence

Table 13. Antigenotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
	induced by Trp-p-1 or Glu-p-2			14, or 30 µmol/plate)			(1996)
S. typhimurium strain TA98	inhibition of *his* gene mutations induced by 4-NQO or AF-2	-	caffeic acid, purity n.p.	1, 2.5, or 5 mg/plate (6, 14, or 30 µmol/plate)	no inhibition		Yamada and Tomita (1996)
E. coli strain B/r WP2	inhibition of mutations induced by UV	-	caffeic acid, purity n.p.	1 or 2 mg/plate (6 or 11 µmol/plate)	no inhibition	No cytotoxicity was observed.	Shimoi et al. (1986)
E. coli strain WP2s	inhibition of Trp gene mutations induced by MX	-	caffeic acid, purity n.p.	100 or 300 µg/plate (0.555 or 1.67 µmol/plate	no inhibition		Watanabe et al. (1994)
9.8.3 Mammalian Systems *In Vitro*							
Chinese hamster V79-6 cells	inhibition of mutations induced by B*a*P 7,8-diol-9,10 epoxide-2	-	chlorogenic acid, 'pure'	up to 500 nmol/mL (177 µg/mL)	inhibition seen	None of the doses were cytotoxic.	Wood et al. (1982)
rat tracheal epithelial cells	inhibition of B*a*P-induced transformation	-	chlorogenic acid, purity n.p.	up to 28.2 µM (9.99 µg/mL)	no inhibition		Arnold et al. (1995)
Chinese hamster V79-6 cells	inhibition of mutations induced by B*a*P 7,8-diol-	-	caffeic acid, 'pure'	up to 500 nmol/mL (90.1 µg/mL)	inhibition seen	None of the doses were cytotoxic.	Wood et al. (1982)

Abbreviations: AFB_1 = aflatoxin B_1; AF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; B*a*P = benzo[*a*]pyrene; B*a*P 7,8-diol-9,10-epoxide-2 = (±)-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; ENU = ethyl nitrosourea; Glu-p-2 = 2-aminopyrido[1,2-*a*:3',2'-*d*]imidazole; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ = 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; $MeIQ_x$ = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; MMC = mitomycin C; MNNG = *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; MNU = methylnitrosourea; MX = 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone; NA = not applicable; n.p. = not provided; 4-NQO = 4-nitroquinoline 1-oxide; PCE = polychromatic erythrocytes; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; p.o. = *per os* = by mouth; Trp-p-1 = 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole; Trp-p-2 = 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; +/- = presence/absence

Table 13. Antigenotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
	9,10 epoxide-2						
rat tracheal epithelial cells	inhibition of B*a*P-induced transformation	-	caffeic acid, purity n.p.	up to 16.6 µM (2.99 µg/mL)	no inhibition		Arnold et al. (1995)
9.8.4 Mammalian Systems *In Vivo*							
mice (strain and age n.p.)	inhibition of methylurea plus nitrate- or MNU-induced micronuclei in bone marrow and colon epithelial cells	NA	chlorogenic acid, purity n.p.	300 mg/kg (847 µmol/kg)	methylurea plus nitrate: inhibition of micronuclei MNU: no inhibition	The authors attributed the positive effect to inhibition of nitrosamine formation.	Aeschbacher and Jaccaud (1989; abstr.)
mice (MS/Ae; 10 to 13-wk-old)	inhibition of colonic cell nuclear aberrations and bone marrow micronuclei induced by nitrosourea	NA	chlorogenic acid, purity n.p.	150 mg/kg (423 µmol/kg) p.o.	inhibition seen in both tissues	The endpoint measured in colon cells is unclear: "Nuclear aberrations" is the term used in the text, but "colonic micronucleus test" appears in a table.	Aeschbacher and Jaccaud (1990)

Abbreviations: AFB_1 = aflatoxin B_1; AF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; B*a*P = benzo[*a*]pyrene; B*a*P 7,8-diol-9,10-epoxide-2 = (±)-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; ENU = ethyl nitrosourea; Glu-p-2 = 2-aminopyrido[1,2-*a*:3',2'-*d*]imidazole; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ = 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; $MeIQ_x$ = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; MMC = mitomycin C; MNNG = *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; MNU = methylnitrosourea; MX = 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone; NA = not applicable; n.p. = not provided; 4-NQO = 4-nitroquinoline 1-oxide; PCE = polychromatic erythrocytes; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; p.o. = *per os* = by mouth; Trp-p-1 = 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole; Trp-p-2 = 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; +/- = presence/absence

Table 13. Antigenotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
mice (Swiss albino; 8 to 12-wk-old)	inhibition of γ-radiation-induced micronuclei in bone marrow PCE	NA	chlorogenic acid, purity n.p.	50, 100, or 200 mg/kg (140, 282, or 564 µmol/kg) p.o. 2 h before, immediately after, or 2 h after radiation	inhibition seen when administered before or immediately after radiation	Authors claim that the 50 mg/kg dose is close to what humans might ingest in a day. Treatment reduced MN-PCE by 45% at all 3 doses.	Abraham et al. (1993)
mice (Swiss albino; 10 to 12-wk-old)	inhibition of bone marrow micronucleus induction in PCE by MNNG, ENU, MMC, or urethane	NA	mixture of several dietary constituents, including chlorogenic acid, purity n.p.	mixture in the diet with and without coffee extract chlorogenic acid dose: 15 or 20 mg/kg (42 or 56 µmol/kg)	mixture plus coffee extract: inhibition seen mixture alone: no inhibition	Mixtures were administered by gavage with and without coffee extract 1 h before clastogen was given i.p. Impossible to isolate chlorogenic acid effects from the mix.	Abraham (1996)
mice (Swiss	inhibition of bone	NA	mixture of	mixture in the	mixture plus coffee extract:	Mixtures were administered	Abraham

Abbreviations: AFB_1 = aflatoxin B_1; AF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; B*a*P = benzo[*a*]pyrene; B*a*P 7,8-diol-9,10-epoxide-2 = (±)-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; ENU = ethyl nitrosourea; Glu-p-2 = 2-aminopyrido[1,2-*a*:3',2'-*d*]imidazole; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ = 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; $MeIQ_x$ = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; MMC = mitomycin C; MNNG = *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; MNU = methylnitrosourea; MX = 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone; NA = not applicable; n.p. = not provided; 4-NQO = 4-nitroquinoline 1-oxide; PCE = polychromatic erythrocytes; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; p.o. = *per os* = by mouth; Trp-p-1 = 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole; Trp-p-2 = 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; +/- = presence/absence

Table 13. Antigenotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
albino; 10 to 12-wk-old)	marrow micronucleus induction in PCE by MNNG, ENU, MMC, or urethane		dietary constituents, including caffeic acid, purity n.p.	diet with and without coffee extract caffeic acid dose: 20 mg/kg (110 µmol/kg)	inhibition seen mixture alone: no inhibition	by gavage with and without coffee extract 1 h before clastogen was given i.p. Impossible to isolate caffeic acid effects from the mix.	(1996)

Abbreviations: AFB_1 = aflatoxin B_1; AF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; B*a*P = benzo[*a*]pyrene; B*a*P 7,8-diol-9,10-epoxide-2 = (±)-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; ENU = ethyl nitrosourea; Glu-p-2 = 2-aminopyrido[1,2-*a*:3',2'-*d*]imidazole; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ = 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; $MeIQ_x$ = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; MMC = mitomycin C; MNNG = *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; MNU = methylnitrosourea; MX = 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone; NA = not applicable; n.p. = not provided; 4-NQO = 4-nitroquinoline 1-oxide; PCE = polychromatic erythrocytes; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; p.o. = *per os* = by mouth; Trp-p-1 = 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole; Trp-p-2 = 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; +/- = presence/absence

9.8.2 Prokaryotic Systems

In prokaryotes, chlorogenic and caffeic acid inhibited the mutagenicity of some mutagens but not others. In the presence of metabolic activation, chlorogenic acid, at the doses indicated in parentheses, inhibited the induction of *his* gene mutations in *S. typhimurium* strain TA98 by pyrolyzate of albumin (0.564 µmol/plate; 200 µg/plate) (Fukuhara et al., 1981), AFB_1 (3-30 mM; 1-9 mg/mL) (San and Chan, 1987), cigarette smoke condensate (up to 20 µmol/plate; 7 mg/plate) (Romert et al., 1994), and extract of oral Swedish moist snuff (up to 20 µmol/plate; 7 mg/plate) (Romert et al., 1994). Both chlorogenic acid (3-10 µmol/plate; 1-5 mg/plate) and caffeic acid (6-30 µmol/plate; 1-5 mg/plate), also in the presence of metabolic activation, inhibited the mutagenicity of 2-aminodipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-p-2) and 3-amino-1,4-dimethyl-5*H*-pyrido-(4,3-*b*)indole (Trp-p-1) (Yamada and Tomita, 1996). Caffeic acid, at 6-50 mM (1-9 mg/mL) or at 0.33-10 µmol/plate (0.059-1.8 µg/plate), inhibited the mutagenicity of AFB_1 (San and Chan, 1987) and unfractionated mainstream smoke (Camoirano et al., 1994), respectively.

However, in strain TA98 in the presence of metabolic activation, chlorogenic acid (0.706-1.41 µmol/plate; 250-500 µg/plate) did not inhibit the mutagenicity of B*a*P or cigarette smoke condensate (Terwel and van der Hoeven, 1985), and neither did chlorogenic acid and caffeic acid, both at concentrations up to 0.05 µmol (18 or 9.0 µg, respectively), inhibit the mutagenicity of 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline ($MeIQ_x$), Trp-p-1, or 3-amino-1-methyl-5*H*-pyrido-(4,3-*b*)indole (Trp-p-2) (Alldrick et al., 1986). Chlorogenic acid was not effective when tested at 0.100 to 100 µM (0.040-35.4 µg/mL) for inhibition of IQ-induced mutations (Ayrton et al., 1992). Caffeic acid was ineffective when tested at 1 to 33 nmol (0.2-6 µg) for inhibition of $MeIQ_x$- and PhIP-induced mutation (Malaveille et al., 1996). In the absence of metabolic activation, chlorogenic acid (3-10 µmol/plate; 1-5 mg/plate) and caffeic acid (6-30 µmol/plate; 1-5 mg/plate) did not inhibit the mutagenicity of 4-NQO or 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) (Yamada and Tomita, 1996).

Chlorogenic and caffeic acids, when heated to simulate the coffee roasting process, did not inhibit the mutagenicity of Trp-p-2 in *S. typhimurium* strain TA98 in the absence of metabolic

activation (Yamaguchi and Iki, 1986). However, the heat-treated compounds did inhibit the mutagenicity of Trp-p-2 when sucrose was added to the medium. Heat-treated chlorogenic acid was slightly more inhibitory than heat-treated caffeic acid.

Both inhibitory and noninhibitory effects were observed in tests using *S. typhimurium* strain TA100. In the presence of metabolic activation, chlorogenic acid, at concentrations up to 20 µmol/plate (7 mg/plate), inhibited cigarette smoke condensate-induced mutations (Romert et al., 1994), and chlorogenic acid and caffeic acid, both at 0.1 or 0.5 µmol (40 or 200 µg, or 20 or 90 µg, respectively), inhibited AFB_1-induced mutations (Francis et al., 1989). Caffeic acid (0.1-0.5 µmol; 20-90 µg), but not chlorogenic acid (0.1-0.5 µmol; 40-200 µg), inhibited the mutagenicity of MNNG in the presence of metabolic activation (Francis et al., 1989). In the absence of metabolic activation, chlorogenic acid and caffeic acid, both used at 0.25 µmol (88.6 or 45.0 µg, respectively) (Huang et al., 1985) or up to 300 µM (106 µg/mL) (chlorogenic acid) or 500 µM (90.1 µg/mL) (caffeic acid) (Wood et al., 1982), inhibited the mutagenicity of B*a*P 7,8-diol-9,10-epoxide-2. In contrast, chlorogenic acid, when tested up to 20 µmol/plate (7 mg/plate), did not inhibit the mutagenicity of an extract of Swedish moist snuff in the presence of metabolic activation (Romert et al., 1994), and caffeic acid, at 0.33-10 µmol/plate (0.059-1.8 mg/plate), did not inhibit 4-NQO-induced mutations in the absence of metabolic activation (Camoirano et al., 1994).

When tested in *S. typhimurium* strain TA1535 in the absence of metabolic activation, chlorogenic acid, at concentrations up to 28 mM (10 mg/mL), inhibited the mutagenicity of MNNG (Chan et al., 1986) and the nitrosation products of nitrosoproline (Stich et al., 1982a; Stich, 1992). Caffeic acid, at concentrations up to 220 mM (40 mg/mL), inhibited the mutagenicity of MNNG (Chan et al., 1986).

In the absence of metabolic activation, neither chlorogenic acid nor caffeic acid, when administered at 6 or 11 µmol/plate (2 or 4 mg/plate, or 1 or 2 mg/plate, respectively), inhibited UV-induced mutations in *E. coli* strain B/r WP2 (Shimoi et al., 1986). In *E. coli* strain WP2s, caffeic acid (0.555 or 1.67 µmol/plate; 100 or 300 µg/plate) did not inhibit the induction of

mutations by 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (MX), in the absence of metabolic activation (Watanabe et al., 1994).

9.8.3 Mammalian Systems *In Vitro*

Both chlorogenic acid and caffeic acid, at concentrations up to 500 µM (177 or 90.1 µg/mL, respectively), inhibited the mutagenicity of B*a*P 7,8-diol-9,10-epoxide-2 in Chinese hamster V79-6 cells in the absence of metabolic activation (Wood et al., 1982). However, neither chlorogenic acid, at concentrations up to 28.2 µM (9.99 µg/mL), nor caffeic acid, at concentrations up to 16.6 µM (2.99 µg/mL), inhibited B*a*P-induced transformation of rat tracheal epithelial cells (Arnold et al., 1995).

9.8.4 Mammalian Systems *In Vivo*

Chlorogenic acid, administered p.o. to male and female mice at 423 µmol/kg (150 mg/kg), inhibited the induction of methylurea plus nitrate of micronuclei (MN) in bone marrow PCE (Aeschbacher and Jaccaud, 1989). The authors attributed the effect to the inhibition of nitrosamine formation. In a similar study using MS/Ae mice, chlorogenic acid at 150 mg/kg (423 mmol/kg) inhibited the induction of colonic cell nuclear aberrations and bone marrow MN-PCE by nitrosourea (Aeschbacher and Jaccaud, 1990).

Male Swiss albino mice were used in an investigation of the inhibition by chlorogenic acid of γ-radiation-induced MN in bone marrow PCE (Abraham et al., 1993). Chlorogenic acid (50.0, 100, or 200 mg/kg; 140, 282, or 564 µmol/kg p.o.) was administered either 2 hours before, immediately after, or 2 hours after radiation treatment. When chlorogenic acid was administered 2 hours before or immediately after radiation, a significant reduction in the frequency of radiation-induced MN was observed at all three doses. No alteration in MN induction was noted when chlorogenic acid was administered 2 hours after the completion of irradiation. The 50 mg/kg dose of chlorogenic acid was described as being close to what humans might ingest in a day (Abraham et al., 1993).

Another antigenotoxicity study was conducted in male Swiss mice in which dietary mixtures, consisting of chlorogenic acid (15-20 mg/kg; 42- 56 µmol/kg) or caffeic acid (20.0 mg/kg; 110 µmol/kg), were administered by gavage with and without coffee extract one hour before a potent known clastogen (i.e., MNNG, ENU, MMC, or urethane) was administered i.p. (Abraham, 1996). Administration of the mixtures without coffee extract had no effect on micronucleus induction, but co-administration of the mixtures and coffee extract produced an enhanced inhibition of micronucleus formation compared to the effect of coffee extract alone. The isolated effects of chlorogenic or caffeic acids could not be determined from this study.

9.9 Immunotoxicity

The details of these studies are presented in **Table 14**.

9.9.1 *In Vitro* Tests

In isolated Wistar-King rat mast cells, chlorogenic and caffeic acid, at 25-100 µM (8.9-35.4 µg/mL and 4.5-18.0 µg/mL, respectively), inhibited histamine release induced by compound 48/80 (Kimura et al., 1985a). Also in isolated rat mast cells, both chlorogenic acid and caffeic acid, at 10-100 µM (3.5-35.4 µg/mL and 1.8-18.0 µg/mL, respectively), inhibited histamine release induced by concanavalin A plus phosphatidylserine (Kimura et al., 1985a); caffeic acid appeared to be more effective than chlorogenic acid in inhibiting histamine release induced by either treatment. Chlorogenic acid, at 140 µM (49.6 µg/mL), reduced serum complement activity in normal human serum (Koethe and Becker, 1992; abstr.), and for caffeic acid, the concentration causing 50% inhibition of complement activity in guinea pig serum complement, was 62.7 µM (11.3 µg/mL) (Nakagami et al., 1995). Caffeic acid, at concentrations up to 10 µM (1.8 µg/mL), also inhibited leukotriene production in mouse peritoneal macrophages (Mirzoeva and Calder, 1996). However, neither chlorogenic nor caffeic acid, at concentrations up to 50 µM (18 µg/mL;

Table 14. Immunotoxicity of Chlorogenic Acid and Caffeic Acid

Test System or Species, Strain, and Age of Animal	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
9.9.1 *In Vitro* Tests					
rat mast cells	chlorogenic acid and compound 48/80, purities n.p.	7.5 µg/mL compound 48/80 plus 10, 25, 50, 75, or 100 µM (3.5, 8.9, 18, 27, or 35.4 µg/mL) chlorogenic acid	25 µM chlorogenic acid inhibited compound 48/80-induced histamine release by more than 50%, while the 3 higher doses inhibited the release by more than 80%.	Mast cells (isolated from the peritoneal cavities of Wistar-King rats), calcium-free Tyrode's solution, compound 48/80, and chlorogenic acid were incubated at 37°C for 10 min. The reaction was terminated by cooling the mixture in ice water.	Kimura et al. (1985a)
	chlorogenic acid, Con A, and PS, purities n.p.	7.5 µg/mL Con A, 5 µg/mL PS, plus 10, 25, 50, 75, or 100 µM (3.5, 8.9, 18, 27, or 35.4 µg/mL) chlorogenic acid	Chlorogenic acid concentrations of 50 µM or higher inhibited Con A plus PS-induced histamine release by more than 80%, while the 2 lower doses inhibited the release by more than 50%.	Mast cells (isolated from the peritoneal cavities of Wistar-King rats), calcium-free Tyrode's solution, Con A, PS, $CaCl_2 \cdot 2H_2O$, and chlorogenic acid were incubated at 37°C for 10 min. The reaction was terminated by cooling the mixture in ice water.	
normal human serum	chlorogenic acid, purity n.p.	140 µM (49.6 µg/mL)	Chlorogenic acid caused a 58% reduction in serum complement activity compared with the control. There was no reduction in the hemolytic activity of C_1 and C_3, 30% reduction in C_4, and 52% reduction in C_2 activity. Chlorogenic acid is presumably acting in serum to enhance the action of C_1 on its natural substrates C_4 and C_2.	CH_{50} assay was used. Chlorogenic acid was incubated with a 1:20 solution of normal human serum for 1 h at 37°C. The authors stated that since chlorogenic acid is present in tobacco smoke, the effect of chlorogenic acid on serum complement may be important in the pathogenesis of inflammation.	Koethe and Becker (1992; abstr.)
human whole blood	chlorogenic acid, purity n.p.	up to 50 µM (18 µg/mL)	Treatment did not influence lipopolysaccharide-stimulated secretion of proinflammatory cytokines (i.e., TNF-α, IL-1β, or IL-6).		Obertreis et al. (1996)

Abbreviations: Con A = Concanavalin A; IL-1β = interleuken-1β; IL-6 = interleuken-6; i.p. = intraperitoneal injection; i.v. = intravenous injection; LTB_4 = leukotriene-B_4; LTC_4 = leukotriene-C_4; n.p. = not provided; PAF = platelet activating factor; PGE_2 = prostaglandin E_2; PS = phosphatidylserine; TNF-α = tumor necrosis factor-α; TPA = 12-O-tetradecanoylphorbol-13-acetate

Table 14. Immunotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age of Animal	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
rat mast cells	caffeic acid and compound 48/80, purities n.p.	7.5 µg/mL compound 48/80 plus 10, 25, 50, 75, or 100 µM (1.8, 4.5, 9.0, 14, or 18.0 µg/mL) caffeic acid	25 µM caffeic acid inhibited compound 48/80-induced histamine release by more than 70%, while the 3 higher doses inhibited the release by more than 80%.	Mast cells (isolated from the peritoneal cavities of Wistar-King rat), calcium-free Tyrode's solution, compound 48/80, and caffeic acid were incubated at 37°C for 10 min. The reaction was terminated by cooling the mixture in ice water.	Kimura et al. (1985a)
	caffeic acid, Con A, and PS, purities n.p.	7.5 µg/mL Con A, 5 µg/mL PS, plus 10, 25, 50, 75, or 100 µM (1.8, 4.5, 9.0, 14, or 18.0 µg/mL) caffeic acid	At 25 µM and higher, caffeic acid inhibited Con A plus PS-induced histamine release by more than 90%. The lowest concentration inhibited the release by more than 50%.	Mast cells (isolated from the peritoneal cavities of Wistar-King rats), calcium-free Tyrode's solution, Con A, PS, $CaCl_2 \cdot 2H_2O$, and caffeic acid were incubated at 37°C for 10 min. The reaction was terminated by cooling the mixture in ice water.	
guinea pig complement serum	caffeic acid, purity n.p.	n.p.	The IC_{50} value (concentration causing 50% inhibition of complement activity) was 11.3 µg/mL (62.7 µM).		Nakagami et al. (1995)
human whole blood	caffeic acid, purity n.p.	up to 50 µM (9.0 µg/mL)	Treatment did not influence lipopolysaccharide-stimulated secretion of proinflammatory cytokines (i.e., TNF-α, IL-1β, or IL-6).		Obertreis et al. (1996)
thioglycollate-elicited mouse peritoneal macrophages	caffeic acid, purity n.p.	up to 10 µM (1.8 µg/mL)	Caffeic acid inhibited leukotriene production (i.e., LTB_4 and LTC_4).	Macrophages were incubated for 2 h with calcium ionophore A23187, which enhanced the release of LTB_4 and LTC_4. Caffeic acid was added 10 min before the stimuli to test its effect on the induced-LTB_4 and LTC_4 synthesis.	Mirzoeva and Calder (1996)

9.9.2 *In Vivo* Tests

Abbreviations: Con A = Concanavalin A; IL-1β = interleuken-1β; IL-6 = interleuken-6; i.p. = intraperitoneal injection; i.v. = intravenous injection; LTB_4 = leukotriene-B_4; LTC_4 = leukotriene-C_4; n.p. = not provided; PAF = platelet activating factor; PGE_2 = prostaglandin E_2; PS = phosphatidylserine; TNF-α = tumor necrosis factor-α; TPA = 12-O-tetradecanoylphorbol-13-acetate

Table 14. Immunotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age of Animal	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
mice (CD-1, female, age n.p.)	chlorogenic acid and TPA, purities n.p.	Topical application of acetone, TPA, plus 0.2 or 1.0 µmol (70 or 350 µg) chlorogenic acid to the ear	Both doses significantly inhibited TPA-induced edema.		Conney et al. (1991)
	chlorogenic acid and arachidonic acid, purities n.p.	Topical application of 5 µmol (2 mg) chlorogenic acid 30 min before 1 µmol arachidonic acid to the ear	Chlorogenic acid did not significantly inhibit arachidonic acid-induced edema.		
monkeys (macaque, gender and age n.p.)	purified natural chlorogenic acid	25 mg (71 µmol) i.v.	No reaction to chlorogenic acid was observed.	The monkeys were first sensitized by topical application of reaginic sera from 16 green-coffee allergic patients. 48 hours later, the monkeys were challenged with chlorogenic acid.	Layton et al. (1968)
mice (CD-1, female, age n.p.)	caffeic acid and TPA, purities n.p.	Topical application of acetone, TPA, plus 0.2 or 1.0 µmol (36 or 180 µg) caffeic acid to the ear	Treatment did not significantly inhibit TPA-induced edema.		Conney et al. (1991)
mice (CD-1, female, age n.p.)	caffeic acid and arachidonic acid, purities n.p.	Topical application of 5 µmol (900 µg) caffeic acid 30 min before 1 µmol arachidonic acid to	Caffeic acid did not significantly inhibit arachidonic acid-induced edema.		Conney et al. (1991)

Abbreviations: Con A = Concanavalin A; IL-1β = interleuken-1β; IL-6 = interleuken-6; i.p. = intraperitoneal injection; i.v. = intravenous injection; LTB_4 = leukotriene-B_4; LTC_4 = leukotriene-C_4; n.p. = not provided; PAF = platelet activating factor; PGE_2 = prostaglandin E_2; PS = phosphatidylserine; TNF-α = tumor necrosis factor-α; TPA = 12-O-tetradecanoylphorbol-13-acetate

Table 14. Immunotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age of Animal	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
		the ear			
mice (ICR, male. age n.p.)	caffeic acid and acetic acid, purities n.p.	50 or 100 mg caffeic acid/kg (280 or 560 µmol/kg) i.p. 30 min prior to 0.25% acetic acid	The peritoneal vascular permeability test was used to determine anti-inflammatory response Caffeic acid significantly inhibited the increase of peritoneal permeability caused by acetic acid.		Chen et al. (1995)
mice (C57BL6, male, age n.p.)	caffeic acid and zymosan, purities n.p.	1 mg zymosan plus 6 mg/kg (33 µmol/kg) caffeic acid, i.p.	Caffeic acid inhibited zymosan-induced LTC_4 production by 50%, but did not affect LTB_4 production.		Mirzoeva and Calder (1996)
rats (Sprague-Dawley, male, age n.p.)	caffeic acid and λ-carrageenan, purities n.p.	1.5% carrageenan and 10 mg (56 µmol) caffeic acid/kg, i.p.	Caffeic acid significantly inhibited carrageenan-induced edema.		Chen et al. (1995)
	caffeic acid and formalin, purities n.p.	3% formalin and 10 mg (56 µmol) caffeic acid/kg, i.p.	Caffeic acid significantly inhibited formalin-induced edema.		
rats (Sprague-Dawley, male, age n.p.)	caffeic acid, bradykinin, histamine, PAF, PGE2, and seratonin, purities n.p.	50 mg Evans blue/kg injection in the penis vein prior to intradermal injection with inflammatory mediators (i.e., 0.5 nM bradykinin, 50 nM histamine, 0.2 nM	Caffeic acid did not affect increased skin permeability induced by inflammatory mediators.		Chen et al. (1995)

Abbreviations: Con A = Concanavalin A; IL-1β = interleuken-1β; IL-6 = interleuken-6; i.p. = intraperitoneal injection; i.v. = intravenous injection; LTB_4 = leukotriene-B_4; LTC_4 = leukotriene-C_4; n.p. = not provided; PAF = platelet activating factor; PGE_2 = prostaglandin E_2; PS = phosphatidylserine; TNF-α = tumor necrosis factor-α; TPA = 12-O-tetradecanoylphorbol-13-acetate

Table 14. Immunotoxicity of Chlorogenic Acid and Caffeic Acid (continued)

Test System or Species, Strain, and Age of Animal	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
		PAF, 3 nM PGE_2 or 5 nM seratonin) and 10 mg/kg (56 µmol) caffeic acid i.p.			

Abbreviations: Con A = Concanavalin A; IL-1β = interleuken-1β; IL-6 = interleuken-6; i.p. = intraperitoneal injection; i.v. = intravenous injection; LTB_4 = leukotriene-B_4; LTC_4 = leukotriene-C_4; n.p. = not provided; PAF = platelet activating factor; PGE_2 = prostaglandin E_2; PS = phosphatidylserine; TNF-α = tumor necrosis factor-α; TPA = 12-O-tetradecanoylphorbol-13-acetate

9.0 µg/mL, respectively), affected lipopolysaccharide-stimulated secretion of proinflammatory cytokines in cultured human whole blood (Obertreis et al., 1996).

9.9.2 *In Vivo* Tests

A single topical application of 0.2 or 1.0 µmol (70 or 350 µg) chlorogenic acid to the ear of mice inhibited TPA-induced edema, but the same molar dose of caffeic acid (36 or 180 µg) had no such effect (Conney et al., 1991); the acids were applied concomitantly with TPA. Neither 5 µmol (2 mg) chlorogenic acid nor 5 µmol (900 µg) caffeic acid inhibited arachidonic acid-induced edema when applied to the ear 30 minutes prior to arachidonic acid (Conney et al., 1991). Intraperitoneal injection of caffeic acid (6 mg/kg; 33 µmol/kg) inhibited zymosan-induced leukotriene C_4 production, but did not affect leukotriene B_4 production in mice (Mirzoeva and Calder, 1996). Intraperitoneal injection of caffeic acid (10 mg; 56 µmol) to rats significantly inhibited edema induced by carrageenan or formalin injected into a paw (Chen et al., 1995). In peritoneal vascular permeability tests used to determine anti-inflammatory response, caffeic acid, at 50 or 100 mg/kg (280 or 560 µmol/kg) i.p., inhibited an acetic acid-induced increase in peritoneal permeability in rats, but at 10 mg/kg (56 µmol/kg) i.p., caffeic acid did not affect the increase in skin permeability induced by bradykinin, histamine, platelet activating factor, prostaglandin E_2, or seratonin (Chen et al., 1995). In monkeys whose skin was first sensitized by topical application of reaginic sera from 16 green coffee-allergic patients, i.v. administration of chlorogenic acid (71 µmol; 25 mg) did not induce allergic reactions (Layton et al., 1968).

9.10 Other Data

9.10.1 Antibacterial Activity

In *in vitro* studies using *Staphylococcus aureus, Streptococcus faecalis, S. typhimurium,* and *E. coli,* caffeic acid exhibited bactericidal activity only after oxidation and under alkaline conditions (Cuq and Jaussan, 1991; cited by IARC, 1993).

9.10.2 Cytotoxicity

Caffeic acid, at 100 µM (18.0 µg/mL), was cytotoxic to cultured human gastric tumor cells (Denizot et al., 1993). Caffeic acid induced apoptosis in HL-60 human leukemia cells at 50-100 µg/mL (9.0-18.0 µg/mL) but not in 3 other human leukemia cell lines, and 24 hour incubation of HL-60 cells with 30 µg/mL (211 µM) caffeic acid was cytotoxic, as measured by trypan blue dye exclusion (Sakagami et al., 1995). At 1 or 3 mM (200 or 500 µg/mL), caffeic acid induced apoptosis in human myelogenous leukemic cells (Sakagami et al., 1997a, b).

In rat 3Y1 diploid fibroblast cells transformed by the E1A gene of human adenovirus type 12, caffeic acid (0.1-100 µM; 0.018-18 µg/mL) attenuated the cytotoxicity of phosphatidylcholine, but not of catechol (Mitsui et al., 1995).

9.10.3 Other *In Vitro* Effects

In *S. cerevisiae,* the mean inhibitory concentrations (IC_{50}) of chlorogenic and caffeic acids for cell proliferation were 215 and 84 mg/L (1190 and 470 µM), respectively (Koch et al., 1993). In an experiment using 160 common drugs and other chemicals, the IC_{50} values in yeast correlated well with LD_{50} values in animals.

Neither chlorogenic nor caffeic acid (20 µM; 7.1 or 3.6 µg/mL) increased the vitamin D_3-induced differentiation of HL-60 leukemia cells (Sokoloski et al., 1997). Caffeic acid at 10 µM (1.8 µg/mL) slightly reduced the colony forming ability of MCF-7 human breast carcinoma cells (Ahn et al., 1997), and at 2 mM (400 µg/mL) inhibited tumor cell invasion and migration when tested on human HT-1080 fibrosarcoma cells (Saito et al., 1997). Caffeic acid (1 mM; 180 µg/mL), when tested on cultured human leukemia HL-60 and ML-1 cells, slightly reduced the concentration of methionine in the medium; culturing these cells in methionine-free medium was found to nearly terminate cell proliferation (Sakagami et al., 1997b). In cultured rat mesangial cells, caffeic acid (concentration not provided) had no significant effect on cell growth or DNA synthesis (Sellmayer and Weber, 1992).

9.10.4 *In Vitro* Effect on Enzymes

Chlorogenic acid was a competitive inhibitor of glucose-6-phosphate (G-6-P) hydrolysis in intact rat liver microsomes (Arion et al., 1997). Further, chlorogenic acid was found to reversibly bind with the G-6-P transporter, T1. The authors stated that chlorogenic acid is the most specific T1 inhibitor described to date.

Caffeic acid is a moderate stimulator of prostaglandin H synthase cyclooxygenase activity and a good reducing substrate for prostaglandin H synthase-compounds I and II (Bakovic and Dunford, 1994). The authors explained this discrepancy in properties as being related to a specific peroxidation mechanism of caffeic acid that includes the formation of an inhibitory complex of caffeic acid with native enzymes followed by a "three-step irreversible ping-pong peroxidation."

9.10.5 *In Vitro* Hepatoprotective Activity

Chlorogenic or caffeic acid (10 or 100 µM each; 3.5 or 35 µg/mL and 1.8 or 18 µg/mL, respectively) significantly reduced CCl_4-induced injury in cultured rat hepatocytes (Basnet et al., 1996). Similarly, caffeic acid (300 µM; 54 µg/mL) partly prevented cytotoxicity induced by hypoxia/reoxygenation or by cyanide in cultured rat hepatocytes (Niknahad et al., 1995).

9.10.6 Inhibition of Oxidation *In Vitro*

Chlorogenic acid (100 µM; 35.4 µg/mL) inhibited lipid peroxidation in rat synaptosomes (Born et al., 1996), while both chlorogenic and caffeic acid, at 10-100 µM (3.5-35.4 µg/mL, or 1.8-18.0 µg/mL, respectively), showed weak inhibitory activity toward lipid peroxidation induced by adenosine 5'-diphosphate (ADP) and ascorbic acid in rat liver mitochondria *in vitro* (Kimura et al., 1984). Vinson et al. (1995) also found that chlorogenic acid exhibited antioxidant activity *in vitro*, but when evaluated for its ability to bind to lipoproteins, it showed weak binding ability. Based on its weak binding ability, the authors concluded that the *in vivo* antioxidant activity of chlorogenic acid may be minimal.

When coincubated with deoxyguanosine, both chlorogenic and caffeic acid enhanced the production of hydroxyl radicals (Shih and Lin, 1993). Chlorogenic acid (Kasai and Nishimura, 1984) and caffeic acid (Stadler et al., 1994), at 1 mM (350 µg/mL), enhanced the hydroxylation of deoxyguanosine in the presence of hydrogen peroxide and iron.

Chlorogenic acid inhibited 2,2-azobis(2-amidinopropane)-mediated oxidation of serum albumin (Born et al., 1996). Caffeic acid (250 µM; 45 µg/mL) greatly suppressed the generation of hydroxyl radicals from the reaction of copper ethylenediamine with hydrogen peroxide (Ueda et al., 1996). Caffeic acid formed ternary copper ethylenediamine· caffeic acid complexes and had moderate hydroxyl radical scavenging ability.

9.10.7 Miscellaneous Effects Identified in Human Studies

When administered orally to humans, both chlorogenic and caffeic acid demonstrated anti-hypercholesterolemic (Yuchi and Kimura, 1986; cited by Pisha and Pezzuto, 1994) and anti-ulcer activities (Okuyama et al., 1983; cited by Pisha and Pezzuto, 1994).

In a study using four male and six female volunteers, 30.5 mg chlorogenic acid (86.1 mmol) was administered in two of four morning meals, which included rolls containing 3.8 mg iron with margarine and water, over a four-day period (Brune et al., 1989). The rolls were labeled with ^{55}Fe and ^{59}Fe. Two weeks later, a blood sample was drawn to determine the content of ^{55}Fe and ^{59}Fe in red blood cells. The same volunteers were also given the same morning meals without chlorogenic acid to determine control iron absorption values. The chlorogenic acid treatment was found to significantly inhibit iron absorption compared to iron absorption levels from the control diet.

10.0 STRUCTURE-ACTIVITY RELATIONSHIPS

Hydroxycinnamic acid derivatives (including caffeic acid and chlorogenic acid) were compared for their ability to inhibit N-nitrosation of dimethylamine and diethylamine and to reduce nitrite levels *in vitro* (Kikugawa et al., 1983). The order of potency for inhibition of nitrosamine formation was caffeic acid > ferulic acid > *p*-coumaric acid > ascorbic acid > cinnamic acid. Chlorogenic acid did not inhibit nitrosamine formation. The order of potency for reducing nitrite levels was caffeic acid > ferulic acid > *p*-coumaric acid > chlorogenic acid > ascorbic acid > cinnamic acid.

Inayama et al. (1984) reported that the degree of antitumor activity in mice by esterified catecholics was higher than that of non-esterified catecholics. Further, catecholylpropionoids, such as caffeic acid, possessed higher antitumor activity than *p*-coumaric acid, cinnamic acid, and their esters. Benzaldehyde induced little antitumor activity, and the hydroxy and dihydroxy derivatives of benzaldehyde and benzoic acid, including the corresponding catecholics, had lower antitumor activity than the catecholylpropionoids.

In a study of some pyrogallol-related compounds, the presence of a pyrogallol (1,2,3-trihydroxybenzene) moiety correlated with the inhibition of UV-induced mutagenicity in *E. coli* B/r WP2, but the mechanism of action was not determined (Shimoi et al., 1986). Chlorogenic and caffeic acids, which do not contain a pyrogallol moiety, did not exert an antimutagenic effect.

The hepatoprotective activity of polyphenolic compounds extracted from *Cynara scolymus* was investigated by measuring their activity against CCl_4-induced toxicity in isolated rat hepatocytes (Adzet et al., 1987). Among caffeoylquinic derivatives, cynarin revealed hepatoprotective properties, but chlorogenic and isochlorogenic acid exhibited no such effects. Caffeic and quinic acids form part of the molecular structure of these compounds. Caffeic acid, but not quinic acid, showed evident hepatoprotective activity. In another study, chlorogenic acid and caffeic acid were less potent than dicaffeoyl quinic acid derivatives in modifying CCl_4 toxicity (Basnet et al., 1996).

Although caffeic acid was shown to induce rat forestomach carcinogenesis in 51-week studies (Hirose et al., 1988; cited by IARC, 1993; Hirose et al., 1992, 1993), the metabolite

ferulic acid did not exert this effect when administered at 1% (10,000 ppm) in the diet for 52 weeks to male F344 rats pretreated with DMH and MNU (Imaida et al., 1990).

In a study investigating the antitumor activity of tannins in mice, condensed tannins and related compounds were inactive, and hydrolyzable tannins and related polyphenols (including caffeic acid) showed only negligible activity (Miyamoto et al., 1992). Oligomeric ellagitannins, particularly dimeric ellagitannins, were the most active.

Due to its phenolic nucleus and an extended side-chain conjugation, ferulic acid, like caffeic acid, is a potent antioxidant (Graf, 1992).

Caffeic acid phenethyl ester (CAPE) (0.1-6.5 nmol applied topically) was found to inhibit TPA-mediated oxidative processes that are essential for skin tumor development in mice (Frenkel et al., 1993). Polyphenolic compounds in general (including chlorogenic and caffeic acid) are presumed to inhibit carcinogenicity by scavenging and trapping potentially DNA-damaging electrophiles, free radicals, and toxic metals (Friedman and Smith, 1984; Tanaka, 1994; all cited by Friedman, 1997; Tanaka et al., 1993).

De Flora et al. (1994) evaluated 88 organic compounds from a variety of chemical classes to determine structural relationships corresponding to their potential antimutagenic potency toward 4-NQO in *S. typhimurium* strain TA100. One structural determinant of mutagenicity, the presence of a sulhydryl group bonded to CH_2, was identified. The presence of a hydroxyl group bonded to a carbon atom and/or the presence of the fragment CH=CH-C=CH (both occurring in caffeic acid) was found to be related to the absence of antimutagenic activity. Further, the authors stated that in antioxidant compounds, nucleophilicity rather than antioxidant properties appears to play a role in inhibiting 4-NQO-induced mutagenicity.

Nakayama et al. (1994) found that polyphenolic compounds bearing the *o*-dihydroxy structure (i.e., nordihydroguaiaretic acid [NDGA], caffeic acid ester, gallic acid ester, quercetin, and catechin) were effective suppressers of H_2O_2-induced cytotoxicity in Chinese hamster V79 cells, *S. typhimurium* strain TA104, and *E. coli* strain PQ37. In contrast, ferulic acid ester compounds bearing the *O*-methyloxyphenol structure were not effective in suppressing such effects.

In an *in vitro* study of the immunotoxic effects of propolis extracts, CAPE was the most potent inhibitor of LTB_4 and LTC_4 synthesis in calcium ionophore A23187-stimulated murine peritoneal macrophages, having similar potency to synthetic lipoxygenase inhibitors (i.e., NDGA and N,N'-dicyclohexyl-*O*-(3,4-dihydroxycinnamoyl)isourea [DCHCU]) (Mirzoeva and Calder, 1996). Caffeic acid was a less potent inhibitor of leukotriene production.

Ferulic acid (Tao et al., 1997), like chlorogenic and caffeic acid (Steele et al., 1994), did not prevent or reduce aberrant crypt foci induced by AOM in F344 rats (Tao et al., 1997). The rats were administered 18 mg AOM/kg i.p. at 7 and 8 weeks of age. Five weeks after the first dose of AOM, 0.1% ferulic acid was administered in the diet for up to 28 days.

11.0 ONLINE DATABASES AND SECONDARY REFERENCES

11.1 Online Databases

Chemical Information System Files

HTSDR
SANSS
TSCATS (Toxic Substances Control Act Test Submissions)

DIALOG Files

Kirk-Othmer Encyclopedia of Chemical Technology

National Library of Medicine Databases

EMIC and EMICBACK (Environmental Mutagen Information Center)

STN International Files

BIOSIS	EMBASE	Registry
CANCERLIT	HSDB	RTECS
CAPLUS	MEDLINE	TOXLINE
CHEMLIST		

TOXLINE includes the following subfiles:

Toxicity Bibliography	TOXBIB
International Labor Office	CIS
Hazardous Materials Technical Center	HMTC
Environmental Mutagen Information Center File	EMIC
Environmental Teratology Information Center File (continued after 1989 by DART)	ETIC
Toxicology Document and Data Depository	NTIS
Toxicological Research Projects	CRISP
NIOSHTIC7	NIOSH
Pesticides Abstracts	PESTAB
Poisonous Plants Bibliography	PPBIB
Aneuploidy	ANEUPL

Epidemiology Information System	EPIDEM
Toxic Substances Control Act Test Submissions	TSCATS
Toxicological Aspects of Environmental Health	BIOSIS
International Pharmaceutical Abstracts	IPA
Federal Research in Progress	FEDRIP
Developmental and Reproductive Toxicology	DART

Databases Available on the Internet

Phytochemeco Database (Agricultural Research Service)

In-House Databases

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ACKNOWLEDGEMENTS

Support to the National Toxicology Program for the preparation of Chlorogenic Acid and Caffeic Acid—Review of Toxicological Literature was provided by Integrated Laboratory Systems, Inc., through NIEHS Contract Number N01-ES-65402. Contributors included: Raymond R. Tice, Ph.D. (Principal Investigator); Bonnie L. Carson, M.S. (Co-Principal Investigator); Karen E. Haneke, M.S.; Maria E. Donner, Ph.D.; Kristine L. Witt, M.S.; Brigette D. Brevard, M.A.; Claudine A. Gregorio, M.A.; and Esther M. Morris, M.S.

APPENDIX A - UNITS AND ABBREVIATIONS

AHH = aryl hydrocarbon hydroxylase

ALT = alanine aminotransferase

AOM = azoxymethane

AST = aspartate aminotransferase

B*a*P = benzo[*a*]pyrene

BBN = *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine

d = day(s)

DHPN = 2,2' -dihydroxy-di-*n*-propylnitrosamine

DMBA = 7,12-dimethylbenz[*a*]anthracene

DMH = 1,2-dimethylhydrazine

GST = glutathione-*S*-transferase

h = hour(s)

i.g. = intragastric

i.p. = intraperitoneal

i.v. = intravenous

LTB_4 = leukotriene-B_4

LTC_4 = leukotriene-C_4

Mg = metric tons

MNNG = *N*-methyl-*N*' -nitro-*N*-nitrosoguanidine

MNU = *N*-methyl-*N*-nitrosourea

mo = month(s)

p.o. = *per os*; by mouth

s.c. = subcutaneous

TPA = 12-*O*-tetradecanoylphorbol-13-acetate

wk = week(s)

yr = year(s)