# Cafestol
# [CASRN 469-83-0]
# and
# Kahweol
# [CASRN 6894-43-5]

# Review of Toxicological Literature

***Prepared for***

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# EXECUTIVE SUMMARY

Cafestol was nominated by a private individual for toxicity and carcinogenicity testing based on an association between exposure and elevated cholesterol levels in humans coupled with the potential to activate the nuclear receptor FXR. Significant exposure to the substance occurs through consumption of coffee. Kahweol, a structurally similar compound also in coffee and found to cause a rise in cholesterol concentrations, has been included in the nomination for testing.

Global coffee production comes from mainly two species, *Coffea arabica* and *Coffea robusta*. Cafestol and kahweol, which are naturally occurring diterpenes found only in coffee, are present in the unsaponifiable lipid fraction. Their content in a coffee drink is influenced by the brew method; brewing releases oil droplets containing cafestol and kahweol from the ground coffee beans. Boiled coffee, such as Scandinavian-style and Turkish-style, contains the highest concentrations, while instant, drip-filtered, and percolated coffee brews contain negligible amounts.

Many uses have been patented for cafestol and kahweol. Coffee bean oil, which contains both compounds, has been reported useful as a sun filter. In combination with a cosmetically or pharmaceutically acceptable carrier, topical compositions containing an effective amount of cafestol have been patented for the prevention or treatment of various skin conditions. The use of the palmitates of cafestol and kahweol for treatment of potentially malignant oral lesions such as leukoplakias is currently under investigation.

In humans, cafestol and kahweol were recovered from the feces. Subjects fed diterpene-rich supplements showed neither compound in urine. Treatment with β-glucuronidase, however, showed that up to 6% of ingested cafestol and 3% of kahweol were excreted in urine as simple conjugates of glucuronic or sulfuric acid.

Cafestol and kahweol have shown anticarcinogenic properties. Feeding green coffee beans to female Sprague-Dawley rats prior to or subsequent to 7,12-dimethylbenz[*a*]anthracene (DMBA) administration inhibited the formation of mammary tumors. Instant coffee yielded the same results as the green coffee. Feeding green coffee beans to female Syrian golden hamsters, followed by application of DMBA to the buccal pouch, resulted in up to a 40% reduction of multiple tumors (tumors of the lip and pouch). Feeding studies with cafestol or kahweol alone also showed decreases in tumor incidences in some of these studies.

The anticarcinogenic property of cafestol and kahweol has been hypothesized to be related to their ability to induce glutathione *S*-transferase (GST). In mice and rats, both substances were found to induce GST activity of the liver and intestinal mucosa. Studies with derivatives of cafestol and kahweol indicate that the furan moiety is the active site for induction of the enzyme activity.

When tested for mutagenicity, cafestol and kahweol were found to be inactive in *Salmonella typhimurium* strain TM677 in the presence and absence of metabolic activation (S9). In an *in vitro* assay, the diterpenes inhibited the covalent binding of aflatoxin B1 metabolites to DNA in a dose-dependent manner using S9 and microsomal subcellular fractions from the livers of rats. In male F344/nctr rats, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-DNA adduct formation in the colon was inhibited.

The cholesterol-raising effect of boiled coffee in humans has been linked with these diterpenes. Studies have shown that an intake of cafestol and kahweol causes an increase in total cholesterol as well as low density lipoprotein (LDL) cholesterol, triglycerides, and alanine aminotransferase (ALT) activity. When coffee solutions or extracts were tested in hamsters, rats, gerbils, rabbits, and rhesus and cebus monkeys, no significant effects on serum total cholesterol and triacylglycerol concentrations were observed.

No data on acute toxicity, subchronic toxicity, chronic toxicity, carcinogenicity, immunotoxicity, reproduction, or teratology, as well as any regulations pertaining to cafestol and kahweol, were located.

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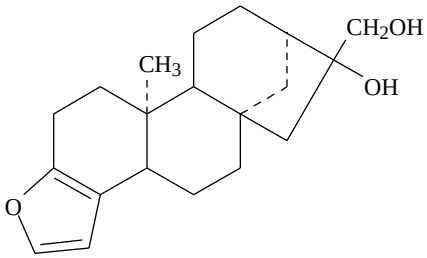
**TABLES**

## 1.0 BASIS FOR NOMINATION

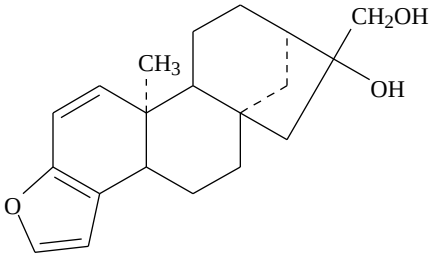
Cafestol was nominated by Dr. C. Weinberger of the National Institute of Environmental Health Sciences (NIEHS) for toxicity and carcinogenicity testing based on its ability "to raise cholesterol levels in humans coupled with its potential to activate the nuclear receptor FXR." Significant exposure occurs through consumption of coffee. Kahweol, a structurally similar compound in coffee also found to elevate serum cholesterol levels, is included in the nomination for testing.

## 2.0 INTRODUCTION

Cafestol
[469-83-0]




Kahweol
[6894-43-5]




### 2.1 Chemical Identification

Cafestol ($C_{20}H_{28}O_3$, mol. wt. = 316.439) is also called:

Cafesterol
Coffeol
5a,8-Methano-5a*H*-cyclohepta[5,6]naphtho[2,1-*b*]furan-7-methanol,
3b,4,5,6,7,8,9,10,10a,10b,11,12-dodecahydro-7-hydroxy-10b-methyl-,
(3b*S*,5a*S*,7*R*,8*R*,10a*S*,10b*S*)-
5a,8-Methano-5a*H*-cyclohepta[5,6]naphtho[2,1-*b*]furan-7-methanol,
3b,4,5,6,7,8,9,10,10a,10b,11,12-dodecahydro-7-hydroxy-10b-methyl-,
[3b*S*-(3bα,5aβ,7β,8β,10aα,10bβ)]-

Kahweol ($C_{20}H_{26}O_3$, mol. wt. = 314.424) is also called:

1,2-Didehydrocafestol
5a,8-Methano-5a*H*-cyclohepta[5,6]naphtho[2,1-*b*]furan-7-methanol,
3b,4,5,6,7,8,9,10,10a,10b-decahydro-7-hydroxy-10b-methyl-,
(3b*S*,5a*S*,7*R*,8*R*,10a*R*,10b*S*)-
5a,8-Methano-5a*H*-cyclohepta[5,6]naphtho[2,1-*b*]furan-7-methanol,
3bα,4,5,6,7,8α,9,10,10a,10b-decahydro-7-hydroxy-10bβ-methyl
5a,8-Methano-5a*H*-cyclohepta[5,6]naphtho[2,1-*b*]furan-7-methanol,
3b,4,5,6,7,8,9,10,10a,10b-decahydro-7-hydroxy-10b-methyl-,
[3b*S*-(3bα,5aβ,7β,8β,10aα,10bβ)]-

(Budavari, 1996; Connolly and Hill, 1991; Registry, 1999)

Cafestol and kahweol have been determined in coffee by novel high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) techniques (Nackunstz and Maier, 1987 abstr.). Both compounds, present in the light petroleum (boiling range 60-70 °C) extract of green coffee beans in the form of esters of fatty acids (Djerassi et al., 1953; Kaufmann and Sen Gupta, 1963; both cited by Lam et al., 1985), were separated in gram quantities without extensive recycling using preparative liquid chromatography (LC) silica cartridges impregnated with 10% silver nitrate (Lam et al., 1985). They can also be identified by gas chromatography-ion trap mass spectrometry (GC-ITDMS) (Lercker et al., 1995). Using gel permeation chromatography on Bio Beads S-X3, it is now possible to simultaneously detect and quantify cafestol and kahweol in their free forms (Kölling-Speer et al., 1999).

## 2.2 Physical-Chemical Properties

| Property | Information | Reference |
|---|---|---|
| **Cafestol** | | |
| Physical State | crystals<br>needles from petroleum ether | Budavari (1996); Connolly and Hill (1991)<br>Glasby (1982) |
| Melting Point ($^{o}$C) | 158-160, 160-162 | Budavari (1996); Connolly and Hill (1991) |
| **Kahweol** | | |
| Physical State | crystals<br>rods from acetone | Connolly and Hill (1991)<br>Glasby (1982) |
| Melting Point ($^{o}$C) | 88-90; 143-143.5 | Connolly and Hill (1991); Sigma-Aldrich (1999) |

Kahweol is a strong oxidizing agent. Upon combustion or decomposition, it emits toxic fumes of carbon monoxide and carbon dioxide (Sigma-Aldrich, 1999).

## 2.3 Commercial Availability

Cafestol is commercially available in esterified form as cafestol acetate (Pelle, 1999). Kahweol can be purchased from the Sigma Chemical Company in St. Louis, MO (Sigma-Aldrich, 1999).

## 3.0 PRODUCTION PROCESSES

Substantially pure cafestol can be prepared in high yields by hydrogenation of kahweol in the presence of a partially deactivated palladium catalyst on a calcium carbonate or active carbon support conditioned by lead (Bertholet, 1987). A mixture of cafestol and kahweol can be obtained from coffee oil by transesterification followed by extraction. The coffee oil is treated

with anhydrous methanol in the presence of a basic catalyst (sodium hydroxide, potassium hydroxide, or potassium carbonate) and extracted with dichloromethane (Bertholet, 1988).

## 4.0 PRODUCTION AND IMPORT VOLUMES

Cafestol and kahweol are not produced or imported into the United States for commercial use. They are diterpenes occurring only in coffee (Kölling-Speer et al., 1999). Almost three-quarters of global coffee production comes from the species *Coffea arabica*, which contains cafestol (about 6 g/kg or 0.6%) and kahweol (3 g/kg or 0.3%) (Debry, 1994; cited by Urgert and Katan, 1996; Nackunstz and Maier, 1987 abstr.). The other major commercial species is *Coffea robusta*, which contains mostly cafestol (2 g/kg or 0.2%) (Urgert and Katan, 1996; Nackunstz and Maier, 1987 abstr.).

## 5.0 USES

Cafestol and kahweol in coffee are used in experiments of physiological and sensory interest (Kölling-Speer et al., 1999). Coffee bean oil, which contains both compounds, has been patented useful as a sun filter (Grollier et al., 1988; cited by Pelle, 1999). Additionally, cafestol possesses antiinflammatory properties (Bertholet, 1987, 1988). In combination with a cosmetically or pharmaceutically acceptable carrier, topical compositions containing an effective amount of cafestol has been patented for the prevention or treatment of any condition in which the skin's lipid barrier is deficient or damaged (e.g., dry skin, pathological cases such as psoriasis and xerosis, and injuries such as burns, wounds, and blisters). The formulations may also enhance percutaneous drug delivery (Pelle, 1999). A mixture of cafestol and kahweol can be used in cosmetic applications (Bertholet, 1987, 1988). The use of their esters, the palmitates, for treatment of potentially malignant oral lesions such as leukoplakias is under investigation (Scully, 1995).

## 6.0 ENVIRONMENTAL OCCURRENCE AND PERSISTENCE

Cafestol and kahweol are characteristic diterpenes found in the unsaponifiable lipid fraction of raw coffee mainly esterified to fatty acids at the C-17 position (Garattini, 1993; Urgert et al., 1997). In determining the identity and levels of various diterpenes in coffee beans of nine wild *Coffea* species in Africa, cafestol was found in all with concentrations ranging from

239 mg/100 g of bean mass to 616 mg/100 g of bean mass (0.239 to 0.616%), which is the same range in commercial species. Variations in kahweol concentrations, however, were found and believed to be related to the geographical distribution of the species—those in West and Central African forests had a low concentration, while species originating from East Africa showed high levels of the compound (de Roos et al., 1997). Relatively large amounts of both chemicals have also been identified in Arabica and Robusta coffee from different geographic regions (Lercker et al., 1995). The total diterpene content ranges from 1.3% to 1.9% (w/w) in the beans of the former and from 0.2% to 1.5% of the latter (Viani, 1988; Ratnayake et al., 1993; both cited by de Roos et al., 1997).

## 7.0 HUMAN EXPOSURE

Individuals are exposed to cafestol and kahweol in coffee. The content of cafestol and kahweol in a coffee drink is significantly influenced by the brew method (Gross et al., 1997). Brewing releases oil droplets containing the two compounds from the ground coffee beans (Ratnayake et al., 1993; cited by Urgert et al., 1995b). The highest concentration of these constituents occurs in Scandinavian-style (cafestol: 7.2 mg/cup [cup = 150 mL]; kahweol: 7.2 mg/cup) and Turkish-style (cafestol: 5.3 mg/cup; kahweol: 5.4 mg/cup) boiled coffee, while instant, drip-filtered, and percolated coffee brews contain negligible amounts. French press coffee has an average cafestol content of 3.5 mg/cup and kahweol content of 4.4 mg/cup, while espresso coffee has 1 mg/cup of each diterpene (Gross et al., 1997; Urgert et al., 1995b). Regular and decaffeinated coffees also have similar diterpene contents. In regular coffee grounds, average levels of 486 mg/100 g (0.486%) cafestol and 469 mg/100 g (0.469%) kahweol were found. In decaffeinated coffee grounds, the values were 485 mg and 411 mg per 100 g (0.485% and 0.411%), respectively (Urgert et al., 1995b). The amount of cafestol and kahweol can be significantly reduced by roasting the green coffee (Kölling-Spear et al., 1999). In contrast, cafestol content was highest in coffee boiled for 10 or more minutes (Nackunstz and Maier, 1987 abstr.).

## 8.0 REGULATORY STATUS

No U.S. government regulations pertaining to cafestol and kahweol were found.

# 9.0 TOXICOLOGICAL DATA

## 9.1 General Toxicology

### 9.1.1 Human Data

A direct relationship between coffee consumption and total cholesterol has been demonstrated (Thelle et al., 1987). The cholesterol-raising effect of boiled coffee has been associated with the diterpenes from coffee oil (Heckers et al., 1994; Mensink et al., 1995; Urgert et al., 1995a; Urgert and Katan, 1996). Paper-filtered coffee does not elevate cholesterol since the lipid content (including diterpenes) is negligible (van Dusseldorp et al., 1991; Ahola et al., 1991; Ratnayake et al., 1993; all cited by Urgert et al., 1996). In a 2-year cross-sectional study of Norwegian subjects controlling for possible confounding variables such as body mass index, number of cigarettes, and physical activity, boiled coffee increased serum cholesterol by 8% (18 mg/dL; 0.47 mmol/L) in men and 10% (21 mg/dL; 0.55 mmol/L) in women (Stensvold et al., 1989). For those drinking filter coffee, the effect was only significant for women.

More than 20 epidemiology studies have been conducted. The effects of cafestol and kahweol on cholesterol, triglyceride, lipoprotein, and alanine aminotransferase (ALT) activity levels from some of these studies are given in section 9.10.2.

### 9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

**Excretion of Cafestol and Kahweol in Humans**

When nine males were fed 50 to 60 mg (0.16-0.19 mmol) of each compound per day for 4 weeks, an average of 6% of the ingested cafestol and 4% of kahweol was recovered from the feces (Urgert et al., 1996a). In seven subjects consuming 40 to 50 mg (0.13-0.16 mmol) of each diterpene per day for 3 weeks in fine coffee grounds, 24% of cafestol and 26% of kahweol were recovered from feces. Subjects fed diterpene-rich supplements showed no free cafestol or kahweol in urine. Treatment with β-glucuronidase, however, showed that up to 6% of ingested cafestol and 3% of kahweol were excreted in urine as simple conjugates of either glucuronic or sulfuric acid. The diterpenes may have also been metabolized into compounds that were not detectable.

### 9.1.3 Acute Exposure

No acute toxicity studies for cafestol and kahweol were available.

### 9.1.4 Short-Term and Subchronic Exposure

No short-term and subchronic toxicity studies were available.

### 9.1.5 Chronic Exposure

No chronic toxicity studies were available.

## 9.2 Reproductive and Teratological Effects

No reproductive or teratological data were available.

## 9.3 Carcinogenicity

No carcinogenicity data were available.

## 9.4 Initiation/Promotion Studies

No initiation/promotion studies were available.

## 9.5 Anticarcinogenicity

The details of the following studies are presented in **Table 1**.

### 9.5.1 Rats

Feeding green coffee beans (Colombian) to female Sprague-Dawley rats prior to or subsequent to 7,12-dimethylbenz[*a*]anthracene (DMBA) administration (peroral; 12 mg in 1 mL olive oil) inhibited the formation of neoplasia, specifically mammary tumors (Wattenberg, 1983). With diets containing 10% green coffee prior to the DMBA treatment, 75% of rats showed tumors, while 20% green coffee in the diet resulted in only 44% of the animals being affected, compared to 91% of animals in the control group. Oral intubation of cafestol palmitate or kahweol palmitate (60 mg) also decreased the neoplastic response (Wattenberg and Lam, 1984). Instant coffee (10% in the diet) had the same results as the 10% green coffee beans. For diets containing 10% green coffee beans administered after DMBA treatment, 50% of rats had mammary tumors compared to 94% of animals in the control group (Wattenberg, 1983).

### 9.5.2 Hamsters

Female Syrian golden hamsters fed a diet consisting of 20% green coffee beans and then painted three times weekly at the right buccal pouch with 0.5% DMBA in mineral oil or just mineral oil for 16.5 weeks showed occasional tumors of the lip and pouch versus the group that were fed a normal diet, where all the animals had multiple tumors (Formby et al., 1987 abstr.; Miller et al., 1988). A 30 to 40% reduction was obtained when hamsters were fed a diet consisting of 0.2 g/kg (0.02%) or 2.0 g/kg (0.2%) of a mixture of equal amounts of cafestol and kahweol prior to receiving any mineral oil treatments to the left buccal pouch (Miller et al., 1991). Application of DMBA and a 2.5% solution of cafestol and kahweol in dimethyl sulfoxide (DMSO) had the same inhibitory effect (McWhorter et al., 1988 abstr.).

## 9.6 Genotoxicity

The details of this study are presented in **Table 2**.

Cafestol palmitate, cafestol acetate, kahweol acetate (concentrations for each ranging from 0.31-5.0 mg/mL), and kahweol palmitate (0.1-1.6 mg/mL) were found to be nonmutagenic in *Salmonella typhimurium* strain TM677 in the presence and absence of metabolic activation (S9), as were the palmitic acid esters of the two diterpenes (0.06-5.0 mg/mL) (Pezzuto et al., 1986). Additionally, in the presence of S9, kahweol palmitate, at the highest concentration, resulted in about 50% survival of bacteria. Without S9, bacterial survival was dose-dependent.

## 9.7 Cogenotoxicity

No cogenotoxicity data were located.

## Table 1. Anticarcinogenicity Studies of Cafestol and Kahweol

| Species, Strain, and Age | Number and Sex of Animals | Chemical Form and Purity | Dose | Observation Period | Results/Comments | Reference |
|---|---|---|---|---|---|---|
| **Rats** | | | | | | |
| Sprague-Dawley, 34-days-old | 16 F/group | green coffee beans (Colombian) containing cafestol and kahweol palmitates, purities n.p. | diets consisting of 10% or 20% green coffee beans fed for 14 days *prior to* DMBA administration (p.o.; 12 mg in 1 mL olive oil on day 15) | 18 wk after DMBA administration | Mammary tumors occurred in 75% and 44% ($p<0.001$) of the animals given the 10% and 20% green coffee beans in the diet, respectively, compared to 91% in the control group. | Wattenberg (1983) |
| Sprague-Dawley, 34-days-old | 16 F/group | instant coffee containing cafestol and kahweol palmitates, purities n.p. | diet consisting of 10% instant coffee fed for 14 days *prior to* DMBA administration (p.o.; 12 mg in 1 mL olive oil on day 15) | 18 wk after DMBA administration | Mammary tumors occurred in 75% of animals versus 100% in the control group. | Wattenberg and Lam (1984) |
| Sprague-Dawley, 34-days-old | 16 F/group | cafestol and kahweol palmitates, purity n.p. | diets consisting of 60 mg administered 3, 2, and 1 day *prior to* DMBA administration (p.o.; 12 mg in 1 mL olive oil on day 15) | 18 wk after DMBA administration | Mammary tumors occurred in 69% of animals fed cafestol palmitate and 56% ($p<0.05$) of those fed kahweol palmitate compared to 84% in the control group. | Wattenberg and Lam (1984) |
| Sprague-Dawley, 34-days-old | 16 F/group | kahweol palmitate, purity n.p. | diet consisting of 60 mg administered 4 h *prior to* DMBA administration (p.o.; 12 mg in 1 mL olive oil on day 15) | 18 wk after DMBA administration | Mammary tumors occurred in 63% ($p<0.01$) of animals versus 100% in the control group. | Wattenberg and Lam (1984) |
| Sprague-Dawley, 7-wk-old | 16 F/group | green coffee beans (Colombian) containing cafestol and kahweol palmitates, purities n.p. | diet consisting of 10% green coffee beans fed *subsequent to* DMBA administration (p.o.; 12 mg in 1 mL olive oil for one wk) | 18 wk after DMBA administration | Mammary tumors occurred in 50% ($p<0.05$) of animals versus 94% in the control group. | Wattenberg (1983) |

Note: Controls were fed a diet without any additions (i.e., test compounds).

## Table 1. Anticarcinogenicity Studies of Cafestol and Kahweol (Continued)

| Species, Strain, and Age | Number and Sex of Animals | Chemical Form and Purity | Dose | Exposure/ Observation Period | Results/Comments | Reference |
|---|---|---|---|---|---|---|
| **Hamsters** | | | | | | |
| Syrian golden, age n.p. | 20 F/group | green coffee beans containing cafestol and kahweol palmitates, purities n.p. | Group 1: normal chow<br><br>Group 2: 20% green coffee beans in chow<br><br>When animals adjusted to diet, 16 from each group were painted 3x/wk with 0.5% DMBA in mineral oil and 4 were painted with just mineral oil (controls) at the right buccal pouch. | 16.5 wk | Occasional tumors of the pouch and lip were found in Group 2 compared to Group 1 where all animals showed multiple tumors. | Formby et al. (1987 abstr.) |
| Syrian golden, age n.p. | 20 F/group | green coffee beans (Colombian) containing cafestol and kahweol, purities n.p. | Group 1: Purina Lab Chow<br><br>Group 2: 20% green coffee beans in chow<br><br>When animals adjusted to diet, 16 from each group were painted 3x/wk with 0.5% DMBA in mineral oil and 4 were painted with just mineral oil (controls) at the right buccal pouch. | 16.5 wk | Gross tumors were found in 75% (9/12) of the animals in Group 1 versus 22% (2/9) of the animals Group 2. The total number of tumors were 29 and 2, respectively. | Miller et al. (1988) |

## Table 1. Anticarcinogenicity Studies of Cafestol and Kahweol (Continued)

| Species, Strain, and Age | Number and Sex of Animals | Chemical Form and Purity | Dose | Exposure/ Observation Period | Results/Comments | Reference |
|---|---|---|---|---|---|---|
| Syrian golden, age n.p. | 20 F/group | 50:50 mixture of cafestol and kahweol, purities n.p. | Group 1: normal diet of 950 g modified 5008 Formulab Chow<br><br>Group 2: diet supplemented with 0.2 g/kg (0.02%) of mixture<br><br>Group 3: diet supplemented with 2.0 g/kg (0.2%) of mixture<br><br>When animals adjusted to diet, 16 from each group were painted 3x/wk with 0.5% DMBA in mineral oil and 4 were painted with just mineral oil (controls) at the left buccal pouch. | 13 wk | The total number of tumors in Groups 2 and 3 (59 and 61, respectively) was 30-40% lower than that of Group 1 (91). Group 3 inhibited 35% the development of DMBA-induced oral neoplasia.<br><br>All tumors were epidermoid carcinomas. | Miller et al. (1991) |
| Syrian golden, age n.p. | 20 F/group | 2.5% solution of cafestol and kahweol in DMSO, purities n.p. | Group 1: DMSO<br><br>Group 2: 2.5% solution<br><br>Groups given 0.5% DMBA in mineral oil 3x/wk at the left buccal pouch; 16 from each group treated with test chemicals on alternate days and 4 treated with test chemicals and mineral oil. | 15 wk | Tumors were common in animals receiving DMBA alone; however, animals receiving DMBA and the diterpenes exhibited a significant delay in tumor development. | McWhorter et al. (1988 abstr.) |

Abbreviations: bw = body weight; DMBA = 7,12-dimethylbenz[*a*]anthracene; DMSO = dimethyl sulfoxide; F = females; h = hour(s); n.p. = not provided; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; p.o. = peroral; wk = week(s)

## Table 2. Genotoxicity Study of Cafestol and Kahweol

| Test System or Species, Strain, and Age | Biological Endpoint | S9 Metabolic Activation | Chemical Form and Purity | Dose | Endpoint Response | Comments | Reference |
|---|---|---|---|---|---|---|---|
| *Salmonella typhimurium* strain TM677 | forward mutation to 8-azaguanine resistance | +/- | cafestol and kahweol and their monoacetates and palmitic acid esters (not specified if mono- or di-); purities n.p. | cafestol, cafestol acetate, and kahweol acetate: 0.31, 0.62, 1.25, 2.5, and 5.0 mg/mL<br>kahweol: 0.1-1.6 mg/mL<br>palmitic acid esters of cafestol and kahweol: 0.06-5.0 mg/mL | Inactive | For kahweol,<br>+S9: ~50% of bacteria survived at the highest concentration<br>-S9: bacterial survival varied dose-dependently (with a range from 0.015-1.0 mg/mL, survival was 91% and 3.2%, respectively). | Pezzuto et al. (1986) |

Abbreviations: n.p. = not provided; "+" = presence; "-" = absence

## 9.8 Antigenotoxicity

The details of these studies are presented in **Table 3**.

A 52.5:47.5 mixture of cafestol and kahweol (92, 460, 2300, and 6200 ppm in the diet of male Sprague-Dawley rats) inhibited the covalent binding of aflatoxin B1 metabolites to DNA in a dose-dependent manner in an *in vitro* assay using S9 and microsomal subcellular fractions from treated rat livers (Cavin et al., 1998). A statistically significant decrease was observed at 2300 ppm and a maximum inhibition of 50% was seen at the highest concentration in the presence of S9. The liver microsomal fractions gave the same results; however, 40% inhibition was observed at 6200 ppm.

In male F344/nctr rats, a 1:1 mixture of cafestol and kahweol (0.2% for 10 days in the diet) strongly and significantly inhibited 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-DNA adduct formation in the colon by 54% (Huber et al., 1997a).

## 9.9 Immunotoxicity

No immunotoxicity data were available.

## 9.10 Other Data

### 9.10.1 Activation of the Nuclear Receptor FXR

The potential carcinogenicity of cafestol is based on its ability to activate the nuclear receptor FXR (Weinberger, 1998). Weinberger et al. (unpublished results) have recently shown that compounds capable of inducing FXR, which have generally been observed to be those having anticarcinogenic activity, lower cholesterol levels. In contrast, compounds with carcinogenic activity counter FXR-dependent transcription and raise cholesterol levels. Cafestol, which raises cholesterol levels, is therefore a putative FXR antagonist and thus a potential candidate carcinogen.

## Table 3. Antigenotoxicity Studies of Cafestol and Kahweol

| Test System or Species, Strain, and Age | Biological Endpoint | S9 Metabolic Activation | Chemical Form and Purity | Dose/Duration | Endpoint Response/Comments | Reference |
|---|---|---|---|---|---|---|
| reaction mixture of calf thymus DNA, aflatoxin B1 ($AFB_1$), and rat liver S9 or microsomal fractions | inhibition of $AFB_1$-DNA binding | + | mixture of cafestol and kahweol palmitates (52.5:47.5), >95% pure | mixture (92, 460, 2300, and 6200 ppm) fed in diets of 5-wk-old male Sprague-Dawley rats) for 28 and 90 days | In both the liver S9 and microsomal fractions of rats treated for 28 days, dose-dependent inhibition of $AFB_1$-DNA adduct formation was observed. With 2300 ppm, a statistically significant decrease ($p<0.05$) in $AFB_1$ binding to DNA was seen in both test systems. With 6200 ppm, a maximum inhibition of 50% of the control value for DNA binding was achieved in liver S9 fractions, while a 40% inhibition was measured in liver microsomal fractions. | Cavin et al. (1998) |
| F344/nctr rats, age n.p. | *in vivo* PhIP-DNA adduct formation | NA | 1:1 mixture of cafestol and kahweol palmitates, purities n.p. | diet consisting of 0.2% mixture for 10 days, followed by a gavage of PhIP (50 mg/kg bw) and sacrifice 24 h later | Treatment with mixture strongly and significantly inhibited adduct formation in the colon by 54%. | Huber et al. (1997a) |

Abbreviations: bw = body weight; h = hour(s); PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; NA = not applicable; n.p. = not provided

### 9.10.2 Effects on Cholesterol, Triglycerides, Lipoprotein, and ALT Activity

#### 9.10.2.1 Human Studies

Details of the following studies are presented in **Table 4**.

In an open randomized study, healthy male and female volunteers who drank coffee containing 148 mg cafestol and kahweol daily for 30 days exhibited a considerable rise in total cholesterol (average mean, 31.6%), low density lipoprotein (LDL) cholesterol (50.2%), and triglyceride concentrations (87%) versus the control group (Heckers et al., 1994).

In three volunteers, consumption of highly purified cafestol (73 mg/day; 0.23 mmol/day) and kahweol (58 mg/day; 0.19 mmol/day) as the corresponding mono- and dipalmitates for 6 weeks increased the serum levels of cholesterol by 66 mg/dL (1.7 mmol/L) and triglycerides by 162 mg/dL (1.83 mmol/L) (Weusten-Van der Wouw et al., 1994).

In a randomized, crossover trial using healthy, normolipemic volunteers, six subjects received 2 g Arabica oil containing 72 mg (0.23 mmol) cafestol per day and 53 mg (0.17 mmol) kahweol per day, and five subjects received 2 g Robusta oil providing 40 mg (0.13 mmol) cafestol per day and 2 mg (0.006 mmol) kahweol per day (Mensink et al., 1995). Compared to a control group given placebo oil, serum triglyceride levels increased 71% in the group receiving Arabica oil and 61% in the group given Robusta oil. Serum cholesterol concentrations were increased by 13% for both oils.

In a randomized, double-blind parallel study, van Rooij et al. (1995) found that Arabica oil, containing 68 mg (0.21 mmol)/kg cafestol and 85 mg (0.27 mmol)/kg kahweol, raised serum total cholesterol by 44.1 mg/dL (1.14 mmol/L) and plasma triglycerides by 72 mg/dL (0.81 mmol/L) but that the effects of Robusta oil, providing 29 mg (0.092 mmol)/kg cafestol and 1 mg (0.003 mmol)/kg kahweol, were not statistically significant.

At a daily dose of 3 g coffee oil for 4 weeks, subjects showed increases of 49 mg/dL (1.3 mmol/L) in serum cholesterol, 73 mg/dL (0.82 mmol/L) in serum triglycerides, and 41 U/L in serum alanine aminotranferase (ALT) activity (upper limit of normal = 53.5 U/L) (Weusten-Van der Wouw et al., 1994). When a coffee oil fraction enriched in non-triglyceride lipids (0.75 g/day, providing a daily dose of 81 mg [0.26 mmol] cafestol and 98 mg kahweol [0.31 mmol]) was given, similar increases resulted. In contrast, 2 g/day of coffee oil stripped of cafestol and kahweol had no effect.

Consumption of coffee including fine particles suspended in the coffee (fines) containing cafestol and kahweol was shown to be associated with an increase in serum cholesterol and ALT activity in volunteers in a randomized controlled parallel study (Urgert et al., 1995a). In the test group—members of which ingested 8 g fines with a mean of 39 mg (0.12 mmol) cafestol and 49 mg (0.16 mmol) kahweol daily for 21 days—the serum cholesterol level increased by 25 mg/dL (0.65 mmol/L), the triglyceride concentration by 27 mg/dL (0.30 mmol/L), and ALT activity increased by 18 U/L (upper limit of normal = 53.5 U/L), compared to control values. Levels returned to baseline 14 weeks after the trial. In a separate study on particle size, coarse coffee grounds, providing a daily intake of 37 mg (0.12 mmol) cafestol and 54 mg (0.17 mmol) kahweol, and fine coffee grounds, providing 48 mg (0.15 mmol) cafestol and 56 mg (0.18 mmol) kahweol per day, both resulted in a mean serum cholesterol concentration of 189 mg/dL (4.9 mmol/L). Mean triglyceride levels and ALT activity in serum, however, were higher with the consumption of the latter.

In a study using unfiltered brewed coffee (cafetière) versus filtered coffee, Urgert et al. (1996b) found elevated levels of total cholesterol (specifically, LDL cholesterol), ALT activity, and triglycerides in individuals who had consumed 0.9 L cafetière coffee (38 mg [0.12 mmol] cafestol, 33 mg [0.10 mmol] kahweol) per day for 24 weeks; the filtered coffee provided <1 mg of the diterpenes. ALT activity was increased 80% above baseline values relative to filtered coffee. All increases, however, were reversible upon withdrawal of treatment.

The elevation of ALT suggests the liver is the target organ of cafestol and kahweol (Weuston-Van der Wouw et al., 1994; Urgert et al., 1996c). However, a study of the chronic intake of coffee (consumption of 5 or more cups of boiled or filtered coffee per day and persons aged 40-42 years) found no increased ALT activity (Urgert et al., 1996c).

The effects of kahweol on serum lipids and liver aminotransferases were studied by Urgert et al. (1997) through comparison of the effects of pure cafestol (60 mg; 0.19 mmol) with a mixture of cafestol and kahweol (60 mg plus 48-54 mg [0.15-0.17 mmol] kahweol) in a crossover trial. In ten male volunteers, consumption of pure cafestol increased total cholesterol by 17%, LDL cholesterol by 19%, and triglycerides by 86%. The mixture of cafestol and kahweol caused further increases of 2%, 4%, and 7%, respectively. Similar responses were obtained from both treatments on ALT activity.

## Table 4. Effects of Cafestol and Kahweol on Cholesterol, Triglyceride, Lipoprotein, and ALT Activity Levels—Human Studies

| Number, Sex, and Age | Chemical Form and Purity | Dose/Duration | Observation Period | Results/Comments | Reference |
|---|---|---|---|---|---|
| 5M and 6F, 24- to 31-yr-old | "pure" 60% cafestol and 40% kahweol in unsaponifiable lipid fraction (crystallized from alcohol forms) | 148 mg of diterpenes daily in 10 mL cream added to 150 mL of freshly prepared filtered coffee | 31 days | All treated subjects showed an increase in total cholesterol (mean of 31.6%), LDL cholesterol (50.2%), and triglyceride concentrations (135% at day 24 and 87% at day 31), whereas the control group (given one cup of the unsupplemented coffee with cream) showed no such trends. | Heckers et al. (1994) |
| 3M, average age of 49.3 yr | mixture of cafestol and kahweol palmitates, purity n.p. | 73 mg (0.23 mmol)/day cafestol and 58 mg (0.19 mmol)/day kahweol for 6 wk | 14 wk | Levels of cholesterol increased by 66 mg/dL (1.7 mmol/L) (71% associated with LDL), triglycerides by 162 mg/dL (1.83 mmol/L), and ALT activity by 31 U/L. | Weusten-Van der Wouw et al. (1994) |
| 11 subjects (6 given Arabica oil, 5 given Robusta oil), sex and ages n.p. | cafestol and kahweol in Arabica and Robusta oils, purities n.p. | Arabica oil: 72 mg (0.23 mmol)/day cafestol; 53 mg (0.17 mmol)/day kahweol<br><br>Robusta oil: 40 mg (0.13 mmol)/day cafestol; 2 mg (0.006 mmol)/day kahweol | 8 wk | Average serum cholesterol levels rose by 13% on both Arabica and Robusta oils. The triglyceride levels rose by 71% and 61%, respectively. | Mensink et al. (1995) |
| 18M, 18F, 19- to 64-yr-old | cafestol and kahweol in Arabica and Robusta oils, purities n.p. | Arabica: 68 mg (0.21 mmol)/kg cafestol, 85 mg (0.27 mmol)/kg kahweol<br><br>Robusta: 29 mg (0.092 mmol)/kg cafestol, 1 mg (0.003 mmol)/kg kahweol<br><br>oils administered as a 7.5 mL emulsion (1 g coffee oil) 2x/day for 6 wk | 3-4 mo | Arabica oil elevated serum total cholesterol by 44.1 mg/dL (1.14 mmol/L) and plasma triglycerides by 72 mg/dL (0.81 mmol/L). The effects from consumption of Robusta oil were not statistically significant. An average serum ALT activity of 17.9 U/L was observed for Arabica oil. | van Rooij et al. (1995) |

## Table 4. Effects of Cafestol and Kahweol on Cholesterol, Triglyceride, Lipoprotein, and ALT Activity Levels—Human Studies(Continued)

| Number, Sex, and Age | Chemical Form and Purity | Dose/Duration | Observation Period | Results/Comments | Reference |
|---|---|---|---|---|---|
| 16 subjects (sex n.p.), average age of 22.2 yr | coffee oil extruded from a blend of Arabic and Robusta beans (88:12 w/w), purities n.p. | 3 g/day: 85 mg (0.27 mmol)/day cafestol and 103 mg (0.328 mmol)/day kahweol for 4 wk | 10 wk | Levels of cholesterol increased by 49 mg/dL (1.3 mmol/L), triglycerides by 73 mg/dL (0.82 mmol/L), and ALT activity by 41 U/L. | Weusten-Van der Wouw et al. (1994) |
| 15 subjects (sex n.p.), average age of 22.2 yr | coffee oil enriched in non-triglyceride lipids (cafestol and kahweol), purities n.p. | 0.75 g/day: 81 mg (0.26 mmol)/day cafestol and 98 mg (0.31 mmol)/day kahweol for 4 wk | 10 wk | Levels of cholesterol increased by 48 mg/dL (1.2 mmol/L), triglycerides by 66 mg/dL (0.75 mmol/L), and ALT activity by 44 U/L. | Weusten-Van der Wouw et al. (1994) |
| 16 subjects (sex n.p.), average age of 21.8 yr | coffee oil stripped of cafestol and kahweol, purities n.p. | 2 g/day: 0 mg/day cafestol and 0.3 mg (1 µmol)/day kahweol for 4 wk | 9 wk | Cholesterol levels increased by 4 mg/dL (0.1 mmol/L), whereas ALT activity decreased by 4 U/L. There was no effect on triglyceride levels. | Weusten-Van der Wouw et al. (1994) |
| Study 1: 6M, 8F, mean age of 24 yr<br><br>Study 2: 9M, 6F, mean age of 26 yr | cafestol and kahweol in coffee grounds, purities n.p. | Study 1: 7.8 g coffee grounds (39 mg [0.12 mmol] cafestol, 49 mg [0.16 mmol] kahweol) per day for 21 days<br><br>Study 2: 7.1 g coarse grounds (37 mg [0.12 mmol] cafestol, 54 mg [0.17 mmol] kahweol) and 6.6 g fine grounds (48 mg [0.15 mmol] cafestol, 56 mg [0.18 mmol] kahweol) per day for 10 days | Study 1: 94 days<br><br>Study 2: 127 days | In Study 1, average serum cholesterol levels rose by 25 mg/dL (0.65 mmol/L) relative to the control group (fed hopjes-caramelvla without coffee grounds). Triglyceride level and ALT activity increased by 27 mg/dL (0.30 mmol/L) and 18 U/L, respectively, versus the control group.<br><br>In Study 2, serum cholesterol levels were very similar (189 mg/dL: 4.89 mmol/L for fine, 4.86 mmol/L for coarse). Higher triglyceride levels and ALT activity were observed with consumption of the fine versus coarse grounds (116 mg/dL vs. 90 mg/dL [1.31 vs. 1.01 mmol/L] and 29 vs. 21 U/L, respectively). | Urgert et al. (1995a) |
| cafestol: 12M, 12F, mean age of 29 yr<br><br>kahweol: 11M, 11F, mean age of 30 yr | cafestol and kahweol in filtered and cafetière coffees, purities n.p. | 0.9 L of filtered (<1 mg of cafestol and kahweol) or cafetière (38 mg [0.12 mmol] cafestol, 33 mg [0.10 mmol] kahweol) coffee per day for 24 wk | 9 mo | Cafetière coffee increased ALT activity and low-density lipoprotein cholesterol level by up to 80% and 9-14%, respectively, above baseline values relative to filtered coffee. Triglyceride concentrations were increased by 26% within 2 wk.<br><br>All increases were reversible on withdrawal of treatment. | Urgert et al. (1996b) |

## Table 4. Effects of Cafestol and Kahweol on Cholesterol, Triglyceride, Lipoprotein, and ALT Activity Levels—Human Studies(Continued)

| Number, Sex, and Age | Chemical Form and Purity | Dose/Duration | Observation Period | Results/Comments | Reference |
|---|---|---|---|---|---|
| Study 1: 88M, 71W, mean age of 41 yr<br><br>Study 2: 79M, 71F, mean age of 41 yr | cafestol and kahweol in filtered and boiled coffee, purities n.p. | consumption of 5 or more cups of coffee per day of filtered coffee (Study 1) or boiled coffee (Study 2) | NA; non-fasting blood obtained by venipuncture of qualified participants | Individuals consuming boiled coffee had higher levels of total cholesterol (231 vs. 219 mg/dL; 5.98 vs. 5.67 mmol/L) and triglycerides (190 mg/dL vs. 170 mg/dL; 2.14 vs. 1.92 mmol/L) compared to the filtered coffee group. No increase in ALT activity, however, was observed in the boiled-coffee group. | Urgert et al. (1996c) |
| 10M, mean age of 24 yr | cafestol and kahweol, 92.2-99.7% pure | 1st treatment period: half given 64 mg (0.20 mmol) cafestol and 1 mg (0.003 mmol) kahweol; other 5 given 60 mg (0.19 mmol) cafestol and 54 mg (0.17 mmol) kahweol for 28 days<br><br>2nd treatment period: switched; first half given 61 mg (0.19 mmol) cafestol and 0 mg kahweol; other 5 given 60 mg (0.19 mmol) cafestol and 48 mg (0.15 mmol) kahweol for 28 days | 183 days | Relative to baseline values, pure cholesterol increased the total cholesterol by 31 mg/dL (0.79 mmol/L, 17%), LDL cholesterol by 22 mg/dL (0.57 mmol/L, 19%), and fasting triacylglycerols by 58 mg/dL (0.65 mmol/L, 86%). Relative to cholesterol alone, the mixture caused increases of 8.9 mg/dL (0.23 mmol/L, 2%), 8.9 mg/dL (0.23 mmol/L, 4%), and 8.0 mg/dL (0.09 mmol/L, 7%), respectively. Kahweol had little additional effect.<br><br>Cafestol alone increased ALT activity by 18 U/L over baseline values. The mixture caused a further increase of 35 U/L. Kahweol had greater effect. | Urgert et al. (1997) |

Note: The upper limit of normal for ALT activity was 53.5 U/L for Urgert et al. (1995a, 1997), 54 U/L for Weusten-Van der Wouw et al. (1994), and 15 U/L for van Rooij et al. (1995).

Abbreviations: ALT = alanine aminotransferase; F = female(s); LDL = low density lipoprotein; M = male(s); mo = month(s); NA = not applicable; n.p. = not provided; U = units; wk = week(s); yr = year(s)

### 9.10.2.2 Animal Studies

Details of the following studies are presented in **Table 5**.

In adult Syrian hamsters fed a high-fat diet and given *ad libitum* coffee solution, plasma cholesterol and triacylglycerol concentrations were significantly increased compared to control animals receiving water (Sanders and Sandaradura, 1992). Studies, however, have failed to verify these results (Urgert and Katan, 1996).

When administered daily by gavage, coffee total lipids (CTL), coffee non-saponifiable matter (NSM), and coffee diterpene alcohols (DTA) extracted from *Coffea arabica* beans all increased the serum cholesterol levels in hamsters fed a diet low in saturated fat and cholesterol (Ratnayake et al., 1995). Given to animals fed a diet high in fat and cholesterol, no significant effects were observed. In both cases, the total serum cholesterol levels of the three coffee lipid extracts did not significantly differ from each other.

Mensink et al. (1992) found no effect on serum cholesterol levels in male hamsters fed freeze-dried boiled coffee in the diet for 35 days. A study with gerbils yielded the same results.

In hamsters and rats fed mash diets (cholesterol-free and high-cholesterol) containing unfiltered boiled coffee for 8 weeks, no statistically significant effect on serum total cholesterol and triacylglycerol concentrations occurred (Beynen et al., 1996).

Wistar rats given *ad libitum* instant or boiled coffee with a purified diet resulted in no elevated serum cholesterol levels (Høstmark et al., 1988).

In a crossover design in which a diet containing 0.5% coffee oil was fed to cebus and rhesus monkeys for at least 6 weeks, no effect on plasma cholesterol or triglyceride concentrations was observed (Terpstra et al., 1995). Furthermore, there was no increase in plasma ALT activity.

In experiments with rabbits, no responses were reported (study details not provided) (Weusten-Van der Wouw et al. 1993; cited by Urgert and Katan, 1996).

## Table 5. Effects of Cafestol and Kahweol on Cholesterol, Triglyceride, Lipoprotein, and ALT Activity Levels—Animal Studies

| Species, Strain, and Age | Number and Sex of Animals | Chemical Form and Purity | Dose | Exposure/ Observation Period | Results/Comments | Reference |
|---|---|---|---|---|---|---|
| Hamsters, Syrian, "adult" | 20M | cafestol and kahweol in coffee solution, purities n.p. | given *ad libitum* with high-fat diet | 28 days | Plasma cholesterol and triacylglycerol concentrations were significantly greater than in controls (given a water solution). The mean levels were as follows:<br>lipid fraction — coffee — control<br>cholesterol — 209 mg/dL (5.40 mmol/L) — 179 mg/dL (4.62 mmol/L)<br>LDL cholesterol — 79.2 mg/dL (2.05 mmol/L) — 64 mg/dL (1.66 mmol/L)<br>triacylglycerols — 186 mg/dL (2.10 mmol/L) — 138 mg/dL (1.56 mmol/L) | Sanders and Sandaradura (1992) |
| Hamsters, Syrian golden, "adult" | 11M/group | coffee total lipids (CTL, 11.4% diterpene esters), non-saponifiable matter (NSM, 88.8% free DTA), and diterpene alcohols (DTA, 48% cafestol and 50% kahweol), purities n.p. | Study 1: low-fat and -cholesterol diet; gavaged with 250 µL olive oil containing 5 mg CTL, 0.5 mg NSM, or 0.5 mg DTA<br>Study 2: high-fat and -cholesterol diet, gavaged with 250 µL coconut oil containing 20 mg CTL, 2 mg NSM, or 2 mg DTA | 21 days | In study 1, CTL and NSM were associated with higher levels of both total and HDL cholesterol versus controls. DTA was associated with higher serum levels of HDL cholesterol.<br>In study 2, CTL, NSM, and DTA were not associated with increased levels of serum lipids. | Ratnayake et al. (1995) |
| Hamsters, Syrian (*Mesocricetus auratus)*, 4- to 6-wk-old | 6M/group | cafestol and kahweol in boiled and filtered coffees, purities n.p. | 1 L of each coffee, yielding 17 g of freeze-dried material | 35 days | No effect on serum cholesterol levels was observed. | Mensink et al. (1992) |

## Table 5. Effects of Cafestol and Kahweol on Cholesterol, Triglyceride, Lipoprotein, and ALT Activity Levels—Animal Studies (Continued)

| Species, Strain, and Age | Number and Sex of Animals | Chemical Form and Purity | Dose | Exposure/ Observation Period | Results/Comments | Reference |
|---|---|---|---|---|---|---|
| Hamsters, Syrian (*Mesocricetus auratus*), 4-wk-old | 13M/group | cafestol and kahweol in boiled coffee, purities n.p. | mash diet containing filtered and unfiltered coffee; 44.1 mg/kg cafestol and 45.4 mg/kg kahweol in unfiltered coffee (cholesterol-free diet); 45.1 mg/kg cafestol and 43.2 mg/kg kahweol (high-cholesterol diet); cafestol and kahweol in filtered coffee not detected | 8 wk | No effect on serum cholesterol and triacylglycerol levels was seen. | Beynen et al. (1996) |
| Rats, Wistar (*Rattus norvegicus*), 3-wk-old | 12F/group | cafestol and kahweol in boiled coffee, purities n.p. | mash diet containing filtered or unfiltered coffee, cafestol and kahweol amounts n.p. | 8 wk | No effect on serum cholesterol and triacylglycerol levels was seen. | Beynen et al. (1996) |
| Rats, Wistar, age n.p. | 12M/group | cafestol and kahweol in instant and boiled coffee, purities n.p. | given *ad libitum* with purified diet (solutions had a caffeine concentration of 420 mg/L) | 4 wk | Rats given coffee had a lower concentration of whole plasma triacylglycerols than control group (given water). Concentration of unesterified and esterified cholesterol and of phospholipids was not significantly effected. | Høstmark et al. (1988) |

## Table 5. Effects of Cafestol and Kahweol on Cholesterol, Triglyceride, Lipoprotein, and ALT Activity Levels—Animal Studies (Continued)

| Species, Strain, and Age | Number and Sex of Animals | Chemical Form and Purity | Dose | Exposure/ Observation Period | Results/Comments | Reference |
|---|---|---|---|---|---|---|
| Gerbils, *Meriones unguiculatus*, 4- to 6-wk-old | 20M/group | cafestol and kahweol in boiled and filtered coffees, purities n.p. | 1 L of each coffee, yielding 17 g of freeze-dried material | 35 days | No effect on serum cholesterol levels was observed. | Mensink et al. (1992) |
| Cebus and Rhesus monkeys, ages n.p. | Cebus: 5M, 11F (2M, 6F in one group; 3M, 5F in a second group)<br>Rhesus: 6M (3/group) | cafestol and kahweol in 0.5% coffee oil, purities n.p. | Cebus: 5.13 mg (0.0162 mmol) cafestol and 6.21 mg (0.0196 mmol) kahweol in the feed per kg bw<br>Rhesus: 5.70 mg (0.0180 mmol) cafestol and 6.90 mg (0.0219 mmol) kahweol in the feed per kg bw | Cebus: 2 x 7.5 wk (crossover design)<br>Rhesus: 2 x 6 wk (crossover design) | No effect on plasma cholesterol or triglyceride concentrations was observed. Furthermore, there was no effect on ALT activity. | Terpstra et al. (1995) |

Abbreviations: bw = body weight; F = female(s); M = male(s); n.p. = not provided; wk = week(s)

### 9.10.3 Effects on the Regulation of Lipid Metabolism (*In Vitro* Models)

Details of the following studies are presented in **Table 6**.

In human skin fibroblasts, cafestol (20 µg/mL; 63 µM) significantly reduced the binding, uptake, and degradation of LDL as well as the synthesis of cholesterol (Halvorsen et al., 1998). An increase, however, occurred in cholesterol esterification. Specifically, the uptake of [$^{125}$I]tyramine cellobiose-labeled LDL ($^{125}$I-TC-LDL) decreased by 50% after 18-hour preincubation with cafestol, compared to controls. Specific binding of radiolabeled LDL was reduced by 54%, while no effect on binding was observed with cafestol during the study. Incorporation of radiolabeled [$^{14}$C]oleic acid into cholesteryl esters was 2.3-fold higher after 24-hour incubation. In contrast, incorporation of [$^{14}$C]acetate into cholesterol was decreased by almost 40%.

In human hepatoma (HepG2) cells, cafestol (20 µg/mL; 63 µM) decreased the uptake of labeled LDL by 15 to 20% and the degradation of LDL by 20 to 30% after 18 hours of preincubation, with maximum effects achieved after 6 and 10 hours, respectively (Rustan et al., 1997). Specific binding of LDL was reduced by 17% after preincubation with the diterpene for 6 hours. Cafestol and a mixture of cafestol and kahweol had no effects on cholesterol synthesis and esterification.

In human intestinal cells, CaCo-2 cells, cafestol increased the rate of uptake and degradation of LDL (Ranheim et al., 1995). At 20 µg/mL (63 µM), it promoted an increase in the rate of uptake and degradation of LDL (50%) and decreased rates of secretion of the cholesteryl ester as well as triacylglycerol. An equimolar mixture of cafestol and kahweol produced a modest increase in the uptake and degradation of cholesterol for concentrations above 5 µg/mL (16 µM). The maximum increase was achieved with 30 µg/mL (95 µM) for both treatments. Specific binding of radiolabeled LDL was increased by 21% after preincubation with cafestol, compared to control cells. In other experiments, Ranheim et al. (1995) found elevated rates of the uptake of LDL by approximately 20% in P19 cells (murine embryonic carcinoma cell line), 3T3 cells (murine fibroblast cell line), and J774 cells (murine macrophage-like cell line) (study details not provided).

## Table 6. Effects of Cafestol and Kahweol on the Regulation of Lipid Metabolism

| Test System or Species, Strain, and Age | Chemical Form and Purity | Dose/Duration | Comments | Reference |
|---|---|---|---|---|
| human skin fibroblasts | cafestol, 99% pure; mixture of cafestol and kahweol (48:47:5 isokahweol), 98% pure | cafestol: 0.5-50 µg/mL (1.6-160 µM) for 18-h preincubation<br>mixture: 1-50 µg/mL for up to 24-h preincubation.<br>Thereafter, cells were incubated up to 24 h. | Effect of cafestol on cell-association of $^{125}$I-TC-LDL: after 6 h, a 9% and 18% reduction with 10 and 20 µg/mL (32 and 63 µM), respectively, was observed. After 18 h, a concentration-dependent decrease resulted (17% to 66% reduction with increasing amounts of 10 to 50 µg/mL [32-160 µM]). The mixture produced similar results but was significantly more potent in inhibiting LDL uptake at 10, 30, and 50 µg/mL.<br>Specific binding of LDL was reduced by 54%; no effect on the binding of radiolabeled LDL during the binding experiment seen.<br>Incorporation of [$^{14}$C]acetate into cholesterol was reduced by 34-38% after 12-24 h of incubation.<br>Incorporation of [$^{14}$C]oleic acid into cholesteryl ester increased 2.3-fold after 24-h incubation. | Halvorsen et al. (1998) |
| human hepatoma cells (HepG2) | cafestol and a mixture of cafestol and kahweol, purities n.p. | 20 µg/mL (63 µM) for 18-h preincubation | Cafestol decreased the uptake of $^{125}$I-TC-LDL by 15-20% and the degradation by 20 to 30% after preincubation, with maximum effects after 6 and 10 h, respectively. Cafestol effects were dose-dependent.<br>Specific binding of LDL was reduced by 17% after 6-h preincubation.<br>Cafestol and the mixture had no effects on cholesterol esterification and cholesterol synthesis. | Rustan et al. (1997) |
| human intestinal (CaCo-2) cells cultured on filter membranes | cafestol, >99% pure; mixture of cafestol and kahweol (48:47:5 isokahweol), purity n.p. | cafestol: 2-50 µg/mL (6.3-160 µM) for 18-h preincubation; 50 µg/mL (160 µM) for 24-h incubation<br>mixture: 5-50 µg/mL 18-h preincubation | Cafestol significantly increased the amount of $^{125}$I-TC-LDL by approximately 25 and 50% after incubations for 12 and 24 h, respectively, versus cells incubated with control medium. The mixture promoted a modest increase in the rate of uptake and degradation for concentrations above 5 µg/mL, compared to the increases induced by cafestol.<br>Specific binding of radiolabeled LDL was increased by 21%.<br>Cholesterol synthesis was increased, and cholesterol esterification was slightly reduced. | Ranheim et al., (1995) |

Abbreviations: h = hour(s); LDL = low density lipoprotein; n.p. = not provided; $^{125}$I-TC-LDL = [$^{125}$I]tyramine cellobiose-labeled LDL

### 9.10.4 Induction of Glutathione Transferase

The anticarcinogenic property of cafestol and kahweol has been postulated to be related to their ability to induce glutathione *S*-transferase (GST) (Schilter et al., 1996). The details of the following studies are presented in **Table 7**.

#### 9.10.4.1 Mice

In female ICR/Ha mice administered 0.01 mmol of cafestol acetate daily for 3 days prior to sacrifice, GST activity was induced in the liver and intestinal mucosa (Pezzuto et al., 1986).

In female ICR/Ha mice, the addition of 20% green coffee beans to the diet was found to increase GST activity in both tissues, with that of the liver 5 times that of controls (Lam et al., 1982). Furthermore, the overall magnitude of enzyme activity in the liver was greater than that in the mucosa of the small bowel. The active ingredients of green coffee beans were found to be almost totally extracted into petroleum ether (PE) when the process was carried out for at least 7 days. This PE extract was found to induce increased GST activity, approximately 40% higher than that of the green coffee beans. Cafestol palmitate (daily administration of 2.5 mg/mouse for 4 days) resulted in a 41% increase in GST activity of the small bowel mucosa versus control. When the dose was increased to 5.0 mg/mouse, GST activity of the small bowel mucosa increased 3.9 times that of controls. With kahweol palmitate, the activity was 5.6 times greater.

In male NMRI mice, 2 mg/g of cafestol palmitate in the diet for 4 days significantly increased GST activity of the cytosolic fraction of the liver; liver weight was increased by approximately 30% (Di Simplicio et al., 1989).

#### 9.10.4.2 Rats

In female Sprague-Dawley rats, 10% coffee beans in the diet for 10 days increased the GST activity of the small intestine 2.1-fold (ratio of test to control) and that of the liver 1.7-fold (Wattenberg and Lam, 1984). Oral intubation of cafestol and kahweol palmitates (45 mg in 1 mL cottonseed oil daily for 4 days) also raised enzyme activity; kahweol palmitate was the more active inducer. In the liver, this was 2.7-fold with kahweol palmitate and 1.9-fold with cafestol palmitate. In the small intestine, the increase was 4.9- and 2.0-fold, respectively.

In male Sprague-Dawley rats, kahweol ester, given by gavage, induced increased GST activity of the liver, forestomach, and small bowel mucosa that was significantly higher than that of the controls in all tissues (study details not provided) (Lam et al., 1989 abstr.).

When fed a mixture of cafestol and kahweol (92, 460, 2300, and 6200 ppm), a dramatic dose-dependent increase in GST Yc2 expression in the livers of the animals was found and clearly detected after 28 days of treatment (Cavin et al., 1998). The effect was also observed in livers obtained from rats after 90 days of treatment. Furthermore, a dose-dependent decrease in the expression of P450s (CYP2C11 and CYP3A2) occurred.

In another rat study, a 1:1 mixture of cafestol and kahweol (doses not given) given prior to PhIP dosing significantly increased hepatic GSTs 2- to 3-fold, particularly the α-class GST 1b isoform, with an increase of 7-fold, but had no effect on colon GST (Huber et al., 1996 abstr., 1997b abstr.). In addition, pretreatment with the mixture prevented GSH depletion (levels enhanced 2.5-fold). In a later experiment conducted by Huber et al. (1998 abstr.), 0.2% of a mixture of cafestol and kahweol in the feed of male F344 rats for 10 days yielded the same results. Further stimulation of the system caused a 2-fold increase in the content of GSH and a 14-fold enhancement of the activity of the π-class GST isoform, which is normally weak. A 0.1% mixture in the feed of the rats gave weaker results, and a 0.02% mixture gave marginal results.

In addition to a dose-dependent increase in liver GST general activity, a dose-dependent induction of the placental form of GST in liver was observed in male and female Sprague-Dawley rats fed a mixture of cafestol and kahweol (92, 460, 2300, and 6200 ppm) for 28 or 90 days, with maximum effect attained within 5 days (Schilter et al., 1996).

Cafestol and kahweol (10 µM and 100 µM) had little effect on the activity of P450 1A2 from rat liver microsomes (Hammons et al., 1996 abstr.).

## 10.0 STRUCTURE-ACTIVITY RELATIONSHIPS

Studies with synthetic derivatives of cafestol and kahweol indicate that the furan moiety is the active site for induction of enzyme activity. Many natural products with this moiety, e.g., citrus limonoids, induce the detoxifying enzyme system GST in animal tissue. However, the induction potency of compounds varies widely; studies with citrus limonoids suggest that other limonoid ring moieties influence the potency of enzyme induction (Lam et al., 1989b).

## Table 7. Effects of Cafestol and Kahweol on Glutathione *S*-Transferase (GST) Activity

| Species, Strain, and Age | Number and Sex | Chemical Form and Purity | Dose/Duration | Results/Comments | Reference |
|---|---|---|---|---|---|
| **Mice** | | | | | |
| ICR/Ha, 7- to 8-wk old | 4F/group | cafestol acetate, purity n.p. | 0.01 mmol administered daily for 3 days prior to sacrifice | GST activity induced by cafestol acetate in the liver and intestinal mucosa. | Pezzuto et al. (1986) |
| ICR/Ha, 7-wk-old | F, number n.p. | cafestol and kahweol palmitate extracted from green coffee beans (Guatemala) in petroleum ether (PE), purities n.p. | initial stages of fractionation: extract equivalent to 20% powdered green coffee beans in diet for 12 days, at which time mice were killed<br>later stages of fractionation: 2.5 mg of test compounds in cottonseed oil; p.o. intubation, 0.2 mL/mouse; animals killed 28 h later | Increased GST activity induced by PE extract (~40% higher than that of green coffee beans). | Lam et al. (1982) |
| ICR/H, 7-wk-old | number and sex n.p. | cafestol palmitate, purities n.p. | single p.o. administration of 2.5 mg/mouse vs. daily administration of dose for 4 days; mice killed 24 h after last dose | With a single administration, GST activity was marginal. By increasing the dose, GST activity of small bowel mucosa increased by 41% versus controls. | Lam et al. (1982) |
| ICR/Ha, 7-wk-old | number and sex n.p. | cafestol and kahweol palmitates, purities n.p. | daily administration of 5.0 mg/mouse for 4 days | GST activity of small bowel mucosa increased to 3.9 times that of controls with cafestol palmitate. GST activity of mice receiving kahweol palmitate was 5.6 times that of controls. | Lam et al. (1982) |
| NMRI, age n.p. | 6M | cafestol palmitate, purity n.p. | 2 mg/g in diet for 4 days | GST activity of the cytosolic fraction of the liver increased significantly. Liver weight increased by ~30%. | Di Simplicio et al. (1989) |

## Table 7. Effects of Cafestol and Kahweol on Glutathione *S*-Transferase (GST) Activity (Continued)

| Species, Strain, and Age | Number and Sex | Chemical Form and Purity | Dose/Duration | Results/Comments | Reference |
|---|---|---|---|---|---|
| **Rats** | | | | | |
| Sprague-Dawley, 42-days-old | 3F/group | cafestol and kahweol in coffee beans, purities n.p. | 10% coffee beans in diet for 10 days | GST activity of liver was increased 1.7-fold (ratio of test to control) and of small intestine 2.1-fold. | Wattenberg and Lam (1984) |
| Sprague-Dawley, 46-days-old | 3F/group | cafestol and kahweol palmitates, purities n.p. | oral intubation; 45 mg in 1 mL cottonseed oil daily for 4 days; experiment terminated 1 day after last dose | GST activity of liver increased 2.7-fold (ratio of test to control) and of small intestine 4.9-fold with kahweol palmitate. Activity was increased 1.9- and 2.0-fold, respectively, with cafestol palmitate. Kahweol palmitate is a more active inducer than the latter. | Wattenberg and Lam (1984) |
| Sprague-Dawley, 5-wk-old | at least 4M/group | mixture of cafestol and kahweol palmitates (52.5:47.5), >95% pure | 92, 460, 2300, and 6200 ppm in diet for various days (5-90 days of treatment) | A significant dose-dependent increase in GST Yc2 expression in the livers observed and clearly detected after 28 days of treatment: a 5-fold induction with 460 ppm, an 11-fold induction with 2300 ppm, and a 16-fold induction with 6200 ppm.<br>CYP2C11 protein expression level decreased dose-dependently and significantly by 35% at 2300 ppm and 88% at 6200 ppm, compared with controls. CYP3A2 protein expression was decreased by 40 and 60%, respectively. | Cavin et al. (1998) |
| Strain and age n.p. | number and sex n.p. | 1:1 mixture of cafestol and kahweol, purities n.p. | pretreatment with mixture, followed by PhIP dosing (amounts n.p.); adduct formation measured 24 h later | Mixture significantly increased hepatic GSTs by 2- to 3-fold and the concentration of α-class GST 1b isoform by 7-fold, but had no effect on colon GST. Pretreatment with mixture prevented GSH depletion (enhancement of 2.5-fold). | Huber et al. (1996 abstr. and 1997b abstr.) |
| F344 | M, number n.p. | 1:1 mixture of cafestol and kahweol, purities n.p. | 0.2% mixture in feed for 10 days; additional tests done with 0.1% and 0.02% mixture | Mixture enhanced overall GST activity by 3-fold and increased the α-class isoform GST 1b by 7-fold. Further stimulation of the hepatic GST system resulted in a 2-fold increase in the content of GSH and a 14-fold enhancement of the π-class GST isoform. Results were statistically significant within 5 days.<br>With 0.1% mixture in feed, effects were weaker. With 0.02% mixture, they were marginal. | Huber et al. (1998 abstr.) |

**Table 7. Effects of Cafestol and Kahweol on Glutathione *S*-Transferase (GST) Activity (Continued)**

| Species, Strain, and Age | Number and Sex | Chemical Form and Purity | Dose/Duration | Results/Comments | Reference |
|---|---|---|---|---|---|
| Sprague-Dawley, 21-days-old | varied from 5M, 5F to 10M, 10F to 15M, 15F per dose | mixture of cafestol and kahweol palmitates (52.5:47.5), >95% pure | 92, 460, 2300, and 6200 ppm for 28 or 90 days | In both M and F rats, treatment for 28 days produced a dose-dependent increase in liver GST general activity and GST-P activity (significant at the higher doses). Treatment for 90 days showed similar results.<br>Induction by mixture was reversible within a month. | Schilter et al. (1996) |
| Strain and age n.p. | number and sex n.p. | 1:1 mixture of cafestol and kahweol, purities n.p. | 10 µM and 100 µM | Mixture had little effect on induction of P450 1A2 activity on liver microsomes. | Hammons et al. (1996 abstr.) |

Abbreviations: F = female(s); GSH = glutathione; GST = glutathione *S*-transferase; GST-P = placental GST; M = male(s); NA = not applicable; n.p. = not provided; PE = petroleum ether; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; p.o. = peroral, per os; wk = week(s)

# 11.0 ONLINE DATABASES AND SECONDARY REFERENCES

## 11.1 Online Databases

Chemical Information System Files

SANSS (Structure and Nomenclature Search System)
TSCATS (Toxic Substances Control Act Test Submissions)

National Library of Medicine Databases

EMIC and EMICBACK (Environmental Mutagen Information Center)

STN International Files

| | |
|---|---|
| BIOSIS | HSDB |
| CANCERLIT | MEDLINE |
| CAPLUS | Registry |
| CHEMLIST | RTECS |
| EMBASE | TOXLINE |

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 |
| NIOSHTIC® | 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

Phytochemical Database (Agricultural Research Service)
Patent Full Text and Image Database (U.S. Patent & Trademark Office)

In-House Databases

CPI Electronic Publishing Federal Databases on CD
Current Contents on Diskette®
The Merck Index, 1996, on CD-ROM

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## APPENDIX A: UNITS AND ABBREVIATIONS

°C = degrees Celsius

µg/L = microgram(s) per liter

µg/mL = microgram(s) per milliliter

µM = micromolar

ALT = alanine aminotransferase

bw = body weight

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

DMSO = dimethyl sulfoxide

F = female(s)

g = gram(s)

g/mL = gram(s) per milliliter

GC = gas chromatography

GC-ITDMS = gas chromatography-ion trap mass spectrometry

GSH = glutathione

GST = glutathione *S*-transferase

GST-P = placental GST

h = hour(s)

HPLC = high-performance liquid chromatography

$^{125}$I-TC-LDL = [$^{125}$I]tyramine cellobiose-labeled LDL

i.p. = intraperitoneal(ly)

kg = kilogram(s)

LC = liquid chromatography

$LC_{50}$ = lethal concentration for 50% of test animals

$LD_{50}$ = lethal dose for 50% of test animals

LDL = low density lipoprotein

M = male(s)

mg/kg = milligram(s) per kilogram

mg/mL = milligram(s) per milliliter

mL/kg = milliliter(s) per kilogram

mM = millimolar

mmol = millimole(s)

mmol/kg = millimole(s) per kilogram

mo = month(s)

mol. wt. = molecular weight

NA = not applicable

n.p. = not provided

PE = petroleum ether

PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine

p.o. = peroral(ly), per os

ppm = part(s) per million

TLC = thin-layer chromatography

wk = week(s)

yr = year(s)