SUMMARY OF DATA FOR CHEMICAL SELECTION

5,6-Benzoflavone
6051-87-2

BASIS OF NOMINATION TO THE CSWG

5,6-Benzoflavone is a potent inducer of P4501A enzymes in the family of microsomal mixed function oxidases. This feature accounts for its present use as an enzyme inducer in assays for mutagenic activity. Because of data suggesting that 5,6-benzoflavone may prevent induction of mammary tumors in laboratory animals, this substance is currently under review at the National Cancer Institute (NCI) as a possible chemopreventive agent. Should 5,6-benzoflavone be developed as a chemopreventive agent, its use would increase dramatically. However, the record on P4501A enzyme inducers is not good; none are currently in use as therapeutic agents because of toxicity. Thus, it is important that a toxicity profile for 5,6-benzoflavone be developed.

INPUT FROM GOVERNMENT AGENCIES/INDUSTRY

The Division of Cancer Prevention (DCP) at NCI requested the nomination of 5,6-benzoflavone for carcinogenicity testing to the National Toxicology Program (NTP).

SELECTION STATUS

ACTION BY CSWG: 12/14/98
- Toxicological evaluation
- Carcinogenicity

Priority: High

Rationale/Remarks:
- Potential for substantial increase in human exposure
- Nominated by NCI's Division of Cancer Prevention (DCP)
- Being evaluated by DCP as a possible chemopreventive agent
- DCP is conducting efficacy studies

CHEMICAL IDENTIFICATION

CAS Registry No.:	6051-87-2
Chemical Abstract Service Name:	1H-Naphtho(2,1-b)pyran-1-one, 3-phenyl- (8CI) (9CI)
Synonyms and Trade Names:	5,6-Benzoflavone, -naphthoflavone, -NF, 3-phenyl-1H-naptho(2,1-b)pyran-1-one
Structural Class:	Flavone, polycyclic heterocyclic

Structure, Molecular Formula, and Molecular Weight:
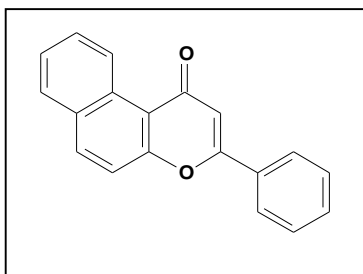
$C_{19}H_{12}O_2$ Mol. wt.: 272.28

Chemical and Physical Properties:

Description:	Fluffy slightly beige crystalline powder (Acros Organics, 1'
Melting Point:	164-166 °C (Acros Organics, 1998a)
Solubility:	Very soluble in benzene; soluble in ether or concentrated sulfuric acid; slightly soluble in alcohol (STN, 1998)

Technical Products and Impurities: 5,6-benzoflavone is available at 99+ percent purity from Acros Organics N.V., a subsidiary of Fisher Scientific (Acros Organics, 1998b) and at 90-95 percent purity from Aldrich (1996).

EXPOSURE INFORMATION

Production and Producers: According to recent chemical catalogs and directories, 5,6-benzoflavone is manufactured or distributed by Aldrich Chemical Company, Inc., and their affiliates, Sigma and Fluka Chemical Corp. 5,6-Benzoflavone is also available from Chem Service, Inc., Fischer Scientific, and Lancaster Synthesis, Inc. (STN, 1998).

Unlike most flavones, 5,6-benzoflavone is a synthetic chemical. No information on production methods was found in the available literature.

Use Pattern: 5,6-Benzoflavone is an inducer of cytochrome P4501A1 and P4501A2, two enzymes in a super-gene family of hemoproteins of the microsomal mixed-function oxidases. This feature accounts for the use of 5,6-benzoflavone to induce microsomal enzymes prepared for mutagenicity assays (Moorthy *et al*., 1995).

An increasing number of laboratories are using a combination of 5,6-benzoflavone and phenobarbital in mutagenicity assays because Aroclor 1254, the traditional P4501A inducer, has been reported to be somewhat toxic and possibly carcinogenic (Ong *et al*., 1980; Paolini *et al*., 1994). The extensive database on this use of 5,6-benzoflavone can be explored further in the scientific literature, including NCI's *Chemical Carcinogensis Research Information System* (CCRIS).

Human Exposure: Presently, exposure to 5,6-benzoflavone is limited to laboratory workers, especially those in genetics labs. No listing was found for 5,6-benzoflavone in the National Occupational Exposure Survey (NOES), which was conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983. 5,6-Benzoflavone is not listed in EPA's Toxic Substances Control Act (TSCA) Inventory. Should 5,6-benzoflavone be developed as a chemopreventive agent, human exposure to this substance would increase dramatically.

Environmental Occurrence: No information on the environmental occurrence of 5,6-benzoflavone was identified in the available literature.

Regulatory Status: No standards or guidelines have been set by NIOSH or OSHA for occupational exposure to or workplace allowable levels of 5,6-benzoflavone. 5,6-Benzoflavone was not on the American Conference of Governmental Industrial Hygienists (ACGIH) list of compounds for which recommendations for a threshold limit value (TLV) or biological exposure index (BEI) are made.

EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Phase 1 Enzyme Induction and the Ah Receptor: Because 5,6-benzoflavone exerts its effects through the Ah receptor, a brief discussion of the enzyme induction associated with the Ah receptor provides valuable background information to fill data gaps for this chemical.

By the early 1960s, the observation had been made that exposure to certain drugs and chemical carcinogens often led to an increased capacity to metabolize a wide variety of xenobiotics. Classic examples of this enzyme induction phenomenon involved administration of the planar aromatic hydrocarbons, benzo[a]pyrene (B[a]P) and 3-methylcholanthrene (3-MC). An important initial finding was that the induction of the metabolizing enzymes was the result of the synthesis of new protein and not due to the allosteric activation of existing enzymes (Swanson & Bradfield, 1993).

B[a]P, 3-MC, and related compounds induce CYP1A1 which encodes cytochrome P4501A1 monooxygenases such as aryl hydrocarbon hydroxylase (AHH). Mammalian P4501A1 catalyzes the oxygenation of polycyclic hydrocarbons to phenolic products and epoxides, some of which are toxic, mutagenic, and carcinogenic. The second, closely related mammalian enzyme, P4501A2, metabolizes certain arylamines. These enzymes were found to be highly inducible in C57BL/6 mice, but not in DBA/2 mice. Inducibility of AHH activity by polyaromatic hydrocarbons (PAHs) in mice appears to be controlled by a single gene locus known as the Ah locus. AHH inducibility is inherited as a simple autosomal dominant trait (Nebert *et al*., 1993; Rowlands & Gustafsson, 1997).

The early rodent experiments involving B[a]P and 3-MC demonstrated that the induced metabolism was an adaptive response in that the upregulated enzymes were able to oxidize the same PAH-inducing agents upon short-term re-exposure. Describing the adaptive response as a detoxification system is problematic, however, because Ah-receptor (AhR)-induced enzymes can actually contribute to the generation of electrophiles that alkylate cellular macromolecules, leading to altered cellular function and genotoxicity (Schmidt & Bradfield, 1996).

The AhR has been identified in the tissues from several mammalian and non-mammalian species, including humans. Several studies have shown that CYP1A1, found on mouse chromosome 9, is under the regulation of the AhR. Activation of the CYP1A1 gene leads to increased P4501A1 mRNA levels, followed by an increase in P450-1A1-related enzyme activities. In Ah-nonresponsive mice, the Ah locus encodes a receptor with a lower affinity for PAHs, resulting in decreased P4501A1 induction. Analysis of the amino acid sequence of AhR has revealed that it belongs to a family within the helix-loop-helix superfamily of proteins, whose other members include the AhR nuclear translocator protein or ARNT, and the *Drosophila* proteins SIM and PER (Rowlands & Gustafsson, 1997).

The work of many laboratories has combined to produce a model for AhR-ARNT signaling. This model holds that the unliganded AhR exists in the cytosol complexed with a dimer of heat shock protein 90 (Hsp90), which maintains the AhR in a ligand-binding conformation and prevents nuclear translocation and/or dimerization with ARNT. The hydrophobic AhR ligands enter the cell by diffusion and are bound by the Hsp90-associated AhR. Ligand binding causes a conformational change resulting in a receptor species with an increased affinity for DNA and a much slower rate of ligand dissociation. This event is associated with nuclear translocation and an exchange of Hsp90 for ARNT. Ultimately, recognition of dioxin-responsive enhancer element sequences by the AhR-ARNT complex results in the transactivation of target genes (Schmidt & Bradfield, 1996).

Human Data: No epidemiological studies or case reports investigating the association of exposure to 5,6-benzoflavone and cancer risk in humans were identified in the available literature.

Investigation of the clinical effects of P4501A induction has been limited because no known potent P4501A inducer is in therapeutic use. The few clinically useful drugs with known P4501A inducing potential in animals have produced a profile of pronounced toxic side-effects in humans at therapeutic doses (McKillop & Case, 1991).

Animal Data: *Acute toxicity.* No information on the LD_{50} or LC_{50} of 5,6-benzoflavone wasfound in the available literature (Acros Organics, 1998a; NLM, 1998).

General toxicity. A modest atrophy of the thymus was observed in mice that received four daily doses of 5,6-benzoflavone at 80 mg/kg. Pronounced thymic involution is one of the characteristic toxic responses to potent P4501A inducing PAHs (Poland & Glover, 1980; McKillop & Case, 1991).

Carcinogenicity. No 2-year carcinogenicity studies of 5,6-benzoflavone in animals were identified in the available literature.

Some long-term studies designed to examine the effects of enzyme induction on the carcinogenicity of PAHs or synthetic steroids have contained control groups exposed to 5,6-benzoflavone. In two studies involving the exposure of various strains of mice to B[a]P or 3-MC, 5,6-benzoflavone was administered alone at a dose level of 150 mg/kg, intraperitoneally (ip), once weekly for 12-14 weeks. The mice were examined for tumors between 6 and 18 months after the first dose. In one study, 5,6-benzoflavone had no tumorigenic effect when compared to vehicle control or untreated mice. In the other study, forestomach papillomas were observed at a pronounced level (over 50% of all animals) in four of the six strains examined in the combined control groups, each consisting of five vehicle control mice and ten 5,6-benzoflavone control mice. The forestomach effects were unrelated to whether the strains of mice were genotypically responsive to the induction of P4501A1 by PAHs (Anderson *et al.*, 1983; Anderson & Seetharam, 1985; McKillop & Case, 1991).

Short-Term Tests: Reviews of the literature searched from 1966 through 1998 produced very little information on the potential genotoxicity of 5,6-benzoflavone. According to Brown and Deitrich (1979), 5,6-benzoflavone over a wide range of concentrations did not produce mutagenicity in *S. typhimurium* TA98, TA100, TA1535, TA1537, or TA1538 with or without Aroclor 1254-induced S-9. 5,6-Benzoflavone was described as having no intrinsic mutagenicity when tested at a 50 & M concentration vs *S. typhimurium* TA98 (Buening *et al.*, 1978).

<u>Metabolism</u>: No information on the metabolism of 5,6-benzoflavone was identified in the available literature.

<u>Other Biological Effects</u>: *Modulation of the Carcinogenicity of Other Chemicals.* Induction of drug-metabolizing enzymes is neither totally beneficial nor totally detrimental (McKillop & Case, 1991).

Several studies have demonstrated protective effects of 5,6-benzoflavone on the carcinogenicity of various chemicals (McKillop & Case, 1991).

- 5,6-Benzoflavone administered to mice resulted in almost total inhibition of B[a]P- induced pulmonary adenoma formation (Wattenberg & Leong, 1970).

- Co-administration of 5,6-benzoflavone to several responsive strains of mice markedly decreased the tumorigenic effect of 3-MC in various tissues (Anderson & Seetharam, 1985).

- 5,6-Benzoflavone inhibited mammary gland tumorigenesis induced by 7,12- dimethylbenz[a]anthracene (DMBA) in female Sprague Dawley rats (Malejka-Giganti *et al.*, 1996).

- 5,6-Benzoflavone inhibited formation of various tumors or preneoplastic lesions induced by aflatoxin B_1 (AFB) in experimental animals (Stresser *et al.*, 1994; CCRIS, 1998).

In an ongoing study funded by the NCI, female Sprague-Dawley rats, 25 per group, were administered 5,6-benzoflavone in the diet at 165 or 1,650 mg/kg bw/day from 53 to 165 days of age. These rats and a positive control group received a single intravenous (iv) injection of methylnitrosourea (MNU) (50 mg/kg bw). High dose rats showed a 2 percent body weight depression at 165 days of age when they were killed, while low-dose rats showed a 7 percent body weight depression. The low dose of 5,6-benzoflavone was more effective in preventing mammary tumors than the high dose (58% vs 35% reduction), in part, perhaps due to the decreased body weight gain (Grubbs, 1998).

5,6-Benzoflavone also affects carcinogenesis in fish. Dietary 5,6-benzoflavone at 500 ppm provided sustained induction of hepatic CYP1A in rainbow trout. Cofeeding 5,6-benzoflavone with AFB for 1-6 weeks inhibited hepatic AFB-DNA adduct formation. 5,6-Benzoflavone fed only once or twice a week still significantly reduced AFB-DNA adduction. Chronic exposure to 5,6-benzoflavone at 500 ppm inhibited tumor response at week 46 (AFB controls, 66.3%; benzoflavone-treated 15.8%). The authors noted, however, that reduced DNA adduct formation at weeks 1-6 correlated poorly with subsequent tumor response (Harttig *et al.*, 1996).

Other studies have indicated an increased incidence of cancer in fish administered 5,6-benzoflavone and a pro-carcinogen. Trout given 500 ppm of 5,6-benzoflavone in the diet six weeks prior to DEN immersion had a significant increase in the incidences of esophageal and gastric adenomas at 48 week compared with the carcinogen-only group (CCRIS, 1998). Trout given 5,6-benzoflavone at 0.005 percent in the diet for 8 weeks after AFB immersion had a significantly greater incidence of liver cancer at one year compared to the carcinogen-only group (CCRIS, 1998).

Modulation of mutagenic activity. The inclusion of 5,6-benzoflavone-treated microsomes in mutagenicity tests results in decreased activation of both cyclophosphamide and AFB to mutagenic products (Wattenberg & Leong, 1970; Hales, 1981; Raina *et al*., 1985). Additional examples of modulation of genotoxicity from 5,6-benzoflavone are presented in Table 1 below.

Table 1. Effects of 5,6-benzoflavone on activity of mutagens

Endpoint:	Modulation of Genotoxicity		
Test system/strain or cell line (locus)	**Dose; study details (activation, solvent, schedule)**	**Result**	**References**
SCEs in V79 Chinese hamster cells	pretreatment of hepatocytes with 5,6-benzoflavone, SCEs induced by B[a]P, DMSO solvent	5,6-benzoflavone decreased B[a]P induced SCEs	Jongen *et al.*, 1988
Transformation of Syrian hamster cells	pretreatment with 5,6-benzoflavone at 1 or 5 µg or 3 times at 1 µg followed by B[a]P or 3- MC vs B[a]P or MC alone	frequencies of transformation greater in cells pretreated with 5,6-benzoflavone	Dipaolo *et al.*, 1971

Induction of reverse mutations in *S. typhimurium* TA98	Ames plate assay: B[a]P w/wo 5,6-benzoflavone-induced S-9, DMSO solvent	S-9 greatly increased revertants/plate	Ong *et al.*, 1980
Induction of reverse mutations in *S. typhimurium* TA1535	Ames plate assay: 2-anthramine w/wo 5,6-benzoflavone-induced S-9, DMSO solvent	S-9 reduced revertants/plate	Ong *et al.*, 1980
Induction of reverse mutations in *S. typhimurium* TA98	Ames plate assay: 3-MC w/wo 5,6-benzoflavone-induced S-9, DMSO solvent	no significant increase in mutation over spontaneous levels	Ong *et al.*, 1980
Mutagenicity in *S. typhimurium* TA98 & TA100	Ames plate assay: AFB or 2-aminofluorene w/wo S5 fraction of Sprague-Dawley rat prostate, rats administered 5,6-benzoflavone, DMSO solvent	increase in mutagenicity directly related to concentration of S-5	Soderkvist *et al.*, 1983

w = with; wo = without

Modulation of other effects possibly associated with cancer induction. AFB-DNA adduction was reduced 46 percent in male F344 rats fed 0.04 percent 5,6-benzoflavone for 7 days before administration of AFB (Stresser *et al.*, 1994).

2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is a mutagenic heterocyclic amine found in cooked meat. PhIP produces tumors of the colon, mammary gland, and prostate in long-term rodent experiments. PhIP requires metabolic activation by cytochrome P4501A2. When the induction of apoptosis in the colonic epithelium of male F344 rats was measured, those given PhIP by gavage and 5,6-benzoflavone in the diet had significantly more apoptosis than the corresponding group given PhIP alone (Hirose *et al.*, 1998).

Treatment of ICR/HA female mice for 18 days with 5,6-benzoflavone at 3 mg/g diet did not alter the metabolism of B[a]P by the forestomach microsomes. This treatment resulted in about a 60 percent reduction of benzo[a]pyrene diol epoxide (BPDE)-DNA adduct formation, however. Comparable *in vivo* studies have shown that treatment of mice with 5,6-benzoflavone resulted in an 80 percent decrease in BPDE-DNA adducts suggesting that 5,6-benzoflavone would protect against B[a]P-induced neoplasia in the mouse forestomach (Ioannou *et al.*, 1981).

2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) is a potent mutagen and carcinogen found in cooked food. To exert its genotoxicity, IQ requires metabolic activation via a two step process involving cytochrome P4501A2-catalyzed N-hydroxylation, followed by formation of an unstable *N*-acetoxyarylamine ester. Nerurkar and coworkers (1995) examined the effects of the Ah locus and acetylator polymorphisms on levels of IQ-DNA adducts formed in C57BL/6 mice. In the kidney, 5,6-benzoflavone pretreatment reduced total adducts by 50 percent in Ah-responsive animals. In the colon of Ah-nonresponsive animals, rapid acetylators had 3-fold more adducts than slow acetylators, 5,6-benzoflavone treated animals, or vehicle controls. In Ah-responsive animals of either acetylator phenotype, 5,6-benzoflavone pretreatment reduced total adducts in the colon by 70 percent. In the bladder, 5,6-benzoflavone pretreatment caused a 2.5 fold increase in adducts but only in Ah-responsive, rapid acetylator mice.

A single 80 mg/kg dose of 5,6-benzoflavone in corn oil administered to female Sprague Dawley rats resulted in significant induction of liver and kidney microsomal ethoxyresorufin-*O*-deethylase (EROD) and methoxyresorufin-*O*-demethylase (MROD) activities at 4-48 hr, with the extent of induction being much higher for EROD. Covalent DNA modification levels were not affected by 5,6-benzoflavone at 4-24 hours, but they were significantly reduced at 48 hours. Renal DNA modification levels were also significantly affected by 5,6-benzoflavone, but to a lesser extent than in the liver (Moorthy *et al*., 1995)

Reproductive effects/fetotoxicity. Administration of 5,6-benzoflavone during pregnancy to the rat results in marked fetotoxicity. Extensive fetal mortality was observed following ip administration of 5,6-benzoflavone at 15 mg/kg/day for 8 days during mid-gestation. Administration of 5,6-benzoflavone on days 9-14 of gestation caused a significant impairment of late fetoplacental growth, although no signs of maternal toxicity were observed (Fuhrman-Lane *et al*., 1983; Shiverick *et al*., 1984).

Teratogenicity. 5,6-Benzoflavone was not toxic and produced no malformations in embryos following administration to pregnant mice. When 5,6-benzoflavone and TCDD were co-administered to pregnant mice, however,

both the teratogenicity and fetolethality of TCDD were increased significantly (Hassoun & Dencker, 1982).

Modulation of tumors in the offspring of pregnant mice. Treatment of pregnant mice with 3-MC causes lung and liver tumors in the offspring, the incidences of which are greatly influenced by the Ah locus regulated induction phenotype for AHH in both the mother and fetuses. To examine the modulating effect of maternal environment on tumor susceptibility, reciprocal crosses between responsive C57BL/6 and non-responsive DBA/2 mice were made, and the pregnant mothers were treated ip on the 17th day of gestation with olive oil, 30 mg/kg of 3-MC, or 30 mg/kg of 5,6-benzoflavone. In fetal lung tissues, the absolute levels and relative induction ratios of AHH activity from D2B6F1 fetuses were very similar to those obtained in B6D2F1 fetuses during the first 24 hours following a transplacental exposure to either inducing agent. This was also the case 48 hours after an injection of 5,6-benzoflavone. However, 48 hours after exposure to 3-MC, the AHH activity in fetal lungs from B6 mothers had declined to practically control values, whereas fetal lungs from D2 mothers still exhibited a high level of AHH activity. Similar induction kinetics for the CYP1A1 gene were obtained in fetal livers. In both organs, treatment with inducing agents for the P4501A1 gene resulted in a rapid and early induction of CYP1A1 RNA by 4 hours. Fetuses from D2 mothers showed a more sustained induction of CYP1A1 RNA following exposure to 3-MC than did fetuses from B6 mothers. These results suggest that the increase in tumor susceptibility observed in the offspring of D2 mothers compared to the offspring of B6 mothers was due, at least in part, to the differences in the persistence of induction of the CYP 1A1 gene locus, and may be the result of differences in the clearance rates of 3-MC from the fetal and maternal compartments or its pharmacokinetic distribution in the two types of maternal environments (Miller *et al*., 1990; Miller, 1994).

Anderson and her coworkers measured metabolism of 3-MC by homogenates of the livers of each of the progency that had been exposed transplacentally to 3-MC or 5,6-benzoflavone. Exposure of fetal mice to a single dose of 3-MC on the 17th day of gestation permanently altered the ability of liver enzymes to metabolize 3-MC in complex but consistent ways, usually resulting in an increase in this metabolic activity. This transplacental imprinting effect was

largely dependent on Ah-regulated induced metabolism of 3-MC by either the mother or the fetus. Pretreatment with 5,6-benzoflavone had no effect in three of the four groups but resulted in decreased adult hepatic metabolism in the non-responsive female offspring. In the responsive animals, 5,6-benzoflavone administration may have abrogated the positive imprinting effect of 3-MC, since none of the responsive males or females differed significantly from controls with regard to liver metabolism of 3-MC. Among non-responsive mice of both sexes this abrogation was clear (Anderson *et al*, 1989; Anderson *et al.*, 1991).

Structure-Activity Relationships: For the structure-activity analysis, two additional flavones, 7,8-benzoflavone and flavone are compared with 5,6-benzoflavone, and the three flavones are compared with two flavonols, quercetin and kaempferol. Flavone is unsubstituted; 5,6-benzoflavone has a benzene ring at the 5,6-position, and 7,8-benzoflavone has a benzene ring at the 7,8-position. Quercetin contains five hydroxyl groups. Kaempferol, a metabolite of quercetin, contains four hydroxyl groups. Structures are shown in Figure 1 below (Edenharder *et al*., 1993).
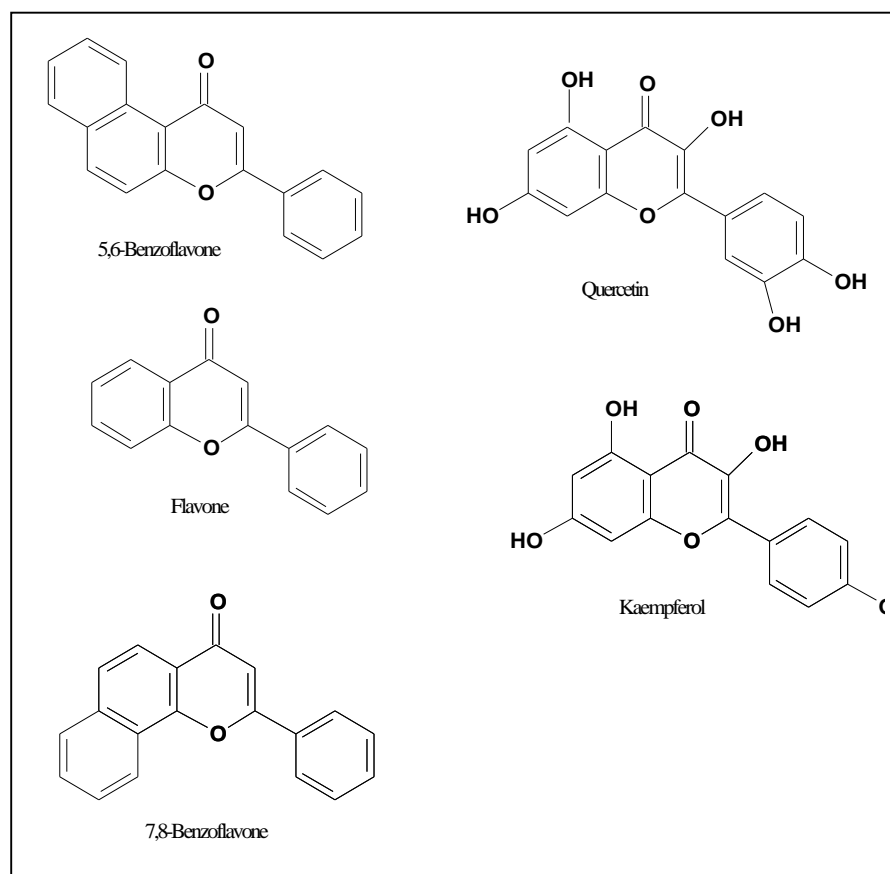
FLAVONES   FLAVONOLS

Figure 1

Information on the carcinogenicity and mutagenicity of quercetin and kaempferol was discussed in detail in a recent summary sheet on *Ginkgo biloba.* Extensive information was found that showed quercetin and kaempferol to be frameshift mutagens. Quercetin consistently showed mutagenic activity in *S. typhimurium* strains TA97, TA98, TA100, and TA102 without metabolic activation. Responses with metabolic activation also tended to be positive, but somewhat dependent on the activation system used and other conditions of the experiment. Kaempferol was tested less extensively than quercetin; this substance was generally positive in TA98, TA100, TA102, and TA1537 with metabolic activation but negative without activation. Quercetin was tested for carcinogenicity in ten different assays; all but two were negative. One positive study was conducted by the NTP; renal tubule adenomas were found in high dose males exposed at 40,000 ppm in the diet for 104 weeks.

The polyhydroxylated flavonols, such as quercetin, show little P4501A inducing potential (McKillop & Case, 1991).

In contrast to the flavonols, flavone, 7,8-benzoflavone, and 5,6-benzoflavone which lack an hydroxyl group at position 3, do not appear to possess mutagenic activity (Sugimura *et al*., 1977; Brown & Dietrich, 1979; Nagao *et al*., 1981; Elliger *et al*., 1984). Neither 5,6-benzoflavone, 7,8-benzoflavone, nor flavone inhibited the mutagenic activity resulting from the metabolic activation of B[a]P and ($\pm$)-*trans*-7,8-dihydroxy-7,8- dihydrobenzo[a]pyrene by rat liver microsomes. These flavones lack a free phenolic group which appears necessary for inhibition (Huang *et al*., 1983).

Although the flavones have not been adequately studied in 2-year animal carcinogenicity studies, some significant differences are seen in tests where they are administered with procarcinogens activated by the microsomal enzyme system. Treatment with 7,8-benzoflavone, or 5,6-benzoflavone before addition of B[a]P or 3-MC enhanced the transformation of Syrian hamster cells. 7,8-Benzoflavone prevented cytotoxicity by the carcinogens, while 5,6-benzoflavone did not (DiPaolo *et al*., 1971). When dibenzo- [*a,l*]pyrene was incubated with calf thymus DNA in the presence of liver microsomes from 5,6-benzoflavone treated rats, DNA adduction was enhanced nearly 20-fold. Inclusion of the selective P4501A1 inhibitor, 7,8-benzoflavone almost

completely (>98%) abolished adduct formation (Arif & Gupta, 1997). 7,8-Benzoflavone was also a potent inhibitor of the initiation of skin tumors by 3-MC and DMBA in female CD-1 mice. 5,6-Benzoflavone inhibited tumor initiation by 3-MC and DMBA, but to a lesser degree than 7,8-benzoflavone. Epidermal AHH was increased by 5,6-benzoflavone but 7,8-benzoflavone produced no effect or slight inhibition of AHH when these two substances were given either topically or ip (Slaga *et al*., 1977).

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