SUMMARY OF DATA FOR CHEMICAL SELECTION

Oligomeric Proanthocyanidins from Grape Seeds and Pine Bark

BASIS OF NOMINATION TO THE CSWG

Grape seed extract and pine bark extract, including the proprietary products Pycnogenol® (pycnogenol) and Masquelier's™ Original OPC[1]s (OPCs), are brought to the attention of the CSWG because of widespread consumer use of these substances as dietary supplements.

Based on the structures of identified active ingredients, the phenolic compounds extracted from grape seeds and pine bark would not be expected to be genotoxic. Indeed, the inventor of pycnogenol states that this product was tested in *Salmonella typhimurium* and was negative. Substantial health claims for grape seed and pine bark extracts, including some studies showing tumor inhibition, make them attractive materials for further study. Antioxidants, especially polyphenols, in red wine have been proposed as an important contributory factor to the protective effect of regular alcohol use against atherosclerotic cardiovascular disease. Grape seed and pine bark extracts may have the beneficial effects of red wine without its detrimental effects.

Because grape seed and pine bark extracts are dietary supplements, the government cannot compel the manufacturer to test the safety of these materials. Given their potential benefits, an independent demonstration of their safety appears warranted. It should be noted that the manufacturer of Masquelier's™ Original OPCs claims to have conducted some testing, but the results were not available for our review.

SELECTION STATUS

ACTION BY THE CSWG: 9/28/00
Studies requested:
Subchronic (90-day) testing

[1]OPC stands for Oligomeric ProanthoCyanidins.

Ames *Salmonella* and micronucleus assays

Reproductive effects and teratogenicity

Priority: High

Rationale/Remarks:

Pycnogenol products based on pine bark and grape seed extract are closely related in term of their active ingredients, polyphenolic antioxidants believed to account for the low incidence of death from coronary heart disease in the French despite diets rich in saturated fat

Testing of Pycnogenol® is recommended as representative of dietary supplements with activivity based on OPCs because it is well characterized

Although they are widely used dietary supplements, the toxicity of these products has not been well characterized

NCI will conduct the Ames and mouse lymphoma assays on one of the extracts.

INFORMATION OBTAINED AFTER THE CSWG MEETING

A recent estimate for the US grape extracts market is $40 to $50 million per year. Perhaps the largest producer of grape extracts in the world is Indena, which sells Leucoselect Phytosome in the US. Indena has completely elucidated the composition of Leucoselect and conducted extensive *in vitro* testing of its antioxidant properties. According to the company, Indena has also conducted animal and human studies to show the effectiveness of Leucoselect in protecting the cardiovascular system. A US producer of grape seed extract, Polyphenolics was founded by Canadaigua Brands, the secon largest supplier of wine in the US. Information on Polyphenolics products is available on their website, anti-oxidant.com (Boswell, 2000).

CHEMICAL IDENTIFICATION

CAS Registry Nos:	No CAS numbers have been assigned to grape seed or pine bark extracts
CAS Names:	No names for grape seed or pine bark extracts have been assigned in the Chemical Abstract Service 8th or 9th Collective Index.

Structure, Molecular Formula, and Molecular Weight: Grape seed and pine bark extracts, including Pycnogenol® and Masquelier's™ Original OPCs, are mixtures of active and inert ingredients. The structures of some compounds proposed as active ingredients are presented in the section on Structure-Activity Analysis.

Description:

Grape Seed Extract & *Grape Seed OPCs:*	Extracts of the seeds of the *Vitis vinifera* grape (American Botanical Council, 2000). Some extracts are also prepared from seeds, skin, and stems of other varieties of red or green grapes (Life Extension Magazine, 1997).
Pycnogenol® and Masquelier's™ *Original Pine Bark OPCs*	Specific blends of procyanidins extracted from bark of the *Pinus maritima*; a light beige colored powder with astringent taste, very soluble in water and ethyl alcohol, insoluble in chloroform, petroleum ether, and ethyl ether (Masquelier, 1987; Packer *et al.*, 1999).
Pine Bark Extracts	Generic versions of pycnogenol which may be made from *Pinus maritima* or other sources of pine bark

Technical Products and Impurities: Grape seed and pine bark extracts are prepared for the common purpose of providing a source of phenolic compounds (monomers and procyanidins). The most common pine bark extract is pycnogenol, which is described in US Patent 4,698,360.[2] Pycnogenol® and Masquelier's™ Original Pine Bark OPCs are virtually identical pine bark extracts that share US Patent 4,698,360 issued on October

[2]In this Summary Sheet, pycnogenol refers to a blend of polyphenolic compounds extracted from the bark of *Pinus maritima*. Pycnogenol® refers to the pycnogenol product sold in the US by Horphag Overseas Ltd.

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6,1987, and hence a litigious history. In the United States, one patent assignee, Horphag Overseas Ltd., registered pycnogenol as a trademark. Thus, the other assignee to patent 4,698,360 cannot market its product in the United States as pycnogenol. The assignee has been acquired by International Nutrition Company, Inc. (INC), which markets its pycnogenol product in the United States under the name Masquelier's™ Original Pine Bark OPCs (INC, 2000; Masquelier, 1987; Scambuster.com, 2000).

The pycnogenol products, Pycnogenol® and Masquelier's™ Original Pine Bark OPCs are sold in the form of 10-100 mg capsules or tablets through various marketing channels (Drugstore.com, 2000; INC, 2000; Horphag, 2000; Infoarea.com, 2000; Lifeplus Vitamins, 2000).

Pycnogenol® is distributed in the US by Henkel Nutrition and Health Group (Anon., 1998). According to Horphag, its pycnogenol products are available at Walmart, Mother Nature, Drugstore.com, GNC, Vitamin Shoppe, Whole Foods, Green Tree, Puritan's Pride, Cyberpharm, TPT, Inc., Martin Health Care Products, Derma E, and other outlets (Horphag Research LTD, 2000).

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In North America, Integrated BioCeuticals, LLC, is INC's main business-to-business distributor for Masquelier's™ Original OPCs raw materials and Primary Services International distributes INC's French pycnogenol extracts under the names Masquelier's™ Original Pine Bark OPCs, and Masquelier's™ Original Grape Seed OPCs. Consumers can obtain these products from BIMINI, BioNutrients, Flora, Healthysource, Life Plus, Naturalife, Nature's Sunshine, Nature's Way, Dr. Nguyen's, Primary Source, Pure, Roes, Shaprite, Source Naturals, and Standard Process (Healthysources, 2000; INC, 2000).

Generic pine bark extracts and grape seed extracts are also manufactured and distributed by various dietary supplement suppliers through vitamin stores, pharmacies, mass marketers, and Internet channels.

Chemical Composition: Standardized grape seed extracts are reported to contain 92-95% OPCs (Wholehealthmd.com, 2000). The second highest concentration of OPCs, 80-85%, is found in pine bark (Wellness Web, 2000).

Although the chemical composition has not been elucidated completely, the main constituents of grape seed and pine bark extracts are phenolic compounds, broadly divided into monomers (catechin, epicatechin, and taxifolin) and condensed flavonoids of various chain lengths that release anthocyanins when heated in acidic conditions. Pine bark extract also contains phenolic acids (such as caffeic, ferulic, and *p*-hydroxybenzoic acids) as minor constituents and glycosylation products, i.e., glucopyranosyl derivatives of either flavanols or phenolic acids as minute constituents (Anon.,1998; Packer *et al.*, 1999).

The cocktail of flavanoids varies from one species to another. The pine possesses a high level of monomers of the catechin type. The grape contains more oligomers, and the predominant monomer is epicatechin (Healthysource, 1999).

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EXPOSURE INFORMATION

Production and Producers:

Manufacturing Process. A wide variety of extraction techniques are employed by grape seed and pine bark extract manufacturers. Depending on the extraction technique used, the procyanidins in the extracts can be present in different sizes or degrees of polymerization. Some manufacturers specifically target smaller oligomers while others target a broad range of monomers, oligomers, and polymers. Some manufacturers desire a small percent of monomers (i.e., catechins) in their final extract while others include a higher percentage of monomers (Omegabiotech.com, 2000).

Masquelier's™ Original Pine Bark OPCs are manufactured according to US Patent 4,698,360. Maritime pine bark is reduced to a coarse powder and extracted with boiling water. Sodium chloride or ammonium sulfate are added up to saturation to the cooled and filtered liquid, and the precipitate formed is discarded. The remaining liquid is extracted with ethyl acetate. The extract is dried and brought back to 1/5 its volume by vacuum distillation. It is then poured into three volumes of chloroform, and stirred mechanically to precipitate the proanthocyadins. The proanthocyadins are collected by filtration purified by redissolution in ethyl acetate and reprecipitated in chloroform. The proanthocyadins are finally washed with chloroform and dried at reduced pressure in a heating chamber (Masquelier, 1987).

For preparation of grape seed extract, catechins and proanthocyanidins can be extracted from winery by-product pomace (Alonso *et al.*, 1991).

Production/import level.

Grape seed extract: In 1997, grape seed extract was reported to be the 7th most popular herbal supplement sold by food, drug, and mass market retail outlets in the US (Blumenthal, 1998). For the period August 1998 to July 1999, grape seed extracts were reported to be the 9th leading herbal supplement in the US with 3.6 million units

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purchased (Sauer,1999). The herb and botanical market was estimated at $4.3 billion in 1999 according to the Nutrition Business Journal (Wilhelm, 2000).

Pine bark extract: Currently, pine bark extracts (*i.e.*, pycnogenol) dominate the US market (mothernature.com, 2000). US sales of Pycnogenol® increased 50 percent from 1996 to 1997. In 1997, Pycnogenol® ranked eight among the best-selling botanical supplements in US pharmacies according to Information Resources, Inc. (Anon., 1998).

No figures on US sales of INC's OPCs or other pine bark extracts were available in the published literature.

Use Pattern: Pycnogenol®, OPC, and grape seed and pine bark extracts are used as nutritional supplements and phytochemicals for various diseases (American Botanical Council, 2000; Packer *et al.*, 1999). According to an unconfirmed Internet source, every day more than 4 million pycnogenol capsules and tablets are taken throughout the world (Pycnogenol: Power Oxidant, 2000). Similar information on grape seed extract was not identified in the available literature.

Grape seed extract is given for general health purposes and to treat microcirculatory maldistribution of blood flow, altered capillary fragility and permeability, and as an anti-inflammatory (American Botanical Council, 2000).

The therapeutic use of pine bark may be traced to ancient medicine in both the Old World and in the Americas. In the 4th century, Hippocrates mentioned its use, and in 1497, pharmacist H. Minner noted that pine bark was helpful for wound healing. In old Europe, pycnogenol was also used to overcome the symptoms of scurvy. In the Americas, natives used pine bark as a food, beverage, and as a remedy for inflamed wounds or ulcers (Packer *et al.*, 1999).

Pycnogenol® is now taken as a dietary supplement to strengthen capillaries and blood vessels; protect blood vessel linings; reduce LDL, or "bad" cholesterol; improve blood

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flow; and reduce platelet aggregation (Anon., 1998). Horphag also cites papers giving other uses of Pycnogenol® on its webpage, including prevention of Alzheimer's disease and memory improvement (Horphag Research LTD, 2000).

The use of pycnogenol for treatment of attention-deficit hyperactivity disorder has been publicized among parent groups for at least 7 years (Greenblat, 1999). Not much information about indication, dosing, safety, efficacy, bioavailability, pharmacokinetics, and pharmacodynamic factors can be conveyed due to a lack of information, including double-blind, controlled studies to support the claimed beneficial effects (Heimann, 1999).

Human Exposure: The primary source of human exposure to grape seed extract, Pycnogenol®, and OPCs is via the manufacturing, distribution, and consumer use of dietary supplements.

According to the US patent, pycnogenol/OPCs may be administered orally, intravenously, or cutaneously. For oral administration, pycnogenol/OPCs is in the form of tablet, sugar coated pills, pellets, capsules, cachets, and drinkable ampoules. Pycnogenol/OPCs may be used in the form of an ointment. The oral dose is generally from 1.5 to 3 mg/kg b.w. per day, which represents a daily dose of 100 - 200 mg a day for a 70 kg man (Masquelier, 1987). All identified sources of pycnogenol supplements were in capsule form.

Grape seed extract in the form of tablets, liquids, or capsules is given at an average daily dose of 50-300 mg (American Botanical Council, 2000; Wholehealthmd.com, 2000).

Environmental Occurrence: OPCs are found in many woody plants. The two most common sources are grape seeds and the white pine of southern Europe. OPCs are also abundant in blackjack oak, horse chestnut, witch hazel, and hawthorn, as well as in apples, berries, barley, bean hulls, chocolate, rhubarb, rose hips, and sorghum (Sterling, 2000). Grape

seed and pine bark extracts are manufactured products and do not occur, *per se*, in the environment.

Regulatory Status: Since 1994, dietary supplements have been regulated under the Dietary Supplement Health and Education Act (DSHEA). For dietary supplements on the market prior to October 15, 1994, the DSHEA requires no proof of safety in order for them to remain on the market. The labeling requirements for supplements allow warnings and dosage recommendations as well as substantiated "structure or function" claims. All claims must prominently note that they have not been evaluated by the FDA, and they must bear the statement "This product is not intended to diagnose, treat, cure, or prevent any disease" (FDA, 1995).

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EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data: No epidemiological studies or case reports investigating the association of exposure to grape seed or pine bark extracts were identified in the available literature.

Pycnogenol has reportedly been taken in Europe under medical supervision for decades with no reports of adverse effects. An unconfirmed Internet source reports that daily doses of up to 35,000 mg of pycnogenol given to humans for six months produced no adverse effects (Healthysource.com, 1999; Pycnogenol: Power Antioxidant, 2000).

Pycnogenol was cited in 17 Adverse Event Reports and grape seed extract was cited in 9 Adverse Event Reports made to the FDA Office of Special Nutritionals as of October 20, 1998, out of a total of 2621 adverse events involving 3451 products. According to FDA, there is no certainty that a reported adverse event can be attributed to a particular product or ingredient (FDA, 1998a,b).

According to an Internet source, there have been reports of a possible decrease in effectiveness of antibiotics, specifically tetracycline and tetracycline derivatives caused by grape seed extracts (BroadcastHealth.com, 2000).

Animal Data: The manufacturer has stated that pycnogenol has been tested at expert centers including the Pasteur Institute in Lyon, France, and BSL Bioservice in Munich, Germany and is nontoxic, nonteratogenic, nonmutagenic, noncarcinogenic, and nonantigenic (Healthysource, 1999; INC, 2000). Details of these studies were not available for review.

Dogs given a daily dose of OPCs from grape seeds equivalent to a 150-lb human taking 19,800 mg/day in a one year study reportedly showed no adverse effects (Innovative Technologies Corporation of America, 1999). No study details were provided.

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Short-Term Tests: According to the manufacturer, Masquelier's™ Original Grape Seed OPCs was tested for mutagenicity in a reverse mutation assay using *Salmonella typhimurium*. In contrast to some bioflavonoids, the product "passed the mutagenicity test without the slightest sign of mutagenicity" (INC, 2000). The test results were not available for our review.

Several procyanidins with different degrees of polymerization (dimers, a trimer, and a polymer) extracted from different natural sources were found to be nonmutagenic in the *Salmonella* mutagenesis assay system (Yu & Swaminathan, 1987).

In contrast, a recent study reported that all dimeric polyphenols and the galloylated metabolites isolated from grape seeds potentiated the mutagenic activity induced by the indirectly acting carcinogen *N*-nitrosopyrrolidine in the presence of an activation system used to activate CYP2E1(Catterall *et al.*, 2000).

Metabolism: Grape seed procyanidins obtained from plants grown in a ^{14}C-enriched environment have been used to study the bioavailability of complex mixtures of flavonoids. Absorption of radiolabeled procyanidins administered orally to mice began 10 minutes after ingestion and slowly declined after 3-7 hours. Procyanidins had a propensity for proline-rich tissues. Thus the aorta was about 10 times more enriched than the lungs and 5 times more than the liver (Packer *et al.*, 1999).

Preliminary data is also available on the bioavailability of pycnogenol in humans. Unmodified proanthocyanidins were detected in the saliva one hour after the ingestion of 150 mg of encapsulated pynogenol (Masquelier, 1987). The urine collected for 24 hours after human subjects were administered single doses of 200 mg of pycnogenol contained ferulic acid and esters of other hydroxycinnamic acids (Packer *et al.*, 1999). The more complex components of pycnogenol, the oligomeric procyanidins appear to undergo biologic modification after ingestion in humans (Packer *et al.*, 1999).

Other Biological Effects: It is not clearly agreed upon what size oligomer or polymer is responsible for what degree of biological activity. Some sources agree that it is only the dimers and trimers that show health benefits while others insist that larger polymers and even monomers can be linked to antioxidant activity (Omegabiotech.com, 2000). The following reports summarize the status of current research.

Tumor Inhibition and Related Studies. Bomser and coworkers (1997) examined the antitumor promoting activity of a polyphenolic fraction of grape seeds (GSP) in CD-1 mouse skin epidermis. Pretreatment of mouse skin with GSP resulted in dose-dependent reductions in 7,12-dimethylbenz[a]anthracene (DMBA)-initiated skin tumor incidence and number of tumors per mouse.

Pine bark extract has been reported to inhibit the tumor promotion process on the epidermis of mice (Kensler *et al.*, 1983). No details of the study were given.

Intragastric administration of pycnogenol inhibited the metabolic activation of tobacco-specific nitrosamine, 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in the lung microsomes of F344 rats. Pycnogenol was not effective in liver microsomes (Huynt & Teel, 1999).

Exposure of pBR322 plasmid DNA to an iron/ascorbic acid system resulted in cleavage/damage of DNA by hydroxyl radical. Pycnogenol significantly monimized this cleavage (Nelson *et al.*, 1998).

Antioxidant/Free Radical Scavenging Properties. Grape seed extract administered to female Swiss-Webster mice caused a dose-dependent inhibition of (TPA)-induced lipid peroxidation and DNA fragmentation in the hepatic and brain tissues, as well as activation of peritoneal macrophages (Bagchi *et al.*, 1998).

Blazso and coworkers tested the *in vivo* $O_2 \cdot -$ scavenging activity of pycnogenol and three of its chromatographic fractions (fraction 1, monomeric flavonoids [taxifolin, catechin,

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epicatechin] and phenolic acids [caffeic, ferulic, and vanillic]; fraction 2, procyanidin dimers, trimers, and tetramers; and fraction 3, oligmers >four subunits). The whole extract and each of its fractions inhibited superoxide-induced reduction of nitroblue tetrazolium to formazan in a dose-dependent fashion. The most active fraction was fraction 3. Other investigators have reported that pycnogenol is an efficient scavenger of both $O_2 \cdot -$ and $HO \cdot$ and that anthocyanidins in general are potent scavengers of $\cdot NO$ and $ONOO^-$ (Packer *et al.*, 1999).

Pycnogenol has been reported to protect the low-density lipoprotein fraction of human plasma from copper-induced oxidation and to interact with cellular antioxidants. Supplementation with pycnogenol in the diet was found to be associated with a significant increase in _-tocopherol levels in rat hearts. Pycnogenol has also been studied in relation to its ability to protect against ultraviolet (UV) radiation-induced injury and as a preventive antioxidant by chelating transition metals (Packer *et al.*, 1999).

Proanthocyanidin-rich grape seed extract (0.1 or 1% in diet) attenuated the development of aortic atherosclerosis in cholesterol-fed rabbits (Yamakoshi *et al.*, 1999).

Cardiovascular Effects. Pycnogenol has been reported to have activities related to cardiovascular functionality, such as a vasorelaxant activity, inhibition of angiotensin-converting enzyme, and the ability to enhance microcirculation (Packer *et al.*, 1999).

In a European study, patients with peripheral circulatory disorders were given pycnogenol (dosage not reported) for 30 days. Pain, limb heaviness, and feelings of swelling decreased significantly during therapy in most patients. Criteria for evaluating a decrease in symptoms were not reported (Cicero *et al.*, 1996).

Inhibition of Inflammatory Responses. Blazso and coworkers demonstrated that oligomeric procyandins in pycnogenol can inhibit localized inflammatory responses (edema) caused by croton oil administered to the mouse ear (Packer *et al.*, 1999).

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Other Effects. Preliminary studies have indicated that pycnogenol significantly inhibits the activity of enzymes which produce free radicals in biological systems, namely horseradish peroxidase, lipoxygenase, and xanthine oxidase (Packer *et al.*, 1999).

Preexposure to grapeseed extract seven days in advance of hepatotoxic doses of acetaminophen appeared to significantly attenuate acetominophen-induced hepatic DNA damage, apoptotic and necrotic cell death of liver cells, and antagonize the influence of acetominophen-induced changes in the antiapoptotic gene, bcl-XL, expression in ICR mice (Ray *et al.*, 1999).

Structure Activity Relationships: In lieu of a traditional SAR analysis, the information suggesting carcinogenicity/anticarcinogenicity of the various components of grape seed and pine bark extracts were examined. These extracts contain phenolic monomers (catechin, epicatechin, and taxifolin) and condensed flavonoids of various chain lengths (procyanidins/proanthocyanidins). Pine bark extract (e.g., Pycnogenol®/OPCs) also contains caffeic, ferulic, and *p*-hydroxybenzoic acids as minor constituents (Anon., 1998; Packer *et al.*, 1999).

Flavonoids are potentially able to quench free radicals by forming resonance-stabilized phenoxyl radicals. Depending on their structure, flavonoids display possibly inhibitory effects on the growth and proliferation of certain malignant cells *in vivo*. These effects are thought to be either direct, due to the electron- and proton-donating capacity of flavonoids, or indirect, due to their ability to alter the activities of key enzymes in cellular response. The antiproliferative effect of monomeric flavonoids was suggested to be partly mediated by inhibition of tyrosine kinase activity, by decrease of c-*jun* m-RNA expression, or by inhibition of c-*jun* N-terminal kinase activation. Moreover catechins were reported to inhibit the interaction of tumor promoters with receptors. Epicatechin derivatives significantly inhibit NADPH cytochrome c reductase activity. The alteration of the NADPH cytochrome c reductase due to flavonoids is believed to play a key role in the inhibition of the mutagenicity induced by aromatic hydrocarbons and aflatoxin B_1 (Packer *et al.*, 1999).

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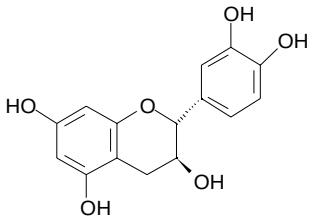
A review of the information on carcinogenicity, mutagenicity, and tumor inhibition/anticarcinogenicity/antimutagenicity found in a search of the National Library of Medicine databases, CCRIS, Genetox, and Toxline and the National Institute of Occupational Safety and Health database, Registry of Toxic Effects of Chemical Substances, is presented below in Table 1 below.

Table 1. Summary of information on active components of pine bark and grape seed extracts

Compound	Carcinogenicity Data	Mutagenicity Data	Inhibition/Anticarcinogenicity/Antimutagenicity Data
Monomers			
catechin [154-23-4] 	Induced glandular stomach adeno-carcinomas in F344 rats given 2% in diet for 104 wk (NLM, 2000) Promoted forestomach and glandular stomach cancers in F344 rats initiated with MNNG (NLM, 2000a)	No conclusions when tested for chromosome aberrations in micronucleus test in mammalian polychormatic erythrocytes (NLM, 2000b) DNA repair in *E. coli* (RTECS, 1999) Sister chromatid exchanges, unscheduled DNA synthesis, DNA inhibition & sex chromosome loss and nondisjunction in human lymphocytes (RTECS, 1999)	Inhibited DMBA-initiated mammary gland tumors in Wistar rats, DMBA-initiated and croton oil promoted skin papillomas in Swiss mice & B[a]P-initiated forestomach tumors in Swiss mice (NLM, 2000a) Ineffective vs EHEN-initiated liver or kidney tumors in Wistar rats, vs NHA-initiated pancreatic adenocarcinomas in Syrian hamsters, & DMBA-initiated and TPA-promoted dermal tumors in CF-1 mice (NLM, 2000a) Slightly reduced mammary gland tumor incidence in C3H mice given 2.5 mg/d in drinking water for 15 months [8/20 vs 12/20] (CCRIS,2000)

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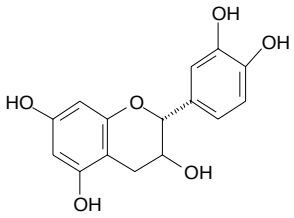
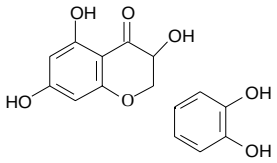
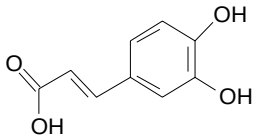
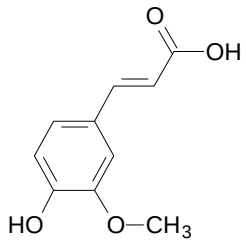
epicatechin [490-46-0]  	No information found	Sister chromatid exchanges in human lymphocytes (RTECS, 1999)	Inhibited multiplicity of DMBA-initiated and croton oil-promoted dermal tumors in Swiss mice and 3-MC-initiated sarcomas in Swiss mice (NLM, 2000a) No effect on mutagenicity of B[a]P or IQ w/wo S-9 (Catterall *et al.*, 2000)
taxifolin [480-18-2]  		Mutagenic in *S. typhimurium* w/wo S-9 (no details) (RTECS, 1999) Positive in *in vitro* cytogenetic analysis (hamster fibroblasts) (RTECS, 1999)	No information found
Condensed flavonoids procyanidins/proanthocyanidins	Keracyanin (related compound) was negative when adminitered in the diet of Wistar rats for 2 years (Tsubura *et al.*, 1983)	Procyanidin dimers, a trimer, and a polymer were nonmutagenic in the *Salmonella* mutagenesis assay system (Yu & Swaminathan, 1987).	No information found

Phenolic acids caffeic acid [331-39-5]  	Induced forestomach papillomas & squamous cell carcinomas in F344 rats when fed at 2% in diet for 104 wk (NLM, 2000a) Induced lung and kidney tumors in B6C3F1 mice when fed at 2% in diet for 96 weeks (NLM, 2000a)	Weakly mutagenic in *Salmonella* Ara test (Ariza *et al.*, 1988) Positive in mouse lymphoma L5178Y (TK+/TK-) assay wo S-9; negative in mouse lymphoma assay w S-9 and in *S. typhimurium* TA98, TA100, TA1535, TA1537 & TA1538 w/wo S-9 (NCI STTP Program reported in NLM, 2000a)	Inhibited AFB1- induced mutagenesis in *S. typhimurium* TA98 with S9 (San & Chan, 1987) and in *S. typhimurium* TA1535 (Chan *et al.*, 1986) Tested in various protocols with variable results (NLM, 2000a) Did not inhibit benzidine mutagenesis in *S. typhimurium* TA-98 w/wo S-9 (Josephy *et al.*, 1985)
ferulic acid [537-98-4] *trans*-ferulic acid [1135-24-6] (4-hydroxy-3-methoxycinnamic acid)  	No information found	Negative in *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 w/wo S-9: (NCI STTP Program reported in NLM, 2000a)	Inhibited DMBA-initiated skin tumors promoted by TPA in CD-1 mice and NQO-initiated tongue carcinomas in F344 rats (NLM, 2000a) Not effective vs B[a]P-initiated lung tumors in Strain-A mice, DMBA-initiated skin tumors promoted by TPA in ICR or NMRI mice, or forestomach tumors initiated by B[a]P gavage in ICR mice (NLM, 2000a) Did not inhibit benzidine mutagenesis in *S. typhimurium* TA-98 w/wo S-9 (Josephy *et al.*, 1985)

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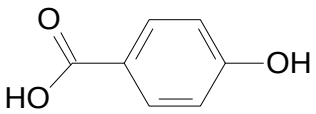
p-hydroxybenzoic acid [99-96-7] 		Negative in *S. typhimurium* TA97, TA98, TA100 & TA102 w/wo S-9 (Kako *et al.*, 1992)	

B[a]P = benzo[a]pyrene; DMBA = 7,12-dimethylbenz[a]anthracene; EHEN = *N*-ethyl-*N*-hydroxyethylnitrosamine; 2-amino-3-methylimidazo-[4,5-f]quinoline = IQ; MNNG = *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; 3-MC = 3-methylcholanthrene; NHA = *N*-nitrosobis(2-hydroxypropyl)amine; NQO = *N*-nitroquinoline-1-oxide; TPA = 12-*o*-tetradecanoylphorbol-13-acetate; w/wo = with or without

Prepared by Technical Resources International, Inc. under contract no. N02-CB-50511 (7/00)

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