

SUMMARY OF DATA FOR CHEMICAL SELECTION

Ginkgo Biloba Extract (GBE)

BASIS FOR NOMINATION TO THE CSWG

Ginkgo biloba extract (GBE) and two ingredients, bilobalide and ginkgolide B, are presented to the CSWG as part of a review of botanicals being used as dietary supplements in the United States. Herbal products represent the fastest growing segment of the vitamin, mineral supplements, and herbal products industry, an industry expected to top \$6 billion in sales by 2001. GBE is an extremely popular herbal supplement; sales in 1996, the last year figures were available, exceeded \$100 million.

Indications are that one in three adults in the United States are now taking dietary supplements. Sweeping deregulation of botanicals now permits GBE to be sold as a dietary supplement to a willing public eager to “improve brain functioning” or “promote radical scavenging activity.”

GBE was selected for review for several reasons. First, GBE is a well defined product, and it or its active ingredients, the ginkgolides, especially ginkgolide B, and bilobalide, have clearly demonstrated biological activity. Second, GBE can be consumed in rather large doses for an extended period of time. Third, some ingredients in GBE are known mutagens; in one case, a suspected high dose carcinogen, quercetin, is intentionally concentrated from the ginkgo leaf to manufacture the final product.

Ginkgo biloba extract

SELECTION STATUS

ACTION BY CSWG: 4/28/98

Studies requested:

- Toxicological evaluation, including 90-day subchronic study
- Carcinogenicity
- Mechanistic studies

Priority: High

Rationale/Remarks:

- Potential for widespread exposure through use as a dietary supplement.
- Test extract standardized to 24% flavone glycosides and 6% terpene lactones.
- Mechanistic studies should explore the relative contribution of each active ingredient and the possible synergism among these ingredients.

Ginkgolide B

SELECTION STATUS

ACTION BY CSWG: 4/28/98

- Toxicological evaluation, including 90 day subchronic study
- Micronucleus assay

Priority: High (for comparison with GBE)/Moderate (for other tests)

Rationale/Remarks:

- Commercially available active component of GBE
- Unique chemical structure
- NCI will conduct - Ames *Salmonella* assay

CHEMICAL IDENTIFICATION

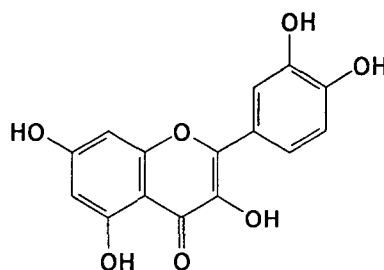
***Ginkgo biloba* extract (GBE)**

<u>CAS Registry Number:</u>	None
<u>Chemical Abstracts Service Name:</u>	None
<u>Definition:</u>	Standardized, concentrated extract of ginkgo leaves containing 24% ginkgo flavone glycosides and 6% terpene lactones
<u>Trade Names:</u>	Egb 761; Ginkgold; Kaveri; LI 1370; rōkan; Tanakan; Tebonin;
<u>Structural Class:</u>	Botanical; phytopharmaceutical

Standardized ingredients of GBE

<u>Common Name:</u>	Quercetin
<u>CAS Registry Number:</u>	117-39-5
<u>Chemical Abstracts Service Name:</u>	4H-1-Benzopyran-4-one, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy- (9CI)

Structure, Molecular Formula and Molecular Weight:



C₁₅ H₁₀ O₇

Mol. wt.: 338.3

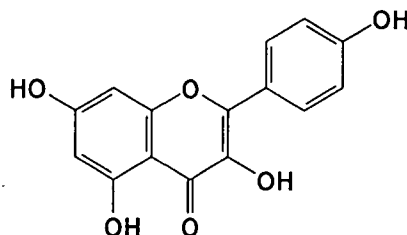
Ginkgo biloba extract

Common Name: Kaempferol

CAS Registry Number: 520-18-3

Chemical Abstracts Service Name: 4H-1-Benzopyran-4-one, 3,5,7-trihydroxy-2-(4-hydroxyphenyl)- (9CI)

Structure, Molecular Formula and Molecular Weight:



$C_{15}H_{10}O_6$

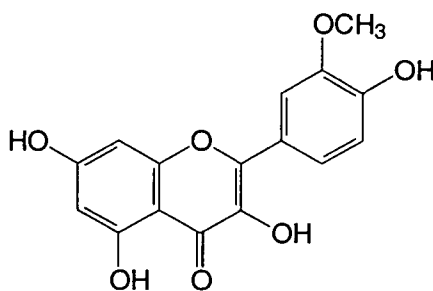
Mol. wt.: 286.2

Common Name: Isorhamnetin

CAS Registry Number: 480-19-3

Chemical Abstracts Service Name: 4H-1-Benzopyran-4-one, 3,5,7-trihydroxy-2-(4-hydroxy-3-methoxyphenyl)- (9CI)

Structure, Molecular Formula and Molecular Weight:



$C_{15}H_{12}O_6$

Mol. wt.: ~314

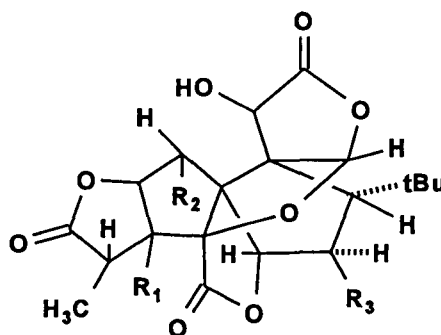
Ginkgo biloba extract

Common Name: Ginkgolides (mixed); ginkgolide A; ginkgolide B

CAS Registry Number: 15291-77-7; 15291-75-5; 15291-75-5

Chemical Abstracts Service Name: Not available

Structure, Molecular Formula and Molecular Weight:



$C_{20}H_{24}O_{10}$ (Ginkgolide B)

Mol. wt.: 424.4 (Ginkgolide B)

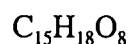
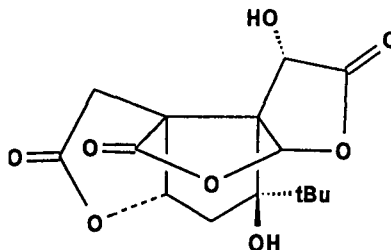
	<u>R1</u>	<u>R2</u>	<u>R3</u>
Ginkgolide A	OH	H	H
Ginkgolide B	OH	OH	H
Ginkgolide C	OH	OH	OH
Ginkgolide J	OH	H	OH
Ginkgolide M	H	OH	OH

Common Name: Bilobalide

CAS Registry Number: 33570-04-6

Chemical Abstracts Service Name: 4H,5aH,9H-Furo(2,3-b)furo(3',2':2,3)cyclopenta(1,2-c)furan-2,4,7(3H,8H)-trione, 9-(1,1-dimethylethyl)-10,10a-dihydro-8,9-dihydroxy-, (5aR-(3aS*,5a α ,8 β ,8aS*,9 α ,10a α))- (9CI)

Structure, Molecular Formula and Molecular Weight:



Mol. wt.: 326.3

Chemical and Physical Properties:

Description:

Ginkgo seed has been listed as a source of medicine since the early Chinese herbals. The leaf has been recommended for medicinal uses as early as 1509 and is still used in the form of teas. The focus of this report is a standardized leaf extract first manufactured and marketed in Europe as a medicine for cardiovascular disease and now available in the United States as a dietary supplement (Huh & Staba, 1992; Salvador, 1995).

Technical Products and Impurities: GBE preparations contain 24% flavone glycosides (quercetin, kaempferol, isorhamnetin) and 6% terpene lactones (ginkgolides, bilobalide), various organic acids, and other constituents (Salvador, 1995). Ginkgolide B accounts for about 0.8% of the total extract, and bilobalide accounts for about 3% of the extract (Vasseur *et al.*, 1994). The first standardized GBE was Egb 761, also called Tebonin, Tanakan, and rökän (Kleijnen & Knipschild, 1992a). In the United States, Nature's Way has exclusive distribution rights to Egb 761 and markets this product under the tradename Ginkgold (Anon, 1992).

Many other companies in Asia, Europe, or the United States manufacture or distribute GBE and dietary supplements containing GBE. GBE is also used in combination products to provide "special nutrients for the brain" (Anon., 1998a). Because herbal remedies are not held to the

Ginkgo biloba extract

same standards of purity and efficacy as medications in the United States, tremendous variability of the same product can occur between manufacturers and from batch to batch (Martin, 1997).

GBE is available from the following bulk distributors and manufacturers: ATZ Chemical, Inc., ACTA Pharmacal, American Ingredients, Inc., Arrow Chemical Inc., Charles Bowman & Co., CPB International, Inc., China Tech, Inc., Eby Sales, Inc., ExtractsPlus, Fabrichem, Inc., G.C.I. Nutrients, Kingschem, Inc., M.M.P., Inc., Mini Star International, Inc., Morse Chemical, Inc., Motherland Herb-Pharm Inc., Pharmline, Inc., QBI, RIA International, and Schweizerhall, Inc. *Ginkgo* extract is available from American Ingredients, Inc., Bio-Botanica Inc., Blue California Co., Botanical Products International, Inc., Extracts Plus, Mini Star International, Inc., Motherland Herb-Pharm Inc., and Vege-Tech Co. *Ginkgo biloba* P.E. is available from Amaz Industries, Inc., American Ingredients, Inc., Anmar International Ltd., Ashland Chemical Company, Eby Sales, Inc., ExtractsPlus, Flavine International Inc., R.W. Greeff & Co., L.L.C., Herbarium, Inc., M.W. International, Inc., Maypro Industries, Inc., Mini Star International Inc., Motherland Herb-Pharm Inc, Starwest Botanicals, Inc., and Stauber Performance Ingredients, Inc. (McCoy, 1998). It could not be ascertained without calling each company which ones produce standardized extract.

(-)-Bilobalide (95% pure) and ginkgolide B (90% pure) are available in research quantities from Sigma (1997).

EXPOSURE INFORMATION

Production and Producers: The ginkgo tree is ancient, the only living representative of the order of *Ginkgoales*, a species that flourished 150 million years ago during the Mesozoic era, reaching its greatest development during the Jurassic and Cretaceous periods (Salvador, 1995). The ginkgo tree is now cultivated extensively in Asia, Europe, North America, New Zealand, and Argentina (Huh & Staba, 1992).

Modern pharmacological research into the active constituents of ginkgo leaves began in the late 1950s. Spearheaded by the phytopharmaceutical company Dr. Willmar Schwabe GmbH, twenty years of research resulted in a standardized, concentrated extract of ginkgo leaves. The 27 step extraction process requires fifty pounds of leaves to yield one pound of extract and takes up to two weeks to complete. Most critical to the extraction process and final product is the standardization of ginkgo flavone glycosides and terpene lactones. The 24% ginkgo flavone glycosides content of GBE constitutes a carefully measured balance of quercetin, kaempferol, and isorhamnetin. The group of constituents unique to GBE, however, are the terpene lactones which constitute 6% of the final extract (Brown, 1996).

In the United States, GBE is marketed to consumers as an herbal supplement. There are four primary distribution channels: drug stores, supermarkets, mass merchandisers, and specialty vitamin shops and nutrition centers (Heller, 1997). The Internet is also becoming an increasingly important distribution channel. Some typical consumer products are listed below in Table 1.

Table 1. Some dietary supplements containing *Ginkgo biloba* extract

Company/Product Name	Product Description
LifePlus/Food for Thought	Tablets containing 50 mg <i>Ginkgo biloba</i> Concentrate and 9 other ingredients
Ultimate Health Inc.	<i>Ginkgo biloba</i> 60 mg (extract 24%)
Vitamin Connection	Nature's Way GINKGOLD tablets, 60 mg
Vitamin Connection	Jarrow Formula's <i>Ginkgo biloba</i> 50:1 tablets, 60 mg
Dynamic Fitness	Capsules containing <i>Ginkgo biloba</i> (extract 24%), 60 mg
Nutra-Source PR-1235	Tablet containing <i>Ginkgo biloba</i> (leaf) 60 mg. (50:1 extract, 24% flavonoids, 6% terpenes lactones; superoxide dismutase activity 5.8×10 to the 5th power/unit/gram)
Reach4Life	Tablets containing 30 mg <i>Ginkgo biloba</i> (24-25% ginkgo heterosides)

Source: Anon., 1998a-d; Nutrasource, 1998; Vitamin Connection, 1998

Production/Import Levels: The US market for retail sales of vitamins, mineral supplements, and herbals is volatile and expanding. Herbal supplements are the major catalysts for the growth in this market. Sales of vitamins, mineral supplements, and herbal products were \$4.4 billion for the year ending July 1997 (Eder, 1997) and are expected to climb to \$6.5 billion by 2001 (Heller, 1997).

Drug stores account for almost half the total food/drug/mass category volume of \$2.1 billion, but this distribution channel is losing market share to supermarkets and mass merchandisers. Supermarket sales of vitamins, including herbal supplements, reached \$586 million for the year ending in November 1997, up from \$507 million the previous year. In 1996, mass merchandisers attained sales of \$575 million, up 26% from the previous year. None of these figures include the nearly equal annual take of specialty vitamin shops, nutrition centers, and other distribution channels (Heller, 1997).

Herbal sales in discount stores totaled \$77 million in 1996, a sales increase of 62% from

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the previous year in a market estimated to be valued at \$227 million for that year (Troy, 1997). In 1995, *Ginkgo biloba* was the number four selling product in health food stores in the United States (Biotek USA, 1998). Sales of *Ginkgo biloba* in the United States exceeded \$100 million in 1996 (Springen & Crowley, 1997).

Between July 19, 1996 and December 30, 1997, the Piers Imports database listed the following imports: 433,719 lb. ginkgo powders or leaves; 68,547 lb. tea; 21,922 lb. dried leaf powder; 17,339 lb. ginkgo tablets; 3,970 lb. crude natural drugs and herbs, and 476 lb. leaves extract. In the same period, approximately 31,000 lb. of ginkgo nuts were also imported (Dialog, 1998).

The European market for herbal medicines is more mature than the US market. Total sales of herbal medicines in the European Union represent approximately one-half of the yearly sales of herbs worldwide. More than 70% of general practitioners in Germany prescribe phytopharmaceuticals; many are covered by national health care insurance. This has led to a phytopharmaceutical market in Germany estimated at \$3 billion annually. In Germany, more than 5 million prescriptions are written for GBE each year, with sales in 1993 amounting to \$280 million (Brown, 1996; Croom & Walker, 1995).

Neither *Ginkgo biloba* nor GBE are listed in EPA's Toxic Substances Control Act (TSCA) Inventory.

Use Pattern: Treatment with *Ginkgo biloba* can be traced to the origins of Chinese medicine 2,800 years ago. In the modern Chinese pharmacopeia, leaves and fruit are still recommended for treating heart and lung problems. The nut, called Pak Ko, is recommended to expel phlegm, stop wheezing and coughing, urinary incontinence and spermatorrhea. The raw seed is said to be anticancerous. It is said to help bladder

ailments, menorrhagia, uterine fluxes, and cardiovascular ailments. The powdered leaf is inhaled for ear, nose, and throat disorders like bronchitis and chronic rhinitis. Locally applied boiled leaves are used for chilblains. The plant has also been used to treat conditions that may have poor circulation as a common symptom, such as brain function impairment, hearing loss, vertigo and tinnitus (Salvador, 1995).

Current interest in *Ginkgo biloba* relates to potential medical applications of the plant extracts. The major active component of GBE was considered, until 1983, to be a flavonoid complex that removed free radicals in the peripheral and/or cerebral vascular systems. More recent evidence suggests that the ginkgolides may be more important bioactive ingredients in the leaf extract, as they are platelet activating factor (PAF) antagonists. An increase in PAF occurs in asthma, graft rejection and in immune disorders that induce toxic shock. Ginkgolide B and related diterpenes inhibit the binding of PAF to receptors on the cell surface of some human leukocytes. Patents have been submitted for the use of ginkgolide B and structurally related PAF antagonists to treat or prevent PAF - O-alkyl-acetyl-glycero-phosphorylcholine (acether) disorders (Huh & Staba, 1992).

In Germany, GBE is licensed for the treatment of cerebral dysfunction, as supportive treatment for hearing loss due to cervical syndrome, and for peripheral arterial circulatory disturbances with intact circulatory reserve (intermittent claudication) (Kleijnen & Knipschild, 1992a).

Like other botanicals, GBE is marketed in the United States as a dietary supplement. Although many botanicals are used to treat or prevent diseases, manufacturers and distributors are prohibited under the Dietary Supplement Health & Education Act of 1994 to include such uses on direct product labeling (Martin, 1997).

Human Exposure: There is potential for ingestion of GBE to a widespread consumer population, since this product is readily available without prescription at a cost highly competitive with prescription medications.

The recommended dose of GBE is 120 to 160 mg daily for persons with intermittent claudication and 240 mg daily for cerebrovascular insufficiency, early stage Alzheimer's disease, resistant depression, and impotence (Brown, 1996).

No listing for *Ginkgo biloba* or GBE was found in the National Occupational Exposure Survey (NOES). NOES was conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983.

Environmental Occurrence: No information was found in the available literature on potential environmental pollution from the manufacture of GBE or dietary supplements containing GBE. Contact with whole ginkgo plants has been associated with severe allergic reactions, including erythema and edema, similar to response to poison ivy (Salvador, 1995).

Regulations: No standards or guidelines have been set by NIOSH or OSHA for occupational exposure to or workplace allowable levels of *Ginkgo biloba* or GBE. *Ginkgo biloba* was not in the American Conference of Governmental Industrial Hygienists (ACGIH) list of compounds for which recommendations for a threshold limit value (TLV) and biological exposure index (BEI) are made.

Herbal remedies, including GBE, are considered by the FDA to be dietary supplements. Under the Dietary Supplement Health and Education Act of 1994, they can be sold legally if they are not labeled or accompanied by any therapeutic or health claims. Herbal

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remedies can be labeled with descriptions of their role in affecting physiological structure or function, but must be labeled with a disclaimer that the product has not been evaluated by the FDA for cure, prevention, or treatment of a disease (Martin, 1997).

For dietary supplements like GBE on the market before October 15, 1994, the Dietary Supplement Health and Education Act requires no proof of safety. A dietary supplement is considered unsafe only if it presents a significant or unreasonable risk of illness or injury under conditions of use recommended or suggested in labeling, or if no conditions of use are suggested or recommended in the labeling, under ordinary conditions of use (Croom & Walker, 1995).

EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data: No epidemiological studies or case reports investigating the association of exposure to GBE or its identified active ingredients and cancer risk were identified in the available literature.

The scientific literature contains few reports of severe, acute plant poisonings. According to Croom and Walker, with the considerable growth in the number of people consuming a larger diversity of botanicals, however, the past may not predict adverse events in the future. It is also likely that the number of acute poisonings caused by herbal remedies is underreported, because current US law does not require manufacturers of dietary supplements to supply adverse reports to the federal government (Croom & Walker, 1995).

In the United States, testing for acute or chronic toxicity is not required of dietary supplements (Croom & Walker, 1995). In Germany, the government has asked manufacturers of *Ginkgo biloba* products to include a label on their oral products that headaches, dizziness, palpitations, gastrointestinal disturbances and allergic skin reactions are possible adverse effects (Salvador, 1995).

At least 40 clinical trials examining the efficacy and safety of GBE for the treatment of cerebral insufficiency have been conducted. Kleijnen and Knipschild (1992b) reviewed this information and found that only 8 trials were of good quality; all 8 reported clinically relevant improvements in patients administered GBE vs. placebo. Dosages in the 120-160 mg range were administered for 4 weeks to 3 months. No serious side effects were reported in any trial. Kleijnen and Knipschild noted that none of the trials could be considered double-blinded despite any statements the original authors had made about methodology. Kleijnen and Knipschild had the opportunity to examine GBE tablets used

in some trials; the drug was easily distinguished from the placebo because of its bitter taste.

Fifteen controlled trials examined GBE use for treatment of intermittent claudication. Only two trials were of acceptable quality. One showed an increase in walking distance after 6 months of treatment. The other showed improvement of pain at rest. Kleijnen and Knipschild (1992a) stated, "So many questions remain unanswered that, in our opinion, further evidence for efficacy is needed for this indication."

A recent clinical trial by Le Bars and coworkers (1997) assessed the safety and efficacy of GBE administered at 120 mg/day for 52 weeks. Modest changes, stabilizing or improving the cognitive performance and social functioning of patients with uncomplicated dementia, were found. Adverse events were as follows in the GBE group vs. the placebo group, respectively: 30% (49/166) vs. 31% (50/161) for all adverse events, 16% (27/166) vs. 12% (19/161) for events related to the study drug, and 12 vs. 9 reported adverse events of severe intensity. Overall, the GBE group reported slightly more gastrointestinal tract signs and symptoms than the placebo group. The findings were compromised by the high dropout rates, 50% in the GBE group and 62% in the placebo group.

Two recent case reports suggest potentially serious adverse effects possibly associated with *Ginkgo biloba*. A 33-year-old woman had taken 120 mg of GBE daily for two years before complaining of diffuse headaches accompanied by diplopia, nausea, and vomiting. An MRI scan of the brain revealed bilateral subdural hematomas. The fluid was removed and identified as unclotted blood. Bleeding time, evaluated while the woman was still taking GBE, was prolonged. Thirty-five days after discontinuation of GBE, bleeding times were within the normal range. At the time the report was written, the patient

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continued not to take *Ginkgo biloba* and remained free of headaches and other neurologic symptoms (Rowin & Lewis, 1996).

Spontaneous bleeding from the iris of the eye occurred in a 70 yr old man one week after he began twice daily ingestion of a Ginkoba™ tablet containing 40 mg of concentrated (50:1) extract. In a 3-month follow-up period, the patient had stopped taking Ginkoba and had no recurrence of bleeding (Rosenblatt & Mindel, 1997).

Animal Data: Acute Studies. The LD₅₀ of orally administered standardized extract in mice is 7.73 g/kg. (This corresponds to 2.3 g/kg of active ingredients, 1.9 g/kg of flavone glycosides, and 464 mg/kg of terpene lactones). For intravenous administration, the LD₅₀ is 1.1 g/kg (Salvador, 1995).

Subacute/Subchronic Studies. In rats and mice, orally administered GBE did not produce evidence of organ damage or impairment of hepatic and renal functions when administered over 27 weeks in doses ranging from 100 to 1,600 mg/kg (Salvador, 1995).

NCI/NTP examined rats exposed to high concentrations of quercetin, a major component of GBE, for 6 or 15 months (Dunnick & Hailey, 1992). At 15 months, increased relative kidney and liver weights were observed in both males and females. At 6 and 15 months, no treatment related toxic lesions were found in the kidneys, and no difference in kidney function was measured by BUN or serum creatinine levels.

Chronic/Carcinogenicity Studies. No 2-year carcinogenicity studies of GBE were identified in the available literature.

Quercetin has been tested for carcinogenicity in several species; this information is

considered relevant to GBE and is presented in Table 2.

Quercetin has also been studied extensively in initiation-promotion studies. These studies have generally been negative (NLM, 1998a).

Table 2. Summary of information on carcinogenicity of quercetin

Species	Carcinogenicity Information
F344 rats, males & females	(-) at 1.25 or 5% in the diet for 104 weeks (NLM, 1998a)
F344 rats, males & females	(+) renal tubule adenomas in high dose males exposed at 40,000 ppm in diet for 104 weeks. (-) in females (NCI/NTP, 1992; Dunnick & Hailey, 1992)
F344 rats, males & females	(-) at 0.1 or 0.2% in diet for 64 weeks (Stoewsand <i>et al.</i> , 1984)
albino rats, males & females	(-); fed 0.25-1% in diet for about 410 days (Ambrose <i>et al.</i> , 1952)
ACI rats, male & female	(-); fed 10% in diet for 850 days (Hirono <i>et al.</i> , 1981)
Norwegian strain rats	(+) for tumors of small intestine & bladder; fed 0.1% in diet for 406 days (Pamukcu <i>et al.</i> , 1980)
F344 rats	(+) for preneoplastic liver foci, hepatomas, and bile duct tumors when fed 0-2% in diet (Hatcher <i>et al.</i> , 1983)
Syrian golden hamsters, males & females	(-) at 10% in diet for 735 days, at 4% in diet for 709 days, or at 1% in diet for 351 days followed by basal diet for 350 days, or at 1% in diet followed by croton oil administration (Morino <i>et al.</i> , 1982)
strain A mouse (lung adenoma assay)	(-); fed 5% in diet (Hosaka & Hirono, 1981)
ddY mice, males	(-); fed 2% quercetin (Saito <i>et al.</i> , 1980)

(+) = positive; (-) = negative

Short-term Tests: No information on the mutagenic activity of GBE or its ginkgoloid or bilobolide constituents was found in the available literature.

Extensive information suggests that quercetin and kaempferol are frameshift mutagens. These studies are summarized briefly. Quercetin has consistently shown mutagenic activity in *S. typhimurium* strains TA97, TA98, TA100, TA102 without metabolic activation. Responses with metabolic activation have also tended to be positive, but

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somewhat dependent on the activation system used (phenobarbital or Aroclor 1254; S9 or S100) and other conditions of the experiment (Hatcher *et al.*, 1983; Stoewsand *et al.*, 1984; NTP, 1992; NLM, 1998a). Kaempferol has been tested less extensively for mutagenic activity than quercetin; this substance was generally positive in TA98, TA100, TA 102, and TA 1537 with metabolic activation but negative without activation (NLM, 1998b).

The genotoxic potential of quercetin has been tested in many other assays; the results are briefly described below.

- positive in *Drosophila melanogaster* (Watson, 1982)
- clastogen in Chinese hamster lung cells and human lymphocytes with or without S9, negative in *in vivo* micronucleus assay (Caria *et al.*, 1995)
- cytotoxic and mutagenic in Chinese hamster lung cells (Nakayasu *et al.*, 1986)
- chromosomal aberrations in CHO cells, negative and positive results reported for SCE (Carver *et al.*, 1983; Kubiak & Rudek, 1990)
- DNA strand breakage in HepG2 and HeLa cells, human lymphocytes (Duthie *et al.*, 1997), and L5178Y cells (Crebelli *et al.*, 1987); negative in colon tumor cell line (Duthie *et al.*, 1997)

Quercetin was administered to Sprague-Dawley rats by intraperitoneal injection or gastric intubation at a single dose of 500-2,000 mg/kg body weight. Moderate mutagenic activity was found in the urine and fecal extracts but not in plasma samples from the treated animals as assessed in *S. typhimurium* strain TA98. The mutagenic activity in the urine accounted for about 0.5% of the administered dose. Higher mutagenic activity was shown in fecal extracts (Crebelli *et al.*, 1987).

Brown and Griffiths (1983) have shown that rats could metabolize quercetin and other 3-

hydroxyl flavonoids to the 3'-O-methyl ether, which is less mutagenic in the Ames test than quercetin. This ability of the rat to form 3'-O-methyl esters may be important in protecting the body against the putative carcinogenic action of quercetin.

The genotoxic potential of kaempferol has also been examined. Kaempferol was positive in the mouse lymphoma L5178Y assay, inducing DNA single strand breaks with or without S9 activation (Meltz & MacGregor, 1981). It also induced mutagenic activity in *Drosophila melanogaster* (Watson, 1982) and chromosome aberrations and micronuclei in V79 cells with S9 (Silva *et al.*, 1997). It induced chromosomal aberrations in CHO cells with or without S9 but was negative for SCE (Carver *et al.*, 1983). In a special study of oxidative damage, kaempferol produced DNA degradation concurrent with lipid peroxidation in rat liver nuclei (Sahu & Gray, 1994).

No information on the mutagenicity of isorhamnetin was found; rhamnetin was mutagenic in *S. typhimurium* (NLM, 1998b) and the mouse lymphoma L5178Y TK+/- assays; rhamnetin also induced single strand breaks in DNA (Meltz & MacGregor, 1981).

Metabolism: A few studies have assessed the pharmacokinetics of GBE (Kleijnen & Knipschild, 1992a). Unpublished data from human experiments suggest that after oral administration of 80 mg Egb 761, the bioavailabilities of ginkgolides A (half life 4 hours) and B (half life 6 hours) are more than 80%, whereas that of ginkgolide C is very low. Bioavailability of bilobalide (half-life 3 hours) is 70% after administration of 120 mg Egb 761 extract. Of the ginkgolides A and B, about 70% and 50%, respectively, are excreted unchanged in the urine. For bilobalide this figure is about 30%.

Moreau-*et al.* (1986) showed that at least 60% of radiolabeled Egb 761 was absorbed in rats after oral administration. Specific activity in blood peaked after 1.5 hours. At 3

hours, the highest amount of radioactivity was measured in the stomach and small intestine. Glandular and neuronal tissues and eyes showed a high affinity for the labeled substance. After 72 hours, exhaled carbon dioxide accounted for about 38% of the administered dose, 22% was excreted in urine, and 29% was present in feces.

The aglycon quercetin is ingested as the glycosides, quercetrin and rutin. Resident microflora of the bowel produce glycosidases capable of releasing quercetin from its sugars. Resident microflora can also cleave the pyrone ring to produce phenyl acetic and phenyl propionic acids. In humans, orally administered quercetrin and rutin were not found in the urine or plasma in an unaltered form. However, 53% of the orally administered dose was recovered as quercetin in the feces within the first 3 days. In animal studies, radiolabeled quercetin was administered orally to rats. Twelve hours later, 80% of the radioactivity was recovered in the carcass, with the major portion (44%) found in the intestinal contents. Of the remaining radioactivity, 15% was respired, 12% was found in lung tissue, 3% was in the wall of the large intestine, less than 1% was in the blood, kidney, and gastric wall, and 4% was in the urine. It has been estimated that unaltered quercetin from ingestion of 25-50 mg daily would result in a distribution of 0.003-0.012 $\mu\text{mol/kg}$ body weight in a typical 70 kg man (Formica & Regelson, 1995).

No information on the metabolism of the ginkgolides or bilobalide was identified in the available literature.

Other Biological Effects: Cerebral insufficiency is an imprecise term that describes a collection of symptoms associated with impaired cerebral circulation sometimes thought to be early indications of dementia (Kleijnen & Knipschild, 1992a). Various compounds present in GBE extract may have a role in cerebral insufficiency by means of several mechanisms of action. Some of these mechanisms, listed below, may also explain the apparent effects

on intermittent claudication.

- increased blood flow, observed in humans
- platelet-activating factor (PAF) antagonism (see discussion on ginkgolides)
- changes in neuron metabolism and a beneficial influence on neurotransmitter disturbances (animals) or a return to normal in the EEG (elderly patients)
- prevention of membrane damage caused by free radicals (*in vitro* studies)

According to Smith and coworkers (1996) the view that *Ginkgo biloba* is a “smart” drug, having memory-enhancing properties in healthy animals and humans, is premature and potentially misguided. In one of the few well-controlled animal studies on memory, Winter (1991) dosed rats with GBE at 100 mg/kg per day, po, for 4 to 8 weeks prior to training and then for an additional 10 weeks. GBE treatment reduced the time to acquisition of tasks and enhanced retention performance. Porsolt and coworkers (1990), however, reported that GBE given to mice and rats at 50 or 100 mg/kg per day for 5 days did not affect performance of rats or mice in a passive avoidance test.

GBE has reduced neural damage in animals. Animals treated with GBE have survived hypobaric hypoxia for longer periods than controls. GBE also reduced behavioral deficits in rats with surgically induced frontal cortex lesions and lessened the neurochemical effects of electroconvulsive shock treatment. Several studies have also shown a positive effect of GBE administration on behavioral recovery following unilateral deafferentation of the vestibular nerve (vestibular compensation). Most of the literature is consistent with the view that GBE can protect against the effects of neural damage. Whether the neuroprotection is from a direct action on the neurons vs. an indirect effect from modulation of blood flow is unclear (Smith *et al.*, 1996).

Various components of *Ginkgo biloba* have also been studied. Most attention has focused on the diterpene ginkgolides as these have been shown to possess very specific and potent antagonist activity against PAF (Braquet, 1987; Braquet & Hosford, 1991).

PAF stimulates the conversion of phospholipids in cells to arachidonic acid, which in turn is metabolized to the prostaglandins and leukotrienes. Prostaglandins and leukotrienes are associated with blood clotting and inflammation, especially in connection with cerebral edema. Several studies have shown that the administration of ginkgolides results in a decrease in platelet aggregation, allergic reaction, and general inflammatory response. The PAF antagonist activity of the ginkgolides is also thought to underlie the traditional use of ginkgo in the treatment of asthma (Houghton, 1994).

Mixed ginkgoloide is a potent PAF antagonist in humans; dosages up to 720 mg as a single dose, 240 mg/day for 2 weeks, or 360 mg for 1 week have been well tolerated in clinical trials. Ginkgolides have been effective in preventing various PAF-related effects in human volunteers, including PAF-induced platelet aggregation and counteracting antigen-induced bronchoconstriction in asthmatic patients. Difficulty in synthesis of ginkgolides has limited their clinical application (Braquet, 1987; Braquet & Hosford, 1991).

Bilobalide is structurally similar to the ginkgolides, but it is not a PAF antagonist. Even so, bilobalide reduced the duration and incidence of 4-O-methylpyridoxine-induced convulsions in mice. O-Demethylase activity was potentiated suggesting a possible mechanism for the anticonvulsant effects of bilobalide (Sasaki *et al.*, 1997). Bilobalide also induced a statistically significant decrease in parasite burden in immunosuppressed Sprague-Dawley rats previously administered *Pneumocystis carinii*. No gross toxic effects or signs of cell damage were detected (Atzori *et al.*, 1993).

Kriegelstein and coworkers (1995) compared the cerebroprotective activity of bilobalide with ginkgolides in rodent models of focal cerebral ischemia, a rat model of global ischemia, and in cultured rat neurons. Both bilobalide and ginkgolides A and B reduced the infarct area on the mouse brain surface when administered before occlusion. When GBE was injected into rats after global forebrain ischemia, local cerebral blood flow was significantly elevated, but neuroprotection was not observed. From these and other tests, the authors concluded that ginkgolides seem to act particularly on neurons whereas bilobalide could act on neurons and astrocytes.

Structure-Activity Relationships: GBE is a complex mixture derived from a natural product.

Its pharmacological action may be multifunctional and dependent on the interactions of several components of the extract. Thus, an analysis of structure-activity relationships for this mixture would be inappropriate. Information on the known toxicities and pharmacological actions of key components of GBE is presented in other sections. The high percentage of quercetin and related mutagenic flavonoids in GBE suggests that it may have an effect in animals at high doses.

It is important to note that the key flavonoids in *Ginkgo biloba* (quercetin, kaempferol, and isorhametin) are structurally related and that kaempferol is a metabolite of quercetin and isorhametin is a metabolite of kaempferol. The flavonoids frequently occur as glycosides. For example, the aglycone quercetin links to rhamnose (quercetrin) or rutinose (rutin) as the 3-O-glycoside (Formica & Regelson, 1995).

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