

SOME DRUGS AND HERBAL PRODUCTS

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**IARC MONOGRAPHS
ON THE EVALUATION
OF CARCINOGENIC RISKS
TO HUMANS**

GINKGO BILOBA

1. Exposure Data

Ginkgo biloba is one of the world's oldest living tree species. It has survived for more than 200 million years and has become popular as an ornamental tree in parks, gardens and city streets. Originating from China, *Ginkgo biloba* is now found all over the world ([Gilman & Watson, 1993](#); [ABC, 2000](#)). Ginkgo seeds can be cooked and eaten as food. They have also been adopted in traditional Chinese medicine for many years. Some of the historical ethnomedical applications of ginkgo leaf extract include the treatment of a variety of ailments and conditions, such as asthma, bronchitis, and fatigue ([Rai et al., 1991](#)). Nowadays, ginkgo leaf extracts are promoted for the improvement of memory, to treat or help prevent Alzheimer disease and other types of dementia, and to decrease intermittent claudication ([Kleijnen & Knipschild, 1992](#); [Kanowski et al., 1996, 1997](#); [Le Bars et al., 1997](#); [Morgenstern & Biermann, 1997](#); [Nicolai et al., 2009](#); [Snitz et al., 2009](#); [Herrschaft et al., 2012](#); [Vellas et al., 2012](#)). Some of these extracts are also used to treat multiple sclerosis, tinnitus, sexual dysfunction, and other health conditions ([Oken et al., 1998](#); [Peters et al., 1998](#); [Lovera et al., 2012](#); [Herrschaft et al., 2012](#); [Evans, 2013](#); [Nicolai et al., 2009](#); [Hilton et al., 2013](#)).

1.1 Identification of the agent

1.1.1 Botanical data

(a) Nomenclature

Botanical name: *Ginkgo biloba* L.

Family: Ginkgoaceae

Genus: *Ginkgo*

Plant part: Leaf

Common names: Fossil tree; Kew tree; Japanese silver apricot; Maidenhair tree

From [ABC \(2000\)](#)

(b) Description

Ginkgo is a perennial plant with little invasive potential, which is resistant to insects and disease. Ginkgo grows slowly up to a height of about 40 m. The ginkgo plant is deciduous, with green leaves that turn golden in autumn. The leaves are simple, with alternate arrangement and lobed margins, fan-shaped with parallel venation, and a blade length of 2–4 inches [5–10 cm]. The female trees bear an inedible foul-smelling fruit containing a hard edible seed ([Gilman & Watson, 1993](#); [ABC, 2000](#)).

1.1.2 Chemical constituents and their properties

The major bioactive constituents found in the leaves of ginkgo are reported to be flavonoids and terpeno lactones, with the flavonoids present

primarily as glycosides ([Ding et al., 2006](#); [van Beek & Montoro, 2009](#)). Major and minor flavonoids are described below. Standardized extracts of ginkgo leaves (CAS No. 122933-57-7; 123009-84-7; 401901-81-3) are frequently formulated to contain ~24% flavonoids and ~6% lactones ([van Beek & Montoro, 2009](#)). Other important constituents found in ginkgo include biflavonoids and traces of alkylphenols, such as ginkgolic acids ([Wagner & Bladt, 1996](#); [DeFeudis, 1991](#); [Schötz, 2002](#); [Siegers, 1999](#); [van Beek & Montoro, 2009](#)). Ginkgo also contains ginkgotoxin, which has been reported to be structurally related to vitamin B6 ([Leistner & Drewke, 2010](#); [Fig. 1.1](#)).

CAS numbers and IUPAC names of the major components found in ginkgo are presented below ([Chemical Abstracts Service, 2014](#)).

(a) *Major flavonoids*

(i) *Quercetin-3-β-D-glucoside*

Chem. Abstr. Serv. Reg. No.: 482-35-9

IUPAC name: 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one

(ii) *Quercitrin*

Chem. Abstr. Serv. Reg. No.: 522-12-3

IUPAC name: 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3S,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxychromen-4-one

Description: Yellow crystalline substance ([Ding et al., 2006](#); [van Beek & Montoro, 2009](#); [O'Neil, 2013](#))

Melting-point: 174 °C

Solubility: Insoluble in cold water

(iii) *Rutin*

Chem. Abstr. Serv. Reg. No.: 153-18-4

IUPAC name: 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-

(1→6)-β-D-glucoopyranosyloxy]-4H-chromen-4-one

Description: Yellow-brownish powder ([Ding et al., 2006](#); [van Beek & Montoro, 2009](#); [O'Neil, 2013](#))

Melting-point: 195 °C ([O'Neil, 2013](#))

Solubility: Soluble in water ([O'Neil, 2013](#))

(b) *Minor flavonoids*

(i) *Quercetin*

Chem. Abstr. Serv. Reg. No.: 117-39-5

IUPAC name: 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one

Description: Yellow crystalline substance ([Ding et al., 2006](#); [van Beek & Montoro, 2009](#); [O'Neil, 2013](#))

Melting-point: 316 °C

Solubility: Insoluble in water

Quercetin was previously evaluated by IARC ([IARC, 1999](#)) as *not classifiable as to its carcinogenicity to humans* (Group 3).

(ii) *Kaempferol*

Chem. Abstr. Serv. Reg. No.: 520-18-3

IUPAC name: 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one

Description: Yellow powder ([Ding et al., 2006](#); [van Beek & Montoro, 2009](#); [O'Neil, 2013](#))

Melting-point: 276 °C

Solubility: Slowly soluble in water

(iii) *Isorhamnetin*

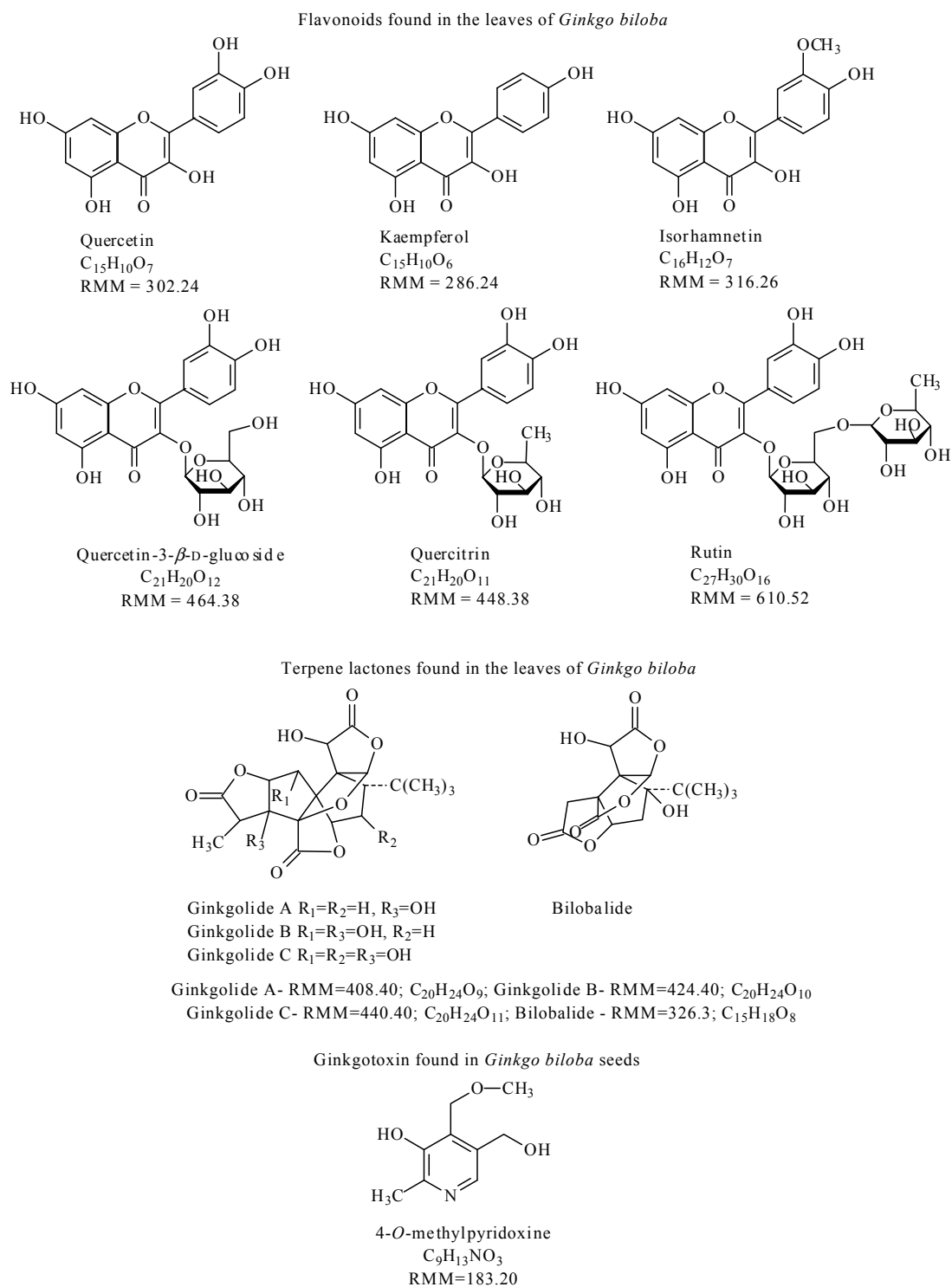
Chem. Abstr. Serv. Reg. No.: 480-19-3

IUPAC name: 3,5,7-trihydroxy-2-(4-hydroxy-3-methoxyphenyl)chromen-4-one

Description: Yellow powder ([Ding et al., 2006](#); [van Beek & Montoro, 2009](#); [O'Neil, 2013](#))

Melting-point: 307 °C

Fig. 1.1 Structural and molecular formulae and relative molecular mass of the major constituents found in *Ginkgo biloba*



RMM, relative molecular mass

From [Ding et al. \(2006\)](#) and [Leistner & Drewke \(2010\)](#)

(c) Lactone components

(i) Ginkgolide A

Chem. Abstr. Serv. Reg. No.: 15291-75-5

IUPAC name: 9H-1,7a-(epoxymethano)-1H,6aH-cyclopenta[c]furo[2,3-b]furo[3',2':3,4]-cyclopenta[1,2-d]furan-5,9,12(4H)-trione, 3-(1,1-dimethylethyl)hexahydro-4,7b-dihydroxy-8-methyl-, [1R-(1 α ,3 β ,3aS*,4 β ,6a α ,7a α ,7b α ,8 α ,10a α ,11aS*)]-)

Description: White crystalline substance ([Ding et al., 2006](#); [van Beek & Montoro, 2009](#); [O'Neil, 2013](#))

Melting-point: 280 °C

(ii) Ginkgolide B

Chem. Abstr. Serv. Reg. No.: 15291-77-7

IUPAC name: 9H-1,7a-(epoxymethano)-1H,6aH-cyclopenta[c]furo[2,3-b]furo[3',2':3,4]-cyclopenta[1,2-d]furan-5,9,12(4H)-trione, 3-(1,1-dimethylethyl)hexahydro-4,7b,11-trihydroxy-8-methyl-, [1R-(1 α ,3 β ,3aS*,4 β ,6a α ,7a α ,7b α ,8 α ,10a α ,11 β ,11aR*)]-)

Description: White crystalline substance ([Ding et al., 2006](#); [van Beek & Montoro, 2009](#); [O'Neil, 2013](#))

Melting-point: ~300 °C

(iii) Ginkgolide C

Chem. Abstr. Serv. Reg. No.: 15291-76-6

IUPAC name: 9H-1,7a-(epoxymethano)-1H,6aH-cyclopenta[c]furo[2,3-b]furo[3',2':3,4]-cyclopenta[1,2-d]furan-5,9,12(4H)-trione, 3-(1,1-dimethylethyl)hexahydro-2,4,7b,11-tetrahydroxy-8-methyl-, [1R-(1 α ,2 α ,3 β ,3aS*,4 β ,6a α ,7a α ,7b α ,8 α ,10a α ,11 α ,11aR*)]-)

Description: White crystalline substance ([Ding et al., 2006](#); [van Beek & Montoro, 2009](#); [O'Neil, 2013](#))

Melting-point: ~300 °C

(iv) Bilobalide

Chem. Abstr. Serv. Reg. No.: 33570-04-6

IUPAC name: (5aR-(3aS*,5a α ,8b,8aS*,9a,10a- α))-9-(1,1-dimethylethyl)-10,10a-dihydro-8,9-dihydroxy-4H,5aH,9H-furo[2,3-b]furo[3',2':2,3]cyclopenta[1,2-c]furan-2,4,7(3H,8H)-trione

(v) Ginkgotoxin

Chem. Abstr. Serv. Reg. No.: 1464-33-1

IUPAC name: 5-Hydroxy-3-(hydroxymethyl)-4-(methoxymethyl)-6-methylpyridine

1.1.3 Technical and commercial products, and impurities

Products containing dried ginkgo leaf, dried ginkgo leaf extract, and standardized dried ginkgo leaf extract are sold worldwide as herbal medicinal products, dietary supplements, and food additives. Tablets and capsules containing between 40–60 mg of extract are sold in the USA as dietary supplements ([Hänsel, 1991](#); [Brestel & Van Dyke, 1991](#)). In Europe, the extract has been sold primarily as a phytopharmaceutical in a variety of dosage forms such as tablets, liquids, and parenteral preparations ([Hänsel, 1991](#); [Brestel & Van Dyke, 1991](#)), and is now available as a herbal medicinal product and food supplement ([Lachenmeier et al., 2012](#)). Commercial products have been shown to contain variable amounts of the active constituents, and some also contain high amounts of ginkgolic acids ([McKenna et al., 2002](#); [Consumer Council, 2000](#); [Harnly et al., 2012](#)). The flavonoids present in ginkgo are primarily in the glycoside form ([Ding et al., 2006](#); [van Beek & Montoro, 2009](#)). The most widely used quality assurance assays (Association of Analytical Communities, AOAC; United States Pharmacopeia, USP, etc) are based on the measurement of free flavonoids obtained directly from the hydrolysis of the flavonoid

glycosides. Addition of cheaper plant material containing large amounts of rutin or other flavonoids and flavonoid glycosides to boost the apparent content of flavonol glycosides has been reported, and more recent tests call for evaluation of flavonoid ratios to detect such adulteration ([Harnly et al., 2012](#)).

Also, enzyme-assisted extraction has led to an increase in the amount of impurities in the extract ([van Beek & Montoro, 2009](#)). Commercial standardized *Ginkgo biloba* extracts are now prepared through a complex series of extractions and back-extractions using different solvents. The purpose is to purify the flavonol glycosides and to remove unwanted compounds ([Harnly et al., 2012](#)).

[Kressmann et al. \(2002\)](#) investigated the pharmaceutical quality and phytochemical composition of several different brands of *Ginkgo biloba* sold as dietary supplements in the USA. In-vitro dissolution characteristics and phytochemical content varied between products, in some cases dramatically (as for the ginkgolic acid content). [This study highlighted the difficulty of estimating exposure to botanical products and their constituent phytochemicals, which may be marketed under the same name, but have very different compositions.]

1.2 Analysis

Identification tests for *Ginkgo biloba* in the USP are based on high-performance thin-layer chromatography (HPTLC). Botanical identity and composition are confirmed by HPTLC, as well as macroscopic and microscopic examination ([USP, 2013](#)). A standardized developing solvent system for flavonoids includes ethyl acetate, anhydrous formic acid, glacial acetic acid, and water (100:11:11:26). The spraying reagents include 5 mg/mL of 2-aminoethyl diphenylborinate in methanol and 50 mg/mL of polyethylene glycol 400 in alcohol. A standardized developing solvent system for terpene

lactones includes toluene, ethyl acetate, acetone and methanol (20:10:10:1.2). Acetic anhydride is used as spraying reagent ([USP, 2013](#)).

The USP requires that a dry extract of *Ginkgo biloba* dried leaf is characterized by containing not less than 22% and not more than 27% of flavonoids calculated as flavonol glycosides via high-performance liquid chromatography (HPLC). The extract should also contain not less than 5.4% and not more than 12% of terpene lactones. *Ginkgo biloba* leaf extract is required to have a ratio of crude plant material to powdered extract between 35:1 and 67:1. A mobile phase composed of methanol, water, and phosphoric acid (100:100:1) is used for the content of flavonol glycosides. A gradient eluent mixture of methanol and water (25:75–90:10) is used for the content of terpene lactones ([USP, 2013](#)).

Validated HPLC methods for flavonoids and terpenes in ginkgo have been published ([Gray et al., 2007](#); [Croom et al., 2007](#)). Quantitative determination of the major components of ginkgo have also been reported using combination methods including HPLC, gas chromatography (GC), nuclear magnetic resonance (NMR), and mass spectrometry (MS) ([Ding et al., 2006](#); [van Beek & Montoro, 2009](#)). Methods that have been recently standardized for the quantitation of the major components of ginkgo include reversed-phase HPLC/ESI-MS (high-performance liquid chromatography/electrospray ionization-mass spectrometry), HPLC/MS-MS, GC-MS, and ¹H-NMR ([Ding et al., 2006](#); [van Beek & Montoro, 2009](#); [Li et al., 2004](#)).

Pharmacopeial standards are mandatory in registered drug products, but not in dietary supplement or food products, so it is difficult to evaluate the composition of marketed non-drug products.

1.3 Uses

1.3.1 Indications

Ginkgo leaves and fruit are used medicinally for a variety of conditions. Among the uses reported for ginkgo are treatment of asthma, bronchitis, cardiovascular diseases, improvement of peripheral blood flow, and reduction of cerebral function ([Perry, 1984](#); [Mouren et al., 1994](#)). Additional uses include allergies, bronchitis, tinnitus, dementia, and memory issues ([Morgenstern & Biermann, 1997](#); [Wang et al., 2010](#); [Holgers et al., 1994](#)). The World Health Organization and the German Commission E have included additional uses for peripheral arterial occlusive diseases ([WHO, 1999](#)).

Previous studies performed using standardized ginkgo extracts have also found therapeutic benefits for early stages of dementia, peripheral arterial occlusive diseases, cerebral insufficiency due to lack of adequate blood flow, and for other related ailments ([Wesnes et al., 2000](#); [Oken et al., 1998](#); [Pittler & Ernst, 2000](#); [Hopfenmüller, 1994](#)). Lately, several clinical trials to evaluate cognitive performance for Alzheimer disease, multiple sclerosis, and for peripheral arterial diseases have been conducted with mixed results ([Vellas et al., 2012](#); [Schneider, 2012](#); [Weinmann et al., 2010](#); [Snitz et al., 2009](#); [Herrschaft et al., 2012](#); [Nicolai et al., 2009](#); [Hilton et al., 2013](#)).

1.3.2 Dosage

According to the Commission E Monographs, 120–240 mg of standardized dry extract in liquid or solid pharmaceutical form for oral intake, is given in two or three daily doses for dementia syndromes, such as primary degenerative dementia, vascular dementia, and mixed forms of both. Doses of 120–160 mg of native dry extract is given in two or three daily doses for improvement of pain-free walking distance in peripheral arterial occlusive disease, and vertigo and tinnitus of vascular and involuntal origin

([Blumenthal et al., 1998](#)). These doses correspond to an estimate of 50 fresh ginkgo leaves to yield one standard dose of the extract. Dried extracts of leaves in the form of tablets, standardized to contain 24% flavone glycosides and 6% terpenes, are available commercially ([Brestel & Van Dyke, 1991](#); [McKenna et al., 2002](#); [Hilton et al., 2013](#); [Kleijnen & Knipschild, 1992](#)).

1.3.3 Trends in use

According to USA National Health and Nutrition Survey (NHANES) data, there has been a steady decline in the prevalence of use in men and women from 1999–2002 (3.9%), and 2003–2006 (3.0%), to 2007–2010 (1.6%) (NHANES, 1999–2010). [Barnes et al. \(2008\)](#) reported that in a survey of users of complementary and alternative medicine that 11.3% of supplement-users had used ginkgo in the previous 30 days.

1.4 Production, sales, and consumption

1.4.1 Production

Ginkgo has been planted on a large scale in France and USA since the 1980s and plantations are found in the south eastern USA with a density of 10 million ginkgo trees per 1000 acres ([Del Tredici, 1991, 2005](#)). A large amount of the ginkgo sold in the USA comes from plantations in China ([Schmid & Balz, 2005](#)).

1.4.2 Sales

Ginkgo biloba is the most frequently prescribed herbal medicine in Germany and one of the most commonly used over-the-counter herbal preparations in the USA ([Diamond et al., 2000](#)). The use of dietary supplements in the USA has significantly increased in the past few years. According to the 2012 Nutrition Business Journal Annual report ([Nutrition Business](#)

[Journal, 2012](#)), ginkgo was the 37th best-selling herbal dietary supplement in the USA in 2011, and the 13th best-selling (by dollar volume) herbal dietary supplement at US\$ 90 million. An estimated 3 million individuals in the USA used ginkgo in 2007, a relatively steady decline from approximately 7.7 million users, and sales of US\$ 151 million in 2002 ([Wu et al., 2011](#)). According to IMS Health MIDAS data (IMS [Health, 2012](#)), total worldwide sales of *Ginkgo biloba* products in 2012 were US\$ 1.26 billion. China accounted for 46% of sales (US\$ 578 million). Fifty-six individuals per 1000 reported using natural health products containing ginkgo in a Canadian survey in 2006 ([Singh & Levine, 2006](#)). Other countries with substantial sales included Germany (US\$ 152 million), Australia (US\$ 61 million), France (US\$ 53 million), Brazil (US\$ 48 million), Republic of Korea (US\$ 40 million), and Viet Nam (US\$ 37 million).

1.4.3 Consumption

Consumption of ginkgo occurs orally or topically in pharmaceutical or dietary-supplement formulations ([Hänsel, 1991](#); [Brestel & Van Dyke, 1991](#); [Blumenthal et al., 1998](#)). Consumers may also be exposed through products containing ginkgo, such as teas, yogurts, and cosmetics that are sold over the internet. [The Working Group also noted that besides the use in medicinal products and supplements, ginkgo has also been used in Europe as ingredient in foods, as in the so-called “wellness” beverages.]

Ginkgo biloba extract is one of four herbal preparations refunded by health insurance in Germany, on prescription by a medical doctor ([AMR, 2013](#)).

1.5 Occupational exposure

No data were available to the Working Group. Workers on ginkgo plantations and in ginkgo-processing plants are probably exposed.

1.6 Regulations and guidelines

According to the 1994 Dietary Supplement Health and Education Act (DSHEA) in the USA, ginkgo is considered a dietary supplement under the general umbrella of “foods” ([FDA, 1994](#)). In the USA, dietary supplements put on the market before 15 October 1994 do not require proof of safety; however, the labelling recommendations for dietary supplements include warnings, dosage recommendations, and substantiated “structure or function” claims. The product label must declare prominently that the claims have not been evaluated by the Food and Drug Administration, and bear the statement “This product is not intended to diagnose, treat, cure, or prevent any disease” ([Croom & Walker, 1995](#)).

In Europe, ginkgo is available either as food supplement or as medicinal product. The Commission E approved different types of ginkgo preparations for human consumption ([Diamond et al., 2000](#)) and published a monograph dedicated to standardized ginkgo leaf extract ([Mills & Bone, 2005](#)). In Europe, most herbal products, including ginkgo, were marketed as medicinal products. This changed when Directive 65/65/EEC ([Council of the European Economic Community, 1965](#)) was implemented, with the requirement of quality, efficacy, and safety data on medicinal products. Many medicinal products did not satisfy those requirements and were nevertheless marketed as food supplements, often just changing the label. This is currently the case for ginkgo, which is widely marketed as a food supplement in Europe ([Lachenmeier et al., 2012](#)).

2. Cancer in Humans

2.1 Background

The available epidemiological studies on ginkgo and cancer consisted of one randomized controlled trial (the Ginkgo Evaluation of

Memory, GEM study), four nested case–control studies from the Vitamins and Lifestyle (VITAL) cohort, and one population based case–control study of cancer of the ovary.

2.2 Randomized controlled trial

See [Table 2.1](#)

The Ginkgo Evaluation of Memory (GEM) study is a randomized, double-blind, placebo-controlled clinical trial on *Ginkgo biloba* extract (EGb 761®) for the prevention of dementia ([DeKosky et al., 2008](#); [Biggs et al., 2010](#)). Participants aged 75 years and older ($n = 3069$) from four clinical centres in the USA were enrolled between 2000 and 2002, and were randomized to either the placebo group, or the group receiving ginkgo as two daily doses of 120 mg of ginkgo extract. Participants were followed until 2008, with a median follow-up of 6.1 years. [The authors did not specify the period of treatment.] Invasive cancer (excluding non-melanoma cancer of the skin) was evaluated as a secondary outcome, and identified from hospital admission and discharge records. Analyses were performed with intention to treat beginning at randomization and at 1 year after randomization. Adherence to study protocol was 64% for the placebo group and 59% for the group receiving ginkgo.

Of the 310 hospitalizations for cancer, 162 occurred in the group receiving ginkgo, and 148 occurred in the group receiving placebo (adjusted hazard ratio, HR, 1.09; 95% CI, 0.87–1.36). For the observation period beginning at randomization, elevated hazard ratios were reported for cancers of the colon and rectum (HR, 1.62; 95% CI, 0.92–2.87), urinary bladder (HR, 1.21; 95% CI, 0.65–2.26) and breast (HR, 2.15; 95% CI, 0.97–4.80) and a decreased hazard ratio was found for cancer of the prostate (HR, 0.71; 95% CI, 0.43–1.17). The hazard ratios for cancer of the lung and combined leukaemia and lymphoma were close to unity (see [Table 2.1](#) for values). In general, hazard ratios for the second observation

period, beginning 1 year after randomization, were generally similar, but with a statistically significant increase in the risk of cancer of the breast (HR, 2.50; 95% CI, 1.03–6.07) and lower risk of cancer of the bladder (HR, 0.99; 95% CI, 0.52–2.61). The hazard ratios from a sensitivity analysis excluding participants who reported cancer 5 years before baseline were within 20%, with the exception of an increase for cancer of the colorectum reaching statistical significance (HR, 2.15; 95% CI, 1.11–4.15) and a attenuation of risk of cancer of the breast, losing statistical significance (HR, 1.55; 95% CI, 0.66–3.63). [The major strengths of the study were randomization of treatment groups and adequate follow-up procedures for identifying cancer cases hospitalized during follow-up; however, incident cancers not resulting in hospitalization may have been missed and the follow-up was short, so the statistical power for cancer was low, and long-term carcinogenic effects due to exposure could not be evaluated. The study findings were difficult to interpret since the sites with statistically significant associations were not consistent in different analyses. Furthermore, the generalizability of the findings was limited by the clinical trial design.]

2.3 Case–control studies

See [Table 2.2](#)

The VITAL cohort includes 77 738 men and women, aged 50–76 years, in Washington state, USA, who completed a postal questionnaire on supplement use, diet, health history, and risk factors between 2000 and 2002 ([White et al., 2004](#)). The overall response rate was about 23%. Detailed data on use of vitamins, mineral supplements, herbal preparations and related products in the past 10 years were obtained via questions regarding brand, current and past use, frequency and duration of use. Dose information was not obtained because of lack of accurate information on potency. Cohort members were followed for 5–6 years, with incident cancer cases or deaths

Table 2.1 Randomized intervention trial on exposure to *Ginkgo biloba* extract

Reference, study location and period	Total subjects	Study design	Organ site	Exposure categories	Exposed cases	Hazard ratio (95% CI)	Covariates Comments
Biggs <i>et al.</i> (2010) , USA 2000–8	3069	Randomized double-blind, placebo-controlled clinical trial	Any	Ginkgo extract: 120 mg, 2× per day; randomization observation period	162	1.09 (0.87–1.36)	Adjusted for clinical centre. GEM study, participants aged ≥ 75 yrs, intention to treat % compliance: placebo, 64%; ginkgo extract, 59% Sensitivity analysis performed excluding participants with a 5-yr history of cancer before enrolment. Median follow-up, 6.1 yr End-point assessment from hospital admissions and discharge records
			Prostate		27	0.71 (0.43–1.17)	
			Lung		26	0.90 (0.53–1.52)	
			Colorectal		31	1.62 (0.92–2.87)	
			Urinary bladder		22	1.21 (0.65–2.26)	
			Breast		18	2.15 (0.97–4.80)	
			Leukaemia and lymphoma		13	1.07 (0.49–2.34)	
			Any		150	1.07 (0.85–1.35)	
			Prostate		25	0.68 (0.41–1.14)	
			Lung		27	1.00 (0.58–1.70)	
			Colorectal		22	1.27 (0.68–2.40)	
			Urinary bladder		18	0.99 (0.52–1.91)	
			Breast		16	2.50 (1.03–6.07)	
			Leukaemia and lymphoma		13	1.17 (0.52–2.61)	

GEM, Ginkgo Evaluation of Memory; yr, year

Table 2.2 Case-control studies of cancer and exposure to *Ginkgo biloba* extract

Reference, study location and period	Total No. cases Total No. controls	Control source (hospital, population)	Exposure assessment	Organ site	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Satia et al. (2009) , Washington State, USA	Lung cancer, 665 76 460 Colorectal cancer, 428 76 084	Nested case-control study: VITAL cohort	Mailed baseline questionnaire; supplement use 10 yrs before baseline	Lung cancer Colorectal cancer	Any pill per day – previous 10 yrs	80 49	1.04 (0.82–1.32) 0.83 (0.59–1.17)	Age, sex, education, smoking Age, sex, education, physical activity, fruit and vegetable consumption, BMI, NSAID use, and sigmoidoscopy Age 50–76 yr; low response rate (21.8%); cases identified by cancer registry (SEER); follow-up until 2006 (mean, 5 yr)
Hotaling et al. (2011) , Washington State, USA	330 76 720	Nested case-control study: VITAL cohort	Same as Satia et al. (2009)	Urothelial carcinoma of the bladder	10 yr daily average	NR	NR (no statistical significant association in multivariate analysis)	Age, sex, race, education, family history of bladder cancer, smoking, and fruit and vegetable intake Age 50–76 yr; cases identified by cancer registry (SEER); follow-up, 6 yr
Brasky et al. (2010) , Washington State, USA	880 34 136	Nested case-control study: VITAL cohort	Same as Satia et al. (2009)	Breast cancer	Former Current 10 yr daily average low (< 4 days/wk or any use < 3 yr) high (≥ 4 days/wk for ≥ 3 yr) Trend	47 56 82 40	1.06 (0.77–1.45) 0.85 (0.64–1.13) 0.98 (0.77–1.25) 0.88 (0.63–1.24) P = 0.51	Adjusted for age, race, education, BMI, height, fruit & vegetable consumption, alcohol consumption, physical activity, reproductive history, history of hysterectomy, hormone therapy, family history of breast cancer and benign breast biopsy, mammography, use of aspirin, ibuprofen, naproxen and multivitamins. Postmenopausal women; excluded women with history of breast cancer or with in-situ breast cancer; participation rate, 23%; mean follow-up, 6 yr

Table 2.2 (continued)

Reference, study location and period	Total No. cases Total No. controls	Control source (hospital, population)	Exposure assessment	Organ site	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Brasky et al. (2011) , Washington State, USA	1602 33 637	Cancer-registry study: VITAL cohort	Same as Satia et al. (2009)	Prostate cancer (invasive)	<i>Use</i>	165	1.03 (0.87–1.22)	Age, race, education, BMI, PSA test, history of prostate disease, family history of prostate cancer, multivitamin use, memory loss, diabetes. Males: age 50–76 yr.; low response rate (~22%); cases identified by cancer registry (SEER); follow-up; 6 yr
					Average, 10 yr	127	1.08 (0.90–1.31)	
					Low (< 4 days/wk or any use < 3 yr)	85	1.04 (0.82–1.31)	
					High (≥ 4 days/wk for ≥ 3 yr)		<i>P</i> = 0.52	
Ye et al. (2007) MA, NH, USA, 1998–2003	668 721	Population	In-person interviews and self-administered dietary questionnaires	Ovarian cancer (epithelial)	Weekly; at least 6 months	11	0.41 (0.20–0.84)	Age, study centre, oral contraceptive use, parity, and Jewish ethnic background RR similar for current versus no-longer-using, no association with exposure duration; however, small number of exposed cases. Participation rate: cases, 52%; controls, 39%
					Ginkgo alone (not other drugs)	6	0.36 (0.14–0.91)	
					<i>Tumour type</i>			
					Mucinous	3	1.17 (0.34–4.05)	
Non-mucinous	8	0.33 (0.15–0.74)						

BMI, body mass index; NR, not reported; NSAID, nonsteroidal anti-inflammatory drugs; PSA, prostate-specific antigen; RR, relative risk; SEER, Surveillance, Epidemiology, and End Results programme of the National Cancer Institute; VITAL; VITamins and Lifestyle Cohort Study; wk, week; yr, year

identified via linkage system to the SEER cancer registry, state death files, and other databases.

Associations between ginkgo intake and cancer were investigated in nested case–control studies with the large VITAL cohort for cancers of the lung and colorectum ([Satia et al., 2009](#)), prostate ([Brasky et al., 2011](#)), breast ([Brasky et al., 2010](#)), and urothelial carcinoma of the bladder ([Hotelling et al., 2011](#)) (see [Table 2.2](#) for more information). No statistically significant increase or decrease in the occurrence of any of the cancers examined was observed in these studies. Hazard ratios were near unity for all associations reported, except cancer of the colorectum and any use of ginkgo (HR, 0.83; 95% CI, 0.59–1.17) and cancer of the breast and current use (HR, 0.85; 95% CI, 0.65–1.13) or high average use (HR, 0.88; 95% CI, 0.63–1.24). [The strengths of these studies were the prospective design, adequate case-ascertainment, broad age range of the study participants, and large size. The major limitations were use of self-reported exposure information, which was not updated after baseline; short follow-up time; low response rates, and inadequate evaluation of exposure–response and exposure–time relationships. These limitations would most likely bias the findings towards the null.]

[Ye et al. \(2007\)](#) conducted a population-based case–control study of 668 incident cases of cancer of the ovary (identified from state registries and tumour boards) and 721 age- and residence-matched general population controls. Approximately 53% of eligible cases and 39% of eligible controls participated. Intake of ginkgo and other herbal remedies, dietary information and other factors was assessed via in-person interview and self-administered questionnaire. Ginkgo intake at least weekly for 6 months or more was associated with a decreased risk of cancer of the ovary (adjusted odds ratio, OR, 0.41; 95% CI, 0.20–0.84). However, the reduced risk was restricted to women with non-mucinous ovarian tumours (OR, 0.33; 95% CI, 0.15–0.74; eight exposed cases). This study also included

cell-culture analyses showing antiproliferative effects of *G. biloba* extract in serous (non-mucinous), but not in mucinous ovarian cancer cells. [The consistency of findings in cell culture and humans was a strength of this study. Limitations of the study were low statistical power, low participation rates, exposure assessment only for 1 year before diagnosis, and lack of information on dose.]

3. Cancer in Experimental Animals

3.1 Mouse

See [Table 3.1](#)

In one study of oral administration, groups of 50 male and 50 female B6C3F₁ mice (age, 6–7 weeks) were given *Ginkgo biloba* extract at a dose of 0 (corn oil vehicle, 5 mL/kg body weight, bw), 200, 600, or 2000 mg/kg bw by gavage, 5 days per week, for 104 weeks. The *G. biloba* extract contained 31.2% flavonol, 15.4% terpene lactones (bilobalide, 6.94%; ginkgolide A, 3.74%; ginkgolide B, 1.62%; ginkgolide C, 3.06%), and ginkgolic acid at 10.45 ppm. Survival of males at 600 and 2000 mg/kg bw was significantly less than that of controls; survival of females at 600 mg/kg bw was significantly greater than that of controls. Mean body weights of males at 600 and 2000 mg/kg bw were less (10% or more) than those of the controls after weeks 85 and 77, respectively; mean body weights of females at 2000 mg/kg bw were generally less (10% or more) than those of the controls between weeks 17 and 69, and after week 93.

In males, the incidence of hepatocellular carcinoma, hepatocellular adenoma or carcinoma (combined), and hepatoblastoma was significantly increased in the groups receiving the lowest, intermediate, and highest dose, and had a significant positive trend. Hepatocellular carcinoma and hepatoblastoma were observed in the same animal on multiple occasions. The

Table 3.1 Studies of carcinogenicity with *Ginkgo biloba* extracts in mice

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance ^a	Comments
B6C3F ₁ (M, F) 104–105 wk NTP (2013) , Hoenerhoff et al. (2013)	0 (control), 200, 600, 2000 mg/kg bw, by gavage in corn oil, 5 days/wk for 104–105 wk 50 M and 50 F/group (age, 6–7 wk)	Hepatocellular adenoma: 17/50*, 37/50**, 41/50**, 48/50** (F) Hepatocellular carcinoma: 22/50*, 31/50***, 41/50**, 47/50** (M) 9/50*, 10/50, 15/50, 44/50** (F) Hepatocellular adenoma or carcinoma (combined): 39/50*, 46/50****, 46/50****, 49/50** (M) 20/50*, 39/50**, 41/50**, 49/50** (F) Hepatoblastoma: 3/50*, 28/50**, 36/50**, 38/50** (M) 1/50*, 1/50, 8/50***, 11/50****(F) Thyroid gland follicular cell adenoma: 0/49, 0/49, 2/50, 2/50 (4%) (M) ^b	* <i>P</i> < 0.001 (trend) ** <i>P</i> ≤ 0.001 *** <i>P</i> ≤ 0.05 **** <i>P</i> ≤ 0.01	<i>Ginkgo biloba</i> study material contained: 31.2% flavonol, 15.4% terpene lactones (bilobalide, 6.94%; ginkgolide A, 3.74%; ginkgolide B, 1.62%; ginkgolide C, 3.06%), ginkgolic acid, 10.45 ppm HPLC/UV profiles identified 37 components: quercetin, 34.08%; kaempferol, 27.7%; isorhamnetin, 5.43%. HPLC/ELS profiles identified 18 components: bilobalide, 17.31%; ginkgolide C, 3.25%; ginkgolide A, 9.06%; ginkgolide B, 2.05%; quercetin, 28.74%; kaempferol, 12.58%; isorhamnetin, 2.24%. Mean body weight of males at 600 and 2000 mg/kg bw were ≥ 10% less than the vehicle-control groups after wk 85 and 77, respectively. Mean body weight of females at 2000 mg/kg bw was ≥ 10% less than the vehicle-control group between wk 17 and 69 and after wk 93

^a The Poly-3 test was used for all statistical analysis in this table.

^b Historical incidence for 2-year gavage studies with corn oil vehicle-control groups (mean ± standard deviation): 1/349 (0.3% ± 0.8%), range, 0–2%; all routes: 7/1143 (0.6% ± 1.0%), range, 0–2%.

bw, body weight; ELS, evaporative light scattering; F, female; HPLC/UV, high-performance liquid chromatography/ultraviolet; M, male; wk, week

Table 3.2 Studies of carcinogenicity with *Ginkgo biloba* extracts in rats

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance ^a	Comments
F344/N (M, F) 104–105 wk NTP (2013)	0 (control), 100, 300, 1000 mg/kg bw, by gavage in corn oil, 5 days/wk for 104–105 wk 50 M and 50 F/group (age, 6–7 wk)	Mononuclear cell leukaemia: 9/50*, 12/50, 22/50**, 21/45** (M) Thyroid follicular cell adenoma: 2/50***, 1/50, 3/50, 5/45 (M) 1/49, 0/50, 3/49, 1/49 (F) ^b Thyroid follicular cell carcinoma: 0/49, 0/50, 1/49, 1/49 (F) ^c Nose respiratory epithelium adenoma: 0/49, 0/49, 2/50, 0/46 (F) ^d	* <i>P</i> = 0.004 (trend) ** <i>P</i> ≤ 0.01 *** <i>P</i> = 0.04	<i>Ginkgo biloba</i> study material contained: 31.2% flavonol, 15.4% terpene lactones (bilobalide, 6.94%, ginkgolide A, 3.74%; ginkgolide B, 1.62%; ginkgolide C, 3.06%), ginkgolic acid. 10.45 ppm HPLC/UV profiles identified 37 components: quercetin, 34.08%; kaempferol, 27.7%; isorhamnetin, 5.43% HPLC/ELS profiles identified 18 components: bilobalide, 17.31%; ginkgolide C, 3.25%; ginkgolide A, 9.06%; ginkgolide B, 2.05%; quercetin, 28.74%; kaempferol, 12.58%; isorhamnetin, 2.24% Mean body weight of males at 300 mg/kg bw, ≥ 10% less than the vehicle-control group after wk 93; at 1000 mg/kg bw, ≥ 10% less than the vehicle-control group after wk 89. Mean body weight of females: at 300 mg/kg bw: ≥ 10% less than the vehicle-control group after wk 93; at 1000 mg/kg bw, ≥ 10% less than the vehicle-control group after wk 89. Body-weight suppression at highest dose could have reduced tumour incidences.

^a The Poly-3 test was used for all statistical analyses in this table.

^b Historical incidence for 2-year gavage studies with corn-oil vehicle-control groups (mean ± standard deviation): 3/298 (1.0% ± 1.1%), range 0–2%; all routes: 8/1186 (0.7% ± 1.0%), range, 0–2%

^c Historical incidence for 2-year gavage studies with corn-oil vehicle-control groups (mean ± standard deviation): 1/298 (0.3% ± 0.8%), range 0–2%; all routes: 5/1186 (0.4% ± 1.0%), range, 0–4%

^d Historical incidence 2-year gavage studies with corn-oil vehicle-control groups (mean ± standard deviation): 0/299; all routes: 1/1196 (0.1% ± 0.4%), range, 0–2%

bw, body weight; ELS, evaporative light scattering; F, female; HPLC/UV, high-performance liquid chromatography/ultraviolet; M, male; wk, week

incidence of thyroid follicular cell adenoma was higher in the group receiving the intermediate dose (2 out of 50; 4%) and highest dose (2 out of 50; 4%) groups than among the historical controls in the National Toxicology Program (NTP) Technical Report study series (historical incidence range, 0–2%; corn oil vehicle controls, 1 out of 349; all routes, 7 out of 1449).

In females, the incidence of hepatocellular adenoma was significantly higher in the groups receiving the lowest, intermediate, and highest dose, and had a significant positive trend. The incidence of hepatocellular carcinoma was significantly higher in the group receiving the highest dose, and had a significant positive trend. The incidence of hepatocellular adenoma or carcinoma (combined) was significantly higher in the groups receiving the lowest, intermediate, and highest dose, and had a significant positive trend. The incidence of hepatoblastoma was significantly higher at the intermediate and highest dose, and had a significant positive trend ([Hoenerhoff et al., 2013](#); [NTP, 2013](#)).

3.2 Rat

See [Table 3.2](#)

In one study of oral administration, groups of 50 male and 50 female F344/N rats (age, 6–7 weeks) were given *G. biloba* extract at a dose of 0 (corn oil vehicle, 2.5 mL/kg bw), 100, 300, or 1000 mg/kg bw by gavage, 5 days per week, for 104 weeks for males or 105 weeks for females. The *G. biloba* extract contained 31.2% flavonol, 15.4% terpene lactones (bilobalide, 6.94%; ginkgolide A, 3.74%; ginkgolide B, 1.62%; ginkgolide C, 3.06%), and ginkgolic acid at 10.45 ppm. Survival of males at 1000 mg/kg bw was significantly less than that of the controls. Mean body weights of males and females at 300 mg/kg bw were less (10% or more) than those of the controls after week 93, and those of males and females at 1000 mg/kg bw were less (10% or more) after week 89.

In males, the incidence of mononuclear cell leukaemia was significantly higher in groups at the intermediate and highest dose, and had a significant positive trend. The incidence of thyroid follicular cell adenoma (2 out of 50, 4%; 1 out of 50, 2%; 3 out of 50, 6%; and 5 out of 45, 10%) had a significant positive trend. In females, the incidences of thyroid follicular cell adenoma (1 out of 49, 0 out of 50, 3 out of 49, 1 out of 49), thyroid follicular cell carcinoma (0 out of 49, 0 out of 50, 1 out of 49, 1 out of 49), and nose respiratory epithelium adenoma (0 out of 49, 0 out of 49, 2 out of 50, 0 out of 46) in the dosed groups were higher than the ranges for historical controls (all routes: 8 out of 1186 [0–2%], 5 out of 1186 [0–4%] and 1 out of 1186 [0–2%], respectively) in the NTP Technical Report study series ([NTP, 2013](#)). [The Working Group noted that body-weight suppression at the highest dose could have reduced the tumour incidence, and that nose respiratory epithelium adenomas are rare spontaneous tumours in F344/N rats.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Several pharmacokinetics studies on the main active components of *Ginkgo biloba* in humans have been reported ([Fourtillan et al., 1995](#); [Wójcicki et al., 1995](#); [Pietta et al., 1997](#); [Mauri et al., 2001](#); [Drago et al., 2002](#); [Wang et al., 2003](#)).

[Wójcicki et al. \(1995\)](#) investigated the pharmacokinetics of flavonoid glycosides in 18 healthy volunteers given one of three different formulations of *Ginkgo biloba* (capsules, drops, and tablets) by oral administration. The area-under-the-curve values were similar for all

formulations. [Drago et al. \(2002\)](#) evaluated the pharmacokinetics of ginkgolide B, the main active ingredient in *G. biloba* extract, in 12 healthy volunteers given different doses (80 mg once daily, or 40 mg twice daily, for 7 days). The results indicated that the maximum concentration time (T_{\max}) was 2.3 hours for both dosages, and the elimination half-life ($t_{1/2\beta}$) and mean residence time (MRT) were longer for the dose of 40 mg given twice daily than that for a single 80 mg dose, although the latter had a higher concentration peak (C_{\max}) ([Drago et al., 2002](#)). After oral administration of a tablet containing *G. biloba* extract in humans, two polyphenols, quercetin and kaempferol were found in urine mainly as glucuronides, and to a lesser extent, sulfates ([Wang et al., 2003](#)).

As part of a clinical study to determine the metabolites of *G. biloba* after human oral consumption, an extract of *G. biloba* leaves was given to six healthy volunteers [sex, age, and weight not specified] as a single dose of 4.0 g per day ([Pietta et al., 1997](#)). Urine samples were collected for 2 days, and blood samples were withdrawn every 30 minutes for 5 hours. The samples were purified through solid-phase extraction with C_{18} cartridges (SPE C_{18} cartridges) and analysed by reversed-phase liquid chromatography–diode array detection for the presence of metabolites. Only urine samples contained detectable amounts of substituted benzoic acids, i.e. 4-hydroxybenzoic acid conjugate, 4-hydroxyhippuric acid, 3-methoxy-4-hydroxyhippuric acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, hippuric acid and 3-methoxy-4-hydroxybenzoic acid (vanillic acid). No metabolites were detected in the blood ([Pietta et al., 1997](#); see [Fig. 4.1](#)).

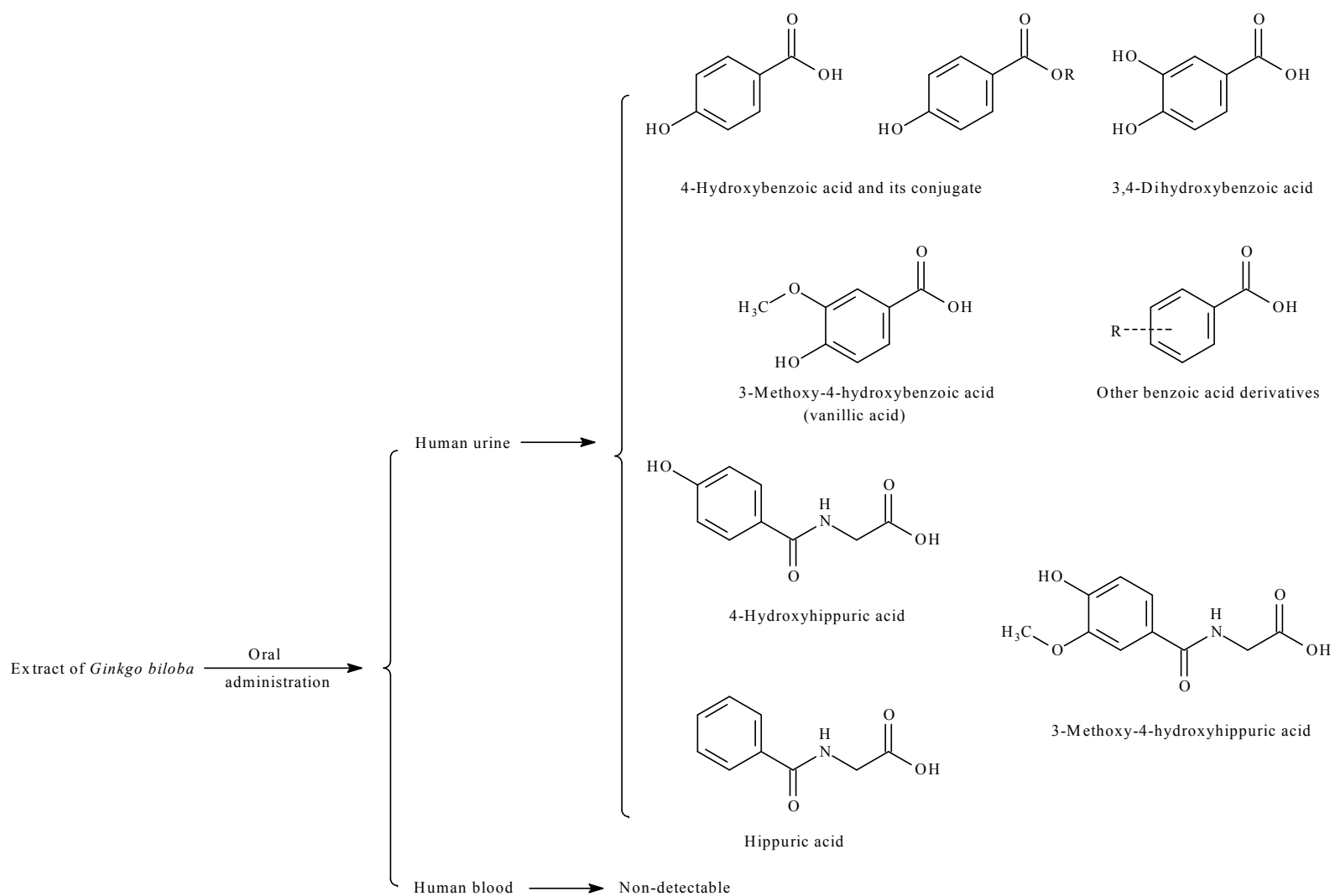
The pharmacokinetics of quercetin and its glycosides (components of *G. biloba*) have been extensively studied in humans ([Prior, 2006](#)). Quercetin glycoside was originally assumed to be absorbed from the small intestine after cleavage of the β -glycoside linkage by colonic microflora

([Griffiths & Smith, 1972](#)). Excretion of quercetin or its conjugates in human urine ranged from 0.07% to 17.4% of intake. Only quercetin glucuronides, but not free quercetin, could be detected in the plasma ([Prior, 2003](#)).

4.1.2 Experimental systems

Several studies have addressed the issue of absorption and excretion of *Ginkgo biloba* in rats and mice ([Moreau et al., 1986](#); [Chen et al., 2007](#); [Ude et al., 2013](#)). In one study in which ^{14}C -labelled *G. biloba* extract (360 mg/kg bw) was administered orally to rats, the amount expired as carbon dioxide only accounted for 16% of the original dose within the first 3 hours. About 38% of the administered dose was exhaled as carbon dioxide after 72 hours; 22% was excreted in the urine, and 29% was excreted in the faeces. Absorption reached at least 60%. A half-life of 4.5 hours and peak of 1.5 hours marked the pharmacokinetics of *G. biloba* in serum, with the characterization of a first-order phase kinetic model. After 48 hours of gradual uptake, radiolabel was primarily found in the plasma. The activity in the erythrocytes was similar to that in the plasma. It was also present in the neuronal, glandular, and ocular tissue, and it was suggested that the upper gastrointestinal tract plays a role in absorption ([Moreau et al., 1986](#)). In rats but not in humans, phenylacetic acid or phenylpropionic acid derivatives were found in the urine after oral administration of *G. biloba* leaf extract ([Pietta et al., 1995](#)). Recent studies in rats have shown that significant amounts of terpene trilactones (ginkgolides A and B, and bilobalide) and flavonoids (quercetin, kaempferol, and isorhamnetin) cross the blood–brain barrier and enter the central nervous system after intravenous and oral administration of *G. biloba* extract ([Chen et al., 2007](#); [Rangel-Ordóñez et al., 2010](#)).

Fig. 4.1 Metabolites of *Ginkgo biloba* in humans



Subjects were given *Ginkgo biloba* extract orally. Urine samples contain detectable amounts of metabolites of *Ginkgo biloba*, but no metabolites were detected in the blood
 Compiled by the Working Group from data in [Pietta et al. \(1997\)](#)

4.1.3 Herb–drug interactions

Despite potential therapeutic effects, the widespread use of *G. biloba* extract may also cause herb–drug interactions, altering drug efficiency or leading to undesired toxic effects of concurrent medications, especially for drugs with narrow therapeutic indices. A growing body of literature has shown that *G. biloba* extract and its constituents may influence the pharmacokinetics of coadministered drugs via altering the expression and activity of drug-metabolizing enzymes and transporters. However, the results from in-vitro studies were not always in agreement with those from in-vivo studies; and two similar clinical studies also showed disparities. For instance, in-vitro studies with human microsomes demonstrated that *G. biloba* extract inhibited cytochrome P450 (CYP) CYP2C9 ([Mohutsky et al., 2006](#); [Etheridge et al., 2007](#)), while clinical studies showed that *G. biloba* extract had no significant effect on CYP2C9 ([Greenblatt et al., 2006](#); [Mohutsky et al., 2006](#); [Uchida et al., 2006](#)). [Robertson et al. \(2008\)](#) reported that *G. biloba* extract induced the activity of CYP3A4 in a clinical study, while other studies reported that *G. biloba* extract did not alter CYP3A4 ([Gurley et al., 2002](#); [Zadoyan et al., 2012](#)), or decreased CYP3A4 activity ([Uchida et al., 2006](#)). [The discrepancy was probably due to multiple factors, for example, different approaches for assessing enzyme activity were applied, different formulations of herbal materials were used, some studies did not include a sufficient sample size to meet statistical requirements; and the sensitivity of detection methods was different; or the studies were conducted in different ethnic groups.]

Clinical studies and case reports have identified herb–drug interactions potentiated by the concurrent use of *G. biloba* extract and prescription drugs, many of which are substrates of CYPs and/or transport P-glycoprotein 1 (multidrug resistance protein 1); these studies are reviewed by [Chen et al. \(2011, 2012\)](#).

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

See [Table 4.2](#)

(a) Mutagenicity

Ginkgo biloba extract (up to 10 000 µg/plate) was mutagenic in *Salmonella typhimurium* strains TA98 and TA100 and *Escherichia coli* strain WP2 *uvrA* pKM101 with or without metabolic activation from rat liver S9 ([NTP, 2013](#)). Two components of *G. biloba* extract, quercetin and kaempferol, were also found to give positive results in these assays and in other assays for mutagenicity with and without metabolic activation ([NTP, 1992, 2013](#)). *G. biloba* constituents that induce mutagenicity include quercetin ([Bjeldanes & Chang, 1977](#); [Hardigree & Epler, 1978](#); [Carver et al., 1983](#); [NTP, 1992](#); [Zeiger et al., 1992](#); [Chan et al., 2007](#)), kaempferol ([Silva et al., 1997](#)), and ginkgolic acids ([Westendorf & Regan, 2000](#)).

(b) Chromosomal damage

No increase in the frequency of micronucleus formation in peripheral blood erythrocytes was observed in male B6C3F₁ mice, but results were equivocal in female B6C3F₁ mice exposed to *G. biloba* extract at a dose of up to 2.0 g/kg bw per day by gavage for 3 months ([NTP, 2013](#)). Quercetin and kaempferol were also found to produce chromosomal alteration in various types of cells ([NTP 1992, 2013](#); [Gaspar et al., 1994](#); [Caria et al., 1995](#); [Silva et al., 1997](#)).

(c) DNA damage

A study has examined and compared the genotoxicity of flavonoids in human somatic cells and germ cells. Human somatic cells (human

Table 4.2 Genetic and related effects of *Ginkgo biloba* extract

Test system	Results		Concentration or dose (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Salmonella typhimurium</i> TA100 and TA98, reverse mutation	+	+	10 000 µg/plate	NTP (2013)
<i>Escherichia coli</i> WP2 <i>uvrA</i> /pKM101 reverse mutation	+	+	10 000 µg/plate	NTP (2013)
Micronucleus formation in peripheral blood erythrocytes of male and female B6C3F ₁ mice in vivo	– (males), EQ (females)	NT	Up to 2.0 g/kg bw per day by gavage for 3 months	NTP (2013)

+, positive; (+), weakly positive; –, negative; bw, body weight; LED, lowest effective dose; NT, not tested; NR, not reported; EQ, equivocal

lymphocytes) and germ cells (human sperm cells) were treated with flavonoids (including quercetin, kaempferol, and rutin, which are present in *G. biloba* extract) at a concentration of 50–500 µM and their DNA damage potentials were examined using the comet assay ([Anderson et al., 1997](#)). Results show that DNA damage was observed in lymphocytes and sperm cells over a similar dose range. [The Working Group noted that genotoxic responses occurred in somatic and germ cells in approximately a one-to-one ratio.] In another study [Duthie et al. \(1997\)](#) showed DNA strand breaks (as measured by comet assay) in human cell lines Caco-2 (colon), HepG2 (liver), HeLa (epithelium) and normal lymphocytes after treatment with quercetin.

4.3 Other mechanistic data relevant to carcinogenicity

4.3.1 Effects on cell physiology

Increased levels of thyroid stimulating hormone (TSH) were observed in rats after treatment with *G. biloba* extract for 14 weeks ([NTP, 2013](#)). In a 3-month study, follicular cell hypertrophy was observed in male and female rats ([NTP, 2013](#)).

G. biloba extract and its constituents including quercetin, kaempferol, and isorhamnetin, may

exert estrogenic activity by directly binding both estrogen receptors α and β ([Oh & Chung, 2004, 2006](#)). The proliferation of MCF-7 cells in response to *G. biloba* extract was biphasic depending on the concentrations of extract and E2 (17 β -estradiol) via estrogen receptor-dependent and independent pathways. In MCF-7 cells, *G. biloba* extract induced cell proliferation at low concentrations of E2, which has little or no estrogenic activity, but blocked the cell proliferation caused by higher concentrations of E2, which shows high estrogenic activity ([Oh & Chung, 2006](#)).

Most studies have focused on the pharmacological effects of *G. biloba*. *G. biloba* extract and its constituents have been shown to be involved in many cellular activities, such as anti-oxidant activity, anti-platelet activating factor, anti-inflammatory effect, inhibition of mitochondrial dysfunction, inhibition of amyloid β aggregation in neuroblastoma cells, and anti-apoptosis activity ([Smith & Luo, 2004](#); [Chan et al., 2007](#); [Shi et al., 2010a, b](#)).

4.3.2 Effects on cell function

No data were available to the Working Group.

4.4 Susceptibility

No data were available to the Working Group.

4.5 Mechanistic considerations

The genotoxicity of *G. biloba* extract could be one of the mechanisms responsible for its possible carcinogenicity. In addition, quercetin and kaempferol, the two flavonoid constituents that are present in high levels in *G. biloba* extract, are mutagenic as examined in several assays in vitro and may thus contribute to the genotoxicity of the *G. biloba* extract.

Quercetin and kaempferol have also been shown to suppress the activities of DNA topoisomerases ([López-Lázaro et al., 2010](#); [Russo et al., 2012](#)). Although some topoisomerase suppressors have therapeutic efficacy in human cancer, the clinical use of topoisomerase inhibitors can also cause formation of secondary tumours, and increased maternal consumption of flavonoids (some are topoisomerase inhibitors) during pregnancy may be associated with infant acute leukaemia ([Ross et al., 1996](#); [Strick et al., 2000](#); [Mistry et al., 2005](#); [Ezoe, 2012](#)) by interfering with DNA repair processes and inducing chromosomal aberrations. It is possible that an inhibitory effect on topoisomerase is the underlying mechanism for quercetin- or kaempferol-associated chromosomal damage.

Genotoxicity or topoisomerase inhibition may be mechanisms of *G. biloba*-associated carcinogenicity.

5. Summary of Data Reported

5.1 Exposure data

Ginkgo biloba, also known as the “fossil tree,” is the oldest living tree. Products containing ginkgo leaf extract have been widely consumed in Europe for many years, and are also popular in

the USA and other parts of the world, including China. Major reported indications are for asthma, bronchitis, cardiovascular diseases, improvement of peripheral blood flow, and reduction of cerebral insufficiency, allergies, tinnitus, dementia, and memory issues. The main parts of the plant used for these applications are the leaves and the seeds. Ginkgo seeds are cooked and eaten as food. Various forms of processed and unprocessed ginkgo leaf are present in dietary supplements, herbal medicinal products and in foods. Considerable sales were reported from China, the USA, Germany, Australia, France, Brazil, the Republic of Korea, Viet Nam, and Canada.

5.2 Human carcinogenicity data

The potential carcinogenicity of *G. biloba* extract has been evaluated in few epidemiological studies: one randomized controlled trial (the Ginko Evaluation of Memory study, GEM), four nested case–control studies from the VITamins And Lifestyle (VITAL) cohort, and one population-based case–control study of cancer of the ovary.

The GEM study was considered to be informative because of its randomized design; however, the population was limited to people aged > 75 years and compliance in the placebo and ginkgo treatment groups was only about 60%. The strengths of the VITAL study were its prospective design, the large number of exposed cases, and the broad age range of the study participants; however, no information was available on use of ginkgo after enrolment.

The VITAL and GEM studies both reported risk estimates for cancers of the breast, colorectum, lung, prostate, and for urothelial cell carcinoma, and the GEM study also reported a risk estimate for leukaemia and lymphoma combined. Increased risk for cancers of the breast and colorectum was reported in the GEM random clinical trial. The findings for cancer of the colorectum were considered to be stronger in this study because,

in a sensitivity analysis that excluded participants reporting a history of cancer 5 years before baseline, the relative risk increased and reached statistical significance, while the relative risk of cancer of the breast was attenuated and no longer statistically significant in the sensitivity analysis. The VITAL cohort did not corroborate the GEM findings for cancers of the colorectum or breast, finding somewhat decreased risk for both types of cancer and ginkgo intake. The differences in findings between the two studies could be due to differences in age or window of exposure. Risks for other types of cancers were either null or somewhat decreased in both studies. The population case-control study found a decreased risk of non-mucinous ovarian cancer, but not mucinous ovarian cancer, but the analysis was based on small numbers of exposed cases.

5.3 Animal carcinogenicity data

A *G. biloba* extract was tested for carcinogenicity in two studies of oral administration in mice and rats. In male and female mice treated by gavage, a *G. biloba* extract containing 31.2% flavonol, 15.4% terpene lactones (bilobalide, 6.94%; ginkgolide A, 3.74%; ginkgolide B, 1.62%; ginkgolide C, 3.06%), and ginkgolic acid at a concentration of 10.45 ppm, produced a significant increase in the incidences of hepatocellular adenoma or carcinoma (combined), hepatocellular carcinoma and hepatoblastoma. In male mice receiving the extract, the incidence of thyroid follicular cell adenoma exceeded the range for historical controls in the study series. In male rats given *G. biloba* extract by gavage, there was a significant positive trend in the incidence of thyroid follicular cell adenoma and a significant increase in the incidence of mononuclear cell leukaemia. In female rats, the incidences of thyroid follicular cell adenoma, thyroid follicular cell carcinoma, and nasal respiratory epithelium adenoma exceeded the range for historical controls in the study series.

5.4 Mechanistic and other relevant data

Components of *G. biloba* extract are extensively metabolized in rodents and humans after oral administration.

G. biloba extract gave positive results in standard bacterial assays for mutation in the absence or presence of exogenous metabolic activation. Two components of *G. biloba* extract, quercetin and kaempferol, also gave positive results in standard tests for genotoxicity. Quercetin, kaempferol, and rutin, a third component of *G. biloba* extract, produced chromosomal damage.

Quercetin and kaempferol are inhibitors of DNA topoisomerases.

Genotoxicity and/or topoisomerase inhibition may be mechanisms of carcinogenicity associated with *G. biloba* extract.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of *Ginkgo biloba* extract.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of *Ginkgo biloba* extract.

6.3 Overall evaluation

Ginkgo biloba extract is *possibly carcinogenic to humans* (Group 2B).

References

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