SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 17 March 2021

doi: 10.2903/j.efsa.2021.6525

Safety and efficacy of a feed additive consisting of a dried extract from the leaves of *Ginkgo biloba* L. (*G. biloba* dry extract) for use in cats and dogs (C.I.A.M.)

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Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of a dried extract prepared from the leaves of *Ginkgo biloba* L. (*G. biloba* dry extract) when used as a sensory additive in feed for cats and dogs. *G. biloba* dry extract is specified to contain at least 24% flavonol glycosides, at least 6% terpene lactones and less than 5 ppm ginkgolic acids. Since uncertainty remains concerning the nature of up to 75% of the extract, the additive is not sufficiently characterised to allow an assessment based on the individual components. In view of the indication of potential carcinogenicity from the results of studies by the National Toxicology Program of the USA, obtained with a *G. biloba* extract comparable with the additive under assessment, the FEEDAP Panel concludes that the additive cannot be considered safe for cats and dogs. In the absence of data, no conclusions can be drawn on the safety for the user. In the absence of evidence that the extract acts as a flavour in animal feed or has an effect on palatability, the FEEDAP Panel was unable to conclude on the efficacy of the additive.

Keywords: sensory additives, *Ginkgo biloba* L., *Ginkgo biloba* dry extract, flavonol glycoside, safety, efficacy

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Question number: EFSA-Q-2018-00318

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Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgments: The Panel wishes to acknowledge the contribution of Jaume Galobart and Jordi Tarrés-Call to this opinion.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal), Bampidis V, Azimonti G, Bastos ML, Christensen H, Fašmon Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brantom P, Chesson A, Westendorf J, Manini P, Pizzo F and Dusemund B, 2021. Safety and efficacy of a feed additive consisting of a dried extract from the leaves of *Ginkgo biloba* L. (*G. biloba* dry extract) for use in cats and dogs (C.I.A.M.). EFSA Journal 2021;19(4):6525, 10 pp. https://doi.org/10.2903/j.efsa.2021.6525

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Table of contents

1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 10(2) of that Regulation specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, within a maximum of seven years after the entry into force of this Regulation.

The European Commission received a request from C.I.A.M. S.r.l.[2] for re-evaluation of the product *Ginkgo biloba* L. extract, when used as a feed additive for cats and dogs (category: sensory additives; functional group: flavouring compounds).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 25 May 2018.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals and the user, and on the efficacy of the product *G. biloba* extract, when used under the proposed conditions of use (see Section 3.2.3).

1.2. Additional information

G. biloba L. extract is currently listed in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 (2b natural products – botanically defined). It has not been previously assessed by EFSA as feed additive.

G. biloba is listed as active medicinal ingredients for all food-producing animal species without a maximum residue limit (MRL).[3]

G. biloba extract from the leaves of *G. biloba* L. (maidenhair tree) is listed in the European list of Cosmetics and Ingredients and Substances as tonic and skin conditioning agent.[4]

For human medicinal uses, the European Medicines Agency (EMA) issued an assessment report and a herbal monograph on *G. biloba* L., folium (EMA, 2015a,b).

Ginkgo dry extract, refined and quantified (*Ginkgonis extractum siccum raffinatum et quantificatum*) is described as refined and quantified dry extract produced from Ginkgo leaf in a monograph of the European Pharmacopoeia (PhEur, 2020, 04/2008:1827).[5]

Folium Ginkgo (*G. biloba* L.) is described in a WHO monograph (WHO, 1999).[6]

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[7] in support of the re-evaluation of *G. biloba* dry extract as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] C.I.A.M. S.r.l., via Piemonte 4, 63100 Ascoli Piceno (AP), Italy.
[3] Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. OJ L 15/1, 20.1.2010, p. 15.
[4] Commission Decision 2006/257/EC of 9 February 2006 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products, OJ L 97, 5.4.2006, p. 1–528.
[5] Technical dossier/Section II/Ref_II_1_03.
[6] Technical dossier/Section II/Ref_II_1_01.
[7] FEED dossier reference: FAD-2010-0328.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the additive's phytochemical markers in animal feed. The Executive Summary of the EURL report can be found in Annex A.[8]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *G. biloba* dry extract, is in line with the principles laid down in Regulation (EC) No 429/2008[9] and the relevant guidance documents: Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b) and Guidance on the assessment of additives intended to be used in pets and other non-food-producing animals (EFSA FEEDAP Panel, 2011).

3. Assessment

The additive under assessment, *G. biloba* dry extract, is prepared from the dried leaves of *G. biloba* L. and is intended for use as a sensory additive (functional group: flavouring compounds) in feed for cats and dogs.

3.1. Origin and extraction

G. biloba L. is a dioecious tree species commonly called the maidenhair tree or simply ginkgo. It is the only living member of the Ginkgoaceae, a family of gymnosperms now extinct and, for this reason, it is often referred to as a 'living fossil'. The fossil records of the Ginkgoaceae indicate a world-wide distribution, but it seems likely that the modern relatives of *G. biloba* originated in China. It is now widely cultivated as an ornamental species in temperate parts of the world. The seeds of gingko are consumed as food, particularly in China and leaf extracts have a history of use in medicine worldwide.

The extract is prepared from dried leaves of *G. biloba* by extended extraction with ethanol at 30°C. After extraction, the insoluble plant biomass is removed by centrifugation and the ethanol extract passed through resin columns able to absorb flavones and terpene lactones. Columns are eluted with ethanol, and the collected liquor is subsequently concentrated by evaporation and spray-dried. Maltodextrin is then added to the dried extract and the mix is ground, sieved and packed for distribution.

3.2. Characterisation

3.2.1. Characterisation of the extract

G. biloba dry extract is identified by the Chemical Abstract Service (CAS) number 90045-36-6 and the European Inventory of Existing Commercial chemical Substances (EINECS) number 289-896-4. The additive is described as a brown fine powder, with a characteristic odour. It has a density of 450–650 kg/m^3. The additive is partially soluble in water and organic solvents.

According to the specification proposed by the applicant, *G. biloba* dry extract contains ≥ 24% flavonol glycosides (mainly quercetin glycosides), ≥ 6% terpene lactones (including ginkgolides A, B and C and bilobalide) and ≤ 5 ppm ginkgolic acids. All these components were selected as the marker compounds. Loss on drying is specified to be ≤ 5%, and ash ≤ 5%. Data were provided for seven batches of the *G. biloba* dry extract, which showed an average content of flavonol glycosides of 26.6% (range 24.5–30.0%).[10] However, certificates of analysis were not provided. No analytical data on the content of terpene lactones and ginkgolic acids in the additive were provided.

The applicant did not provide the full characterisation of the additive, despite being requested. Furthermore, the specification proposed for the additive does not define maximum levels for flavonol glycosides, ginkgolides A, B and C and bilobalide. Although the additive contains maltodextrin, the amount added is unknown and, as a result, uncertainty remains concerning the nature of up to 75% of the composition of the extract.

[8] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2010-0328_ginko_extract_c_.pdf

[9] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

[10] Technical dossier/Section II/Annex II_1_02_ Analysis batches.

The applicant provided a commercial information sheet for the *G. biloba* dry extract,[11] which included the limits applied for the marker substances (including terpene lactones and ginkgolic acids), chemical impurities and microbiological contamination. Specifications for chemical impurities include heavy metals (lead ≤ 3.0 mg/kg, cadmium ≤ 1.0 mg/kg and mercury ≤ 0.1 mg /kg), mycotoxins (aflatoxin B1 ≤ 5.0 μg/kg, aflatoxins B1, B2, G1 and G2 ≤ 10.0 μg/kg), residual solvents (ethanol ≤ 0.5%, below the thresholds proposed by International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (EMA, 2010)) and pesticide residues (which are declared to comply with the maximum limits of Regulation (EU) No 396/2005[12]). Specifications for microbial contamination include aerobic bacteria ≤ 50,000 colony forming unit (CFU)/g, fungi (yeast/moulds) ≤ 500 CFU/g, bile tolerant Gram-negative bacteria ≤ 100 CFU/g, *Salmonella* spp. absent in 25 g, *Escherichia coli* absent in 1 g. However, analytical data supported by certificates of analysis were not provided. The FEEDAP Panel notes that the specification for aerobic bacteria is very high.

Particle size analysis (by sieving) of the additive showed that 90% of particles passed a 300-μm sieve, and 43–60% of three batches of *G. biloba* dry extract passed a 50-μm sieve. No data were provided on the dusting potential of the additive.

3.2.2. Stability

The applicant stated that the typical shelf-life of *G. biloba* dry extract is at least three years when stored in closed containers protected from heat, light and humidity.[13] Stability studies provided showed that the content of flavonol glycosides was on average 93% of the initial content in three batches of *G. biloba* dry extract after 3-year storage (storage conditions not reported).[14]

3.2.3. Conditions of use

G. biloba dry extract is intended for use in feedingstuffs, premixtures and complementary feed for cats and dogs, up to the maximum use level of 40 mg/kg complete feed.

3.3. Safety for the target species and the user

No specific studies were provided on absorption, distribution, metabolism and excretion with the extracts under assessment or with the individual constituents.

Tolerance studies and/or toxicological studies made with the additive under application were not submitted. In addition, the additive was not sufficiently characterised to allow an assessment based on the individual components.

A *G. biloba* extract has been included in the testing program of the National Toxicology Program (NTP) of the U.S.A. (NTP, 2013). A standardised commercial *G. biloba* extract[15] dissolved in maize oil was administered by gavage to groups of male and female (50M/50F) F344/N rats at 100, 300 or 1,000 mg/kg body weight (bw) and B6C3F1/N mice at 200, 600 or 2,000 mg/kg bw for five days a week for two years. Control groups received maize oil. At the end of the study, tissues were taken from all animals and complete histopathology was performed.[16] The extract used may not be the same as the additive under application but is likely to be sufficiently similar for the results of those studies to be relevant.

Every group of animals exposed to the *G. biloba* extract had increased rates of a variety of lesions in the liver, thyroid gland, and nose; also, male and female mice experienced several different lesions in the forestomach. The lesions seen included hypertrophy in the liver and thyroid gland in rats and

[11] Technical dossier/Section II/Annex II_1_01_Data_sheet_Gingko biloba extract.

[12] Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC Text with EEA relevance. OJ L 70, 16.3.2005, p. 1–16.

[13] Technical dossier/Section II/ Annex II_4_01_Stability statement of supplier.

[14] Technical dossier/Section II/ Annex II_4_02_Stability shelf life.

[15] Test item containing flavonol glycosides 31.2%, mainly quercetin 16.71%, kaempferol 12.2% and isorhamnetin 2.37%; terpenoids 15.4%, mainly bilobalide 6.94%, ginkgolide C 3.06%, ginkgolide A 3.74%, and ginkgolide B 1.62%; alkylphenols (ginkgolic acids, cardanols) 10.45%.

[16] Tissues sampled and examined histopatologically: adrenal gland, bone with marrow, brain, clitoral gland, oesophagus, eyes, gall bladder (mice), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder and uterus.

mice, liver hyperplasia in rats, hyperplasia and atrophy of the epithelium within the nose of rats. There was also inflammation, hyperplasia, hyperkeratosis and ulceration of the forestomach of mice.

Under the conditions of 2-year gavage studies, there was some evidence of carcinogenic activity of the tested *G. biloba* extract in both sexes of F344/N rats, based on increased incidences of thyroid gland follicular cell adenoma. Increased incidences of mononuclear cell leukaemia and hepatocellular adenoma in male rats and respiratory epithelium adenoma in females, may also have been related to the *G. biloba* extract administration, although there is not a consistent relationship with dose.

There was clear evidence of carcinogenic activity of the administered *G. biloba* extract in the liver of both sexes of B6C3F1/N mice, based on increased incidences of hepatocellular carcinoma, hepatoblastoma and hepatocellular adenoma in females. Increased incidences of thyroid gland follicular cell adenoma were also seen in male mice which may be related to the *G. biloba* extract administration. The NTP report also gives the results of two genotoxicity studies, which indicate that genotoxicity cannot be ruled out for the same *G. biloba* extract. This conclusion is supported by a study by Westendorf and Regan (2000) who reported DNA-strand breaks following treatment of rat hepatocytes with gingkolic acids.

NTP reported that "*Ginkgo biloba* extract (1,000–10,000 μg/plate) was mutagenic in *Salmonella* Typhimurium strains TA98 and TA100 and in *E. coli* strain WP2 uvrA/pKM101, with and without 10% induced rat liver S9 mix. No increase in the frequency of micronucleated erythrocytes was observed in peripheral blood of male B6C3F1/N mice administered *G. biloba* extract (125 to 2,000 mg/kg per day) for three months by gavage. In female mice administered these doses, the results of the micronucleus test were judged to be equivocal based on a significant trend test and no individual dose groups being significantly elevated over the vehicle control group. A significant ($p < 0.001$) dose-related decrease in the percentage of circulating PCEs was observed in male mice, suggesting that increasing doses of *G. biloba* extract induced bone marrow toxicity. In female mice, a significant ($p = 0.001$) decrease in the percentage of circulating polychromatic erythrocytes (PCEs) was also observed, although the response in females was less well correlated with dose than in males."

In view of the indication of potential carcinogenicity from the results of NTP studies on an extract assumed to be representative of the one under assessment, the FEEDAP Panel concludes that the additive cannot be considered safe for cats and dogs.

No specific data were provided by the applicant regarding the safety of the additive for the user and, consequently, no conclusions can be drawn on the additive' potential to be dermal/eye irritant or skin sensitiser. The additive contains 43–60% of the particles < 50 μm. In the absence of data on their dusting potential, it is not possible to estimate exposure of users to dust.

In the absence of adequate data, the FEEDAP Panel cannot conclude on the safety of the additive under assessment for the users.

3.4. Efficacy

Ginkgo is not listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2010) or by the Flavour and Extract Manufactures Association (FEMA).

In the absence of evidence that the extract acts as a flavour in animal feed or has an effect on palatability, the FEEDAP Panel is unable to conclude on the efficacy of the additive.

4. Conclusions

Since uncertainty remains concerning the nature of up to 75% of the extract, the additive is not sufficiently characterised to allow an assessment based on the individual components.

In view of the indication of potential carcinogenicity from the results of studies by the NTP of the USA obtained with a *G. biloba* extract comparable with the additive under assessment, the FEEDAP Panel concludes that the additive cannot be considered safe for cats and dogs.

In the absence of data, no conclusions can be drawn on the safety for the user.

In the absence of evidence that the extract acts as a flavour in animal feed or has an effect on palatability, the FEEDAP Panel is unable to conclude on the efficacy of the additives.

5. Documentation as provided to EFSA / Chronology

Date	Event
05/11/2010	Dossier received by EFSA. *Ginkgo biloba* L. extract for cats and dogs. Submitted by C.I.A.M. S.r.l.
24/04/2018	Reception mandate from the European Commission
22/05/2018	Application validated by EFSA – Start of the scientific assessment
22/06/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety for the target species, safety for the user, efficacy*
23/08/2018	Comments received from Member States
27/08/2018	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
11/10/2019	The applicant informed the European Commission on the impossibility to provide the information requested in line with Article 8(1)(2) of Regulation (EC) No 1831/2003
17/03/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

bw body weight
CAS Chemical Abstracts Service

CFU	colony forming unit
EINECS	European Inventory of Existing Commercial chemical Substances
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
MRL	maximum residue limit
NTP	National Toxicology Program
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for *Ginkgo biloba* L. extract

In the current application authorisation is sought under Article 10(2) for the botanically defined *Ginkgo biloba* L. extract under the category / functional group (2 b) "sensory additives"/"flavouring compounds", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the *feed additive* is sought to be used for cats and dogs.

The *feed additive* is a brown fine powder containing a mixture of chemical components naturally present, such as *flavonoids*: *flavonols (quercetin*, *kaempferol* and *isorhamnetin)* in the form of glycosides; diterpene lactones: *ginkgolides A, B, C, J, and M*; sesquiterpene lactone - *bilobalide*; and *ginkgolic acids*. The standardised extracts contain 22 to 27% of the *flavonol* glycosides; 2.8 to 3.4% of *ginkgolides A, B and C*; 2.6 to 3.2% of *bilobalide*, and less than 5 mg of *ginkgolic acids*/kg extract. According to the Applicant, the phytochemical markers of the *feed additive* - as specified by European Pharmacopoeia Monograph 04/2008:1827 - are (1) the sum of various *flavonoids quantified against quercetin*, (2) *terpene lactones* (e.g. *ginkgolides A, B and C*, *bilobalide*) and (3) *ginkgolic acids*.

The *feed additive* is intended to be incorporated directly into *feedingstuffs* or through *premixtures*. The Applicant proposed no minimum or maximum level of the *feed additive*; however, a maximum content of 40 mg *feed additive* /kg *feedingstuffs* was suggested by the Applicant.

For the determination of Ginkgo flavonoids in the *feed additive* the Applicant applied an inhouse analytical method based on the European Pharmacopoeia Monograph 04/2008:1827.

The mentioned European Pharmacopoeia Monograph is related to, refined and quantified dry extract produced from Ginkgo leaf and describes assays for identification, based on thin layer chromatography (TLC) and for quantification, based on high performance liquid chromatography (HPLC).

The EURL recommends for official control the methods included in the above mentioned European Pharmacopoeia Monograph for the quantification of the selected phytochemical markers in the *feed additive*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.