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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of tannic acid when used as feed flavouring for all animal species[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Tannic acid is a synonym for hydrolysable tannins, which are widely distributed in nature. The use of tannic acid as a feed additive up to 15 mg/kg feed is safe for all animal species. The use of tannic acid as a feed additive under the proposed conditions of use presents no safety risk to consumers. In the absence of data, it would be prudent to regard the additive as potentially hazardous to workers by exposure to the skin, eyes and mucous membranes or by inhalation exposure. Tannic acid is naturally present in many plant species. The use of tannic acid as a feed additive is considered safe for the environment. Tannic acid is recognised as a food flavouring agent and is included in the European Union list of food flavourings. As its function in feed is essentially the same as that in food, no further demonstration of efficacy is necessary.

KEY WORDS

sensory additive, flavourings, tannic acid, safety, efficacy

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SUMMARY

Following a request from the European Commission, the 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 tannic acid for all animal species. Tannic acid is a synonym for hydrolysable tannins, which are widely distributed in nature.

Based on a literature review, the safety of the use of tannic acid as a feed additive up to 15 mg/kg feed could be established for all animal species.

The use of tannic acid as a feed additive under the proposed conditions of use presents no safety risk to consumers.

In the absence of data, it would be prudent to regard the additive as potentially hazardous to workers by exposure to the skin, eyes and mucous membranes or by inhalation.

The use of tannic acid as a feed additive is considered safe for the environment.

Tannic acid is recognised as a food flavouring agent and is included in the European Union list of food flavourings. As its function in feed is essentially the same as that in food, no further demonstration of efficacy is necessary.

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BACKGROUND

Regulation (EC) No 1831/2003[4] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7. In particular, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[5] for authorisation of tannic acid, when used as feed additive for all animal species (category: sensory additives; functional group: flavourings) under the conditions mentioned in Table 1.

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 4(1) (authorisation of a feed additive or new use of a feed additive) and 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.[6] According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 9 August 2011.

Tannic acid is authorised under Directive 2001/83/EC on the Community code relating to medicinal products for human use for the treatment of mild diarrhoea, inflammation of oral mucosa and skin and haemorrhoids.[7]

Tannic acid is listed by the Council of Europe (2000), and appears in the European Union list of flavouring substances for use in food with the FLAVIS number 16.080[8] and in the European Union Register of Feed Additives.

The JECFA (WHO, 1990) assessed tannic acid as a filtering aid and not as part of the flavouring evaluation program.

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall 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, consumer, user and the environment and the efficacy of tannic acid when used under the conditions described in Table 1.

[4] 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.

[5] On 13 March 2013, EFSA was informed by the applicant that FFAC EEIG was liquidated on 19 December 2012 and their rights as applicant were transferred to FEFANA asbl (EU Association of Specialty Feed Ingredients and their Mixtures, Avenue Louise, 130A, Box 1, 1050 Brussels, Belgium).

[6] EFSA Dossier reference: FAD-2010-0123.

[7] Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. OJ L 311, 28.11.2001, p. 67.

[8] Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	Tannic acid			
Registration number/EC No/No (if appropriate)				
Category(ies) of additive	2. Sensory additives			
Functional group(s) of additive	b) flavouring compounds			
Description				
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)	
Tannic acid (CAS-No 72401-53-7[9])	$C_{76}H_{52}O_{46}$	96%	HPLC[10]	
Trade name (if appropriate)	-			
Name of the holder of authorisation (if appropriate)	-			
Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg or Units of activity or CFU/kg of complete feedingstuffs (select what applicable)		
All species and categories	-	-	-	-
Other provisions and additional requirements for the labelling				
Specific conditions or restrictions for use (if appropriate)	-			
Specific conditions or restrictions for handling (if appropriate)	All feedingstuffs, as part of a premixture only			
Post-market monitoring (if appropriate)	-			
Specific conditions for use in complementary feedingstuffs (if appropriate)	-			
Maximum Residue Limit (MRL) (if appropriate)				
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues	
-	-	-	-	

[9] Tannic acid is registered by the Community Register Feed and the Food Register under CAS No 72401-53-7. The US Pharmacopeia (US Pharmacopoeia, 2009), Fenaroli's Handbook of Flavor Ingredients (Fenaroli, 2010), Food Chemicals Codex (FCC, 2008), the International Organization of the Flavor Industry and the applicant's member assign to this product the CAS No 1401-55-4.

[10] Available at the website of the EURL.

ASSESSMENT

1. Introduction

Tannic acid is a synonym for hydrolysable tannins, which are widely distributed in nature (e.g. *Quercus infectoria*, *Caesalpinia spinosa* or the genus *Rhus*) (WHO, 2009). It is astringent in taste.

Tannic acid has been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (WHO, 1990) as a filtering aid and not as a flavouring substance. This assessment presumed that any residual tannins were removed from food after use. The European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) has not, to date, considered tannic acid as a flavouring substance for food. However, tannic acid does appear in the European Union (EU) list of flavouring substances for use in food with the EU Flavour Information System (FLAVIS) number 16.080[11]. This inclusion was made following the listing of tannic acid as flavouring substance by the Council of Europe (2000), although no formal safety assessment was made.

The Feed Flavourings Authorisation Consortium (FFAC), which supplies flavours to the feed industry, has requested authorisation for the use of tannic acid as an additive in feed (category: sensory additives, flavouring compounds) for use in all animal species.

2. Characterisation

2.1. Characterisation of the active substance

Tannic acid is a low molecular weight polymer of gallic acid and 3-galloylgallic acid esterified with glucose (European Pharmacopoeia, 2010) of variable composition. It is a yellow-brown powder generally obtained by extraction with water or organic solvents from a number of well known botanical sources (e.g. *Castanea sativa*, *Quercus infectoria* or *Rhus* spp.).

Tannic acid has the Chemical Abstracts Service (CAS) number 72401-53-7 and a notional molecular formula of $C_{76}H_{52}O_{46}$ in the EU Register of Feed additives and the Union List of Food Flavourings. However, the United States Pharmacopeia (2005), Fenaroli's Handbook of Flavor Ingredients (Burdock, 2010), the Food Chemicals Codex (FCC, 2008) and the International Organization of the Flavor Industry (IOFI) assign to this product the CAS number 1401-55-4. The applicant considers both CAS numbers to be synonyms for the same substance.

2.2. Proposal for classification

The applicant specifies that the tannic acid content of the additive is 96 % or above, which complies with the US Pharmacopeia and Food Chemicals Codex (tannic acid ≥ 93 % on a dry matter (DM) basis and water less than 12 %). Gravimetric analysis (by the hide powder precipitation test) of five batches from two botanical sources (*Caesalpinia spinosa* and *Quercus infectoria*) showed that this specification was exceeded in all cases (ranging from 96.2 to 98.4 % of DM)[12].

The remaining material, as stated by the applicant, contains low molecular weight fractions such as gallic acid, digallic acid and/or lower galloyl esters of quinic acid such as mono- or digalloyl quinic acid. No analytical data were submitted to support this statement.

The applicant specifies that the heavy metals content, expressed as the lead equivalent, is ≤ 40 mg/kg, the arsenic content is < 3 mg/kg and the lead content is < 2 mg/kg. Analysis of six batches shows

[11] Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1.

[12] Technical Dossier/Supplementary information October 2013/Annex_Supplier_Caesalpinia spinosa.pdf and Annex_Supplier_Quercus infectoria.pdf

compliance with these values. However, cadmium and mercury were not individually measured. The additive was shown to be free of resinous substances (test of turbidity)[13], and residual solvents (acetone or ethyl acetate) were below the levels set by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (EMA, 2010). The applicant provided evidence for the absence of microbial contamination (filamentous fungi, yeast, Enterobacteriaceae, *Escherichia coli* < 10 colony-forming units/g, *Salmonella* not detectable in 10 g) in three batches[14].

Particle size distribution of four batches from a single source was determined by laser diffraction. Over 50 % of the particles have a diameter below 50 µm. Dusting potential was determined using a Heubach dustometer[15] for the same four batches of product and gave a mean value of 9.2 g/m^3.

2.3. Manufacturing process

Tannic acid is extracted with water, acetone or ethyl acetate from a variety of botanical sources after grinding into a fine powder (e.g. *Quercus infectoria*, the seed pods of *Caesalpinia spinosa* and selected species of *Rhus*). After grinding, the solvents are evaporated. After the evaporation steps, the powder is washed and dried.

2.4. Stability

The applicant proposes a shelf-life of five years for this additive when stored in closed containers under recommended conditions (in a cool and dry place). This was not supported by experimental evidence.

2.5. Conditions of use

The additive is intended to be used in feed for all animal species at the recommended use levels of 5 to 15 mg/kg complete feed[16]. The additive is not intended to be used in water for drinking[17].

2.6. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of tannic acid. The Executive Summary of the EURL report can be found in the Appendix.

3. Safety

The FEEDAP Panel notes that tannic acid can be extracted from a variety of sources with different characteristics. The assessment has been made on published literature on tannins present in common sources, such as oak leaves and sweet chestnuts, although it is recognised that many other sources with different impurities could be produced and are on the market.

The relevance of this assessment to the product specified by the applicant is uncertain; however, it may be assumed that it could apply to all products meeting the specifications of the US Pharmacopeia or the Food Chemical Codex.

3.1. Safety for the target species

The applicant did not provide tolerance studies with target animals. The assessment is based on published literature.

[13] Technical Dossier/Section II/ Page 7.
[14] Technical Dossier/Supplementary information October 2013/Annex Microbiology.
[15] Technical Dossier/Supplementary information January 2014/Annex_i_PSD_and_Dust-pot.pdf
[16] Technical Dossier/Supplementary information October 2013.
[17] Technical Dossier/Supplementary information January 2012.

3.1.1. Ruminants

Oak toxicosis has long been recognised as a problem in cattle, and to a lesser extent in horses, when animals have access to and ingest immature leaves and recently fallen acorns. The toxic effects have been attributed to the hydrolysable tannins present, which reach their highest concentrations in such immature tissues. However, a comparison of calves deliberately fed oak leaves with others given a matching amount of purified tannic acid showed clear differences in the extent and pattern of toxicity. The oak-fed calves developed the expected signs of renal failure, whereas the tannic acid-fed calves remained healthy (Plumlee et al., 1998). Only when calves were given much higher levels of tannic acid (> 4 400 mg/kg body weight (bw)) did clinical symptoms appear, in the form of methaemoglobinaemia, rather than as renal failure.

Rumen metabolism appears to prevent overt toxicity from tannic acid (Murdiati et al., 1992). Consequently, adult ruminants can tolerate levels of hydrolysable tannins in feed in the range 15 000–25 000 mg/kg feed without any detectable loss in performance characteristics, whether measured as growth or milk production (Silanikove et al., 1996; Frutos et al., 2004; Krueger et al., 2010; Liu et al., 2013). Pre-ruminant calves appear to be more vulnerable to toxicosis, but the concentrations of tannic acid required to elicit clinical symptoms are substantially higher (more than 1 500 mg/kg feed) than those proposed for flavour purposes (Plumlee et al., 1998).

In addition to studies concerned directly with oak toxicosis, a number of other publications have dealt with the addition of tannic acid to the diets of non-ruminants. Since the removal of antibiotics for growth promotion in the EU, many of these have focused on the potential of tannic acid preparations to improve performance or control infections. Although such studies cannot be considered as tolerance studies *per se*, they generally involve feed concentrations of tannic acid substantially higher than those proposed for flavour purposes and thus do provide information on tolerated levels. The source of tannic acid was, in most cases, commercial products derived from sweet chestnut (*Castanea sativa*).

3.1.2. Pigs

The growth and feed intake of pigs for fattening with a start weight of approximately 30 kg until slaughter (100 kg) were unaffected by the inclusion of 2 000 mg of a tannic acid preparation from sweet chestnut/kg feed (Prevolnik et al., 2012). Similarly, in a three-week feeding trial, average daily body weight gain in piglets with a start weight of around 15 kg was not significantly affected by the presence of 1 500 mg of a tannic acid preparation/kg diet, and no effects on haematological parameters were seen. However, it should be noted that the test diets also included organic acids and that the tannic acid diet gave numerically lower values than the basal diet (Stukelj et al., 2010). This suggested that younger animals might be more sensitive to the presence of tannic acid. This suggestion was supported by a study in piglets of a start weight ~ 6 kg given diets supplemented for a total of 28 days with 125, 250, 500 or 1 000 mg tannic acid/kg feed. This study shows a linear increase in feed to gain ratio and a linear decrease of daily body weight gain, erythrocyte count, haemoglobin and haematocrit with increasing levels of tannic acid (Lee et al., 2010). It should be noted that the statistical methodology followed does not allow for comparison of the means of the different treatments. Nevertheless, the haematological parameters appeared to be affected by the 1 000 mg/kg treatment, whereas the effect was less clear at lower doses. For the zootechnical parameters, the adverse effects seem to start at 250 mg/kg.

3.1.3. Poultry

One study with chickens for fattening showed that the inclusion of tannic acid in diets has no adverse effects on growth or feed intake at concentrations up to 2 000 mg of a tannic acid preparation from chestnut wood/kg feed for 42 days (Schiavone et al., 2008). However, other reports have shown that, at concentrations greater than 1 000 mg tannic acid from chestnut wood/kg feed, growth and feed intake are reduced in a 41-day trial (Jamroz et al., 2009) and up to 30 000 mg tannic acid/kg feed for 35 days impaired the immune function of chickens (Marzo et al., 1990). In hens receiving 10 000, 20 000 or 40 000 mg tannic acid/kg feed, no effects on feed consumption, egg production, feed

utilisation, fertility or hatchability were observed for those birds receiving the lowest dose. Feed intake and egg production were significantly reduced in birds given the two highest doses (Blakeslee and Wilson, 1979).

3.1.4. Rabbits

In two studies with New Zealand male rabbits, inclusion of a tannic acid preparation (from chestnut wood) for 21 days at 5 000 mg or 10 000 mg/kg feed showed no adverse effects on growth or feed conversion, which were significantly better than the control group that was not given tannic acid (Liu et al., 2011, 2012).

3.1.5. Interactions with other components of the diet

Tannic acid interacts with proteins, which may result in lower digestibility of the proteins (Karamać, 2009). Tannic acid (5, 10, 15 and 20 g/kg rat diet) has been shown to interfere with the absorption of iron, but at concentrations higher than those proposed for flavouring use (Afsana et al., 2004; Karamać, 2009).

3.1.6. Conclusions on safety for the target species

The published studies show that levels of tannic acid preparations up to 15 000 mg/kg feed for adult ruminants and 10 000 mg/kg feed for laying hens do not result in adverse effects in these species. Rabbits tolerate up to 10 000 mg/kg, pigs for fattening tolerate up to 1 500 mg/kg and chickens for fattening tolerate up to 1 000 mg/kg without adverse effects. Young animals seem to be more sensitive (> 1 500 mg/kg feed was harmful to calves). In the study with weaned piglets, animals receiving 125 mg/kg showed no sign of adverse effects. In all cases, the values shown to be safe are several orders of magnitude higher than the proposed use as feed flavouring.

Considering that the data support the safety for pigs, poultry and cattle with a wide margin of safety, the FEEDAP Panel concludes that the use of tannic acid as a feed additive up to 15 mg/kg feed is safe for all animal species.

3.2. Safety for the consumer

3.2.1. Absorption, distribution, metabolism and elimination

Tannic acid *per se* is a polymer which is poorly absorbed in the digestive tract (Nakamura et al., 2003). Tannic acid is degraded in the gut by bacteria or enzymes and its degradation products are absorbed. *In vitro* studies carried out in ruminal fluid collected from cattle showed that tannic acid was converted to gallic acid, pyrogallol and resorcinol. After 48 or 72 hours of incubation, neither tannic acid nor gallic acid could be detected (Singh et al., 2001). This degradation is attributed to microbiota abundantly present in ruminants (Goel et al., 2005; Mingshu et al., 2006). The enzymes responsible for it are the tannases (tannin acyl hydrolases), which degrade tannin in the gastrointestinal tract of several animal species, mainly ruminants and some fish species (Mandal and Ghosh, 2013). Tannases were also shown to be present in faecal bacteria (*Streptococcus gallolyticus*) common in the gastrointestinal tract of other species (cattle, horses, pigs, dogs and sheep) (Sly et al., 1997).

The main metabolites of tannic acid detected in serum in the rat were 4-*O*-methylgallic acid, pyrogallol and resorcinol after oral administration. These metabolites were excreted into urine as free or sulphated gallic acid (3,4,5-trihydroxybenzoic acid) (0.01 %), free or sulphated 4-*O*-methylgallic acid (0.10 %), pyrogallol (1,2,3-trihydroxybenzene) (0.24 %) and resorcinol (1,3-dihydroxybenzene) (2.06 %). Approximately 60 % of the ingested tannic acid was found in faeces within 54 hours, together with small amounts of gallic acid, pyrogallol and resorcinol (Nakamura et al., 2003).

Gallic acid, a hydrolysis product of tannic acid, administered orally to rats was excreted in urine mainly as 4-*O*-methylgallic acid, but unchanged gallic acid was also found (EFSA, 2012a, b). A study on the metabolism of gallic acid in rats showed that, besides 4-*O*-methylgallic acid and free gallic

acid, several pyrogallol derivatives were excreted in urine after oral administration of gallic acid: free and glucuronated pyrogallol and its methylated derivative 2-*O*-methylpyrogallol, excreted either free or after conjugation with glucuronic acid or sulphate (Yasuda et al., 2000). Pharmacokinetic studies in rats showed that [^{14}C]resorcinol is mainly eliminated in urine as glucuronide conjugate and that there is no indication of bioaccumulation in tissues (liver, skin, fat, muscle). After a single oral dose (112 mg/kg bw) to F344 rats, [^{14}C]resorcinol was rapidly absorbed, metabolised and excreted. Most of the applied dose (90.8–92.8 %) was eliminated in urine within 24 hours as glucuronide conjugate (65 %) and other conjugated metabolites (i.e. monosulphate, mixed sulphate-glucuronide and diglucuronide conjugate). Comparable results were obtained at higher doses (225 mg/kg bw, single dose or daily dose administered for five consecutive days) (Kim and Matthew, 1987). When [^{14}C]resorcinol was administered subcutaneously to Sprague–Dawley rats (10, 50 or 100 mg/kg bw), 98 % of the applied dose was excreted via urine within 24 hours, mainly as glucuronide conjugate (Merker et al., 1982).

Several *in vivo* studies on the metabolism of gallic acid, tannic acid and hydrolysable tannin from *Terminalia oblongata* in sheep have been described by Murdiati et al. (1992). In one of the experiments, gallic acid or tannic acid (both at a dose rate of 0.38 g/kg per day for four days) were administered to cannulated sheep via the rumen or abomasum. Another experiment involved administration of tannic acid or gallic acid (0.5 g/kg twice daily) into the rumen for 14 days. In a third experiment, animals were fed a diet containing 2.3 % hydrolysable tannin for 17 days. Digesta, blood and urine were collected at several time points for the analysis of metabolites. Pyrogallol and resorcinol were found in abomasal digesta of all the animals dosed with either tannic acid or gallic acid and also in ileal digesta of sheep. After rumen administration of gallic acid, resorcinol glucuronide was identified in urine. Administration of tannic acid into the rumen resulted in the presence of diphenyl lactone glucuronide pyrogallol, phloroglucinol and resorcinol in the urine in addition to resorcinol glucuronide. A similar metabolic profile was observed in the urine of sheep fed *Terminalia oblongata*. The urinary elimination of the metabolites attained a plateau after some days and subsequently declined. The authors attribute this reduction of excretion to the increase of degradation of gallic acid in the rumen throughout the study.

There are few *in vivo* metabolic studies in non-ruminants, which are insufficiently documented and use analytical methodologies that may not have detected trace levels of metabolites, as they do not conform with modern standards. In these the metabolites 4-*O*-methylgallic acid and pyrogallol were identified in the urine of rabbits fed a diet containing 0.5 % gallic acid (Booth et al., 1959). The same metabolites were identified in the urine of hens fed with tannic acid or gallic acid (Potter and Fuller, 1968).

In humans, after oral administration of gallic acid, 4-*O*-methylgallic acid and gallic acid were detected in plasma and urine as both free and conjugated derivatives (Shahrzad and Bitsch, 1998).

3.2.2. Toxicology

A large number of studies on the mutagenicity/genotoxicity of tannic acid is available, but many of the tests either are not validated for evaluating genotoxic risk or do not conform with modern protocols as outlined in the current Organisation for Economic Co-operation and Development (OECD) methodological guidelines. Only studies on hydrolysable tannins have been evaluated.

Tannic acid was not mutagenic in five bacterial reverse mutation tests (Mohtashamipur and Norpoth, 1984; Rashid et al., 1985; Nagabhushan et al., 1991; Watanabe et al., 1998; Chen and Chung, 2000) and one SOS chromotest in *E. coli* (Kevekordes et al., 1999). Positive results for mutagenicity were obtained in some *in vitro* tests in mammalian cells, including comet assays (Chu, 2002) and tests for chromosomal aberrations (Stich and Powrie, 1982; Stich and Dunn, 1986) and micronuclei (Stich and Dunn, 1986; Sanyal et al., 1997). When tannic acid was administered at single doses of 250, 500 or 750 mg/kg bw by oral gavage and at 500 mg/kg bw given intraperitoneally (i.p.) in an *in vivo* bone marrow micronucleus test in BALB/c mice, a negative result was obtained. The proportion of

circulating erythrocytes that were polychromatic was unaffected by treatment, but the bone marrow would have been exposed following the i.p. dose, and it is concluded that tannic acid was not mutagenic *in vivo* (Gimmler-Luz et al., 1998). The *in vitro* results indicate a potential for tannic acid to be mutagenic, but the results of the *in vivo* test indicate that the mutagenicity was not expressed *in vivo*.

A 13-week sub-chronic toxicity study was performed in groups of 12 male and 12 female F344 rats using doses of 0.025, 0.05, 0.1, 0.2 and 0.4 % in drinking water (Ogasawara et al., 1990). There was no unscheduled mortality and no treatment-related effects on body weight gain, feed consumption, haematology or organ weights. A slight decrease in water intake was seen in the 0.4 % group. There were some statistically significant differences between some of the treated groups and controls in several of the blood biochemistry parameters measured in terminal blood, but the lack of a dose-response relationship and the inconsistency between the two sexes suggested that none of the differences reflected any toxicological process. Microscopic examination of organs revealed liver necrosis in treated males, which the authors regarded as "of minor importance". However, the available report of the study did not indicate which groups were affected or provide any detail of the severity of the lesions.

A carcinogenicity study was performed in groups of 50 male and 50 female F344 rats (Onodera et al., 1994). The preparation used in this study consisted of 85.69 % tannic acid, 8.84 % gallic acid and 7.5 % moisture. The preparation was administered via drinking water at 0.25 and 0.5 % (corresponding to mean doses of tannic acid of 131 and 243 mg/kg bw per day in males, and 159 and 291 mg/kg bw per day in females, respectively) for 104 weeks, followed by a further 10 weeks during which all groups were given distilled water to drink. Animals were observed daily for clinical signs. There was no sampling of blood or urine. Autopsies were performed, weights of organs were measured and a wide range of tissues plus any tumours were examined microscopically. Intake of drinking water was slightly decreased in a dose-related manner in all treatment groups, and body weight gain was depressed at both doses in the females, which showed lower (7 and 8 % lower in the low- and high-dose groups, respectively) body weights than the control group at the end of the experiment. There were no mortalities. No increased incidence of tumours or differences in the site or type of tumours were observed compared with the control group. There was also no treatment-related effect on the incidence of non-neoplastic lesions. The results for clinical signs and organ weights were not reported.

No studies were available on reproductive toxicology.

3.2.3. Exposure

No information on the exposure has been provided by the applicant and, to the knowledge of the FEEDAP Panel, no information is available.

Tannic acid is authorised in the EU as a food flavouring, although no data on exposure are available for the European population. The Food and Drug Administration estimates that the added tannic acid in the USA can reach 100 mg/kg in various foods[18].

Taking this into account and given that the maximum proposed use level in animal feed is 15 mg/kg complete feed and that no accumulation in tissues is to be expected, it is unlikely that use in animal nutrition would significantly add to background exposure.

3.2.4. Conclusions on the safety for the consumer

Tannic acid is poorly absorbed *per se* from the gut lumen, but is extensively degraded in the gastrointestinal tract. Its metabolites in target species, experimental animals and humans are very similar and appear to be efficiently excreted. The expected lack of residues in food of animal origin

[18] Available online: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=scogslisting&id=348

suggests that it is unlikely that consumers will receive an appreciable amount of the parent compound or its metabolites. Toxicology studies of tannic acid are limited to evaluation of genotoxicity, short-term repeat-dose toxicity and carcinogenicity. Results from the *in vitro* genotoxicity tests indicate potential genotoxicity *in vitro*, but there was an absence of genotoxicity *in vivo* and no evidence of carcinogenicity following oral exposure. The results of the 90-day toxicity study showed necrosis in the livers of treated male rats (but not in females), but similar lesions were not found in the 104-week rat carcinogenicity study. The absence of liver lesions in females in the 90-day study and in both sexes in the carcinogenicity study suggests that these lesions were not produced as a result of treatment with tannic acid.

The FEEDAP Panel concludes that the use of tannic acid as a feed additive under the proposed conditions of use presents no safety risk to consumers.

3.3. Safety for the user

No experimental data on the safety for the user were provided. Potential hazards for skin and eye contact are recognised in the material safety data sheets[19]. The four batches of the additive that were tested had large numbers of fine particles and high dusting potentials, indicating that workers could be exposed by inhalation to a dust of tannic acid.

Consequently, the FEEDAP Panel considers it prudent to regard the additive as potentially hazardous to workers by exposure to the skin, eyes and mucous membranes or by inhalation.

3.4. Safety for the environment

Tannic acid is naturally present in feed materials. It is highly unlikely that its use as a feed additive would increase its concentration in the environment to any measurable extent; therefore, no risk to the safety of the environment is foreseen.

4. Efficacy

Tannic acid is listed in the EU list of food flavourings. Since its function in feed is essentially the same as that in food, no further demonstration of efficacy is necessary.

CONCLUSIONS

The use of tannic acid up to the proposed maximum level of 15 mg/kg complete feed is safe for all animals.

The use of tannic acid as a feed additive under the proposed conditions of use presents no safety risk to consumers.

In the absence of data, it would be prudent to regard the additive as potentially hazardous to workers by skin, eyes and mucous membranes or by inhalation.

The use of tannic acid as a feed additive is considered safe for the environment.

Tannic acid is recognised as a food flavouring agent and is included in the EU list of food flavourings. As its function in feed is essentially the same as that in food, no further demonstration of efficacy is necessary.

DOCUMENTATION PROVIDED TO EFSA

1. Chemically defined flavourings – Tannic acid. December 2010. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

[19] Technical Dossier/ Section II/ Annex II.2. MSDS Tannic acid.

2. Chemically defined flavourings – Tannic acid. Supplementary information. January 2012. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

3. Chemically defined flavourings – Tannic acid. Supplementary information. October 2013. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

4. Chemically defined flavourings – Tannic acid. Supplementary information. February 2014. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG).

5. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Chemically defined flavourings – Tannic acid.

6. Comments from Member States received through the ScienceNet.

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APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Chemically Defined Flavourings - Group 30 (Tannic acid)[20]

In the current application authorisation is sought under articles 4(1) and 10(2) for the *feed additive Tannic acid (Flavis 16.080)* under the "sensory additives", functional group 2(b) "flavouring compounds", according to the classification system of Annex I of Regulation (EC) No 1831/2003. *Tannic acid* belongs to the *Chemically Defined Flavourings - Group 30 (CDG 30)* - described in Annex I of Commission Regulation (EC) No 1565/2000 as – "*miscellaneous substances*". Authorisation is sought for the use of the *feed additive* for all species and categories.

Tannic acid is a yellow brown granular powder with a minimum purity of 93 %. *Tannic acid* is intended to be incorporated in *feedingstuffs* or drinking *water*, only in combination with other flavouring substances as *mixture of flavouring compounds*. The Applicant suggested no minimum or maximum, but normal contents of flavouring compound in *feedingstuffs* range from 0.1 to 100 mg/kg.

For the identification of *Tannic acid* in the *feed additive*, the Applicant proposed the internationally recognised European Pharmacopoeia method (Ph. Eur. 6th edition, monograph 1477), based on colorimetric or precipitation tests. Additionally, the Applicant proposed for the determination of *Tannic acid* in the *feed additive* the internationally recognised FAO JECFA monograph for food additives, in which: (i) identification is based on Thin Layer Chromatography (TLC), with Retention factors (R_f) of the sample and reference standard have to be the same; and (ii) quantification is based on gravimetric method.

Even though no performance characteristics are provided, the EURL recommends for official control the European Pharmacopoeia method (Ph. Eur. 6th edition, monograph 1477) and the FAO JECFA monograph for the determination of *Tannic acid* in the *feed additive*.

For the identification of *Tannic acid* (as gallic acid) in the *mixture of flavouring compounds*, the Applicant proposed a qualitative method based on Reversed Phase High Performance Liquid Chromatography (RP-HPLC) coupled to an UV detector measuring at 270 nm. In order to demonstrate the transferability of the proposed analytical method, the qualitative method was tested successfully in a second independent laboratory using four commercial premixtures, in the frame of a verification study.

Based on the satisfactory experimental evidence provided, the EURL recommends for official control the method submitted by the Applicant, based on Reversed Phase High Performance Liquid Chromatography (RP-HPLC), for the identification of *Tannic acid* (as gallic acid) in the *mixture of flavouring compounds*.

The Applicant did not provide any experimental method or data for the identification of *Tannic acid* in *feedingstuffs* and *water*. Therefore the EURL cannot evaluate nor recommend any method for official control to identify *Tannic acid* in *feedingstuffs* or *water*.

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) is not considered necessary.

[20] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0123.pdf

GLOSSARY

bw	body weight
CAS	Chemical Abstracts Service
DM	dry matter
EFSA	European Food Safety Authority
EU	European Union
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FFAC	Feed Flavourings Authorisation Consortium
FLAVIS	EU Flavour Information System
IOFI	International Organization of the Flavor Industry
i.p.	intraperitoneal
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
SCF	Scientific Committee on Food
WHO	World Health Organization