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Safety and efficacy of Diarr-Stop S Plus® (Na_2EDTA, tannin-rich extract of *Castanea sativa*, thyme oil and oregano oil) as a feed additive for pigs for fattening

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

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 Diarr-Stop S Plus®, a mixture of sodium salt of ethylenediaminetetraacetic acid (Na_2EDTA), a tannin-rich extract of *Castanea sativa*, thyme oil and oregano oil. The additive is intended for use with pigs for fattening at a recommended dose of 1,000 mg/kg feed to reduce the incidence of dysentery caused by *Brachyspira hyodysenteriae* and so improve performance. The additive is considered safe for pigs for fattening at the recommended dose of 1,000 mg/kg feed. None of the active constituents of Diarr-Stop S Plus® raised safety concerns for consumers when considered individually and at the concentrations delivered to feed using the recommended dose. As no interactions between constituents impacting on consumer safety are expected, the use of Diarr-Stop S Plus® in feed for pigs for fattening is considered safe for consumers without a withdrawal period. The additive should be considered as a skin and eye irritant, but the FEEDAP Panel could not conclude on the potential for dermal sensitisation. Because of the particle size, exposure of users by inhalation is considered unlikely. In the absence of appropriate data on the ecotoxicological effects of Na_2EDTA, the assessment of risk for terrestrial and aquatic compartments cannot be completed. None of the submitted efficacy studies, all based on observations on commercial premises, comply with the minimum requirements for an experimental design for the demonstration of efficacy. Consequently, the FEEDAP Panel is unable to conclude on the efficacy of the additive Diarr-Stop S Plus® when used in feed for pigs for fattening.

Keywords: Na_2EDTA, tannin, *Castanea sativa*, thyme oil, oregano oil, *Brachyspira hyodysenteriae*, pigs for fattening

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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 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.

The European Commission received a request from Pharmatéka Bt.[2] for authorisation of the product Diarr-Stop S Plus® (sodium salt of ethylenediaminetetraacetic acid (Na_2EDTA), *Castanea sativa* mill, thyme oil and oregano oil), when used as a feed additive for pigs for fattening (category: zootechnical additives; functional group: gut flora stabilisers).

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). 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 14 November 2011.

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, consumer, user and the environment and on the efficacy of the product Diarr-Stop S Plus® (Na_2EDTA, *C. sativa* mill, thyme oil and oregano oil), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive Diarr-Stop S Plus® is a preparation containing a mixture of the Na_2EDTA, a tannin-rich extract from *C. sativa*, thyme oil and oregano oil.

Diarr-Stop S Plus® has not previously been assessed for use as a feed additive in the European Union (EU) or elsewhere. However, its individual components have been assessed for use in food/feed for a variety of purposes. Calcium disodium EDTA is an authorised food additive in the EU (E 385) with an acceptable daily intake (ADI) set by the Joint Food and Agriculture Organization of the United nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) of 2.5 mg/kg body weight (bw) per day (WHO, 1966, 1974). No ADI for EDTA or its sodium salts has been established. However, from the ADI established for calcium disodium EDTA, a value of 1.9 mg/kg bw per day can be calculated for EDTA itself. This value was used in the determination of safe levels of ferric sodium EDTA as a source of iron for humans (EFSA ANS Panel, 2010).

Tannic acid (synonymous with hydrolysable tannin) is listed by the Council of Europe (2000), and appears in the EU list of flavouring substances for use in food[3] with the EU Flavour Information System (FLAVIS) number [16.080] and in the EU Register of Feed Additives. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) adopted an opinion on the safety and efficacy of tannic acid when used as feed flavouring for all animal species (EFSA FEEDAP Panel, 2014) and an earlier inconclusive opinion on a tannin-rich extract from *C. sativa* as a zootechnical additive when used with piglets and rabbits for fattening (EFSA, 2005).

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[4] in support of the authorisation request for the use of Diarr-Stop S Plus® as a feed additive.

[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] Pharmatéka Bt., Szíjgyártó utca 10, H-1048, Budapest, Hungary.
[3] 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.
[4] FEED dossier reference: FAD-2010-0406.

The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[5] and the applicable EFSA guidance documents.

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 and other scientific reports, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of Diarr-Stop S Plus® in animal feed. The Executive Summary of the EURL report can be found in Annex A.[6]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Diarr-Stop S Plus® is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c).

3. Assessment

The additive Diarr-Stop S Plus® is a preparation containing a mixture of the sodium salt of ethylenediaminetetra acetic acid (Na_2EDTA), a tannin-rich extract from *C. sativa*, thyme oil and oregano oil. It is intended to be used as a zootechnical additive (functional group: gut flora stabilisers) to positively influence the intestinal microbiota of pigs for fattening, particularly by reducing the incidence of dysentery caused by the bacterial spirochete *Brachyspira hyodysenteriae*, and so improve performance.

3.1. Characterisation

3.1.1. Characterisation of the additive

Diarr-Stop S Plus® is a simple blend of Na_2EDTA, a tannin-rich extract and two essential oils together with an emulsifier (glyceryl polyethyleneglycol ricinoleate), and glucose and wheat flour as carrier. The two essential oils are first mixed with the emulsifier and then added to the glucose, the tannin extract, Na_2EDTA and the wheat flour. All of the components are purchased from other commercial sources and blended in-house. The intended composition of the additive and the specification for the main components is shown in Table 1.

Table 1: Composition of Diarr-Stop S Plus® and specifications for the main components

Constituent	Concentration (% w/w)	Specifications	Analysed % w/w (range)
Na_2EDTA	24.0	21.6–26.4%	23.8 (22.8–24.0)
Tannin-rich extract	10.0		
Tannic acid		5.3–8.0%	6.7 (6.0–7.0)
Thyme oil	1.4		
Oregano oil	0.8		
Thymol		0.3–0.8%	0.78 (0.60–0.98)
Carvacrol		0.3–0.8%	0.65 (0.51–0.88)
Emulsifier	2.2		
Glucose	10.0		
Wheat flour	51.6		

[5] 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.

[6] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0406-DiarrStop.pdf

The content of the active components was confirmed by the analysis of six to nine batches. Individual compounds and tannin were directly determined, but this was not possible for the botanical extracts. In these cases, the major component were measured (Table 1). The dry matter (DM) content of seven batches ranged between 88.2% and 94.4%. A further three batches used in the determination of shelf life were also analysed. The mean content of EDTA was 23.4% (22.9–23.8%), tannic acid 7.3% (7.2–7.5%), thymol 0.67% (0.64–0.68) and carvacrol 0.47% (0.46–0.49). In addition, the concentration of a number of other terpenoids deriving from the essential oils was also measured (linalool 0.55%, p-cymene 0.60% and γ-terpinene 0.02%).

Action limits are set for the presence of heavy metals (Pb < 5 mg/kg, Hg < 0.1 mg/kg, Cd < 0.5 mg/kg) and for arsenic (< 2 mg/kg). Analysis of three batches of the additive showed that all values for heavy metals and arsenic were below these limits.[7] Routine testing for microbial contaminants showed that total aerobic bacterial counts and counts for total Enterobacteriaceae were < 100 colony-forming unit (CFU)/g, *Escherichia coli, Staphyloccocus aureus* and *Clostridium perfringens* < 10 CFU/g, and *Salmonella* sp. were absent in 25 CFU/g. Fungal counts were also < 100 CFU/g additive.[7] Three batches of the additive were analysed for aflatoxin B1 (0.35–0.42 mg/kg),[8] deoxynivalenol (< 0.04 mg/kg)[9] and dioxins (0.033–0.035 WHO-polychlorinated dibenzodioxin/dibenzofuran PCDD/F -TEQ ng/kg).[10]

The particle size of the additive was determined in two batches of the additive by sieving analysis and in three further batches by laser diffraction.[11] The data showed that the mean diameter of particles was approximately 700 μm and that essentially there were no particles with a diameter < 100 μm. No data on dusting potential is given.

3.1.2. Characterisation of the active substances

3.1.2.1. Na_2EDTA

Disodium ethylenediamine tetraacetate (Chemical Abstracts Service (CAS) number: 6381-92-6, 139-33-3); European Inventory of Existing Commercial Chemical Substances (EINECS) number: 205-358-3, International Union of Pure and Applied Chemistry (IUPAC) name: Disodium{[2-(bis-carboxymethyl-amino)-ethyl]-carboxymethylamino}-acetate; synonyms: ethylenediaminetetraacetic acid disodium salt; ethylenedinitrilotetraacetic acid disodium salt) has an empirical formula of $C_{10}H_{14}N_2Na_2O_8$ and a molecular weight of 372.24. Its structural formula is shown in Figure 1.

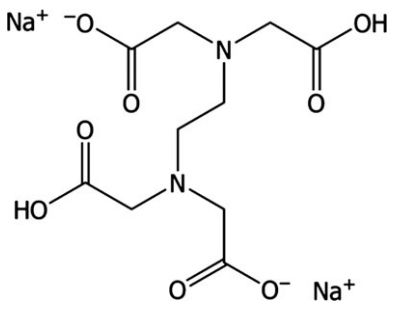

Figure 1: Structural formula of Na_2EDTA

Na_2EDTA is a white powder, soluble in water (100 g/L at 20°C) with a bulk density of 700 g/L. The free acid is a hexadentate ligand and chelating agent, particularly for divalent cations.

The Na_2EDTA used in the additive has a specified minimum content of 99.0% and a maximum content of nitrilotriacetic acid, a byproduct of its manufacture, of < 0.1%.[12]

3.1.2.2. Tannin-rich extract from *C. sativa*

The extract is obtained by aqueous extraction of sweet chestnut (*C. sativa*) and is commercially available. It is specified to contain 75.3% hydrolysable tannins, 17% of non-tannin substances and

[7] Technical dossier/Section II/Annex 2.
[8] Technical dossier/Section II/Annex 3.
[9] Technical dossier/Section II/Annex 4.
[10] Technical dossier/Section II/Annex 5.
[11] Technical dossier/Section II/Annex 6 and Technical dossier/Supplementary information August 2014/Particle size analysis.
[12] Technical dossier/Section II/Annex 10.

7.1% moisture. The analysis of one batch of a commercial product showed that the tannin content was 75.6% (ash: 1.2% and moisture 6%).[13]

3.1.2.3. Thyme oil

Thyme oil (CAS number 8007-46-3) is an essential oil obtained by steam distillation from *Thymus vulgaris*. It is a pale yellow to reddish brown liquid, immiscible in water. The main component is the monoterpene thymol (synonym: 2-isopropyl-5-methylphenol), which has the CAS number 89-83-8, the empirical formula $C_{10}H_{14}O$ and a molecular weight of 150.22. The thymol content in thyme oil ranges between 35% and 55%. The analysis of nine batches of the additive gave a mean value of 7.78 g/kg additive, implying a mean thymol concentration of 55% for the oil used in the additive.[14]

Other components typically present in thyme oils are monoterpenes or closely related compounds, notably p-cymene (15–28%), terpinene (5–10%), linalool (4–6.5%), carvacrol (1–4%), myrcene (1–3%) and terpinen-4-ol (0.2–2.5%).[15] No certificates of analysis were provided.

3.1.2.4. Oregano oil

Oregano oil is an essential oil obtained by steam distillation from *Origanum vulgare* (CAS number 8007-11-2). It is a yellowish to dark brownish liquid, immiscible in water. The main component is carvacrol (60–80%) (synonyms: 5-isopropyl-2-methylphenol, 2-methyl-5-(1-methylethyl)-phenol)), which has the CAS number 499-75-2, the empirical formula $C_{10}H_{14}O$ and molecular weight of 150.22. The structural isomer thymol is also typically present at 2–5% of the oil.[16] Analysis of carvacrol in the final product gave a mean value of 6.54 g/kg additive, implying a mean concentration in the oil used in the formulation of about 80%.[14]

3.1.3. Stability and homogeneity

Three batches of the additive were stored in sealed containers either at 25°C/60% relative humidity (RH) or 40°C/75% RH for periods of 18 months and 6 months, respectively.[17] No losses of Na_2EDTA, tannic acid or the two major terpenoids (thymol and carvacrol) were observed over these periods. A reduction in the minor components derived from the essential oils were seen, particularly p-cymene and linalool which both showed an > 50% reduction in content by the end of the experiments.

The mean content of Na_2EDTA appeared stable after 3 months of storage at 25°C/60% RH when three batches of an unspecified pelleted feed were examined (24.2% vs 23.4% Na_2EDTA).

No information was provided on the stability in premixtures or on the stability of the other substances present in the additive when incorporated into premixes or feed.

To assess the distribution of the additive in feed, three batches of the additive were mixed with pig feed at a concentration of 1,000 mg Diarr-Stop S Plus®/kg feed. For each batch, eight samples of the supplemented feed were taken: two from the top, two from the bottom and two from the middle of the mixing vessel, plus another two samples taken randomly. The concentration of Na_2EDTA in each sample was analysed and coefficients of variation (CV) calculated. The average CV across the three batches of the additive was 1.5% (ranging from 0.9% to 1.8%) indicating the homogeneous distribution of the additive in feed.[18]

3.1.4. Conditions of use

Diarr-Stop S Plus® is intended to be used in feed for pigs for fattening with a minimum weight of 35 kg until slaughter with a recommended use level of 1,000 mg/kg complete feed. No withdrawal period is foreseen.[19]

[13] Technical dossier/Supplementary information May 2014/Section II/Annex 15.
[14] Technical dossier/Section II/Annex 1.
[15] Technical dossier/Supplementary information January 2015.
[16] Technical dossier/Supplementary information May 2014/Section II_Identity.
[17] Technical dossier/Supplementary information January 2015/Annexes/StabStudDSSPOri.pdf
[18] Technical dossier/Supplementary information January 2015/Annexes/HomStudDSSPOri.pdf
[19] Technical dossier/Supplementary information January 2015/Answers.pdf

3.2 Safety

3.2.1. Safety for the target species

3.2.1.1. Safety for suckling and weaned piglets

A total of 12 sows (Big Hungarian White × Hungarian Landrace) were distributed to one of three treatments based on parity, farrowing date and litter size, and their litters used in the tolerance trial.[20] The experiment was divided into two phases, suckling (8–30 days) and rearing (31–79 days). The three treatments resulted from the supplementation of a basal diet (maize–wheat–barley–soybean meal) with Diarr-Stop S Plus® at 0, 1,000 mg/kg (1× recommended dose) or 10,000 mg/kg complete feed (10× recommended dose). Diets were provided *ad libitum*. Contents of Na_2EDTA were confirmed by analysis (260 mg Na_2EDTA/kg for the 1× recommended dose and 2,510 mg Na_2EDTA/kg for the 10× recommended dose).[21] On days 8 and 30 of age, individual piglet weight and litter feed intake were measured.

At weaning, only the piglets which weighed more than 6 kg were transferred to the rearing pens, where piglets from the same treatment were distributed to five pens per treatment (three males and three females per pen). Piglets from the four litters belonging to the same treatments were mixed. At the transfer, the 50:50 sex ratio was adjusted according to the weight at weaning in order to have similar initial body weight in the rearing phase in the three treatments (control: 7.4 ± 0.7; 1×: 8.2 ± 1.0; 10×: 8.2 ± 1.6). The rearing period lasted for an additional 49 days, until piglets were 79 days old. During this period and at the end of the trial, piglets were individually weighed and feed intake per pen was measured.

Blood samples were taken from all animals[22] (n = 90) at day 1 and day 49 of the rearing period for measurement of haematological[23] and biochemical parameters.[24] Data were analysed by analysis of variance (ANOVA) and statistical stated at $p < 0.05$.

A total of six piglets died during the suckling period, one each in the control and 1× treatments and four in the 10× group. Crushing was the main reason for the deaths. No significant differences were observed in weight gain (~ 220 g/day) or final weight at weaning (mean of control animals 6.7 kg, ×1 group 7.9 kg and ×10 group 7.4 kg). Although feed intake was measured, results were not statistically examined as most nutrition was provided by the sow.

No differences in weight/weight gain (approximately 31 kg/470 g per day) and feed intake or in any of the haematological and biochemical parameters measured were observed in the rearing phase.[25] No mortality was reported in any of the treatments during this phase of growth.

3.2.1.2. Safety for pigs for fattening

A combined tolerance and residue study was carried out with a total of 36 pigs for fattening (Hungarian Large White × Hungarian landrace, 29 kg initial weight).[26] The animals were distributed to one of three treatments (six males and six females individually housed per treatment), resulting from the supplementation of a basal diet (maize–wheat–barley–soybean meal) with Diarr-Stop S Plus® at 0, 1,000 mg/kg (1× recommended dose) or 10,000 mg/kg complete feed (10× recommended dose) (doses not confirmed by analysis). Diets were provided *ad libitum*. General health was monitored and animals and feed weighed at biweekly intervals. Blood, urine and faeces were collected from each animal at the start of the trial, after 6 weeks when animals weighed around 59 kg and at the end of the experiment (approx. weight 102 kg). The experiment lasted 92 days. However, for the groups receiving Diarr-Stop S Plus®, four animals were given the treated diets until the end of the experiment, another four until 2 days before the end of the experiment and the remaining four until 4 days before the end of the experiment. These animals received the control feed when the treated feed was withdrawn. Blood, urine and faecal samples were collected at the beginning, after 1.5 months and at the end of the experiment. At the end of the experiment all animals were

[20] Technical dossier/Section III/Annexes 1–3, 8 and 9.
[21] Technical dossier/Supplementary information December 2015/Annex 3.
[22] Technical dossier/Supplementary information December 2015/Annex 2a and 2b.
[23] Platelets, haematocrit, red blood cells, haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration (MCHC) and white blood cells: total and differential counts.
[24] Aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), urea, creatinine, phosphorous, calcium, iron and iron-binding capacity.
[25] Technical dossier/Supplementary information December 2015/Annexes 4, 5 and 6.
[26] Technical dossier/Supplementary information May 2014/Section III/Annexes 4, 5, 6, 8 and 9.

sacrificed, carcasses evaluated, and samples of liver, kidney, large and small intestine, back fat, thigh muscle and rib bone were collected from each animal. The content of EDTA was measured in the blood, urine and faecal samples and in the various tissues as part of the metabolism/residue determination (see Section 3.2.2). Data were analysed by ANOVA and significance assumed at $p < 0.05$.

No animals died in the course of the experiment. No significant differences were observed on final weight (average: 102 kg) or weight gain (average: 767 g/day). However, feed intake was significantly reduced in the 10× group in comparison with the control (2.04 vs 2.39 kg/day, $p < 0.05$) which resulted in a significantly better feed to gain ratio (2.75 vs 3.07, $p < 0.05$).

Samples of rib bones and liver were obtained from the pigs for fattening in the tolerance study described above. The bones were analysed for Ca, Zn and Pb contents, while livers were analysed for Cu, Mn, Zn and Pb contents.[27] No effects were observed in the levels of the trace elements studies with the exception of Mn in liver, which was significantly reduced in pigs receiving the additive irrespective of the dose (4.01, 3.66 and 3.41 mg/kg for 0, 1× and 10×, respectively; all $p < 0.05$).

Samples of the small and large intestine were analysed for morphology (length and width of the villi, thickness of the endothelium, cellular infiltration of the propria layer, positive epithelioglandular cells in the Lieberkühn's crypts).[28] No differences were observed in any of the measured parameters between treatments.

3.2.1.3. Microbial studies

The applicant provided a series of *in vitro* studies with Na_2EDTA alone and none with the additive under application. These focussed on the survival/proliferation of strains of *B. hyodysenteria* in the presence of Na_2EDTA and the effects on other bacterial components of the gut microbiota.

3.2.1.4. Conclusions on safety for the target species

The Panel recognises that the tolerance study provided for pigs for fattening does not fulfil the requirement for a tolerance study (no blood haematology and biochemistry). However, taking all of the data into consideration and recognising that both weaned piglets, considered to be the most sensitive stage of growth, and pigs for fattening showed no adverse effects when given diets containing 10 times the recommended dose of Diarr-Stop S Plus®, the FEEDAP Panel considers that the additive is safe for pigs for fattening at the recommended dose of 1,000 mg/kg complete feed.

3.2.2. Safety for the consumer

No toxicological studies are available made with the additive itself and so safety for the consumer is assessed by considering the different components of the additive separately.

3.2.2.1. EDTA

A no observed adverse effect level (NOAEL) of 500 mg/kg bw per day has been identified for Na_2EDTA and Na_3EDTA from a 90-day study in rats and in a long-term (2-year) study in rats and mice. Higher doses in the 90-day study (2,500 mg/kg bw per day) and in a range-finding study preceding the 2-year study caused diarrhoea, loss of bodyweight, decreased packed cell volume (PCV) and Hb, and parakeratosis in the oesophagus and forestomach. Studies in animals showed no adverse effects on reproductive performance at doses of EDTA up to 250 mg/kg bw per day. Teratological effects of EDTA seen with high doses (1,000 mg/kg bw per day and greater) were primarily attributed to interference with zinc homeostasis and its consequences for development and could be avoided by inclusion of excess Zn in the diet (European Union Risk Assessment Report (EU-RAR), 2004).

Although no evidence of genotoxicity were found for EDTA in somatic cells, a positive result was seen in a micronucleus test with spermatids, indicating that aneugenic effects may be induced in specific phases of spermatogenesis (late spermatocytogenesis). However, the effect was seen only with the use of an extremely high dose in the median lethal dose (LD_{50}) range. As the induction of aneuploidy is based on a threshold mode of action, it was concluded that the potential for induction of aneuploidy will not be expressed at low doses (EU-RAR, 2004).

[27] Technical dossier/Supplementary information May 2014/Section III/Annex 15.
[28] Technical dossier/Supplementary information May 2014/Section III/Annex 17.

Residue deposition in the target species

A combined tolerance and residue study was carried out in pigs for fattening, with three experimental subgroups: a control, an experimental group receiving Diarr-Stop S Plus® at the proposed dose (1,000 mg Diarr-Stop S Plus®/kg complete feed) and an experimental group receiving Diarr-Stop S Plus® at 10 times the proposed dose (10,000 mg Diarr-Stop S Plus®/kg complete feed) (for the experimental design see Section 3.2.1.2). At slaughter, without prior withdrawal of the additive, all the analysed tissues showed mean concentrations of EDTA at the ng/g levels, with liver the organ with highest concentrations, for both dosages (436 and 1,382 ng/g), followed by kidney (220 and 707 ng/g), meat (163 and 522 ng/g) and fat (31 and 172 ng/g). With 2 or 4 days withdrawal before slaughter, the EDTA concentrations in the tissues appreciably decreased, to about half the values above or lower.

Taking the current ADI of 2.5 mg/kg bw per day established for calcium, disodium EDTA and from this the calculated value of 1.9 mg/kg bw per day for the EDTA moiety, compared with the estimated maximum exposure of 9 μg/kg bw per day, the FEEDAP Panel has no concern over the safety of this component of Diarr-Stop S Plus® for the consumer.

3.2.2.2. Tannin-rich extract of sweet chestnut

The FEEDAP Panel delivered an opinion on a tannin-rich extract (EFSA, 2005), which is stated to be the same source of tannin used in the preparation of Diarr-Stop S Plus®. In this opinion it was concluded that 'higher molecular weight fragments are probably not absorbed to any detectable extent and that only free or released gallic and ellagic acids are available for uptake. Both can be detected in serum and urine in their original form (alone or as conjugates) and, in the case of gallate, as simple methoxyl derivatives. Humans are naturally exposed to hydrolysable tannins and their breakdown products and although it is not possible to conclude on the extent to which the products of hydrolysable tannins are deposited in animal tissues, this is likely to be low'.

The FEEDAP Panel reviewed the published data on toxicology studies made with hydrolysable tannins from various sources in course of an application for use as a feed flavour (EFSA FEEDAP Panel, 2014). Results from the *in vitro* genotoxicity tests indicated potential genotoxicity *in vitro*, but there was an absence of genotoxicity *in vivo* and no evidence of carcinogenicity following oral exposure.

A single report of a 90-day repeat dose subchronic study in which rats were given tannic acid via the drinking water (0.025–0.4%) was available only as an abstract. Necrosis in the livers of treated male rats (but not in females) was reported. However, the authors of the study considered this as incidental because of the absence of liver lesions in females in the 90-day study and in both sexes in the carcinogenicity study and consequently proposed the top dose as a NOAEL. This would equate to approximately 360 mg/kg bw per day using the default values for conversion proposed by the EFSA Scientific Committee (EFSA Scientific Committee, 2012). The FEEDAP Panel is not able to confirm the NOAEL for this study in the absence of the full study report.

Hydrolysable tannins are degraded in the digestive tract of livestock and the breakdown products absorbed are eliminated by well recognised routes. Thus, it is unlikely that tannin or its metabolites accumulate to any extent in tissues and products of animal origin. Even was this the case, the available toxicity studies gives some reassurance that there would be no untoward effects from residues of either tannin or its metabolites following consumption of animal products.

3.2.2.3. Thyme oil and oregano oil

The FEEDAP Panel assessed thymol and carvacrol, the major components of the two oils, in the context of an examination of their use as feed flavourings. Both were considered safe for the consumers when used in pig feed at a maximum concentration of 12 mg/kg (EFSA FEEDAP Panel, 2012d).

The levels of thymol and carvacrol delivered using the additive at the maximum recommended dose (1,000 mg/kg feed) are 8.6 mg/kg feed (thymol) and 7.2 (carvacrol) mg/kg feed. If the highest value from the batch to batch variation (see Section 3.1.1) is used then the values are 10.8 and 9.7 mg/kg feed, which are still below the upper concentration in feed considered safe for consumers set in the FEEDAP opinion. For the other measured components of the essential oils, assuming the use of the maximum dose, linalool would be present at 5 mg/kg feed, γ-terpinene at 0.2 mg/kg feed and p-cymene at 6 mg/kg feed. These compounds have been previously assessed by the FEEDAP Panel in the course of an assessment made for use as a flavour in feed (EFSA FEEDAP Panel, 2012e, 2015). For linalool, γ-terpinene and p-cymene, it was concluded that the use in feed of up to 20, 1.5 and 25 mg/kg feed, respectively, would be safe for the consumers.

3.2.2.4. Conclusions on safety for the consumer

None of the active constituents of Diarr-Stop S Plus® raised safety concerns when considered individually and at the concentrations delivered to feed using the recommended dose. Since no interactions between constituents impacting on consumer safety are expected, the use of Diarr-Stop S Plus® at the recommended dose of 1,000 mg/kg in feed for pigs for fattening is considered safe for consumers without a withdrawal period.

3.2.3. Safety for the user

3.2.3.1. Effects on eyes and skin

A skin irritation test was performed with Diarr-Stop S Plus® on reconstructed human epidermis following OECD guideline 439.[29] The results showed that the additive has the potential to be an irritant of human skin. Therefore, it should also be considered an eye irritant.

No information was provided on the sensitisation properties of the additive.

3.2.3.2. Effects on the respiratory system

The additive has negligible amount of particles of inhalable size (< 100 μm diameter). Exposure of the lower respiratory system is therefore considered unlikely.

3.2.3.3. Conclusions on safety for the user

The additive should be considered as a skin and eye irritant. Exposure by inhalation is considered unlikely. In the absence of data, the FEEDAP Panel could not conclude on dermal sensitisation.

3.2.4. Safety for the environment

Most of the constituents are natural compounds whose concentrations are not expected to increase because of the use of Diarr-Stop S Plus® as a feed additive. According to the EFSA guidance for assessing the safety of feed additives for the environment (EFSA, 2008), the addition of a naturally occurring substance that will not result in a substantial increase in its concentration in the environment is exempt from further assessment. Consequently, the tannin extract, the two essential oils and the excipients are not further considered.

EDTA is the major constituent of Diarr-Stop S Plus® and is not a naturally occurring substance. The addition of Diarr-Stop S Plus® at the recommended dose results in 240 mg Na_2EDTA added per kilogram of feed. Therefore, an initial environmental risk assessment was performed based on this dose and the physicochemical values shown in Table 2.

Table 2: Physicochemical properties of Na_2EDTA[30]

Properties	Na_2EDTA
CAS	6381-92-6
Molecular weight	372.24
Solubility in water (g/L)	500
K_{oc} (a)	3.75×10^3
Log K_{ow}	−11.7

OC: organic carbon.

(a): $K_{oc} = Kp_{soil}/Foc_{soil}$; Kp_{soil} = 75 L/kg (EU-RAR, 2004); Foc_{soil}: 0.02 kg/kg^{-1} (EFSA, 2008) K_{ow}: octanol/water partition coefficient.

From these data a soil concentration (PEC_{soil}) of 4,308.9 μg/kg was calculated for the use of 240 mg Na_2EDTA/kg feed for pigs for fattening according to the EFSA guidance document (EFSA, 2008). This is considerably above the threshold value of concern of 10 μg/kg. A groundwater concentration (PEC_{gw}) of 64.9 μg/L and a surface water concentration (PEC_{sw}) of 21.66 μg/L also were calculated, both of which are above the threshold of 0.1 μg/L. This indicates the need for a phase II environmental risk assessment for the compartments of concern.

[29] Technical dossier/Section III/Annex 22.
[30] Technical dossier/Section II/Section II_Identity.

3.2.4.1. Phase II assessment

The European Union Risk Assessment Report (EU-RAR, 2004) on EDTA states that '... Na_2EDTA both in the process of intake to the body and discharge to the environment can be considered identical to that of H_4EDTA and Na_4EDTA, so its toxicity can also be perceived according to the same mechanism'. It is to be expected that the fate and behaviour of EDTA in different salts would be similar. However, the ecotoxicological profile of H_4EDTA and Na_4EDTA differs. The applicant has not submitted any new data on the ecotoxicological effects of Na_2EDTA and therefore a PNEC calculation for Na_2EDTA cannot be made. Consequently, in the absence of appropriate data on the ecotoxicological effects of Na_2EDTA, the assessment of risk for terrestrial and aquatic compartments cannot be completed.

3.3. Efficacy

The four studies provided as evidence of the efficacy of Diarr-Stop S Plus® were made in commercial farms, without replication. None of the submitted studies comply with the minimum requirements for an experimental design for the demonstration of efficacy. Consequently, the FEEDAP Panel is unable to conclude on the efficacy of the additive Diarr-Stop S Plus® when used in feed for pigs for fattening.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[31] and Good Manufacturing Practice.

4. Conclusions

The additive is considered safe for pigs for fattening at the recommended dose of 1,000 mg/kg complete feed.

None of the active constituents of Diarr-Stop S Plus® raised safety concerns when considered individually and at the concentrations delivered to feed using the recommended dose. As no interactions impacting on consumer safety are expected, the use of Diarr-Stop S Plus® at the recommended dose of 1,000 mg/kg in feed for pigs for fattening is considered safe for consumers without a withdrawal period.

The additive should be considered as a skin and eye irritant. Exposure by inhalation is considered unlikely. In the absence of data, the FEEDAP Panel could not conclude on the dermal sensitisation.

In the absence of appropriate data on the ecotoxicological effects of Na_2EDTA, the assessment of risk for terrestrial and aquatic compartments cannot be completed.

None of the submitted studies comply with the minimum requirements for an experimental design for the demonstration of efficacy. Consequently, the FEEDAP Panel is unable to conclude on the efficacy of the additive Diarr-Stop S Plus® when used in feed for pigs for fattening.

Documentation provided to EFSA

1) Diarr-Stop S Plus® for pigs for fattening. November 2010. Submitted by Pharmatéka Bt.
2) Diarr-Stop S Plus® for pigs for fattening. Supplementary information. August 2012. Submitted by Pharmatéka Bt.
3) Diarr-Stop S Plus® for pigs for fattening. Supplementary information. May 2014. Submitted by Pharmatéka Bt.
4) Diarr-Stop S Plus® for pigs for fattening. Supplementary information. August 2014. Submitted by Pharmatéka Bt.
5) Diarr-Stop S Plus® for pigs for fattening. Supplementary information. January 2015. Submitted by Pharmatéka Bt.
6) Diarr-Stop S Plus® for pigs for fattening. Supplementary information. December 2015. Submitted by Pharmatéka Bt.

[31] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

7) Diarr-Stop S Plus® for pigs for fattening. Supplementary information. January 2016. Submitted by Pharmatéka Bt.
8) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Diarr-Stop S Plus.
9) Comments from Member States.

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Abbreviations

ADI	acceptable daily intake
ALP	alkaline phosphatase
ANOVA	analysis of variance
ANS Panel	EFSA Panel on Additives and Nutrient Sources added to Food
AST	aspartate aminotransferase
BW	body weight
CAS	Chemical Abstracts Service
CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CFU	colony-forming unit
CV	coefficient of variation
DM	dry matter
EDTA	ethylenediaminetetraacetic acid
EINECS	European Inventory of Existing Commercial Chemical Substances
EU-RAR	European Union Risk Assessment Report
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization of the United Nations
FEED Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed
FGE	food group evaluation
FLAVIS	The EU Flavour Information System
FL-no	FLAVIS number
GGT	gamma-glutamyl transferase
GC–MS	gas chromatography–mass spectrometry
Hb	haemoglobin
HPLC-UV	high-performance liquid chromatography with ultraviolet detection
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	median lethal dose
LOQ	limit of quantification
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
Na_2EDTA	sodium salt of ethylenediaminetetraacetic acid
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCDD/F	polychlorinated dibenzodioxins and dibenzofurans
PCV	packed cell volume
PEC	predicted environmental concentration
PNEC	predicted no effect concentration
RH	relative humidity
Rrec	recovery rate
RSDip	relative standard deviation for intermediate precision
RSDr	relative standard deviation for repeatability
SCAN	Scientific Committee on Animal Nutrition
SCF	Scientific Committee on Food
TEQ	toxic equivalent
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory (EURL) for feed additives on the method(s) of analysis for Diarr-Stop S Plus

In the current application, authorisation is sought under article 4(1) for Diarr-Stop S Plus under the category/functional group 4(b) 'zootechnical additives'/'gut flora stabilisers' according to the classification system of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the feed additive for pigs for fattening.

Diarr-Stop S Plus is a brown powder preparation containing wheat meal, dextrose/meritose and glycerol polyethyleneglycol ricinoleate, and four active substances (19–29% sodium ethylene-diaminetetraacetate, Na_2EDTA; 5–15% tannin/tannic acid as *Castanea sativa* mill; 0.6–0.9% thymol as thyme oil; and 0.5–0.8% carvacrol as origanum oil).

The feed additive is to be used directly into feedingstuffs. The Applicant suggested an inclusion level of Diarr-Stop S Plus ranging from 900 to 1,100 mg/kg complete feedingstuffs.

For the determination of the sodium ethylenediaminetetraacetate (Na_2EDTA) in the feed additive, the Applicant submitted a single-laboratory validated and further verified method based on high-performance liquid chromatography with ultraviolet detection (HPLC-UV). The following performance characteristics were reported in the frame of the validation and verification studies: – a relative standard deviation for repeatability (RSDr) ranging from 1.7% to 5.0%; – a relative standard deviation for intermediate precision (RSDip) ranging from 4.4% to 5.0%; and – a recovery rate (Rrec) ranging from 100% to 106%.

For the determination of tannin/tannic acid in the feed additive, the Applicant applied the ring trial validated method (ISO 9648) based on spectrophotometry and reported RSDr of 6.7% for a tannin/tannic acid content of 5.8% in the feed additive. This precision value is in good agreement with the performance characteristics reported by the ISO method.

For the determination of thymol and carvacrol in the feed additive, the Applicant submitted a single-laboratory validated and further verified method based on gas chromatography with flame ionisation detection (GC-FID). The following performance characteristics were reported in the frame of the validation and verification studies: – RSDr ranging from 1.9% to 9.2%; – RSDip ranging from 7.7% to 12.6%; – a recovery rate (Rrec) 93%; and a limit of quantification (LOQ) of 0.005 mg/kg feed additive for the both analytes.

Based on the experimental evidence available, the EURL recommends the single-laboratory validated and further verified HPLC-UV and GC-FID methods submitted by the Applicant together with the ISO method for the quantification of ethylenediaminetetraacetate (Na_2EDTA), thymol, carvacrol and tannin/tannic acid in the feed additive (Diarr Stop S Plus).

The accurate determination of Diarr-Stop S plus in feedingstuffs is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine Diarr-Stop S plus in feedingstuffs.

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.