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## Safety and efficacy of a feed additive consisting of tocopheryl phosphate mixture (TPM) for all animal species (Avecho biotechnology limited)

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### Abstract

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 tocopheryl phosphate mixture (TPM) as nutritional feed additive for all animal species. The additive has not been authorised for use in animal nutrition. TPM is produced by chemical synthesis and is a mixture of two different phosphorylated tocopheryl compounds in approximate 2:1 weight ratio: all-*rac*- $\alpha$ -tocopheryl di-hydrogen phosphate (TP) and all-*rac*-di- $\alpha$ -tocopheryl hydrogen phosphate (T<sub>2</sub>P). It is intended to be used as nutritional additive (as a source of vitamin E) in feed for all animal species and categories. Considering the limited information on the ADMER for the components of the additive and the uncertainties on the potential aneugenicity and clastogenicity of the additive, the Panel cannot conclude on the safety of the additive for the target species and for the consumer. TPM is not a skin irritant nor a skin sensitiser but should be considered irritant to the eyes and the upper respiratory tract. Owing to the uncertainty on the potential aneugenicity and clastogenicity of the additive, it is not possible to conclude on safety for the user. The FEEDAP Panel cannot conclude on the safety of TPM for the environment due to lack of data on environmental impact of T<sub>2</sub>P. TPM is a bioavailable source of  $\alpha$ -tocopherol. The data available, however, do not allow the Panel to establish the relative bioequivalence of TPM as vitamin E. Therefore, the Panel is not in the position to conclude on the efficacy of TPM for all animal species.

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**Keywords:** vitamin E, tocopheryl phosphate mixture, safety, efficacy, nutritional additive

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## 1. Introduction

### 1.1. Background and terms of reference

Regulation (EC) No 1831/2003<sup>1</sup> 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 feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Avecho Biotechnology Limited<sup>2</sup> for the authorisation of the additive consisting of tocopheryl phosphate mixture, when used as a feed additive for all animal species (category: nutritional additives; functional group: vitamins, pro-vitamins and chemically well-defined substances having similar effect).

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 4 April 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, consumer, user and the environment and on the efficacy of the feed additive consisting of tocopheryl phosphate mixture, when used under the proposed conditions of use (see **Section 3.1.4**).

### 1.2. Additional information

Tocopheryl phosphate mixture (TPM) is not authorised in the EU as a feed additive.

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety and efficacy of vitamin E, in the form of all-*rac*- $\alpha$ -tocopheryl acetate, RRR- $\alpha$ -tocopheryl acetate and RRR- $\alpha$ -tocopherol, when used as a feed additive for all animal species (EFSA FEEDAP Panel, 2010). In 2012, the FEEDAP Panel issued an opinion on the safety and efficacy of synthetic  $\alpha$ -tocopherol when used as a technological additive (antioxidant) for all animal species (EFSA FEEDAP Panel, 2012b) and another opinion on the safety and efficacy of tocopherol-rich extracts of natural origin, tocopherol-rich extracts of natural origin/delta-rich and synthetic tocopherol for all animal species (EFSA FEEDAP Panel, 2012c). The FEEDAP Panel has issued several opinions on the renewal of authorisation of different additives consisting of vitamin E (EFSA FEEDAP Panel, 2021a–f).

Vitamin E (3a700) in the form of all-*rac*- $\alpha$ -tocopheryl acetate, RRR- $\alpha$ -tocopheryl acetate and RRR- $\alpha$ -tocopherol is currently authorised as a nutritional additive for all animal species.<sup>3</sup>  $\alpha$ -Tocopherol is also authorised for use as a technological additive (functional group: antioxidants) in feed for all animal species (1b307).<sup>4</sup>

All-*rac*- $\alpha$ -tocopheryl acetate is described in the European Pharmacopoeia 10.0 (PhEur), monograph 0439 (PhEur, 2020).

The Scientific Committee for Food (SCF) established a tolerable upper intake level (UL) for vitamin E as 270 mg/day for adults and rounded to 300 mg/day (SCF, 2003). The EFSA Panel on Dietetic Products, Nutrition and Allergy issued an opinion on dietary reference values for vitamin E as  $\alpha$ -tocopherol (EFSA NDA Panel, 2015). The EFSA Panel on Food Additives and Nutrient Sources Added to Food (EFSA ANS

<sup>1</sup> Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

<sup>2</sup> Originally, the application was sent by Triveritas Ltd, Bank Barn, CA8 9JY How Mill, Brampton, UK on behalf of Phosphagenics Ltd, Unit A8, 2A Westall Road, VIC 3168, Clayton, Australia. During the assessment, the applicant became Avecho Biotechnology Limited (Unit 8A, 2A Westall Road, Hallmarc Business Park, 3,168 Clayton, Victoria, Australia), represented in the EU by Cyton AH Biosciences GmbH (part of the Knoell group, Eastsite XII, Konrad-Zuse-Ring 25, 68,163 Mannheim, Germany).

<sup>3</sup> Commission Regulation (EU) No 26/2011 of 14 January 2011 concerning the authorisation of vitamin E as a feed additive for all animal species, OJ L 11, 15.1.2011, p. 18–21.

<sup>4</sup> Commission Regulation (EU) No 2015/1152 of 14 July 2015 concerning the authorisation of tocopherol extracts from vegetable oils, tocopherol-rich extracts from vegetable oils (delta rich) and alpha-tocopherol as feed additives for all animal species. OJ L 187, 15.7.2015, p. 5.

Panel) issued an opinion on the evaluation of tocopherol-rich extract (E 306),  $\alpha$ -tocopherol (E 307),  $\gamma$ -tocopherol (E 308) and  $\delta$ -tocopherol (E 309) as food additives (EFSA ANS Panel, 2015).

Tocopherol-rich extract (E 306),  $\alpha$ -tocopherol (E 307),  $\gamma$ -tocopherol (E 308) and  $\delta$ -tocopherol (E 309) are authorised as food additives.<sup>5</sup> Vitamin E is authorised for use in food for nutritional purposes,<sup>6</sup> for use in cosmetics as antioxidant<sup>7</sup> and as a veterinary medicinal product.<sup>8,9</sup>

## 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<sup>10</sup> in support of the authorisation request for the use of TPM 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.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the TPM in animal feed. The executive summary of the EURL report can be found in Annex A.<sup>11</sup>

### 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of TPM is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012a), Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

## 3. Assessment

Vitamin E represents a group of fat-soluble compounds that contains four tocopherols ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) and four tocotrienols ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) with antioxidant activity. Among those,  $\alpha$ -tocopherol has the highest biological activity.

The product subject of this application is TPM constituted by  $\alpha$ -tocopheryl di-hydrogen phosphate and di- $\alpha$ -tocopheryl hydrogen phosphate which are phosphorylated esters of  $\alpha$ -tocopherol (vitamin E). It is intended to be used as nutritional additive (functional group: vitamins, pro-vitamins and chemically well-defined substances having similar effect) in feed for all animal species and categories. TPM is produced by chemical synthesis.<sup>12</sup>

<sup>5</sup> Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council Text with EEA relevance. OJ L 83, 22.3.2012.

<sup>6</sup> Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009, OJ L 181, 29.6.2013, p. 35.

<sup>7</sup> Commission Decision of 9 February 2006 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products (2006/257/EC). OJ L 97, 5.4.2006, p. 1

<sup>8</sup> 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, 20.1.2010, p. 1.

<sup>9</sup> Commission Regulation (EC) No 997/1999 of 11 May 1999 amending Annexes I, II and III of Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. OJ L 122, 12.5.1999, p. 24.

<sup>10</sup> FEED dossier reference: FAD-2018-0057.

<sup>11</sup> The full report is available on the EURL website: [https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2017-0057-tocopheryl\\_phosphate.pdf](https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2017-0057-tocopheryl_phosphate.pdf)

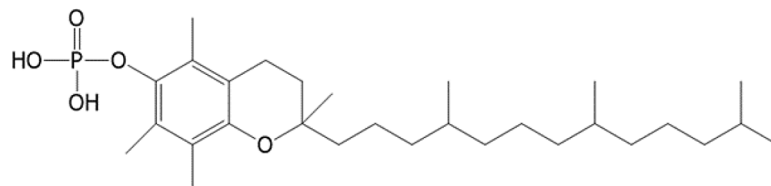
<sup>12</sup> Commission Implementing Regulation (EU) 2017/873.

### 3.1. Characterisation

#### 3.1.1. Characterisation of the active substances

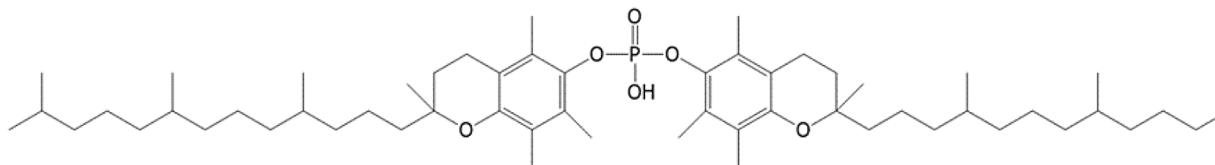
TPM is a mixture of two different phosphorylated tocopheryl compounds in approximate 2:1 weight ratio: all-*rac*- $\alpha$ -tocopheryl di-hydrogen phosphate (TP) and all-*rac*-di- $\alpha$ -tocopheryl hydrogen phosphate (T<sub>2</sub>P).

All-*rac*- $\alpha$ -tocopheryl di-hydrogen phosphate International Union of Pure and Applied Chemistry (IUPAC) name: all-*rac*-2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-3,4-dihydro-2H-1-benzopyran-6-yl dihydrogen phosphate; synonym: tocopheryl phosphate, has the Chemical Abstracts Service (CAS) No 38976-17-9. The chemical formula is C<sub>29</sub>H<sub>51</sub>O<sub>5</sub>P, the molecular weight is 510.69 g/mol. The structural formula is given in Figure 1.



**Figure 1:** Structural formula of all-*rac*- $\alpha$ -tocopheryl di-hydrogen phosphate (TP)

All-*rac*-di- $\alpha$ -tocopheryl hydrogen phosphate (IUPAC name: Bis[all-*rac*-2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-3,4-dihydro-2H-1-benzopyran-6-yl] hydrogen phosphate; synonym: di-tocopheryl phosphate) has the CAS No 635703-86-5. The chemical formula is C<sub>58</sub>H<sub>99</sub>O<sub>6</sub>P, the molecular weight is 923.38 g/mol. The structural formula is given in Figure 2.



**Figure 2:** Structural formula of all-*rac*-di- $\alpha$ -tocopheryl hydrogen phosphate (T<sub>2</sub>P)

#### 3.1.2. Manufacturing process

TPM is manufactured by chemical synthesis

[REDACTED]

#### 3.1.3. Characterisation of the additive

The additive is specified to contain 50–70% TP, 20–40% T<sub>2</sub>P, < 10% all-*rac*- $\alpha$ -tocopherol, < 6% phosphoric acid and < 1% total related substances,<sup>15</sup> in all cases on an 'as is' basis.<sup>16</sup>

The analysis of five batches<sup>17</sup> showed, on 'as is' basis, an average content of 59.3% TP (58.5–60.1), 30.8% T<sub>2</sub>P (29.9–31.4%), 1.4% all-*rac*- $\alpha$ -tocopherol (0.2–2.36%), [REDACTED] phosphoric acid

<sup>13</sup> Technical dossier/Supplementary information January 2019/Annexes 1 and 2.

<sup>14</sup> Technical dossier/Section II/Section 2.3.

<sup>15</sup> Technical dossier/Section II/Section 2.1.3, Annex II.1 and Annex II.5 section 6.9.

<sup>16</sup> Total related substances correspond to all unidentified impurities on HPLC chromatograms during the quantitative analysis of TPM.

<sup>17</sup> Technical dossier/Section II/Annex II.6 Appendix I. Analytical method for TP and T<sub>2</sub>P was HPLC using phosphate in isopropanol as mobile phase and UV detection.

(██████████), 0.46% total related substances (0.1–0.7%)<sup>18</sup> and ██████████ water.<sup>19</sup> On a dry matter basis, the amount of identified material is, on average, 101.1% (range 99.4–102.6%).

### 3.1.3.1. Undesirable substances

Cadmium, lead, mercury and arsenic were analysed in six batches of the additive. Cadmium and mercury were below the limit of detection (LOD). Lead ranged from below LOD to ██████████ mg/kg and arsenic ranged ██████████ mg/kg.<sup>20</sup> Two batches were further analysed for aluminium (0.16 and 0.21 mg/kg), antimony (0.6 mg/kg), molybdenum (0.01 and 0.02 mg/kg), nickel (0.11 and 0.12 mg/kg), iron (below the LOD and 2 mg/kg), copper (below the LOD and 0.03 mg/kg) and tin, sodium and bismuth (all three below the LOD).<sup>21</sup>

The detected amounts of the above described impurities do not raise safety concerns.

### 3.1.3.2. Physical properties of the additive

The additive (TPM) is a waxy light–dark brown powder ██████████. The pH ranges between 3 and 4.<sup>22</sup> Melting point is ██████████, ignition point is >120°C.<sup>23</sup> The partition coefficient (log P), the acid dissociation constant (Ka) and the adsorption/desorption coefficient (Koc) of TP and T<sub>2</sub>P (one batch each analysed)<sup>24</sup> indicated that TP is ██████████ in water whereas T<sub>2</sub>P is ██████████ in water. The bulk density measured in three batches ranged from 547 to 560 kg/m<sup>3</sup>.<sup>25</sup>

The dusting potential showed high variability. The analysis of one batch of the additive, in accordance with EN15051 method, showed that the inhalable, thoracic and respirable dust mass fractions were 72, 14 and 1 mg/m<sup>3</sup>, respectively.<sup>26</sup> Analyses of two additional batches using the same method showed a much lower dustiness: No respirable dust was detected and the inhalable and thoracic mass fractions ranged 0.4–1.2 and 0.1–0.5 mg/m<sup>3</sup>, respectively.<sup>27</sup>

Regarding the particle size distribution of the additive under application, the analysis of one batch by laser diffraction, shows a proportion of particles < 10, < 50 and < 100 μm diameter of ██████████, respectively.<sup>28</sup> ██████████ of the particles have a diameter ranging from ██████████. No information was provided on the presence of small particles including nanoparticles.

### 3.1.3.3. Stability and homogeneity

The shelf-life of TPM (three batches) was studied when stored in a plastic bag inside a polypropylene container at 25°C for 24 months and at 40°C for 6 months.<sup>29</sup> Losses (TP + T<sub>2</sub>P) at the end of 24 months ranged 1.6–2.8%. Losses at the end of 6 months at 40°C ranged 1.8–2.7%.

The stability of TPM in premixtures was studied at an inclusion level of 25,000 mg/kg in a premix containing cereal carrier fines, soybean meal and oil<sup>30</sup> when stored in paper bags with internal plastic liner at 25, 30 and 40°C for 12, 12 and 6 months, respectively. Three different TPM batches were tested in the same premixture. Average losses (TP + T<sub>2</sub>P) after 6 months were 17, 20 and 30%, for samples stored at 25, 30 and 40°C, respectively. Average losses at 12 months were 25 and 29% for samples stored at 25 and 30°C, respectively.<sup>31</sup>

<sup>18</sup> Technical dossier/Supplementary information January 2019/FAD-2017-0057 SIn Addendum 220,618 responses: Total related substances correspond to all unidentified impurities on HPLC chromatograms during the quantitative analysis of TPM.

<sup>19</sup> Technical dossier/Section II/Annex II.2. Analysis of TP and T<sub>2</sub>P by HPLC.

<sup>20</sup> Technical dossier/Annex II.2 and II.3. LOD in mg/kg of cadmium, lead and mercury was ██████████.

<sup>21</sup> Technical dossier/Annex II.3. LOD in mg/kg was 0.01 for sodium and bismuth and 0.02 for the rest.

<sup>22</sup> Technical dossier/Section II 2.2.2.1.

<sup>23</sup> Technical dossier/Section II/Annex II.26.

<sup>24</sup> Technical dossier/Section II/Annex II.12.

<sup>25</sup> Technical dossier/Section 2.2.2.1.

<sup>26</sup> Technical dossier/Section II/Annexes II.9 and II.10.

<sup>27</sup> Technical dossier/Supplementary information January 2019/Annex 4.

<sup>28</sup> Technical dossier/Section III/Annex III.31 Supplementary information January 2019/FAD-2017-0057 SIn Addendum 220,618 responses and Annex 4.

<sup>29</sup> Technical dossier/Supplementary information January 2019/FAD-2017-0057 SIn Addendum 220,618 Responses 15Jan19, reply to question 3.

<sup>30</sup> Technical dossier/Annex II.8.

<sup>31</sup> Technical dossier/Section II/Annex II.21.

<sup>32</sup> Technical dossier/Section II/Annex II.22 and Supplementary information January 2019/ FAD-2017-0057 SIn Addendum 220,618 responses and Annex 5.

<sup>33</sup> Technical dossier/Section II/Annex II.22.

The stability of TPM in premixtures was studied at 125,000 mg/kg inclusion level in a premix containing beta-carotene, selenium, cereal carrier fines and white oil, when stored in paper bags with internal plastic liner at 25, 30 and 40°C (for each inclusion level) for 6 months. Five batches were tested in the same premixture. Average losses (TP + T<sub>2</sub>P) were 14, 12 and 45%, respectively.<sup>34</sup>

The stability of TPM in feedingstuffs was studied in cattle pelleted feed (based on wheat and barley) supplemented at 500 mg/kg feed.<sup>35</sup> Five batches were tested in a cattle feed and two batches in another. Samples were stored in plastic bags at 25, 30 and 40°C for 3 months. Average losses (TP + T<sub>2</sub>P) after 3 months were 3, 11 and 25%, respectively.

The stability of TPM (one batch) in feedingstuffs was studied in a poultry pelleted feed (consisting of wheat, soybean meal and canola meal) supplemented at 10 mg/kg feed.<sup>36</sup> Samples were stored in plastic bags at 25, 30 and 40°C for 3 months. No losses were detected after storage at 25 or at 30°C. Average losses (TP + T<sub>2</sub>P) after 3 months at 40°C were 14%.

The capacity of the additive to distribute homogeneously in feed was studied in two batches of the premixture supplemented at 25,000 mg/kg described above;<sup>37</sup> and in the cattle pelleted feed supplemented at 500 mg/kg described above. The coefficients of variation were 3.0 and 3.3% for the premixtures and 3.9% in the pelleted feed.

### 3.1.4. Conditions of use

The additive is intended for use in feeds to meet the vitamin E requirements for all animal species. It should be added to feedingstuffs via premixtures.<sup>38</sup> No maximum or minimum use levels nor a time limit for the duration of the supplementation are proposed.<sup>39</sup>

The applicant proposed a relative biopotency of TPM of 0.96 of that of all-*rac*- $\alpha$ -tocopheryl acetate calculated from the molecular weight of the substances (1 mg TPM would correspond to 0.96 international units (IU) of vitamin E).<sup>40</sup>

## 3.2. Safety

The additive is specified to contain 50–70% TP, 20–40% T<sub>2</sub>P and < 10% all-*rac*- $\alpha$ -tocopherol. TP is naturally present in tissues and foodstuffs (Ogru et al., 2003; Negis et al., 2005), hence is a natural derivative of  $\alpha$ -tocopherol. The T<sub>2</sub>P present in the additive and representing about 30% of the additive is considered to be a xenobiotic substance.

### 3.2.1. Absorption, distribution, metabolism and excretion of $\alpha$ -tocopherol, TP and T<sub>2</sub>P

The ADME of  $\alpha$ -tocopherol was reviewed in detail in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2010). The intestinal absorption of tocopherols varies between 20% and 80% depending on intake level and with lower absorption at higher dietary supply. The  $\alpha$ -tocopherol form is transported via the lymphatic system into the bloodstream, where it is distributed into lipoproteins for passage into the liver. After hepatic uptake,  $\alpha$ -tocopherol is secreted again into the circulation. A continuous intake of vitamin E is required in order to maintain vitamin E concentrations in cellular membranes throughout the body. Tocopherols are mainly distributed in adipose tissue, liver and muscle (Machlin and Gabriel, 1982). Relatively low deposition of vitamin E occurs in the body; when tocopherol is ingested in excessive amounts, it generally converts to water-soluble lactone, esterifies to glucuronic acid and is excreted in the urine and faeces (Brigelius-Flohé and Traber, 1999).

Tocopheryl phosphate can be dephosphorylated into  $\alpha$ -tocopherol by enzymes like cholesterol esterase or phosphatases. Conversion of TP into  $\alpha$ -tocopherol has been demonstrated *in vitro* by alkaline phosphatase (Rezk et al., 2004), alkaline phosphatase from intestinal mucosa (Negis et al., 2005) or from the spleen (Topi and Alessandrini, 1953), as well as from homogenates of mouse skin (Nakayama et al., 2003). Once TP is dephosphorylated to tocopherol, it follows the same pathways as tocopherols. It has been also demonstrated that TP can be produced from tocopherol

<sup>34</sup> Technical dossier/Section II/Annex II.23.

<sup>35</sup> Technical dossier/Section II/Annex II.24a.

<sup>36</sup> Technical dossier/Section II/Annex II.24b.

<sup>37</sup> Technical dossier/Section II/Annex II.25a.

<sup>38</sup> Technical dossier/Supplementary information January 2019/ FAD-2017-0057 SIn Addendum 220,618 responses.

<sup>39</sup> Technical dossier/Section II.2.5.1.

<sup>40</sup> One IU of vitamin E corresponds per definition to 1 mg all-*rac*- $\alpha$ -tocopheryl acetate.



both *in vitro* and *in vivo* in rats (Gianello et al., 2005). This may be, in part, the reason why TP has been measured naturally in tissues and various food items.

The applicant submitted data on the plasma concentration of TP and  $\alpha$ -tocopherol in weaned piglets after oral administration of TP or all-*rac*-tocopheryl acetate (TAc) (van Kempen et al., 2018). In the study, weaned piglets fitted with jugular catheters received a single test meal (350 g, 6% fat content) containing either deuterated (trimethyl-d<sub>9</sub>) TAc or TP at 75 IU/kg body weight (n = 8 per treatment). Twelve serial blood samples were obtained starting pre-meal until 78 h post-meal. The results showed that TP was absorbed as such and transformed into  $\alpha$ -tocopherol after a relative short time. TP was not present in plasma 48 h after ingestion. The authors suggest that the conversion of absorbed TP to  $\alpha$ -tocopherol may not be complete.

Little information is available regarding the fate of T<sub>2</sub>P after oral administration.

The applicant provided a study in mice to investigate the metabolic fate of T<sub>2</sub>P. The study consisted of

<sup>41</sup>

. Considering the limitations in the design, reporting and results obtained, the study is of limited relevance for the assessment and thus, not further considered.

The applicant submitted efficacy studies conducted in weaned piglets, pigs for fattening and dairy cows which provided some data on the plasma content of  $\alpha$ -tocopherol, TP and T<sub>2</sub>P after oral administration of TPM.

The studies in weaned piglets and pigs for fattening were conducted in commercial farms and had a similar design.<sup>42</sup> The two studies aimed at studying the effect of TPM on the performance of the animals and included the measurement of plasma levels of all-*rac*- $\alpha$ -tocopherol, TP and T<sub>2</sub>P.

In the study conducted in weaned piglets,<sup>43</sup> a total of 1,512 28-day-old males (Large white  $\times$  Landrace duroc-based hybrid sire line) were randomly distributed in 112 pens containing 13–14 pigs/pen (representing 14 pens per treatment). The study considered four control groups receiving 20, 40, 80 and 160 mg all-*rac*- $\alpha$ -tocopheryl acetate (TAc)/kg feed and four other groups receiving either 20 mg TAc + 5 mg TPM/kg feed, 20 mg TAc + 10 mg TPM/kg feed, 20 mg TAc + 20 mg TPM/kg feed and 20 mg TAc + 40 mg TPM/kg feed. Vitamin E background concentration in feed was not analysed. Only the supplemental values of TPM were confirmed analytically (starter 4.3, 7.3, 20.3 and 35.0 mg TPM/kg feed; weaner 4.8, 10.9, 17.2 and 38.0 mg TPM/kg feed).<sup>44</sup> The study lasted 35 days and the blood samples were obtained on days 14 and 34 from at least 1 pig/pen (14 samples per treatment, the same pig was sampled every time).

Plasma concentration of  $\alpha$ -tocopherol on day 14 in groups supplemented only with vitamin E showed no differences between the treatments (ranging from 0.77 mg/L to 1.08 mg/L). On day 34, the plasma levels of  $\alpha$ -tocopherol in those control groups increased with increasing levels of TAc (from 0.87 mg/L in group 20 mg/kg diet to 1.83 mg/L in group 160 mg/kg diet) but did not show a significant increase in the animals receiving 20 mg TAc + TPM (range 0.78–0.98 mg/L). As regards plasma concentration of TP on day 14, it was higher in the group receiving TPM at 40 mg/kg (32.0  $\mu$ g/L) compared to the other TPM supplemented groups (range 4.2–11.3  $\mu$ g/L); and on day 34, it was higher in the groups treated with 20 and 40 mg TPM/kg (17.9 and 24.4  $\mu$ g/L, respectively) compared to the other TPM supplemented groups (9.9 and 3.8  $\mu$ g/L in the groups fed with 10 and 5 mg TPM/kg, respectively). In the TPM supplemented groups, plasma concentrations of T<sub>2</sub>P were below the LOQ (2.0 ng/mL). The groups allowing comparison between vitamin E sources, 40 mg TAc/kg feed and 20 mg TAc + 20 mg TPM/kg feed, showed concentrations of  $\alpha$ -tocopherol that were not statistically different (0.95 mg/L and 0.89, respectively) after 34 days.

In the study conducted in pigs for fattening,<sup>45</sup> a total of 1,120 female grower pigs (crossbreed) of 70 days of age were randomly distributed in seven treatment groups containing eight replicates (pens)

<sup>41</sup> Technical dossier/Supplementary information November 2021/Annex I.

<sup>42</sup> Technical dossier/Section IV/Annexes IV.10 and IV.11.

<sup>43</sup> Technical dossier/Section IV/Annex IV.10.

<sup>44</sup> Technical dossier/Section IV/Annex IV.10, table 4 of page 34.

<sup>45</sup> Technical dossier/Section IV/Annex IV.11.

per group and 20 animals per replicate. Treatment groups consisted of one control group devoid of vitamin E (no TAc added; vitamin E background concentration in feed not analysed), three positive control groups receiving 10, 20 and 40 mg TAc/kg feed, and three groups receiving 5, 10 and 20 mg TPM/kg feed (in this late group, the level was increased to 30 mg TPM/kg feed in the finisher phase). The supplemental values of TPM were analytically measured and showed the following values: grower feed 3.1, 10.1 and 13 mg TPM/kg; finisher feed 3.7, 5.8 and 24 mg TPM/kg. The study lasted 70 days. Plasma content of  $\alpha$ -tocopherol, TP and T<sub>2</sub>P were measured on day 0 and day 62.

Regarding  $\alpha$ -tocopherol content in plasma, it showed a statistically significant dose–response increase in the positive controls and in the TPM supplemented groups. TP levels increased significantly in a dose-related manner with TPM supplementation, albeit of marginal biological relevance ( $\mu$ g/L). In TPM-treated groups, plasma concentrations of T<sub>2</sub>P were below the LOQ (2.0 ng/mL). The groups allowing comparison between vitamin E sources, 10 mg all-*rac*- $\alpha$ -tocopheryl acetate/kg feed and 10 mg TPM/kg feed showed values of alpha-tocopherol that were not statistically different (1.64 mg/L and 1.53, respectively) (see Table 1).

**Table 1:** Plasma concentrations of vitamin E ( $\alpha$ -tocopheryl acetate and TP) in pigs supplemented with  $\alpha$ -tocopheryl acetate (positive control) or with TPM, versus a negative control, at days 0 (D0) and 62 (D62)

Test item	Control	$\alpha$ -tocopheryl acetate			TPM		
		0	10	20	40	5	10
$\alpha$ -Tocopherol D0 (mg/L)	2.15	1.81	1.96	2.66	2.58	2.58	1.94
$\alpha$ -Tocopherol D62 (mg/L)	1.21 <sup>a</sup>	1.64 <sup>ab</sup>	1.91 <sup>bc</sup>	2.26 <sup>c</sup>	1.27 <sup>a</sup>	1.53 <sup>ab</sup>	1.75 <sup>b</sup>
TP D62 ( $\mu$ g/L)	ND	ND	ND	ND	3.0 <sup>a</sup>	8.8 <sup>a</sup>	39.3 <sup>b</sup>

ND: not determined.

Different superscripts within a row indicate statistically significant differences between treatment groups.

(1): The level of TPM was increased to 30 mg/kg feed in the finisher phase.

The study in dairy cows/heifers aimed at studying the effect of supplementing the diet with TPM (500 mg/day delivered in 1 kg single mixed ration) on the milk quality and fertility (data not submitted).<sup>46</sup> In this study, plasma concentrations of  $\alpha$ -tocopherol, TP and T<sub>2</sub>P were measured in 6 cows and 13 heifers. The animals received TPM from 30 days prior to the expected farrowing (together with 0.144 g vit E /kg feed); and for up to a total of 150 days post calving (together with 0.591 g vit E /kg feed). The form of vitamin E used was not specified. Data on the cows not supplemented were provided only for day 0. In the group of supplemented cows/heifers, plasma levels of  $\alpha$ -tocopherol and T<sub>2</sub>P were analysed at days 0, 30 and 150<sup>47</sup> while TP was measured on days 30 and 150. No data were available of the control group. The results showed numerical increases on the  $\alpha$ -tocopherol content in plasma with time (cows from 4.79 mg/L on day 0 to 5.71 on day 30, heifers from 4.73 mg/L on day 0 to 16.28 on day 150). However, since no data on the control group was made available, no conclusion is possible regarding the increase on the  $\alpha$ -tocopherol in blood. The plasma contents of TP and T<sub>2</sub>P were below the LOQ (2  $\mu$ g/L) or below the LOD (0.5  $\mu$ g/L), respectively, at any time point.

### 3.2.1.1. Conclusions on the ADME of $\alpha$ -tocopherol, TP and T<sub>2</sub>P

The ADME of  $\alpha$ -tocopherol present in the additive is well known and previously described in FEEDAP Panel opinions. From the data available, it can be concluded that TP is absorbed and converted into  $\alpha$ -tocopherol in pigs. No significant deposition of TP in edible tissues and products of target species is expected due to its conversion to  $\alpha$ -tocopherol, but no data were provided regarding TP residues in food products and excretion in faeces/urine. The data showed no presence of T<sub>2</sub>P in blood after oral administration of TPM to pigs and cows. Therefore, absorption of T<sub>2</sub>P as such (if any) appears to be very limited and no significant deposition in edible tissues and products of target species is expected. The data provided, however, do not allow to conclude on the fate of the T<sub>2</sub>P present in the additive.

<sup>46</sup> Technical dossier/Section IV/Annex IV.15.

<sup>47</sup> Analysis of alpha-tocopherol and TP in cows on day 150 was not done.

### 3.2.2. Toxicological studies

The applicant submitted genotoxicity studies (reverse mutation assay and *in vivo* micronucleus test) and a 90-day oral toxicity study testing the product under assessment.<sup>48</sup> In addition, a reverse mutation assay and an *in vivo* chromosome aberration test were submitted with an item containing

[REDACTED]

#### 3.2.2.1. Genotoxicity studies

In a reverse mutation assay,<sup>50</sup> TPM was investigated for the potential to induce reverse mutations at the histidine locus in the genome of five tester strains of *Salmonella Typhimurium* (TA98, TA100, TA102, TA1535 and TA1537) in the presence and absence of a metabolic activation system (mammalian microsomal enzymes, S9 mix) in compliance with the OECD TG 471. The test was conducted up to a maximum level of 1,000 µg/ml, as higher concentrations caused evident cytotoxicity. The plate incorporation method was used in two independent experiments. The test item did not induce any increase in the mean number of revertants. Accordingly, TPM was considered to be non-mutagenic under the conditions of the study described in this report.

The potential of the additive under assessment to induce micronuclei in the bone marrow was investigated in the Swiss mouse, in accordance with the OECD TG 474 in a GLP compliant test.<sup>51</sup> A suspension of test item was administered to two groups of 10 mice (five male, five female) at 2,000 mg/kg (10.23 mL/kg). Corn oil (10 mL/kg) was used as a negative control. Negative control and test item-treated groups were killed at 24 or 48 h after dosing. 9,10-dimethyl-1,2-benzanthracene (DMBA) was used as a positive control and this group was killed 48 h after dosing. The test item did not induce any increase in the frequency of micronuclei in the bone marrow of mice under the conditions of the study. However, as no evidence of target exposure was provided, this result is not sufficient to conclude on the genotoxic potential of the substance at sites of contact.

Another reverse mutation assay<sup>52</sup> using an item containing [REDACTED] was performed in accordance with OECD Guideline 471, testing concentrations equivalent to [REDACTED]. No increase of mutation rate was observed. This study provides supportive evidence for hazard identification/characterisation of the additive under assessment.

The same diluted test item was tested in an *in vitro* cytogenetics assay using duplicate cultures of Chinese hamster ovary (CHO) cells in two independent experiments<sup>54</sup> performed in compliance with OECD TG 473 (1997).

[REDACTED]

Considering that the test item used is not optimal and there are no data on numerical aberrations, the available data are considered insufficient to conclude and are reported as supportive information.

#### 3.2.2.2. Repeat dose 90-day oral toxicity

A 90-day oral toxicity study in rats, conducted in accordance with OECD TG 408 and following the GLP principles, was performed with the additive under assessment.<sup>55</sup>

[REDACTED]

<sup>48</sup> Technical dossier/Supplementary information January 2019/FAD-2017-0057 SIn Addendum 220,618 responses.

<sup>49</sup> Technical dossier/Section III/Annex III.22 and III.23.

<sup>50</sup> Technical dossier/Section III/Section 3.2.2.2 and annex III.21.

<sup>51</sup> Technical dossier/Section III/Section 3.2.2.2 and annex III.24.

<sup>52</sup> Technical dossier/Section III/Section 3.2.2.2 and annexes III.22 and III.18.

<sup>53</sup> Technical dossier/Section III/Section 3.2.2.2 .

<sup>54</sup> Technical dossier/Section III/Annexes III.23 and III.18.

<sup>55</sup> Technical dossier/Section III/Section 3.2.2.4 and annexes III.26, III.27, III.28 and III.29 and Annex III.19; Supplementary information January 2019/ FAD-2017-0057 SIn Addendum 220,618 responses and Annex 7.

56

[REDACTED], the FEEDAP Panel identified a lowest observed adverse effect level (LOAEL) of [REDACTED] mg TPM/kg bw per day for males and females, respectively. A No observed adverse effect level (NOAEL) could not be identified.

### 3.2.2.3. Conclusions of the toxicology studies

The additive under assessment is considered not mutagenic. The data available do not allow to conclude on the potential aneugenicity and clastogenicity of the additive. From a 90-day oral toxicity study in rats, LOAELs of [REDACTED] mg TPM/kg bw per day, for males and females, respectively, were derived.

### 3.2.3. Safety for the target species

No safety studies in the target animals were provided by the applicant. The only available information is from the 90-day study in rats from which it was not possible to derive an NOAEL. Although TP seems to be absorbed in the intestine and converted into alpha-tocopherol, there is no information on the fate of T<sub>2</sub>P in the organism. This fact, together with the impossibility to derive an equivalence factor between TPM and all-*rac*- $\alpha$ -tocopheryl acetate (see Section 3.3 Efficacy),<sup>57</sup> the uncertainties regarding the genotoxic potential and the absence of experimental safety data in target animals prevent the FEEDAP Panel to perform an assessment of the safety of the additive for the target species.

Therefore, the FEEDAP Panel cannot conclude on the safety of TPM for the target species.

### 3.2.4. Safety for the consumer

In 2003, the SCF established an upper tolerable level (UL) for vitamin E for adults as 300 mg/day (SCF, 2003), and based on the body weight, the UL for children (1–3 years) and adolescents was set at 100 and 260 mg/day, respectively.

The data made available by the applicant on the ADME of the active substances present in the additive under assessment do not allow to evaluate in full the metabolic fate of TP and T<sub>2</sub>P. The relative equivalence in vitamin E units of the additive cannot be established (see Section 3.3). Moreover, uncertainty remains on the genotoxic potential of the active substances of the additive and no data on the residues were submitted by the applicant. Therefore, the FEEDAP Panel is not in the position to conclude on the safety of the additive for the consumers.

### 3.2.5. Safety for the user

An acute skin irritation GLP study performed with TPM according to OECD TG 439 was submitted.<sup>58</sup> No potential for skin irritancy was observed.

<sup>56</sup> [REDACTED]

<sup>57</sup> One IU of vitamin E being defined as 1 mg all-*rac*- $\alpha$ -tocopheryl acetate.

<sup>58</sup> Technical dossier/Supplementary information January 2019/Annex 9.

An acute eye irritation GLP study performed with TPM according to OECD TG 437 was submitted.<sup>59</sup> The results indicated a serious potential for eye irritation.

A local lymph node assay was performed to assess the skin sensitisation potential of TPM.<sup>60</sup> The study was performed as GLP compliant and followed the OECD TG 429. The results showed no sensitisation potential.

No data were provided on the effects of the additive on the respiratory tract. The dusting potential showed that exposure of users by inhalation, although limited, is possible. Considering that the additive is an eye irritant, the Panel considers that irritation of respiratory mucosae following exposure by inhalation is likely.

### 3.2.5.1. Conclusions on safety for the user

TPM is not a skin irritant nor a skin sensitiser but should be considered irritant to the eyes and the upper respiratory tract. Owing to the uncertainty on the genotoxic potential of the additive, it is not possible to conclude on safety for the user.

### 3.2.6. Safety for the environment

$\alpha$ -Tocopherol is a natural compound, and its degradation route is of low environmental impact, as for other low-toxicity vitamins. Tocopherol phosphate occurs naturally in feedingstuffs and tissues. It is absorbed and converted to  $\alpha$ -tocopherol (van Kempen et al., 2018).

T<sub>2</sub>P does not naturally occur in the environment. Although the applicant claims that it is readily transformed to  $\alpha$ -tocopherol after absorption, no substantial evidence of this was provided. The FEEDAP Panel noted that, given the characteristics of T<sub>2</sub>P ( ), it could accumulate in the environment.

The PECsoil for the T<sub>2</sub>P fraction was calculated considering the common use levels described in the dossier (50 mg TPM/kg feed in cattle  $\times$  0.3 = 17 mg T<sub>2</sub>P/kg feed; 40 mg TMP/kg feed in poultry and pigs  $\times$  0.3 = 13 mg T<sub>2</sub>P/kg feed),<sup>61</sup> the log Koc proposed by the applicant of ,<sup>62</sup> and taking the lamb for fattening as worst-case scenario at a supplemental level of 40 mg TMP/kg feed. The calculated PEC soil resulted in 277  $\mu$ g/kg soil dry weight (dw), well above the trigger value of 10  $\mu$ g/kg. In line with the requirements of the FEEDAP guidance on the safety of the additive for the environment (EFSA, 2008b), a Phase II assessment would be needed, but no data were provided by the applicant. Therefore, in the absence of data, the FEEDAP Panel cannot conclude on the safety of TPM for the environment.

## 3.3. Efficacy

The applicant provided two studies in weaned piglets (one with TPM<sup>43</sup> and the other only with TP (van Kempen et al., 2018)), one in pigs for fattening (with TPM)<sup>45</sup> and one in dairy cows/heifers (with TPM)<sup>46</sup> that have been described in Section 3.2.1. Those studies provided limited information on the bioavailability and relative equivalence of TPM compared to other sources of vitamin E.

The applicant submitted also a study in chickens for fattening to evaluate the effect of different levels of TPM (containing each in addition 20 mg all-*rac*- $\alpha$ -tocopheryl acetate/kg) on the performance and meat quality.<sup>63</sup> The study included a negative control and a positive control group supplemented with TAc and had a duration of 28 days. However, the endpoints measured included only zootechnical performance parameters and did not allow to calculate the relative bioequivalence with vitamin E.

The FEEDAP Panel noted that from the data/information provided, it can be concluded that TP can be converted into  $\alpha$ -tocopherol in weaned piglets, and a dose-related increase in the blood concentration of  $\alpha$ -tocopherol was found with increasing levels of TPM in the studies done with pigs (significant in pigs for fattening, numerical in weaned piglets). Therefore, the additive can be regarded as a source of vitamin E for all animal species. However, the data were not sufficient (e.g. only one group per study allowing comparison, diets and trial design and the endpoints measured) to allow the Panel to establish the relative bioequivalence of TPM as vitamin E.<sup>57</sup> Consequently, the proposed

<sup>59</sup> Technical dossier/Supplementary information January 2019/Annex 11.

<sup>60</sup> Technical dossier/Supplementary information January 2019/Annex 10.

<sup>61</sup> Technical dossier/Section III/Subsection 3.1.1.

<sup>62</sup> Technical dossier/Supplementary information August 2019/2. Annex – FAD-2017-0057 Sin Addendum 200,519 Responses FINAL.

<sup>63</sup> Technical dossier/Section IV/Annex IV.12.

relative bioequivalence of 0.96 for all animal species as calculated by the applicant (see Section 3.1.4) cannot be confirmed.

Therefore, there is insufficient evidence for the efficacy of TPM for all animal species.

### 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<sup>64</sup> and Good Manufacturing Practice.

## 4. Conclusions

Considering the limited information on the ADMER for the components of the additive and the uncertainties on the potential aneugenicity and clastogenicity of the additive, the Panel cannot conclude on the safety of the additive for the target species and for the consumer.

TPM is not a skin irritant nor a skin sensitiser but should be considered irritant to the eyes and the upper respiratory tract. Owing to the uncertainty on the potential aneugenicity and clastogenicity of the additive, it is not possible to conclude on safety for the user.

The FEEDAP Panel cannot conclude on the safety of TPM for the environment due to lack of data on environmental impact of T<sub>2</sub>P.

TPM is a bioavailable source of  $\alpha$ -tocopherol. The data available, however, do not allow the Panel to establish the relative bioequivalence of TPM as vitamin E. Therefore, the Panel is not in the position to conclude on the efficacy of TPM for all animal species.

## 5. Documentation provided to EFSA/chronology

Date	Event
25/10/2017	Dossier received by EFSA. Tocopheryl phosphate mixture (TPM) for all animal species. Submitted by Triveritas Ltd on behalf of Phosphagenics Ltd. During the assessment, the applicant became Avecho Biotechnology Limited.
14/11/2017	Reception mandate from the European Commission
04/04/2018	Application validated by EFSA – Start of the scientific assessment
08/05/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: method of analysis, manufacturing process, characterisation of the additive, stability, conditions of use, toxicological studies, safety for the target species, safety for the consumer, safety for the user, safety for the environment and efficacy.</i>
04/07/2018	Comments received from Member States
26/07/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
16/11/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
1 March 2022	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: Efficacy.</i>
14/01/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
29/06/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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<sup>64</sup> Regulation (EC) No 1831/2005 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.

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## Abbreviations

CAS	Chemical Abstracts Service
CV	coefficient of variation
DM	dry matter
EURL	European Union Reference Laboratory
IU	International Unit
LOD	limit of detection
LOQ	limit of quantification
MSDS	Material safety data sheets
RH	relative humidity
SCF	Scientific Committee on Food



TAc	tocopheryl acetate
TMP	tocopheryl phosphate mixture
WHO	World Health Organization
VKM	Norwegian Scientific Committee for Food Safety

## **Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for tocopheryl phosphate mixture (TPM)**

In the current application, authorisation is sought under article 4(1) for Tocopheryl Phosphate Mixture (TPM), under the category/functional group 3(a) 'nutritional additives'/vitamins, pro-vitamins and chemically well-defined substances having similar effect' according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the feed additive for all animal species and categories. The feed additive is a mixture of two different forms of phosphorylated tocopherol namely alpha-tocopheryl di-hydrogen phosphate (TP) at a content ranging from 50% to 70% w/w and alpha-di-tocopheryl hydrogen phosphate (T<sub>2</sub>P) at a content ranging from 20% to 40% w/w as well as small amounts of residual alpha-tocopherol; phosphoric acid and water. The feed additive is intended to be used in feedingstuffs through premixtures for all animal species without minimum or maximum limits.

Consequently, for the characterisation of Tocopheryl Phosphate Mixture (TPM), the Applicant submitted two single-laboratory validated method and based on high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection for the determination of TP and T<sub>2</sub>P in the feed additive, and HPLC coupled to fluorescence detection (FD) for their determination in premixtures and feedingstuffs.

Upon request of the EURL, the Applicant provided suitable evidence for the transferability of the proposed methods for the determination of TP and T<sub>2</sub>P in the feed additive, premixtures and feedingstuffs.

Consequently, the EURL considers the following methods submitted by the Applicant suitable for official control: a HPLC-UV to quantify TP and T<sub>2</sub>P in the feed additive and the HPLC-FD method to quantify them in premixtures and 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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.