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Safety and efficacy of Axtra® PHY 20000 TPT2 (6-phytase) as a feed additive for poultry and porcine species

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Abstract

Axtra® PHY 20000 TPT2 is a solid preparation that contains a 6-phytase produced with a genetically modified strain of *Trichoderma reesei*. The production strain and its recombinant DNA were not detected in Axtra® PHY 20000 TPT2. From the results obtained in tolerance studies, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the additive is safe for the target species at 2,000 FTU/kg feed. The studies provided to address the safety for the consumer were performed with the fermentation product that is used to formulate the additive and the results do not indicate any reason for concern for consumer safety arising from the use of the product as a feed additive. The studies provided to address the safety for the user were performed with the fermentation product that is used to formulate the additive and have been assessed in a previous opinion. Considering the results of those studies and the substances used during the formulation of Axtra® PHY 20000 TPT2, this formulation is not considered a dermal sensitiser. However, it should be considered a potential irritant to skin, eyes and the respiratory tract, and owing to the nature of the active substance, it should be considered a potential respiratory sensitiser. However, the exposure by inhalation is expected to be negligible. No risks to the environment are expected from the use of Axtra® PHY 20000 TPT2 as a feed additive. Based on the results of efficacy studies, the Panel concluded that the additive has the potential to be efficacious at 250 FTU/kg feed.

Keywords: zootechnical additives, digestibility enhancers, phytase, safety, efficacy, poultry, pigs

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Summary

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of Axtra® PHY 20000 TPT2 as a feed additive for poultry and porcine species. Axtra® PHY 20000 TPT2 is a solid preparation that contains a 6-phytase produced with a genetically modified strain of *Trichoderma reesei*. The European Food Safety Authority (EFSA) adopted an opinion on the safety and efficacy of a liquid formulation of the same additive which addressed the safety of the genetic modification of the production strain, the safety of the additive for consumers and users and the safety and efficacy of the product for the target species (poultry and pigs).

The description of the genetic modification and the assessment of its safety have been performed in a previous opinion and it was concluded that the recipient organism is safe and the introduced sequences give rise to no safety concerns. Newly submitted data showed that neither the production strain nor its recombinant DNA was detected in Axtra® PHY 20000 TPT2.

The applicant provided the tolerance studies in chickens and turkeys for fattening, laying hens, weaned piglets and sows that have been assessed for the evaluation of the liquid formulation. From the results obtained in those studies, the Panel concluded that the additive is safe for the target species at 2,000 FTU/kg feed. The Panel considered that the conclusions drawn from those studies are valid for the new formulation of the additive; consequently, the additive is considered safe for the target species at 2,000 FTU/kg feed.

The studies provided to address the safety for the consumer were performed with the fermentation product that is used to formulate the additive and have been assessed in a previous opinion. Based on the results obtained in a bacterial reverse mutation assay, an *in vitro* mammalian chromosome aberration test and a subchronic repeated dose oral toxicity study, the Panel concluded that the results of the studies do not indicate any reason for concern for consumer safety arising from the use of the product as a feed additive.

The studies provided to address the safety for the user were performed with the fermentation product that is used to formulate the additive and have been assessed in a previous opinion. The intermediate product tested was shown to be non-toxic by inhalation and without irritant or (dermal) sensitising properties. The Panel considered that the substances added to this intermediate during the formulation of Axtra® PHY 20000 TPT2 are not likely to change the sensitisation properties, but may influence the irritancy. Therefore, Axtra® PHY 20000 TPT2 is not considered a dermal sensitizer, but should be considered a potential irritant to skin, eyes and the respiratory tract. Because of the proteinaceous nature of the active substance, the additive is considered a potential respiratory sensitiser, but the exposure is expected to be negligible.

No risks to the environment are expected from the use of Axtra® PHY 20000 TPT2 as a feed additive.

The applicant submitted efficacy studies that have been previously assessed by the FEEDAP Panel. Based on the results of those studies, the Panel concluded that the additive is efficacious at 250 FTU/kg feed. The Panel considers that those conclusions apply also to the new formulation of the additive.

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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 Danisco UK Ltd[2] for authorisation of the product Axtra® PHY 20000 TPT2 (6-phytase), when used as a feed additive for all poultry and porcine species (category: zootechnical additives; functional group: digestibility enhancers).

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). The particulars and documents in support of the application were considered valid by EFSA as of 1 March 2016.

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 Axtra® PHY 20000 TPT2 (6-phytase), when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) adopted an opinion on the safety and efficacy of the liquid form of the additive Axtra® PHY (Axtra® PHY 15000 L) produced with a genetically modified strain of *Trichoderma reesei* (ATCC SD-6528) which addressed the safety of the genetic modification of the production strain, the safety of the additive for consumers and user and the safety and efficacy of the product for the target species (EFSA FEEDAP Panel, 2015). This formulation is currently authorised for use as a feed additive for all poultry and porcine species (other than suckling piglets).[3] The applicant has now requested the authorisation of a new formulation of the additive.

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 Axtra® PHY 20000 TPT2 as a feed additive. 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.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance 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 Axtra® PHY 20000 TPT2 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),

[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] Danisco (UK) Ltd, PO Box 777, SN 8 1XN, Marlborough, Wilshire, United Kingdom.
[3] Commission implementing regulation (EU) 2016/899 of 8 June 2016 concerning the authorisation of a 6-phytase produced by *Trichoderma reesei* (ATCC SD-6528) as a feed additive for all poultry species and all porcine species (other than suckling piglets) (holder of authorisation Danisco (UK) Ltd).
[4] FEED dossier reference: FAD-2015-0048.
[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/jrcsh/files/finirep-fad-2015-0048-axtra-phy-20000tpt2.pdf

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, 2008a), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c), Technical Guidance: Microbial Studies (EFSA, 2008b), Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA, 2008c) and Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011).

3. Assessment

The additive Axtra® PHY is authorised as a zootechnical additive (functional group of digestibility enhancers) for all poultry and porcine species. The current opinion assesses a solid formulation of this additive, Axtra® PHY 20000 TPT2, for the same target species.

3.1. Characterisation[7]

3.1.1. Characterisation of the active substance

Axtra® PHY 20000 TPT2 is a preparation that contains a 6-phytase (phytase, EC 3.1.3.26) which is produced by a genetically modified strain of *T. reesei* with the deposit number ATCC SD-6528.[8] The description of the genetic modification and the assessment of its safety have been performed in a previous opinion (EFSA FEEDAP Panel, 2015). The Panel considered that the recipient organism is safe and the introduced sequences give rise to no safety concerns.

3.1.2. Manufacturing process

The phytase is obtained by a multi-step process consisting of fermentation, concentration and purification steps. The resulting fermentation product is mixed with potassium sorbate and sodium benzoate. This liquid fermentation product is then granulated with sodium sulfate, polyvinyl alcohol, talc and sodium phytate. The applicant declared that no antibiotic substances are used in the production process.

3.1.3. Characterisation of the product

The new formulation under assessment is a solid granulate with a guaranteed minimum activity of 20,000 phytase units[9] (FTU)/g. Other side activities are xylanase, β-glucanase and amylase activities.[10] The batch-to-batch variation was studied in five batches[11] and the mean value was 26,160 FTU/g, ranging from 23,716 to 29,422 FTU/g (coefficient of variation (CV) of 8.0%). The additive contains the fermentation product, sodium sulfate, polyvinyl alcohol, talc and sodium phytate.

Three batches of Axtra® PHY 20000 TPT2 were analysed for chemical and microbiological contamination and antimicrobial activity.[12] The analyses of chemical contamination included arsenic (< 2.0 mg/kg), cadmium (< 0.5 mg/kg), lead (< 5 mg/kg) and mercury (< 0.1 mg/kg). The levels of mycotoxins, including aflatoxin (< 5 μg/kg), ochratoxin (< 2 μg/kg), zearalenone (< 50 μg/kg), deoxynivalenol (< 0.5 mg/kg) and fumonisin (< 50 μg/kg), were also determined. The host strain was grown in laboratory conditions in four different media (in darkness at 25°C for 12 days) and extracts from the growth media were analysed for secondary metabolites.[13] None of the trichothecenes (trichodermin, trichodermol or harzianum A) was detected nor was gliotoxin. Microbiological analysis included total viable counts (≤ 375 colony-forming units (CFU)/g), coliforms (< 10 CFU/g), *Escherichia coli* and *Salmonella* spp. (not detected in 25 g). Antimicrobial activity was not detected.[14] The production strain and recombinant DNA were not detected in three batches of the additive.[15]

[7] This section has been amended following the confidentiality claims made by the applicant.
[8] Technical Dossier/Section II/Annex II.13 and Supplementary information July 2016.
[9] One FTU is defined as the amount of enzyme that releases 1 μmol of inorganic orthophosphate from a sodium phytate substrate per minute at pH 5.5 and 37°C.
[10] Technical dossier/Section II/Annex II.8.
[11] Technical dossier/Section II/Annex II.2.
[12] Technical dossier/Section II/Annex II.3.
[13] Technical dossier/Section II/Annex II.12.
[14] Technical dossier/Section II/Annexes II.3, II.4 and II.5.
[15] Technical dossier/Section II/Annexes II.3. and II.6, and Supplementary information July 2016/Annex S13.

Particle size distribution and the dusting potential were studied in three batches.[16] The dusting potential ranged from 0 to 15 mg/m^3. Particles below 500 μm diameter were 90% and below 400 μm were 10%. No particles were detected below 194 μm. The product has a bulk density 1,303 kg/m^3.[17]

3.1.4. Stability and homogeneity

The shelf life of three batches of Axtra® PHY 20000 TPT2 was studied at 25°C (60% RH) and 40°C (75% RH)[18] stored in their commercial packaging for up to 18 months. The initial mean phytase content of the batches was ~ 28,350 FTU/g and the mean recovery of phytase activity after 18 months was 77.5% at 25°C and 28.6% at 40°C. This result would support the claim by the applicant of a shelf life of 15 months when stored below 25°C.

Three batches of the additive were added to a vitamin–mineral complete premixture for poultry (including choline chloride) to provide ~ 143,000–177,000 FTU/kg premixture.[19] Samples were stored in closed plastic containers at 25°C (60% RH) for up to 6 months. Recovery values of enzyme activity after 6 months ranged from 86% to 100% of the initial activity.

Three batches of the additive were mixed in a complete feed (mash form) based on maize and soya bean meal (intended to provide 387–458 FTU/kg feed).[20] Samples were kept in closed paper bags at 25°C (60% RH) for 3 months. Mean enzyme activity recovery values after 3 months ranged from 85% to 92%. The stability to feed processing was studied in three batches of the additive that were added to complete feed based on maize and soya bean meal at 405–459 FTU/kg feed.[21] Recovery values of enzyme activity after pelleting at 95°C ranged from 87% to 98%. Three batches of the additive were added to complete feed, based on maize and soya bean meal (intended to provide 379–436 FTU/kg feed) and pelleted at 95°C.[22] Samples were stored in closed paper bags at 25°C for 3 months. Recovery values of enzyme activity ranged from 89% to 100%.

The capacity to homogeneously distribute of the additive was studied in two batches of mash feed by analysing 10 subsamples. The CV was ~ 10% in either case.

3.1.5. Conditions of use

The additive is to be used in chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding, laying hens, turkeys for breeding purposes, weaned piglets, sows for reproduction, pigs for fattening, minor porcine species and minor poultry species at the dose of 250 FTU/kg feed.

3.2. Safety

Safety aspects regarding the use of the liquid formulation of the additive in feed, including the safety for the target species, consumers, for the users and for the environment have been previously established (EFSA FEEDAP Panel, 2015). No new information has been submitted, with the exception of the data showing the absence of the production strain and recombinant DNA in the final solid formulation.

Considering the manufacturing process and composition of the new formulation and the test items used in the studies performed to assess the safety for the target species and the safety for the consumer, the Panel considers that the conclusions drawn for the liquid formulation apply also to the solid. Therefore, Axtra® PHY 20000 TPT2 is considered to be safe for the target species at a dose of 2,000 FTU/kg feed and its use as a feed additive raises no concerns for the consumers. The Panel also considers that the new formulation will not have an impact on the conclusions drawn regarding the safety for the environment; therefore, Axtra® PHY 20000 TPT poses no risks to the environment. However, the difference in the formulation may have an impact on the safety for the user.

[16] Technical dossier/Section II/Annex II.9 and Supplementary information July 2016/Annex S3.
[17] Technical dossier/Section II/Annex II.3.
[18] Technical dossier/Section II/Annex II.22.
[19] Technical dossier/Section II/Annex II.23 and Supplementary information July 2016/Annexes II.S5 and S6.
[20] Technical dossier/Section II/Annex II.24 and Supplementary information July 2016/Annexes II.S7 and S8.
[21] Technical dossier/Section II/Annex II.26 and Supplementary information July 2016/Annexes II.S11 and S12.
[22] Technical dossier/Section II/Annex II.25 and Supplementary information July 2016/Annexes II.S9 and S10.

3.2.1. Safety for the user

In the previous opinion, the Panel evaluated an acute inhalation toxicity (OECD Guideline 403), an acute dermal and eye irritant potential (OECD Guidelines 404 and 405, respectively) and a skin sensitisation potential of the test item (OECD Guideline 429), (EFSA FEEDAP Panel 2015). In these tests, the intermediate product tested was shown to be non-toxic by inhalation and without irritant or (dermal) sensitising properties. No new data have been submitted.

The Panel considers that the substances added to this intermediate during the formulation of Axtra® PHY 20000 TPT2 are not likely to change the sensitisation properties. Therefore, Axtra® PHY 20000 TPT2 is not considered a dermal sensitizer. Based on the information provided in the safety data sheets of the ingredients used to formulate it (sodium sulfate, polyvinyl alcohol, talc and sodium phytate), Axtra® PHY 20000 TPT2, should be considered a potential irritant to skin, eyes and the respiratory tract. Because of the proteinaceous nature of the active substance, the additive is considered a potential respiratory sensitiser, but since the dusting potential is low and the large size of the particles, the exposure is expected to be negligible.

3.3. Efficacy

The FEEDAP Panel evaluated in the previous opinion efficacy studies performed in chickens and turkeys for fattening, weaned piglets, pigs for fattening and sows (EFSA FEEDAP Panel, 2015). Based on the results, the FEEDAP Panel concluded that 'the additive is efficacious in chickens and turkeys for fattening, laying hens, piglets, pigs for fattening and sows at the dose of 250 FTU/kg feed. These conclusions can be extended to chickens reared for laying, to turkeys reared for breeding and to turkeys for breeding purposes, and extrapolated to minor poultry species and minor porcine species at the dose of 250 FTU/kg'. No new information has been provided. Considering that the active substance in the new formulation is the same, the Panel considers that those conclusions apply also to the new formulation of the additive.

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[23] and Good Manufacturing Practice.

4. Conclusions

The production strain and its recombinant DNA were not detected in Axtra® PHY 20000 TPT2.

The additive is safe for the target species at the dose of 2,000 FTU/kg feed.

The use of Axtra® PHY 20000 TPT2 as a feed additive does not raise concerns for the safety of the consumer.

Axtra® PHY 20000 TPT2 is not considered a dermal sensitizer but should be considered a potential irritant to skin, eyes and the respiratory tract. Because of the proteinaceous nature of the active substance, the additive is considered a potential respiratory sensitizer.

The use of Axtra® PHY 20000 TPT2 in animal nutrition is considered safe for the environment.

Axtra® PHY 20000 TPT2 has the potential to be efficacious in improving the availability of phytate phosphorus in the target species at the minimum dose of 250 FTU/kg feed.

Documentation provided to EFSA

1) Axtra® PHY 20000 TPT2. December 2015. Submitted by Danisco UK Ltd.
2) Axtra® PHY 20000 TPT2. Supplementary information. July 2016. Submitted by Danisco UK Ltd.
3) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Axtra® PHY 20000 TPT2.
4) Comments from Member States.

[23] 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.

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EFSA (European Food Safety Authority), 2008b. Technical Guidance: microbial Studies. EFSA Journal 2008;6 (10):836, 3 pp. doi:10.2903/j.efsa.2008.836

EFSA (European Food Safety Authority), 2008c. Technical Guidance: extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition. EFSA Journal 2008;6(9):803, 5 pp. doi: 10.2903/j.efsa.2008.803

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Technical guidance: tolerance and efficacy studies in target animals. EFSA Journal 2011;9(5):2175, 15 pp. doi:10.2903/j.efsa.2011.2175

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for zootechnical additives. EFSA Journal 2012;10(1):2536, 19 pp. doi:10.2903/j.efsa.2012.2536

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance for establishing the safety of additives for the consumer. EFSA Journal 2012;10(1):2537, 12 pp. doi:10.2903/j.efsa.2012.2537

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. doi:10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015. Scientific Opinion on the safety and efficacy of Axtra® PHY 15000 L (6-phytase) as a feed additive for poultry and porcine species. EFSA Journal 2015;13(11):4275, 31 pp. doi:10.2903/j.efsa.2015.4275

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011. Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use. EFSA Journal 2011;9(6):2193, 54 pp. doi:10.2903/j.efsa.2011.2193

Abbreviations

CFU	colony-forming unit
CV	coefficient of variation
EURL	European Union Reference Laboratory
FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed
OECD	Organisation for Economic Co-operation and Development

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis of Axtra® PHY 20000 TPT2

In the current application authorisation is sought under article 4(1) for Axtra® PHY 20000 TPT2, under the category/functional 4(a) "zootechnical additives"/"digestibility enhancers" according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for several animal species. The active agent of Axtra® PHY 20000 TPT2 is 6-phytase (EC 3.1.3.26), produced by fermentation of *Trichoderma reesei*. According to the Applicant, Axtra® PHY 20000 TPT2 is a dry formulation with a guaranteed minimum enzyme activity of 20000 FTU/g. It is intended to be used in premixtures and/or complete feedingstuffs to obtain 6-phytase activities of 250 FTU/kg feedingstuffs. The Applicant expresses the phytase enzymatic activity in FTU/g units, where "one phytase unit (FTU) is the amount of enzyme which releases one micromole of inorganic phosphate from sodium phytate per minute at pH 5.5 and 37°C".

For the quantification of phytase in the feed additive, premixtures and feedingstuffs, the Applicant submitted a single-laboratory validated and further verified colorimetric methods similar to the EN ISO 30024 standard method. Based on the experimental data available, the EURL recommends for official control these colorimetric methods for the quantification of phytase activity in the feed additive, 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) is not considered necessary.