SCIENTIFIC OPINION

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Safety and efficacy of a feed additive consisting of endo-1,4-beta-xylanase (produced by *Aspergillus oryzae* DSM 33700) (RONOZYME® WX (CT/L)) for all poultry species and all *Suidae* (DSM nutritional products ltd)

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Abstract

The additive RONOZYME® WX (CT/L) contains endo-1,4-beta-xylanase produced with a genetically modified strain of the filamentous fungus *Aspergillus oryzae*; the additive is currently authorised for poultry for fattening, weaned piglets, pigs for fattening, lactating sows and laying hens. The applicant has requested to change the production strain, substituting strain *A. oryzae* DSM 26372 with *A. oryzae* DSM 33700, and to extend the use of the additive to all poultry species and all *Suidae*. RONOZYME® WX (CT/L), manufactured with the production strain *A. oryzae* DSM 33700, did not give rise to safety concerns with regard to the genetic modification of the production strain. No viable cells of the production strain nor its DNA were detected in an intermediate product representative of both final formulations of the additive. RONOZYME® WX (CT/L) was considered safe for all poultry species and all *Suidae* at the recommended inclusion levels. The use of RONOZYME® WX CT and L manufactured with the production strain *A. oryzae* DSM 33700 raised no concerns for consumers. RONOZYME® WX L is not an eye irritant; however, no conclusions could be drawn on the potential of RONOZYME® WX CT to be an eye irritant. Both formulations are not irritant to the skin, but due to the lack of data, the FEEDAP Panel was not able to conclude on the potential of both formulations of the additive to be skin sensitisers. Due to the proteinaceous nature of the active substance, the additive is considered a respiratory sensitiser. The additive manufactured by *A. oryzae* DSM 33700 raises no safety concerns for the environment. The additive has the potential to be efficacious in all poultry species and all *Suidae* at 100 and 200 FXU/kg complete feed, respectively.

Keywords: zootechnical additives, digestibility enhancers, endo-1,4-beta-xylanase, RONOZYME® WX CT/L, safety, efficacy

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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 feed additive shall submit an application in accordance with Article 7. Also, Article 13(3) of that Regulation lays down that if the holder of an authorisation proposes changing the terms of the authorisation by submitting an application to the Commission, accompanied by the relevant data supporting the request for the change, the Authority shall transmit its opinion on the proposal to the Commission and the Member States. In addition, Article 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest one year before the expiry date of the authorisation.

The European Commission received a request from DSM Nutritional Products Ltd represented in the EU by DSM Nutritional Products Sp. z o.o[2] for the authorisation, modification of the current authorisation and renewal of the authorisation of the additive consisting of endo-1,4-beta-xylanase (produced by *Aspergillus oryzae* DSM 33700) (RONOZYME® WX (CT/L)), when used as a feed additive for all poultry species and all *Suidae* (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), under Article 13(3) (modification of the authorisation of a feed additive) and under Article 14(1) (renewal of the authorisation). The dossier was received on 23 February 2022 and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2022-00156. The particulars and documents in support of the application were considered valid by EFSA as of 5 September 2022.

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 endo-1,4-beta-xylanase (produced by *A. oryzae* DSM 33700) (RONOZYME® WX (CT/L)), 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) delivered in 2012 an opinion on the safety and efficacy of RONOZYME® WX CT/L produced by *A. oryzae* DSM 10287 when used as a feed additive for poultry, piglets (weaned) and pigs for fattening (EFSA FEEDAP Panel, 2012a) and in 2016 assessed the proposal made by the applicant in order to change the production strain to *A. oryzae* DSM 26372 (EFSA FEEDAP Panel, 2016). EFSA issued two opinions on the safety and efficacy of this product when used in laying hens (EFSA FEEDAP Panel, 2017a, 2019a) and an opinion on its safety and efficacy for sows for reproduction (EFSA FEEDAP Panel, 2019b).

The additive (4a1607i) is currently authorised for use in poultry for fattening, weaned piglets and pigs for fattening,[3] lactating sows[4] and laying hens.[5] The applicant has requested the renewal of the authorisation for the species/categories for which there is an authorisation, the extension of use to all

[1] 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.
[2] DSM Nutritional Products Ltd, Wurmisweg 576 4303 Kaiseraugst Switzerland, represented in the EU by DSM Nutritional Products Sp. z o.o., Tarczyńska 113 96–320 Mszczonów Poland.
[3] Commission Implementing Regulation (EU) 2017/1006 of 15 June 2017 amending Implementing Regulation (EU) No 1206/2012 as regards the change of the production strain of the preparation of endo-1,4-beta-xylanase, produced by *Aspergillus oryzae* (DSM 10287) as feed additive for poultry for fattening, weaned piglets and pigs for fattening (holder of authorisation DSM Nutritional Products Ltd). OJ L 339, 16.6.2017, p. 9.
[4] Commission Implementing Regulation (EU) 2020/995 of 9 July 2020 concerning the authorisation of a preparation of endo-1,4-beta-xylanase produced by *Aspergillus oryzae* (DSM 26372) as a feed additive for lactating sows (holder of authorisation DSM Nutritional Products Ltd represented by DSM Nutritional Products Sp. Z o.o). OJ L 273, 20.8.2020, p. 17.
[5] Commission Implementing Regulation (EU) 2020/1034 of 15 July 2020 concerning the authorisation of a preparation of endo-1,4-beta-xylanase produced by *Aspergillus oryzae* (DSM 26372) as a feed additive for laying hens (holder of authorisation DSM Nutritional Products Ltd represented by DSM Nutritional Products Sp. Z o.o). OJ L 227, 16.7.2020, p. 34.

poultry species and all *Suidae*, and a modification of the production strain (substituting strain *A. oryzae* DSM 26372 with another strain belonging to the same species of filamentous fungi (DSM 33700)).

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[6] in support of the authorisation request for the use of endo-1,4-beta-xylanase (produced by *A. oryzae* DSM 33700) (RONOZYME® WX (CT/L)) as a feed additive.

In accordance with Article 38 of the Regulation (EC) No 178/2002[7] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[8] a non-confidential version of the dossier has been published on Open.EFSA.

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 15 May 2023 to 5 June 2023 for which no comments were received.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 5 September 2022 to 5 December 2022 for which the received comments were considered for the assessment.

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, to deliver the present output.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the active substance in animal feed are valid and applicable for the current application.[9]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of endo-1,4-beta-xylanase (produced by *A. oryzae* DSM 33700) (RONOZYME® WX (CT/L)) is in line with the principles laid down in Regulation (EC) No 429/2008[10] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b); Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017b); Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEEDAP Panel, 2017c); Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017d); Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a); Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b); Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019c) and Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2021).

3. Assessment

This assessment regards the renewal of the authorisation for the product RONOZYME® WX (CT/L) (endo-1,4-beta-xylanase; IUBMB EC 3.2.1.8, xylanase) when used as a zootechnical additive (functional group of digestibility enhancers) in poultry for fattening, laying hens, weaned piglets, pigs for fattening and lactating sows. It also regards the request for an extension of use to all poultry

[6] Dossier reference: FEED-2021-2507.

[7] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

[8] Decision available online: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

[9] Evaluation report available on the EU Science Hub https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en

[10] 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.

species and all *Suidae* and a change in the production strain, substituting the strain *A. oryzae* DSM 26372 with another strain belonging to the same species (DSM 33700). The product will be hereafter referred to its trade names RONOZYME® WX CT (solid form) and RONOZYME® WX L (liquid form).

3.1. Characterisation

3.1.1. Characterisation of the production organism

The endo-1,4-beta-xylanase present in the additive is obtained by fermentation with a genetically modified strain of *A. oryzae* which is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH with the deposition number DSM 33700.[11]

The taxonomic identification of the production strain as *A. oryzae* was achieved by phylogenetic analysis. Although the whole genome sequence (WGS) of the production strain was available, the analysis did not consider a large set of core genes (e.g. BUSCO), but was based only on the coding region of three marker genes (*caM*, *benA* and *rpb2*) and the internal transcribed spacer (ITS) region of the rDNA gene. Comparison of the *caM*, *benA*, *rpb2* and ITS sequences of the production strain with the orthologous sequences from the reference strain *A. oryzae* RIB40 and the closely related species *Aspergillus miniscleotigenes*, *Aspergillus aflatoxiformans* and *Aspergillus flavus* confirmed the identification of DSM 33700 as *A. oryzae*.

3.1.1.1. Information related to the genetically modified microorganism[12]

Characterisation of the recipient or parental microorganism

The parental strain is *A. oryzae* A1560 (synonym IFO 4177).

Characterisation of the donor organisms

[redacted] The donor organism for the [redacted] xylanase gene [redacted] is *Thermomyces lanuginosus*. [redacted]

Description of the genetic modification

The *A. oryzae* production strain was developed from the parental strain A1560 (IFO 4177) by classical mutagenesis and genetic modification steps.

[redacted] Thus, a combination of WGS data, [redacted] was used for the characterisation of the integration region in the production strain.

[redacted]

[11] Annex 2.1.5.
[12] Annex 2.1.6.

[redacted]

3.1.2. Manufacturing process

According to the applicant, the manufacturing process has not been substantially modified as compared to the one previously reported for the additive, with the exception of the change of the production strain (see above).

The endo-1,4-beta-xylanase is produced by submerged batch-fed fermentation of the production strain *A. oryzae* DSM 33700.[13] The enzyme endo-1,4-beta-xylanase is recovered from the fermentation broth by steps involving re-coat vacuum drum filtration, filter press filtration, centrifugation, ultrafiltration and/or evaporation and germ filtration ([redacted]).[14] The resulting fermentation product is blended with water, potassium sorbate, glycerol and sorbitol to prepare the final liquid formulation (RONOZYME® WX L) or it is subject to the granulation-coating process to obtain the solid formulation (RONOZYME® WX CT).

The applicant states that no antimicrobial substances are used in the manufacturing process.

3.1.3. Characterisation of the additive

The additive is currently authorised in two different formulations, solid (RONOZYME® WX CT) and liquid (RONOZYME® WX L), ensuring 1,000 FXU[15]/g and 650 FXU/mL, respectively. The applicant provided data to characterise both formulations of the additive produced with the new strain.

RONOZYME® WX CT is a coated solid form which ensures a minimum activity of xylanase of 1,000 FXU/g. The batch-to-batch variation of the enzyme activity was studied in six batches and the mean value was 1,143 FXU/g, ranging from 1,040 to 1,203 FXU/g.[16] This formulation contains endo-1,4-beta-xylanase concentrate (1%), cellulose ([redacted]), kaolin[17] ([redacted]), sodium sulfate ([redacted]), dextrin ([redacted]), calcium carbonate ([redacted]), vegetable oil ([redacted]) and water ([redacted]).[18]

RONOZYME® WX L is a light-yellow liquid form which ensures a minimum activity of xylanase of 650 FXU/g. The batch-to-batch variation of the enzyme activity was studied in five batches and the mean value was 678 FXU/g, ranging from 675 to 685 FXU/g.[19] It contains endo-1,4-beta-xylanase concentrate (0.5%), potassium sorbate ([redacted]), glycerol ([redacted]), sorbitol ([redacted]) and water ([redacted]).[18]

The applicant set specifications for chemical and microbiological contamination which include arsenic (< 3 mg/kg), cadmium (< 0.5 mg/kg), lead (< 5 mg/kg), mercury (< 0.5 mg/kg), total viable count (< 5×10^4 colony forming units (CFU)/g), total coliforms (< 30 CFU/g), *E. coli* (no detection in 25 g) and *Salmonella* spp. (no detection in 25 g). Analytical data to confirm the specifications were provided for six and five batches of the solid and liquid form, respectively, showing the following values:[20] arsenic ranged between 0.72 and 0.96 mg/kg in the solid form and was < 0.3 mg/kg in the liquid one; cadmium ranged between 0.07 and 0.13 mg/kg in the solid form and was < 0.05 mg/kg in the liquid one; mercury was < 0.05 mg/kg in both formulations of the additive and lead ranged between 0.61 and 1.3 mg/kg in the solid form and was < 0.5 mg/kg in the liquid one; total viable counts were < 100–400 and < 100–200 CFU/g for the solid and liquid form, respectively; total coliforms were < 4–20 and ≤ 4 CFU/g for the solid and liquid form, respectively; *E. coli* and *Salmonella* spp. were not detected in 25 g of either form.[21] Enterobacteriaceae, yeasts and filamentous fungi were also measured in both formulations of the additive and showed results ≤ 10 CFU/g.[22,23]

The detected amounts of the above-described impurities and microbial contamination do not raise safety concerns.

[13] RONO WX_SECTION 2.3, Annex 2.3.1 and RFI Annex 8.2.
[14] RFI Annex 8.3.
[15] One xylanase unit (FXU) is the amount of enzyme which liberates 7.8 μmol of reducing sugars (xylose equivalents) from azo-wheat arabinoxylan per minute at pH 6.0 and 50°C.
[16] Annex 2.1.2 and RFI Annex 5.
[17] Currently under re-evaluation.
[18] Annex 2.1.1.
[19] Annex 2.1.2.
[20] Annex 2.1.1 and RFI Annex 5.
[21] RFI Annex 7. Limit of detection: 0.5 mg Pb/kg, 0.3 mg As/kg, 0.05 mg Cd/kg, 0.05 mg Hg/kg, 100 CFU total viable count/g, 1 CFU *E. coli* in 25 g and 1 CFU *Salmonella* in 25 g.
[22] Limit of detection: 10 CFU/g.
[23] RFI Annex 6.

The absence of antimicrobial activity was demonstrated according to the FEEDAP Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b) in culture supernatants from three independent fermentation batches.[24] The applicant also investigated the presence of antimicrobial activity in three batches of the intermediate concentrate and five batches of each form of the additive.[25] No antimicrobial activity was detected in those batches; however, details of the method used were not provided, and therefore, the study was not further considered.

Some *Aspergillus* species are known to be capable of producing mycotoxins and other secondary metabolites. [redacted] Three batches of the intermediate concentrate ([redacted]), used to formulate the final products,[12] were analysed for the presence and quantification of 3-nitropropionic acid (BNP), cyclopiazonic acid (CPA) and aflatoxin B1, and showed values below the limit of detection (LOD) of the corresponding analytical method.[26]

The presence of viable cells of the production strain was investigated in three batches of the intermediate concentrate ([redacted]) that is used to formulate the two final formulations of the additive.[12] Samples of [redacted] were analysed in triplicate [redacted] No growth was detected.

The presence of DNA from the production strain was tested in triplicate in [redacted] of the same batches of the intermediate concentrate analysed for the presence of viable cells.[12] [redacted] The LOD in samples spiked with genomic DNA of the production strain was 10 ng of genomic DNA per g of product. DNA of the production strain was not detected in the samples.

3.1.4. Physico-chemical properties of the additive, stability and homogeneity

The enzyme itself (same xylanase),[27] the manufacturing process and the composition of the two formulations of the additive have not been modified. Therefore, the change in the production strain is not expected to have an impact on the physical properties of the additive, stability and capacity of the xylanase to homogeneously distribute in the feed/premixtures. Physico-chemical properties, stability and homogeneity of both formulations of the additive were addressed in previous opinions and are still considered valid (EFSA FEEDAP Panel, 2012a,b, 2016). However, the applicant submitted updated data on the shelf-life of both formulations of the additive obtained with *A. oryzae* DSM 33700, which are reported below.

The shelf-life of RONOZYME® WX CT was studied when stored at 25°C and 40°C for 6 (three batches) and 12 months (two batches), and at 25°C for 24 months (three batches) in standard unopened original packaging. After 6 months, recoveries ranged between 88 and 99% and between 83% and 86% for the samples kept at 25°C and 40°C, respectively. After 12 months, no losses were observed at 25°C while recoveries ranged between 73% and 92% for the samples kept at 40°C. After 24 months, recoveries at 25 and 40°C ranged between 78% and 100%.[28]

The shelf-life of RONOZYME® WX L was studied when stored at 10, 25 and 40°C for 6 (three batches) and 12 months (two batches), and at 10 and 25°C for 24 months in glass vials sealed with metal caps. No losses were observed at 10 and 25°C after 6 or 12 months or at 10 °C after 24 months. Recoveries ranged between 85% and 91% for the samples kept at 25°C for 24 months and between 84 and 86% and 56 and 60% for the samples kept at 40°C after 6 and 12 months, respectively.[29]

[24] RFI Annex 3. Indicator strains: [redacted]
[25] Annex 2.1.2 and Annex 2.1.9.
[26] Annex 2.1.9. BNP, CPA and aflatoxin B1 were determined using liquid chromatography. Limit of detection (LOD) in mg/kg were 0.47 for BNP, 0.003 for CPA and 0.0003 for aflatoxin B1.
[27] RFI Annex 16.
[28] Annex 2.4.3. and RFI Annex 9.2.
[29] Annex 2.4.4. and RFI Annex 9.1.

3.1.5. Conditions of use

RONOZYME® WX (CT/L) produced by *A. oryzae* DSM 26372 is currently authorised for use in feed as a zootechnical additive for poultry for fattening and laying hens at the minimum recommended inclusion level of 100 FXU/kg complete feed, and for weaned piglets, pigs for fattening and lactating sows at the minimum recommended inclusion level of 200 FXU/kg complete feed.

Other provisions as stated in the authorisations:

1) In the directions for use of the additive and premixtures, the storage conditions and stability to heat treatment shall be indicated.
2) For users of the additive and premixtures, feed business operators shall establish operational procedures and organisational measures to address potential risks resulting from its use. Where those risks cannot be eliminated or reduced to a minimum by such procedures and measures, the additive and premixtures shall be used with personal protective equipment, including breathing protection and skin protection.

In the authorisation for poultry for fattening, weaned piglets and pigs for fattening the other provisions also include:

1) Recommended maximum dose per kilogram of complete feedingstuff for:
   - poultry for fattening: 200 FXU;
   - piglets (weaned): 400 FXU;
   - pigs for fattening: 400 FXU.
2) For use in weaned piglets up to ~ 35 kg.

The applicant has not asked to modify these conditions of use but requested to substitute the current production strain (*A. oryzae* DSM 26372) with a new strain of the same species of filamentous fungi (DSM 33700).

In addition, the applicant requested the extension of use to all poultry species and all *Suidae* at the minimum recommended inclusion levels of 100 and 200 FXU/kg complete feed, respectively.

3.2. Safety

The applicant performed a literature search in order to provide evidence that, in the light of the current knowledge, the additive remains safe under the approved conditions for the target species, consumers, users and the environment. The applicant searched in three relevant databases (Web of Science Core Collection, Biosis Citation Index and Medline). The search was limited to publications from 2001 to 2021 and the search terms and search strategy were provided.[30] The main search terms included production organism in combination with the enzyme or terms relevant for consumers, users, target animals and the environment safety. The literature search retrieved 53 publications which were reviewed for their relevance for the safety for the target species, humans or the environment. Most of the publications were excluded from the assessment because the safety of the product was not assessed. The other publications retrieved did not report any safety issues, were EFSA opinions or addressed the potential of *Aspergillus* to produce toxins. However, the outcome of this literature search is of limited relevance considering the modification regarding the production strain of the additive under assessment.

3.2.1. Safety of the production organism

The production strain *A. oryzae* DSM 33700 differed from the parental strain (*A. oryzae* A1560) by expressing an endo-1,4-beta-xylanase gene [redacted] from *T. lanuginosus*. The introduced sequences raise no safety concern. [redacted], cyclopiazonic acid and aflatoxin B1 (the latter two compounds were below the limit of detection in the intermediate concentrate). Viable cells of the genetically modified production strain and its DNA were not detected in an intermediate concentrated product representative of the two final formulations of the additive. The product RONOZYME® WX (CT/L) manufactured with *A. oryzae* DSM 33700 does not give rise to safety concerns with regard to the genetically modified production strain.

[30] Annex 3.9.

3.2.2. Toxicological studies

The applicant submitted genotoxicity studies and a subchronic oral toxicity study to support the safety of the additive, which are presented below. All the toxicological studies were performed with the xylanase produced by *A. oryzae* DSM 33700 (16,100 FXU/g, total organic solids (TOS) 10.3%) from which the two final RONOZYME® WX formulations are obtained. The test item is considered representative for the two final formulations of the additive.

3.2.2.1. Bacterial reverse mutation test

The test item was tested for the induction of reverse mutations in *Salmonella* Typhimurium tester strains TA98, TA100, TA1535 and TA1537 and in *E. coli* WP2 uvrA (pKM101).[31] The experimental protocol was in line with Organisation for Economic Co-operation and Development (OECD) Testing Guideline (TG) 471 and claimed to be compliant with good laboratory practices (GLP). The test item was dissolved in water and tested both in the presence and absence of the metabolic activation system (S9-mix) from liver of rats pretreated with a combination of phenobarbital and β-naphthoflavone at, at least, five concentration levels up to 5,000 μg TOS/mL in two independent studies. Positive and negative controls were included. A 'treat and wash' procedure was used on a scientific basis justified by the applicant for all treatments in this study, since the test item was expected to contain significant levels of histidine.

No cytotoxicity or precipitate was seen in the first experiment at concentrations up to 5,000 μg TOS/mL. No indication of mutagenic activity was observed in any experimental condition, while a significantly increased number of revertant colonies was observed in the positive controls.

The FEEDAP Panel concludes that the test item did not induce gene mutations in bacteria under the experimental conditions used in this study.

3.2.2.2. *In vitro* mammalian cell micronucleus test

The test item was evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage.[32] The experimental protocol was in line with OECD TG 487 and claimed GLP compliant. The maximum tested concentrations were established with a preliminary cytotoxicity experiment. Tests were conducted both in the presence and absence of a post-mitochondrial supernatant fraction (S9) obtained from the livers of rats treated with a combination of phenobarbital and β-naphthoflavone. Cells were stimulated for 48 h with phytohaemagglutinin (PHA) to produce exponentially growing cells and then treated for 3 h (followed by a 17-h recovery period) with 9.77, 19.53, 39.06, 78.13, 156.25, 312.5, 625, 1,250, 2,500 and 5,000 μg TOS/mL Xylanase, dissolved in water (purified by reverse osmosis) both in the absence and in the presence of S9-mix. In a parallel assay, cells were treated for 20 h in the absence of S9-mix with no recovery period. No reductions were observed in the cytokinesis-block proliferative index (CBPI) at any concentration tested.

In the main study, cells were treated with 0, 1,250, 2,500 and 5,000 μg of TOS/mL of xylanase and 2 replicate cultures per treatment and 1,000 binucleated cells per replicate (i.e. 2,000 cells per dose) were scored for micronuclei. No significant reductions were observed in the CBPI at any concentration tested in all parts of the study.

No evidence of chromosomal damage or aneuploidy was observed as frequencies of micronucleated binucleate cells (MNBN) were not significantly different from concurrent negative controls and fell within historical control ranges for all treatments with the test item in the presence or absence of S9-mix metabolic activation. The positive controls performed as expected, showing statistically significant increase in the induction of MNBN cells which was observed following 3 or 20 h treatment, in the presence or absence of S9-mix.

The FEEDAP Panel concludes that the test item did not induce chromosome damage *in vitro* in mammalian cells under the experimental conditions employed in this study.

3.2.2.3. Subchronic oral toxicity study

Han Wistar (RccHan™;WIST) rats (10/sex/group) received the test item by oral gavage in water at dose levels of 0 (control), 107, 353.2 or 1,070.2 mg TOS/kg bw per day (equivalent to 16,727.9,

[31] RFI Annex 13.
[32] Annex 3.5.

55,202.1 and 167,279 FXU/kg bw per day) respectively, for 90 consecutive days.[33] The study was conducted in compliance with OECD TG 408 and claimed GLP compliant.

No effects were observed on survival, behaviour, body weight, feed and water intake, haematology, clinical parameters, gross pathology and histological examination. In addition, there was no evidence that the test item had any effect on the thyroid stimulating hormone levels tested in the study, on oestrus cycles or on testicular pathology.

A no observed adverse effect level (NOAEL) of 1,070.2 mg TOS/kg bw per day (equivalent to 167,279 FXU/kg bw per day), the highest dose tested, was derived from this study.

3.2.2.4. Conclusion on the toxicological studies

The FEEDAP Panel concludes that the xylanase used for the formulation of the additive showed no genotoxicity potential. Moreover, the results obtained in a subchronic oral toxicity study raised no concerns regarding the product and allowed to derive a NOAEL of 167,279 FXU/kg bw per day.

3.2.3. Safety for the target species

The safety of RONOZYME® WX (CT/L) produced by *A. oryzae* DSM 10287 or DSM 26372 for the target species was evaluated in previous opinions (EFSA FEEDAP Panel, 2012a, 2017a, 2019b). Based on the results obtained, the FEEDAP Panel concluded that the additive is safe for chickens and turkeys for fattening, and sows for reproduction at 200 FXU/kg complete feed, for laying hens at 100 FXU/kg complete feed and for weaned piglets at 400 FXU/kg complete feed. The conclusions were extended to all poultry species and pigs for fattening.

The product under assessment shares the manufacturing process, the composition and the enzyme xylanase with the previous products produced by *A. oryzae* DSM 10287 or DSM 26372. In order to support the safety for the target species of the additive obtained with *A. oryzae* DSM 33700, the applicant referred to the 90-day toxicity study that has been described above (see Section 3.2.2). The NOAEL identified (167,279 FXU/kg bw per day) was used to calculate the maximum safe level in feed for the different target species in accordance with the procedure described in the Guidance on the safety for the target species (EFSA FEEDAP Panel, 2017b). The calculated maximum safe concentrations in feed are presented in Table 1.

Table 1: Maximum safe concentration of the additive in feed

	Body weight (kg)	Feed intake (Kg DM/day)	Maximum safe concentration (FXU/kg complete feed(1))
Chicken for fattening	2	0.158	18,634
Turkey for fattening	3	0.176	25,092
Laying hen	2	0.106	27,775
Piglet	20	0.88	33,456
Pig for fattening	60	2.20	40,147
Lactating sow	175	5.28	48,790

(1): Complete feed containing 88% dry matter.

The maximum safe levels obtained are higher than the maximum recommended level currently authorised for poultry for fattening, laying hens, weaned piglets, pigs for fattening and lactating sows, and the minimum recommended level for the new target species. Therefore, the FEEDAP Panel concludes that RONOZYME® WX (CT/L) produced by *A. oryzae* DSM 33700 is safe for the currently authorised species under the approved conditions of use.

The current application requests also for an extension of use of the additive to all poultry species and all *Suidae*. The Panel considers that the conclusions from chickens for fattening and laying hens can be extended/extrapolated to all poultry species. Similarly, the conclusions reached in weaned piglets and sows can be extended/extrapolated to all *Suidae*. Therefore, the FEEDAP Panel concludes that the additive is safe when used under the authorised and proposed conditions of use for all poultry and all *Suidae*.

[33] RFI Annex 12.

3.2.4. Safety for the consumer

The results of the genotoxicity studies and the subchronic oral toxicity study conducted with the xylanase produced by *A. oryzae* DSM 33700, which is representative of the final formulations of the additive, do not indicate any reason for concern for consumer safety arising from the use of the product as feed additive in poultry and *Suidae* species.

Therefore, the FEEDAP Panel concludes that the use of RONOZYME® WX in feed for all poultry and all *Suidae* is of no concern for the consumer.

3.2.5. Safety for the user

3.2.5.1. Effect on respiratory system

Data on the particle size distribution of RONOZYME® WX CT were provided in a previous opinion and showed that 98% of the particles had a diameter between 150 and 1,200 μm and less than 1% of particles were below 150 μm (EFSA FEEDAP Panel, 2012a). No new data was provided on the physico-chemical properties of the additive. Based on the proteinaceous nature of the active substance, the additive is considered a respiratory sensitiser.

3.2.5.2. Effect on skin and eyes

The applicant provided two skin irritation studies and two eye irritation studies conducted with the final formulations of the additive (RONOZYME® WX L and RONOZYME® WX CT).

Skin irritation studies were performed according to the OECD Testing Guideline (TG) 439.[34,35] The results of the studies showed that RONOZYME® WX L and RONOZYME® WX CT are not skin irritant.

Eye irritation studies were performed according to the OECD Testing TG 437.[36,37] The results of the studies showed that RONOZYME® WX L is not an eye irritant. For RONOZYME® WX CT, the *In Vitro* Irritancy Score (IVIS) was measured to be 6.7, meaning that 'No stand-alone prediction' of eye irritation can be made under the conditions of the test.

No data on dermal sensitisation was provided for both formulations of the additive therefore, the FEEDAP Panel is not able to conclude on the potential of both formulation of the additive to be skin sensitisers.

3.2.5.3. Conclusions on safety for the user

Based on the studies submitted, both formulations of the additive were not irritant to skin. RONOZYME® WX L is not an eye irritant; however, no conclusions can be drawn on the potential of RONOZYME® WX CT to be an eye irritant. Due to the lack of data, the FEEDAP Panel is not able to conclude on the potential of both formulations of the additive to be dermal sensitisers. Due to the proteinaceous nature of the active substance (xylanase), the additive is considered a respiratory sensitiser.

3.2.6. Safety for the environment

Viable cells of the production strain and its recombinant DNA were not detected in an intermediate product representative of both final formulations of the additive. The additive does not raise safety concerns for the environment with regard to the genetic modification of the production strain. The active substance of the additive is a protein, and as such will be degraded/inactivated during passage through the digestive tract of animals or in the environment. Therefore, no risks for the environment are expected and no further environmental risk assessment is required.

3.3. Efficacy

The production strain DSM 33700 of the product under evaluation is different from the previous strains DSM 10287 and DSM 26372 of *A. oryzae* (see Section 3.1.1). However, the enzyme under assessment (endo-1,4-beta-xylanase) is the same.[27] Consequently, the efficacy results obtained testing the endo-1,4-beta-xylanase produced using *A. oryzae* DSM 10287 or DSM 26372 assessed in previous

[34] RFI Annex 15.1.
[35] RFI Annex 15.2.
[36] RFI Annex 15.3.
[37] RFI Annex 15.4.

opinions (EFSA FEEDAP Panel, 2012a,b, 2017a, 2019a,b) are considered applicable to the endo-1,4-beta-xylanase produced by *A. oryzae* DSM 33700. The product is authorised in poultry for fattening and laying hens at 100 FXU/kg complete feed and in weaned piglets, pigs for fattening and lactating sows at 200 FXU/kg complete feed.

The applicant requested to maintain the current conditions of use for those species and also the extension of use to all poultry species and all *Suidae* at a minimum recommended level of 100 and 200 FXU/kg complete feed, respectively.

Considering that the mode of action of the enzyme present in the additive can be reasonably assumed to be the same in the different poultry species and in the different *Suidae* species, the FEEDAP Panel concludes that the additive is efficacious in all poultry species and in all *Suidae* at a minimum recommended level of 100 FXU/kg and 200 FXU/kg complete feed, respectively.

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[38] and Good Manufacturing Practice.

4. Conclusions

RONOZYME® WX CT (solid form) and RONOZYME® WX L (liquid form), manufactured with the production strain *A. oryzae* DSM 33700, do not give rise to safety concerns with regard to the genetic modification of the production strain. No viable cells of the production strain and no DNA were detected in an intermediate product representative of both final formulations of the additive.

RONOZYME® WX (CT/L) is considered safe for all poultry species and all *Suidae* at the authorised and proposed conditions of use (100 and 200 FXU/kg complete feed, respectively).

The use of RONOZYME® WX CT and L manufactured with the production strain *A. oryzae* DSM 33700 raises no concerns for consumers.

Both formulations of the additive are not irritant to skin. RONOZYME® WX L is not an eye irritant; however, no conclusions can be drawn on the potential of RONOZYME® WX CT to be an eye irritant. Due to the lack of data, the FEEDAP Panel is not able to conclude on the potential of both formulations of the additive to be skin sensitisers. Both formulations of the additive should be considered a respiratory sensitiser.

The additive raises no concerns to the environment.

The additive has the potential to be efficacious in all poultry species and in all *Suidae* at the minimum recommended levels of 100 FXU/kg and 200 FXU/kg complete feed, respectively.

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[38] 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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Abbreviations

CFU	colony forming units
EURL	European Union Reference Laboratory
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
ITS	internal transcribed spacer
PCR	polymerase chain reaction
WGS	whole genome sequence