European Food Safety Authority

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SCIENTIFIC OPINION

Opinion on a request from the European Commission related to the enzyme preparation of trade name "Danisco Xylanase G/L (endo-1,4-beta-xylanase)" as a feed additive for laying hens and chickens and ducks for fattening.[1]

Updated Scientific Opinion of the Panel on Genetically Modified Organisms

(Question No EFSA-2009-00498)

Adopted on 02 July 2009

PANEL MEMBERS[*]

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SUMMARY

Following a request from European Commission, the Panel on Genetically Modified Organisms was asked to issue a scientific opinion on the enzyme preparation of trade name "Danisco Xylanase G/L (endo-1,4-beta-xylanase)" as a feed additive for laying hens and chickens and ducks for fattening, based on new data provided by the applicant in the supplementary dossier.

The presence/absence of recombinant DNA from the production microorganism in the Danisco Xylanase G/L feed additive was assessed by a PCR method amplifying a fragment of 506 bp and reaching a detection limit of 5 ng mL^{-1} sample. In samples from the original production process, DNA had been detected. Subsequently, two modifications were introduced in the production process. Using the same detection method, recombinant DNA was no longer detected either in the enzyme preparation corresponding to the final marketable product or in the concentrate.

[1] For citation purposes: Scientific Opinion of the Panel on Genetically Modified Organisms on a request from the European Commission related to the enzyme preparation of trade name "Danisco Xylanase G/L (endo-1,4-beta-xylanase)" as a feed additive for laying hens and chickens and ducks for fattening. *The EFSA Journal* (2009) 1183, 1-8

[*] This opinion is not shared by 0 members of the Panel. / (conflict of interest) 0 members of the Panel did not participate in (part of) the discussion on the subject referred to above.

Request from the European Commission related to "Danisco Xylanase G/L" as a feed additive

Key words: Zootechnical additive, digestibility enhancer, enzyme, *Trichoderma reesei*, xylanase, chickens for fattening, ducks, laying hens, efficacy, safety, tolerance, genetically modified microorganism.

TABLE OF CONTENTS

BACKGROUND AS SUBMITTED BY EC

Regulation (EC) No. 1831/2003 establishes rules governing the Community authorisation of additives for animal nutrition and, in particular, defines the conditions that a substance/product should meet to be granted the authorisation.

The Scientific Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority adopted on 19th September 2007 and on 22nd November 2007 two opinions on the safety and efficacy of the enzyme preparation with the trade name "Danisco Xylanase G/L" as feed additive for chickens for fattening, laying hens, ducks for fattening and turkeys for fattening. The first opinion was adopted in cooperation with the Panel on Genetically Modified Organisms (GMO Panel). In the opinion it was concluded that "fragments of recombinant DNA are present in the final concentrate only in trace amounts". This was considered by the European Commission as not complying with Article 7(3)(i) of Regulation (EC) No. 1831/2003. Therefore, the Commission gave the possibility to the company, Danisco Animal Nutrition, to submit supplementary data. The applicant provided new data to be evaluated by the European Food Safety Authority (EFSA). In its second opinion, adopted on 21st May 2008, the GMO Panel concluded that, since no new additional information was provided by the company, "the presence of trace amounts of recombinant DNA cannot be excluded".

The European Commission received supplementary information from the applicant company, Danisco Animal Nutrition, for the enzyme preparation "Danisco Xylanase G/L" as feed additive for laying hens and chickens and ducks for fattening.

TERMS OF REFERENCE AS SUBMITTED BY EC

The European Commission requests the European Food Safety Authority to issue an opinion on the enzyme preparation with the trade name "Danisco Xylanase G/L" as feed additive for laying hens and chickens and ducks for fattening taking into account its earlier opinions on 19th September 2007 and 21st May 2008 and the new data provided.

ASSESSMENT

1. Introduction

The European Food Safety Authority has received a request from the European Commission to update the Opinion on the Danisco Xylanase G/L (endo-1,4-beta-xylanase) feed additive, adopted in September, 2007 (EFSA, 2007). The opinion concluded that trace amounts of DNA are present in the enzyme concentrate and that, since no sequences which cause concern are introduced in the final production strain, the presence of this low concentration of recombinant DNA in the final product does not raise any particular safety concern. The European Commission considered, however, that this was not in compliance with Article 7(3)(i) of Regulation (EC) No. 1831/2003 and gave the company the possibility to submit additional information. An updated opinion was adopted by the GMO Panel on 21st May 2008 taking into consideration the additional information submitted by the company (EFSA, 2008). In this opinion, it was concluded that "no recombinant DNA was detected in the enzyme preparation when it was diluted to the activity level corresponding to the final marketable product". However, recombinant DNA was detected in the enzyme concentrate which was about 39 times more concentrated. Since no additional purification steps of the enzyme preparation which could have removed the traces of DNA have been reported, the presence of trace amounts of recombinant DNA below the quantification limit in the final product cannot be excluded. However, since no sequences which cause concern are introduced, the genetic modification and the presence of trace amounts of recombinant DNA in the final product do not raise any safety concern."

The company provided information concerning the presence/absence of recombinant DNA in lots of Danisco Xylanase in three different reports. One report was dated 6 August 2007, the second 12th December 2007, the third 16th January 2009. The data presented in the report of 6th August 2007 were assessed in the opinion on the Danisco Xylanase G/L (endo-1,4-beta-xylanase) feed additive, adopted in September 2007 (EFSA, 2007). In the updated opinion adopted in May 2008, the supplementary information provided in the report of 12th December 2007 was assessed and compared with the previous set of data. The present opinion is based on the supplementary information provided on the report of 16th January 2009 and on a comparison of the three sets of data. In addition, information provided in response to a request by the GMO Panel and received in May 2009 has also been considered.

2. Information relating to the product purification process

The xylanase enzyme is recovered from the fermentation broth by biomass separation involving a series of filtration steps. In the report of 16th January 2009, the lots were produced by the production process including two modifications compared to the original one. First the holding time after fermentation was reduced to maximum 7 days, limiting cell lysis. Secondly, an ultrafiltration step after cell separation replaced the evaporation step.

3. Assessment of the presence of recombinant DNA and of the potential risk of gene transfer

The comparison of the three data sets is presented in Table 1. In the report of 6th August 2007 three lots of enzyme concentrate were tested. The information of May 2009 confirmed that these lots were produced by the original production process without the two modifications (reduced holding time and an ultrafiltration step after cell separation) introduced later. The three lots were produced on an industrial scale. In the information of May 2009, the concentrations of the lots of August 2007 were recalculated based on the current unit definition. They contained an average xylanase activity of 190756 U g^{-1}. The presence of trace amounts of recombinant DNA was shown in all lots from August 2007 tested. The strength of the signal from the amplified fragment was considerably weaker than that of the control sample consisting of 5 ng of spiked target DNA per mL.

In the report of 12th December 2007, three lots of Danisco Xylanase produced on a pilot scale were tested. In these lots the enzyme concentrate was diluted so that the enzyme activity corresponded to that of the intended commercial preparation. Stabilizers and preservatives were added. In the three lots produced on the same day, with a guaranteed minimum activity of 40000 U g^{-1} of xylanase and an average of about 47833 U g^{-1}, no recombinant DNA was detected. No information on the production process used was provided.

In the report of 16th January 2009, two lots of Danisco Xylanase produced at industrial scale and one lot produced at pilot scale were tested. The lots were produced by the production process including the two modifications, as described above. The lots were tested in a concentrated form with an activity ranging from 215 118 to 228 432 U g^{-1} and in the enzyme preparation corresponding to the final marketable product with an activity ranging from 53 780 to 57 108 U g^{-1}. No recombinant DNA was detected in any of the samples.

In all experiments the same PCR method was used to amplify a fragment of 506 bp. The limit of detection was 5 ng of spiked target DNA per mL of sample. Proper controls were used to confirm that the stabilizers and preservatives did not affect the performance of the PCR reaction and that DNA was not lost during sample preparation. No recombinant DNA was detected in the set of samples produced with the process including the two modifications. These samples include enzyme preparation with the activity level corresponding to the final marketable product as well as the concentrate.

Table 1. **Comparison of data presented in three different reports provided to the European Food Safety Authority concerning the presence of recombinant DNA in the Danisco Xylanase G/L (endo-1,4-beta-xylanase) feed additive.**

Date of report	Analysed product [(a)]	Concentration of the xylanase[(b)]	Presence of recombinant DNA
6/8/2007	Industrial scale Enzyme concentrate (obtained after ultrafiltration) Original production process	181340 U g^{-1} 203900 U g^{-1} 187028 U g^{-1}	Detectable at a level below 5 ng mL^{-1}
12/12/2007	Pilot scale Enzyme concentrate diluted and formulated to correspond to the final commercial product Production process not defined	47 833 U g^{-1}	Not detected [(c)]
16/1/2009	Industrial scale (2 samples); pilot scale (1 sample) Enzyme concentrate (obtained after ultrafiltration) Modified production process	228 432 U g^{-1} 215 118 U g^{-1} 222 000 U g^{-1}	Not detected
16/1/2009	Industrial scale (2 samples); pilot scale (1 sample) Enzyme concentrate diluted and formulated to correspond to the final commercial product Modified production process	57 108 U g^{-1} 53 780 U g^{-1} 55 000 U g^{-1}	Not detected[(b)]

(a): Fermentation and purification processes and PCR method used for analysis were identical in all sets of data.
(b): All concentrations have been calculated using the current unit definition.
(c): PCR reaction was done on the liquid preparation.

4. Safety for the environment

The production microorganism is removed from the product, the recombinant DNA in the final commercial product is below the limit of detection (5 ng mL^{-1}) and no sequences which cause concern are introduced during the genetic modification. Therefore, no further environmental risk assessment is required.

CONCLUSIONS

The presence/absence of recombinant DNA from the production microorganism in the Danisco Xylanase G/L feed additive was assessed by a PCR method amplifying a fragment of 506 bp and reaching a detection limit of 5 ng mL^{-1} sample. In samples from the original production process, DNA had been detected. Subsequently, two modifications were introduced in the production process. These consist of reduced holding time after fermentation limiting cell lysis and replacement of the original evaporation step by ultrafiltration. Using the same detection method, recombinant DNA was no longer detected either in the enzyme preparation corresponding to the final marketable product or in the concentrate.

Since the production microorganism is removed from the product, the recombinant DNA in the final commercial product is below the limit of detection (5 ng mL^{-1}) and no sequences which cause concern are introduced due to the genetic modification, no further environmental risk assessment is required.

DOCUMENTATION PROVIDED TO EFSA

1. Enclosure. February 2009. Submitted by European Commission.

   Company supplementary information on the EFSA opinion for Xylanase G/L (endo-1,4-beta-xylanase) as a feed additive. January 2009. Danisco Animal Nutrition.

2. Supplementary information on Danisco Xylanase G/L. May 2009. Submitted by Danisco Animal Nutrition.

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http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178654544327.htm

EFSA, 2008. Opinion on a request from the European Commission related to the enzyme preparation of trade name "Danisco Xylanase G/L (endo-1-4-beta-xylanase)" as a feed additive for laying hens and chickens and ducks for fattening. The EFSA Journal 714, 1-7.
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