SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 7 March 2018

doi: 10.2903/j.efsa.2018.5233

Statement complementing the EFSA Scientific Opinion on application (EFSA-GMO-DE-2011-95) for the placing on the market of genetically modified maize 5307 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Crop Protection AG taking into consideration an additional toxicological study

EFSA Panel on Genetically Modified Organisms (GMO),
Hanspeter Naegeli, Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Mikołaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli, Jean-Michel Wal, Konstantinos Paraskevopoulos and Anna Lanzoni

Abstract

The GMO Panel was previously not in the position to complete the food/feed safety assessment of maize 5307 due to an inadequate 28-day toxicity study necessary for an appropriate assessment of eCry3.1Ab protein. Following a mandate from the European Commission, the GMO Panel assessed a supplementary 28-day toxicity study in mice on the eCry3.1Ab protein (1,000 mg/kg body weight (bw) per day) to complement its scientific opinion on application EFSA-GMO-DE-2011-95 for the placing on the market of the maize 5307 for food and feed uses, import and processing. The supplementary 28-day toxicity study did not show adverse effects. Taking into account the previous assessment and the new information, the GMO Panel concludes that maize 5307, as assessed in the scientific opinion on application EFSA-GMO-DE-2011-95 (EFSA GMO Panel, 2015) and in the supplementary toxicity study, is as safe and nutritious as its conventional counterpart in the scope of this application.

Keywords: GMO, maize 5307, food and feed safety, import and processing, eCry3.1Ab, 28-day study

Requestor: European Commission

Question number: EFSA-Q-2017-00052

Correspondence: GMO@efsa.europa.eu

Panel members: EFSA Panel on Genetically Modified Organisms (GMO), Hanspeter Naegeli, Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Mikołaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli and Jean-Michel Wal.

Acknowledgements: The Panel wishes to thank the members of its standing Working Group on Food/Feed Risk Assessment for the preparatory work on this scientific opinion.

Suggested citation: EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Guerche P, Jones H, Manachini B, Messéan A, Nielsen EE, Nogué F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Paraskevopoulos K and Lanzoni A, 2018. Statement complementing the EFSA Scientific Opinion on application (EFSA-GMO-DE-2011-95) for the placing on the market of genetically modified maize 5307 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Crop Protection AG taking into consideration an additional toxicological study. EFSA Journal 2018;16(4):5233, 9 pp. https://doi.org/10.2903/j.efsa.2018.5233

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

The GMO Panel was previously not in the position to complete the food/feed safety assessment of maize 5307 due to an inadequate 28-day toxicity study necessary for an appropriate assessment of eCry3.1Ab protein.

On 23 December 2016, the European Commission requested the GMO Panel to complement its scientific opinion on application EFSA-GMO-DE-2011-95 for the placing on the market of the maize 5307 for food and feed uses, import and processing (EFSA GMO Panel, 2015) taking into consideration a supplementary toxicity study provided by the applicant after the adoption of the EFSA opinion.

The GMO Panel assessed the supplementary 28-day oral repeated dose toxicity study in mice on the eCry3.1Ab protein at 1,000 mg/kg body weight (bw) per day. The study was conducted in accordance with OECD TG 407 and with the principles of Good Laboratory Practice. No adverse effects were observed in this study.

Taking into account the previous assessment and the new information, the GMO Panel concludes that maize 5307, as assessed in the scientific opinion on application EFSA-GMO-DE-2011-95 (EFSA GMO Panel, 2015) and in the supplementary toxicity study, is as safe and nutritious as its conventional counterpart in the scope of the application.

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

On 16 April 2015, the GMO Panel adopted a scientific opinion on application EFSA-GMO-DE-2011-95 for the placing on the market of maize 5307 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (EFSA GMO Panel, 2015). In this scientific opinion, the GMO Panel considered that the information available for maize 5307 was not sufficient to reach a final overall conclusion since 'the EFSA GMO Panel cannot conclude on the safety of the eCry3.1Ab protein'. This was due to the fact that 'the 28-day rat oral toxicity study on eCry3.1Ab provided to support the safety assessment of this newly expressed protein was not considered adequate'.

On 6 May 2015, the European Food Safety Authority (EFSA) received a letter from the applicant where they disputed the claim that the 28-day toxicity study was inadequate and indicated that such a study was compliant with applicable OECD protocols and entirely adequate for a proper food/feed risk assessment by toxicologists. On 19 May 2015, EFSA replied reiterating that the study showed a major deviation from the applicable OECD protocol TG 407 (2008) leading to the impossibility of deriving a scientifically meaningful conclusion on the toxicological profile of maize 5307. Furthermore, in this letter, EFSA offered to the applicant to consider a post-adoption teleconference to further clarify the scientific rationale leading to the conclusions of the scientific opinion.[1]

Following the publication of the scientific opinion on maize 5307, on 1 June 2015, the applicant requested EFSA a post-adoption teleconference. Following exchanges with the applicant (EFSA to the applicant on 16 June 2015, 22 June 2015 and 30 June 2015; the applicant to EFSA on 19 June 2015 and 24 June 2015), the meeting was held on 2 July 2015. In this context, EFSA provided clarifications on the original 28-day study weaknesses; Syngenta acknowledged that the study contained an unusual deviation from OECD TG 407 (2008) and confirmed that a new 28-day toxicity study in rodents on the eCry3.1Ab protein would have been provided. This was reiterated in a letter sent to EFSA on 3 July 2015. EFSA provided the applicant a summary of the post-adoption teleconference on 17 July 2015.

On 23 December 2016, the European Commission mandated EFSA to assess a new 28-day toxicity study on the eCry3.1Ab protein received from the applicant on 8 December 2016, and to complement its original scientific opinion on maize 5307 taking into consideration this additional information. EFSA acknowledged the receipt of the mandate on 23 March 2017.

The GMO Panel asked the applicant clarifications on this supplementary toxicity study on 22 May 2017, 20 October 2017 and 17 November 2017. Following a request by the applicant, on 6 June 2017, EFSA invited the applicant to a clarification teleconference regarding EFSA question sent on 22 May 2017 (teleconference held on 12 June 2017). The applicant replied to EFSA on 24 July 2017, 30 October 2017 and 14 December 2017.

In the frame of contract OC/EFSA/UNIT/GMO/2014/02, the contractor performed preparatory work and delivered a report on the methods applied by the applicant to perform the study.

This statement reports the GMO Panel assessment of this supplementary toxicity study and the related additional information provided by the applicant following EFSA's requests.

According to the mandate received from European Commission on 23 December 2016, this statement complements the EFSA scientific opinion on maize 5307 (EFSA GMO Panel, 2015), which is the report requested under Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003 and is part of the EFSA Overall Opinion in accordance with Articles 6(5) and 18(5) of that Regulation.

2. Data and methodologies

2.1. Data

In delivering this statement, the GMO Panel took into account the supplementary toxicity study provided by the applicant in the context of this mandate and the EFSA scientific opinion on application EFSA-GMO-DE-2011-95 (EFSA GMO Panel, 2015).

2.2. Methodologies

The GMO Panel carried out a scientific risk assessment of this supplementary toxicity study taking into account the appropriate principles described in its guidelines for the risk assessment of genetically modified (GM) plants and derived food and feed (EFSA GMO Panel, 2011).

[1] http://www.efsa.europa.eu/en/supporting/pub/1025e

3. Assessment

The 28-day oral repeated dose toxicity study in mice on the eCry3.1Ab protein provided in the context of the current mandate was conducted in accordance with OECD TG 407 (2008) and with the principles of Good Laboratory Practice.

Groups of Crl:CD1(ICR) mice (12/sex per group; males singly-housed and females caged in pairs; approximately 6–7 weeks old at study start) were administered by gavage respectively: the eCry3.1Ab protein (in 0.5% [w/v] carboxymethylcellulose) at a targeted nominal dose of 1,000 mg/kg body weight (bw) per day (eCry3.1Ab protein group); the vehicle alone (vehicle control group) or bovine serum albumin (BSA) at a targeted nominal dose of 1,000 mg/kg bw per day (BSA control group).

The test substance used in this 28-day study provided by the applicant contained 80.7% of an eCry3.1Ab protein produced by a recombinant system (*Escherichia coli*). This protein was considered by the GMO Panel to be equivalent to the protein expressed in maize 5307.[2]

The eCry3.1Ab protein dosing formulation (suspension),[3,4] and the BSA protein dosing formulation were prepared daily. Samples of the eCry3.1Ab protein and BSA dosing formulations were taken on day 1 and then weekly for concentration analysis (along with vehicle) and for homogeneity analyses.

The GMO Panel noted that stability tests on the test substance (i.e. the lyophilised eCry3.1Ab protein) were not performed as part of this study. The applicant provided data demonstrating that the test substance was stable in its function (i.e. insecticidal) in a formulation and in storage conditions as used in this study.[3] The GMO Panel therefore considers that this is not a major deviation compromising the study.

Feed and water were provided *ad libitum*. During the treatment period, the animals were checked twice daily for mortality and clinical signs. Detailed clinical observations were conducted on all animals once daily after dosing during the treatment period. Body weights were recorded pretreatment (week 1) and then weekly; body weight gains were calculated relative to test day 1. Feed consumption was determined weekly.

At the end of the treatment period, blood samples were taken and haematological and clinical chemistry analyses were performed. All animals were sacrificed and underwent a detailed necropsy examination with selected organs weighed. Organs and tissues from all animals were collected and subjected to a comprehensive histopathological examination. A pathology peer review was conducted.

For all continuous endpoints, mean and standard deviation were calculated per group (males and females combined); in addition, mean, standard deviation, median, minimum and maximum, and lower and upper quartiles were reported for each sex.

In the frame of a preliminary analysis, a significant cage effect was noted in socially housed females for a few endpoints[5]; these effects were either related to isolated endpoints or to endpoints derived from others which did not show a significant cage effect. Therefore, the analysis of data from individual animals is considered appropriate.

The eCry3.1Ab protein group was statistically compared to the BSA control group. The latter was also compared to the vehicle control group in order to assess potential effects of the higher protein intake.[6] Furthermore, the eCry3.1Ab protein group was statistically compared to the vehicle control group.

For all the continuous parameters, a two-sided multi-way analysis of variance (ANOVA) at the 5% significance level was conducted for the two sexes combined (factors: sex, block-within-sex,[7] three level treatment, i.e. test substance, vehicle, and BSA, and the two-way interaction between sex and treatment in order to account for potential sex–dose interactions). A two-way ANOVA (factor: block and treatment) separately for each sex was performed when needed.[8] The data were explored

[2] EFSA GMO Panel, 2015 and Additional information 24/7/2017 and 31/10/2017.
[3] Additional information 31/10/2017.
[4] The purity of the test substance was taken into account in the formulation.
[5] Relative organ weight: thymus; clinical chemistry: albumin/globulin ratio, globulin, glucose, urea, alkaline phosphatase, triglycerides; haematology: mean corpuscular haemoglobin, mean corpuscular volume, red blood cell distribution width.
[6] With respect to body weight, including cumulative body weight gain, feed consumption, feed utilisation, haematology and clinical chemistry, organ weights (absolute and relative to terminal body weight).
[7] When an unbalanced occurrence of missing data between sexes was observed (bile acid, calcium, chloride, potassium and sodium) a pooled model without the block-within-sex term was used.
[8] Sex-specific organ weights (relative and absolute, in the latter the terminal body weight was added to the model as a covariate); parameters showing unequal variation across the sexes (glucose, triglycerides, eosinophil count and reticulocyte count). In order to take into account the social housing, in females the cage was used as the experimental unit in the model for food consumption and food utilisation. Furthermore, males and females are analysed separately if a significant interaction term (Sex*Dose) was identified in the pooled analysis.

graphically for outliers and no remarkable deviations were identified. Therefore, no outliers were excluded from the analysis. p values for the fixed effect treatments were presented and the estimated differences among pairwise comparisons were also reported. In addition, estimated means and confidence intervals for all endpoints were converted from the scale of their natural units (e.g. mg/dL) to standardised effect size (i.e. normalised to standard deviation).

The few statistically significant differences between the BSA and vehicle control groups were considered by the GMO Panel to be within normal biological variability. Therefore, both these groups were considered suitable to be used as controls for the comparison and evaluation of data from the eCry3.1Ab protein group.

The results of the dose confirmation analyses revealed that the measured concentration for eCry3.1Ab and BSA protein in 0.5% carboxymethylcellulose were within the acceptance criteria of ± 20% (–10.7–10.0%) of their theoretical concentrations respectively, based on nominal dosing suspensions at 100 mg/mL. The results of the homogeneity analyses indicated that the preparations were homogeneously mixed.

No mortality occurred during the treatment period. The GMO Panel considered that the isolated clinical findings observed in the eCry3.1Ab protein group were incidental and not related to the test substance.

Compared to the BSA control group, feed utilisation was statistically significantly lower in males in week 3, higher in males in week 4 and lower in females in week 2 of the study. There were no differences in the mean overall feed consumption throughout the study nor in body weight and body weight gain. The fluctuations in feed utilisation are therefore considered not related to the test substance.

A statistically significantly lower absolute liver weight was seen in animals given the test substance as compared to BSA control group. This difference was considered of no toxicological relevance since the relative liver weight was unchanged.

A statistically significantly lower glucose level was noted in males given the eCry3.1Ab protein (10.66 ± 1.151 mmol/L) as compared to BSA controls (12.23 ± 1.352 mmol/L); this difference was not considered to be adverse.

Macroscopic examinations at necropsy revealed no gross pathological findings related to the treatment with the eCry3.1Ab protein. Microscopic examinations of selected organs and tissues identified no test substance-related differences in the incidences and severity of the histopathological findings between the groups.

The GMO Panel noted the following deviations from OECD TG 407 requirements: coagulation analysis, functional observational battery and locomotor activity tests were not performed.

Regarding the lack of the coagulation analysis, the GMO Panel considered that the integrated assessment of related parameters (e.g. no difference in the platelet count between animals given the eCry3.1Ab protein and those given BSA, or in the spleen and bone marrow histopathology between groups) indicates that an effect on coagulation parameters is unlikely. The GMO Panel therefore concludes that this is not a major deviation compromising the safety assessment of the eCry3.1Ab protein.

Regarding the lack of functional observational battery and locomotor activity tests (tests performed to identify potential neurotoxicity of a test substance), the GMO Panel used a weight of evidence approach to evaluate potential neurotoxic effects of the eCry3.1Ab protein: (1) in this 28-day study, no test substance-related findings were, according to the study report, noted in the detailed clinical examinations (performed daily on individual animals outside the cage); (2) no test substance-related effects were seen in an acute oral toxicity study in mice given 1720 mg eCry3.1Ab protein/kg bw; (3) no similarity of the eCry3.1Ab protein to known neurotoxicants was identified in bioinformatics analysis (EFSA GMO Panel, 2015); (4) according to current knowledge, there is no indication of neurotoxicity for Cry proteins (e.g. EFSA GMO Panel, 2017a,b; 2016a,b,c). The GMO Panel therefore considers that the lack of functional observational battery and locomotor activity tests is not a major deviation compromising the safety assessment of the eCry3.1Ab protein.

The GMO Panel concludes that no adverse effects were observed in this 28-day mouse toxicity study on eCry3.1Ab protein (1,000 mg/kg bw per day).

4. Conclusions

The 28-day toxicity study on eCry3.1Ab protein assessed by the GMO Panel did not show adverse effects. Taking into account the previous assessment and the new information, the GMO

Panel concludes that maize 5307, as assessed in the scientific opinion on application EFSA-GMO-DE-2011-95 and in the supplementary toxicity study, is as safe and nutritious as its conventional counterpart in the scope of this application.

Documentation provided to EFSA

1) Letter from the applicant to EFSA dated 5 May 2015 (received on 6 May 2015) with comments regarding the Scientific opinion of the EFSA GMO Panel on maize 5307 (application EFSA-GMO-DE-2011-95).
2) Letter from EFSA to the applicant dated 19 May 2015 replying to the comments on application EFSA-GMO-DE-2011-95 and inviting the applicant to a post-adoption tele-conference following the EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products.[1]
3) Letter from the applicant to EFSA dated 1 June 2015 requesting a post-adoption teleconference.
4) Letter from EFSA to applicant dated 16 June 2015 inviting the applicant to a post-adoption teleconference to be held on 2 July 2015 including a draft agenda.
5) E-mail from the applicant to EFSA dated 19 June 2015 accepting the EFSA invitation to a post-adoption teleconference to be held on 2 July 2015.
6) E-mail from EFSA to applicant dated 22 June 2015 inviting the applicant to provide a list of items for discussion for the post adoption teleconference to be held on 2 July 2015.
7) Letter from the applicant to EFSA dated 24 June 2015 providing items for discussion the post adoption teleconference to be held on 2 July 2015.
8) Letter from EFSA to applicant dated 30 June 2015 submitting the official invitation letter to the post adoption teleconference to be held on 2 July 2015.
9) Letter from EFSA to applicant dated 30 June 2015 submitting the agenda of the post adoption teleconference on 2 July 2015.
10) Letter from the applicant to EFSA dated 3 July 2015 providing a summary of the discussion the post adoption teleconference on 2 July 2015 and committing to conduct a new 28-day study.
11) Letter from EFSA to the applicant dated 17 July 2015 summarising the outcome of the clarification post-adoption teleconference.
12) Mandate from EC to EFSA received on 23 December 2016, for the assessment of additional information related to the application for authorisation of food and feed containing, consisting and produced from genetically modified maize 5307 (application EFSA-GMO-DE-2011-95).
13) Acknowledgment letter from EFSA to EC sent on 23 March 2017.
14) Letter from EFSA to the applicant dated 22 May 2017 requesting additional information on maize 5307.
15) Letter from EFSA to the applicant dated 6 June 2017 calling the applicant for a clarification teleconference.
16) Email from EFSA to applicant dated 19 June 2017 summarising the conclusions of the post-adoption teleconference held on 12 June 2017.
17) Letter from the applicant to EFSA received on 24 July 2017 providing additional information requested.
18) Letter from EFSA to the applicant dated 20 October 2017 requesting additional information on maize 5307.
19) Letter from the applicant to EFSA received on 30 October 2017 providing additional information requested.
20) Letter from EFSA to the applicant dated 17 November 2017 requesting additional information on maize 5307.
21) Letter from the applicant to EFSA received on 14 December 2017 providing additional information requested.

References

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011. EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011;9(3):2150, 37 pp. https://doi.org/10.2903/j.efsa.2011.2150

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2015. Scientific Opinion on application (EFSA-GMO-DE-2011-95) for the placing on the market of genetically modified maize 5307 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Crop Protection AG. EFSA Journal 2015;13(5):4083, 29 pp. https://doi.org/10.2903/j.efsa.2015.4083

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2016a. Scientific Opinion on an application by Dow AgroSciences (EFSA-GMO-NL-2013-116) for placing on the market of genetically modified insect-resistant soybean DAS-81419-2 for food and feed uses, import and processing under Regulation (EC) No 1829/2003. EFSA Journal 2016;14(12):4642, 23 pp. https://doi.org/10.2903/j.efsa.2016.4642

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2016b. Scientific opinion on application (EFSA-GMO-NL-2011-96) for the placing on the market of genetically modified insect-resistant and herbicide-tolerant cotton GHB119, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience AG. EFSA Journal 2016;14(10):4586, 27 pp. https://doi.org/10.2903/j.efsa.2016.4586

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2016c. Scientific Opinion on an application by Syngenta (EFSA-GMO-DE-2011-99) for the placing on the market of maize Bt11 x 59122 x MIR604 x 1507 x GA21 and twenty subcombinations, which have not been authorised previously independently of their origin, for food and feed uses, import and processing under Regulation (EC) No 1829/2003. EFSA Journal 2016; 14(8):4567, 31 pp. https://doi.org/10.2903/j.efsa.2016.4567

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2017a. Scientific opinion on the assessment of genetically modified maize 1507 x 59122 x MON810 x NK603 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2011-92). EFSA Journal 2017;15(11):5000, 29 pp. https://doi.org/10.2903/j.efsa.2017.5000

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2017b. Scientific Opinion on application EFSA-GMO-BE-2013-118 for authorisation of genetically modified maize MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 and subcombinations independently of their origin, for food and feed uses, import and processing submitted under Regulation (EC) No 1829/2003 by Monsanto Company. EFSA Journal 2017; 15(8):4921, 32 pp. https://doi.org/10.2903/j.efsa.2017.4921

OECD, 2008. Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents. OECD Publishing, Paris. https://doi.org/10.1787/9789264070684-en

Abbreviations

ANOVA	analysis of variance
BSA	bovine serum albumin
bw	body weight
GM	genetically modified
GMO	Genetically Modified Organism
OECD	Organisation for Economic Co-operation and Development