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Statement complementing the EFSA Scientific Opinion on application (EFSA-GMO-NL-2009-75) for placing on the market of genetically modified oilseed rape Ms8 × Rf3 × GT73 and subcombinations, which have not been authorised previously (i.e. Ms8 × GT73 and Rf3 × GT73) independently of their origin, for food and feed uses, import and processing, with the exception of isolated seed protein for food, under Regulation (EC) No 1829/2003), taking into consideration additional information

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Abstract

The EFSA Panel on Genetically Modified Organisms (GMO) previously assessed oilseed rape Ms8 × Rf3 × GT73 and its subcombinations Ms8 × GT73 and Rf3 × GT73 according to the scope as defined in the application EFSA-GMO-NL-2009-75, and was not in the position to complete the safety assessment of products rich in protein, such as rapeseed protein isolates or products of this nature in animal feeding. Following a mandate from the European Commission, the GMO Panel assessed a 28-day toxicity study in mice with the glyphosate oxidoreductase (GOXv247) protein, provided to complement information related to application EFSA-GMO-NL-2009-75 for the placing on the market of oilseed rape Ms8 × Rf3 × GT73 and its subcombinations Ms8 × GT73 and Rf3 × GT73, for food and feed uses, import and processing, with the exception of isolated seed protein for food. The 28-day toxicity study on *Escherichia coli*- produced GOXv247 protein did not show adverse effects in mice, at the gavage doses up to 1000 mg/kg body weight (bw) per day. Taking into account its previous assessment on EFSA-GMO-NL-2009-75 and the outcome of the 28-day toxicity study in mice with the GOXv247 protein provided in this mandate, the GMO Panel, based on a weight of evidence approach, concludes that food and feed containing, consisting and produced from genetically modified oilseed rape Ms8 × Rf3 × GT73 and its sub combinations Ms8 × GT73 and Rf3 × GT73, are as safe as its conventional counterpart, according to the scope as defined in the application EFSA-GMO-NL-2009-75.

Keywords: GMO, oilseed rape Ms8 × Rf3 × GT73, subcombinations Ms8 × Rf3 and Ms8 × GT73, food and feed safety, import and processing, glyphosate oxidoreductase (GOXv247) protein, 28-day toxicity study

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Summary

The EFSA Panel on Genetically Modified Organisms (GMO) previously assessed oilseed rape Ms8 × Rf3 × GT73 and its subcombinations Ms8 × GT73 and Rf3 × GT73 according to the scope as defined in the application EFSA-GMO-NL-2009-75, and was not in the position to complete the safety assessment of products rich in protein, such as rapeseed protein isolates or products of this nature in animal feeding, because of the lack of a 28-day toxicity study in rodents with the glyphosate oxidoreductase (GOXv247) protein, in line with the applicable guidelines.

On 16 November 2018, the European Commission requested the GMO Panel to complement its original scientific opinion on application EFSA-GMO-NL-2009-75 for the placing on the market of oilseed rape Ms8 × Rf3 × GT73 and its subcombinations Ms8 × GT73 and Rf3 × GT73, for food and feed uses, import and processing, with the exception of isolated seed protein for food (EFSA GMO Panel, 2016), taking into account the additional information submitted by the co-applicants BASF Agricultural Solutions Belgium NV and Monsanto Company, consisting of a 28-day toxicity study in mice with the GOXv247 protein.

The GMO Panel assessed the supplementary 28-day toxicity study in mice with the GOXv247 protein up to 1,000 mg/kg body weight (bw) per day. The study was conducted in accordance with OECD TG 407 (OECD, 2008) and with the principles of Good Laboratory Practice. No adverse effects were observed in this study.

Taking into account its previous assessment on EFSA-GMO-NL-2009-75 (EFSA GMO Panel, 2016) and the outcome of the 28-day toxicity study in mice with the GOXv247 protein provided in this mandate, the GMO Panel, based on a weight of evidence approach, concludes that food and feed containing, consisting and produced from genetically modified oilseed rape Ms8 × Rf3 × GT73 and its sub combinations Ms8 × GT73 and Rf3 × GT73, are as safe as its conventional counterpart, according to the scope as defined in the application EFSA-GMO-NL-2009-75.

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1. Introduction

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

On 13 April 2016 the GMO Panel adopted its Scientific Opinion (EFSA GMO Panel, 2016) on application EFSA-GMO-NL-2009-75, jointly submitted by Bayer CropScience AG and Monsanto Company under Regulation (EC) No 1829/2003 for placing on the market of genetically modified oilseed rape Ms8 × Rf3 × GT73 and its sub combinations Ms8 × GT73 and Rf3 × GT73 independently of their origin and not previously authorised, for food and feed uses, import and processing, with the exception of isolated seed protein for food. The GMO Panel confirmed the conclusions given in the context of previous assessments on the safety of the single oilseed rape events Ms8, Rf3 and GT73 (i.e. products with trace levels of GOXv247 protein in GT73 event), and did not find indications of safety concern of oilseed rape Ms8 × Rf3 × GT73 food and feed with trace levels of the GOXv247protein (e.g. oil, pollen, toasted meal). However, the GMO Panel was not in the position to complete the safety assessment of products rich in protein, such as rapeseed protein isolates or products of this nature in animal feeding from oilseed rape Ms8 × Rf3 × GT73 and its sub combinations Ms8 × GT73 and Rf3 × GT73, according to the scope as defined in the application EFSA-GMO-NL-2009-75, because of the lack of a 28-day toxicity study in rodents with the GOXv247 protein, in line with the applicable guidelines.

Following the publication of the scientific opinion on oilseed rape Ms8 × Rf3 × GT73 and its sub combinations Ms8 × GT73 and Rf3 × GT73 (EFSA GMO Panel, 2016), on 19 January 2017 EFSA received a letter from the applicant requesting a post adoption teleconference. The meeting was held on 2 February 2017. In this context, EFSA reconfirmed the conclusions of the GMO Panel on the risk assessment of application EFSA-GMO-NL-2009-75, owing to the lack of relevant data supporting the safety of the GOXv247 protein (i.e. 28-day toxicity study). EFSA provided the applicant a summary of the post-adoption teleconference on 8 February 2017.

On 16 November 2018, the European Commission requested EFSA to complement the original Scientific Opinion of the GMO Panel (EFSA GMO Panel, 2016) taking into account additional information submitted by the co-applicants BASF Agricultural Solutions Belgium NV and Monsanto Company on 23 October 2018, consisting of a 28-day toxicity study in mice with the GOXv247 protein. EFSA acknowledged the receipt of the mandate on 17 December 2018.

The GMO Panel asked the applicant clarifications on this supplementary toxicity study on 21 December 2018; 31 October 2019; 12 February 2020. EFSA received information to the requests on 2 August 2019; 10 January 2020; 14 April 2020.

In the frame of contract OC/EFSA/GMO/2014/01 (Lot2), the contractor performed preparatory work and delivered a report on the methods applied by the applicant to perform the 28-day toxicity study.

This statement reports the GMO Panel assessment of this supplementary 28-day toxicity study and the related additional information provided by the applicants following EFSA's requests.

According to the mandate received from EC on 16 November 2018, this statement complements the EFSA scientific opinion on oilseed rape Ms8 × Rf3 × GT73 and its sub combinations Ms8 × GT73 and Rf3 × GT73 (EFSA GMO Panel, 2016), 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 28-day toxicity study and the related additional information provided by the applicant in the context of this mandate and the EFSA scientific opinion on application EFSA-GMO-NL-2009-75 (EFSA GMO Panel, 2016).

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 GM plants and derived food and feed (EFSA GMO Panel, 2011).

3. Assessment

To complement the toxicological assessment of the glyphosate oxidoreductase (GOXv247) protein expressed in oilseed rape Ms8 × Rf3 × GT73 and its subcombinations Ms8 × GT73 and Rf3 × GT73, the applicant provided a 28-day toxicity study in mice on GOXv247 conducted in accordance with OECD TG 407 (OECD, 2008) and to the principles of Good Laboratory Practice.

Groups of CD-1 mice, (16/sex per group), 7 weeks old at the start of dosing were administered by oral gavage respectively: the GOXv247 protein (in water) at a targeted nominal dose of 1,000, 100 or 10 mg/kg body weight (bw) per day (GOXv247 protein groups); or 1,000 mg/kg bw per day of bovine serum albumin (BSA) (control group). The GMO Panel noted that the applicant did not use a control group given the vehicle alone. Based on the comparison between the BSA control and the laboratory historical control data provided by the applicant,[1] the GMO Panel considered BSA as an appropriate control in this study.

The test substance contained 95% of an *Escherichia coli*-produced GOXv247 protein, with a deduced amino acid sequence identical to the GOXv247 protein expressed in oilseed rape GT73 and to the *E. coli*-produced GOXv247 protein used in previous safety studies (e.g. acute toxicity test)[1]; moreover, amino acid sequence analysis of the *E. coli*-produced GOXv247 used in this 28-day toxicity study by mass spectrometry (MS) matched the deduced sequence as defined by the *GOXv247* gene. Additional experimental analyses showed that this protein had the expected molecular weight and immunoreactivity to GOXv247 specific antibodies and was not glycosylated. The functional activity of the *E. coli*-produced GOXv247 protein used in this study was not tested.

In-life procedures and observations and terminal procedures were conducted in accordance to OECD TG 407 (OECD, 2008), except that a functional observation battery was not performed. There was no indication of neurological effects based on the routine observations of clinical signs in either the 28-day toxicity study or a previously evaluated acute toxicity study (EFSA 2004). Therefore, the GMO Panel concludes that this deviation from OECD TG 407 (OECD, 2008) does not compromise the safety assessment of the GOXv247 protein.

A one-way analysis of variance (ANOVA) (factor: dose) was conducted for the two sexes separately; in case a statistically significant dose effect was identified, each of the three dose groups was compared with the control group using Dunnett's test. In addition, and independently from the ANOVA, a two-sample t-test was used to compare the high-dose group with the control group.

Based on the results of concentration analysis, the applicant confirmed that the administered doses were 1,000, 100 and 10 mg/kg bw per day. The results of the substance analysis tests indicated that the dosing preparations were homogeneous and exhibited acceptable stability. There was a single death in the high dose female group, which was associated with evidences of a gavage error. Statistically significant increases in body weight gain were noted in males in the low dose group but not at the higher doses, and food consumption was increased in mid-dose females but not at the high dose; these findings are not considered to be treatment related as they exhibited no dose response.

Statistically significant changes were seen in sorbitol dehydrogenase activity levels in serum (reduced in high dose males); absolute thyroid/parathyroid weights (reduced in all female test groups); thymus weights (reduced in high dose females). These findings are within the normal range of variation seen in this strain and age of the mice and are not considered to be adverse. Total white blood cells (WBC) counts were increased in males treated with the high dose, but this was related to 2 out of 16 mice with very high neutrophil counts and are not considered to be treatment related. There were no treatment-related findings in the gross or microscopic pathology examinations.

The GMO Panel concludes that no adverse effects were observed in mice in this 28-day toxicity study on *E. coli*- produced GOXv247 protein, at gavage doses up to 1,000 mg/kg bw per day.

Since the equivalence between the test substance used in the 28-day toxicity study and the oilseed rape GOXv247 protein was not complete (i.e. functionality of the *E. coli*-produced GOXv247 protein was not tested), the GMO Panel followed a weight of evidence approach to address the safety of the GOXv247 protein, considering the various aspects detailed below.

a) *Use of urea-denatured protein as the test substance:* The applicant indicates that GOXv247 is a membrane-bound protein produced in *E. coli* in an insoluble form as inclusion bodies and, therefore, a solubilisation step is required for its purification. Earlier work with detergent solubilisation produced only a relatively impure (22%) material[1]. The urea-based purification

[1] Additional information 2/8/2019.

process allowed to obtain 95% pure protein and therefore is considered a reasonable and efficient approach to produce an adequate amount of high purity material for the 28-day study.

b) *Functional equivalence of the test substance starting material with the plant protein:* The *E. coli* paste (starting material of the GOXv247 used in the 28-day study) was tested for GOXv247 specific activity and found to show an enzymatic function similar to that of GOXv247 in GT73 seed extracts (specific activity 5.8 vs 6.6 μmol/min per mg protein and similarly specific versus the tested substrates). The presence of GOXv427 active material in the paste consolidates the conclusions on the structural equivalence of the *E. coli* and plant GOXv247 described above.

c) *Relevance of GOXv247 function in human and animal bodies:* The potential for the active GOXv247 protein once ingested to transform a food/feed component or an endogenous molecule into a hazardous compound, or to adversely affect levels of an essential nutrient is considered to be low:

   i) GOXv247 is a membrane-bound protein, requiring FAD co-factor and oxygen donors/acceptors for full activity. It is unlikely that suitable conditions consistent with supporting significant GOXv247 activity will be present in the gastrointestinal tract or in the event of absorption.
   ii) Many oxidase and oxido-reductase enzymes are naturally occurring in mammals. In the tests provided by the applicant, GOXv247 has been shown to have a high substrate specificity for glyphosate and closely related molecules, but not for the tested endogenous amino acids. It is unlikely that ingested GOXv247 will metabolise molecules other than glyphosate in a manner that could not also be performed by endogenous enzymes.
   iii) Ingested GOXv247 is likely to be degraded/denatured in the acid environment of the stomach and by enzymes in the gastrointestinal tract. It is unlikely that significant amounts of active GOXv247 will be systemically absorbed. The GOXv247 protein was degraded in the pepsin resistance test in studies provided by the applicant and previously assessed by the GMO Panel.

d) *Bioinformatic analysis:* The potential for the GOXv247 protein to be a toxin has been addressed by *in silico* comparison with known toxins in the recent updated bioinformatic analysis provided in the context of a renewal application recently adopted by the GMO Panel (EFSA GMO Panel, 2020).

Overall, the GMO Panel also concludes, based on a weight of evidence consideration of the 28-day toxicity study, molecular characterisation, enzymatic properties and likely degradation on ingestion, that GOXv247 expressed in oilseed rape Ms8 × Rf3 × GT73 and its subcombinations Ms8 × GT73 and Rf3 × GT73 will not cause any adverse effects in animals or humans consuming food and feed containing, consisting and produced from this crop.

4. Conclusions

The 28-day toxicity study on GOXv247 protein assessed by the GMO Panel did not show adverse effects.

Taking into account its previous assessment on EFSA-GMO-NL-2009-75 (EFSA GMO Panel, 2016) and the outcome of the 28-day toxicity study in mice with the GOXv247 protein provided in this mandate, the GMO Panel, based on a weight of evidence approach, concludes that food and feed containing, consisting and produced from genetically modified oilseed rape Ms8 × Rf3 × GT73 and its subcombinations Ms8 × GT73 and Rf3 × GT73, are as safe as its conventional counterpart, according to the scope as defined in the application EFSA-GMO-NL-2009-75.

5. Documentation as provided to EFSA (if appropriate)

Letter from the European Commission received on 16 November 2018 requesting EFSA to complement its original scientific opinion on oilseed rape Ms8 × Rf3 × GT73 (application EFSA-GMO-NL-2009-75), including the sub combinations in scope of the application, on the basis of new information received from the applicants, BASF Agricultural Solutions Belgium NV and Monsanto Company.

Acknowledgement letter dated 17 December 2018 from EFSA to the European Commission.

EFSA requested additional information to the applicant on 21 December 2018.

EFSA received additional information from the applicant on 2 August 2019.

EFSA requested additional information to the applicant on 31 October 2019.
EFSA received additional information from the applicant on 10 January 2020.
EFSA requested additional information to the applicant on 12 February 2020.
EFSA received additional information from the applicant on 14 April 2020.

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Abbreviations

BSA	bovine serum albumin
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
MS	mass spectrometry
OECD	Organisation for Economic Co-operation and Development