Assessment of genetically modified oilseed rape GT73 for renewal authorisation under Regulation (EC) No 1829/2003 (application EFSA-GMO-RX-002) EFSA Panel on Genetically Modified Organisms (GMO), Panel members

. Efsa Panel On Genetically Modified Organisms (gmo), Fernando Alvarez, Michele Ardizzone, Tommaso Raffaello, Hanspeter Naegeli, Jean-Louis Bresson, Tamas Dalmay, Ian Crawford Dewhurst, Michelle M Epstein, Leslie Firbank, et al.

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 2 July 2020

doi: 10.2903/j.efsa.2020.6199

Assessment of genetically modified oilseed rape GT73 for renewal authorisation under Regulation (EC) No 1829/2003 (application EFSA-GMO-RX-002)

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Abstract

Following the submission of application EFSA-GMO-RX-002 under Regulation (EC) No 1829/2003 from Monsanto Company, the Panel on Genetically Modified Organisms of EFSA (GMO) was asked to deliver a scientific risk assessment on the data submitted in the context of the renewal of authorisation application for the herbicide-tolerant genetically modified oilseed rape GT73. The data received in the context of this renewal application contained post-market environmental monitoring reports, a systematic search and evaluation of literature, updated bioinformatic analyses and additional documents or studies performed by or on behalf of the applicant. The GMO Panel assessed these data for possible new hazards, modified exposure or new scientific uncertainties identified during the authorisation period and not previously assessed in the context of the original application. Under the assumption that the DNA sequence of the event in oilseed rape GT73 considered for renewal of authorisation is identical to the sequence of the originally assessed event, the GMO Panel concludes that there is no evidence in renewal application EFSA-GMO-RX-002 for new hazards, modified exposure or scientific uncertainties that would change the conclusions of the original risk assessment on oilseed rape GT73.

Keywords: Oilseed rape, GT73, renewal, Regulation (EC) No 1829/2003

Requestor: European Commission (DG SANTE)

Question number: EFSA-Q-2016-00478

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Acknowledgments: The Panel wishes to thank the members of its standing Working Groups on Molecular Characterisation, Food/Feed and Environmental Risk Assessment for the preparatory work on this scientific opinion, and the EFSA staff members Yann Devos, Andrea Gennaro, Irene Muñoz-Guajardo, Anna Lanzoni, Konstantinos Paraskevopoulos and Matthew Ramon for the support provided to this scientific opinion.

Suggested citation: EFSA Panel on Genetically Modified Organisms (GMO), Naegeli H, Bresson J-L, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Moreno FJ, Mullins E, Nogué F, Rostoks N, Sánchez Serrano JJ, Savoini G, Veromann E, Veronesi F, Álvarez F, Ardizzone M and Raffaello T, 2020. Scientific Opinion on the assessment of genetically modified oilseed rape GT73 for renewal authorisation under Regulation (EC) No 1829/2003 (application EFSA-GMO-RX-002). EFSA Journal 2020;18(7):6199, 14 pp. https://doi.org/10.2903/j.efsa.2020.6199

ISSN: 1831-4732

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

Summary

Following the submission of application EFSA-GMO-RX-002 under Regulation (EC) No 1829/2003[1] from Monsanto Company, the Panel on Genetically Modified Organisms of EFSA (GMO) was asked to deliver a scientific risk assessment opinion on the data submitted in the context of the renewal of authorisation application for the herbicide-tolerant genetically modified (GM) oilseed rape GT73. The scope of the renewal application EFSA-GMO-RX-002 covers feed containing or consisting of GM oilseed rape GT73, and products other than food and feed containing or consisting of it, excluding cultivation within the European Union (EU).

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-RX-002, additional information provided by the applicant, scientific comments submitted by the EU Member States and relevant scientific publications. The data received in the context of the renewal application EFSA-GMO-RX-002 contained: post-market environmental monitoring reports, an evaluation of the literature retrieved by a systematic search, updated bioinformatics analyses and additional studies performed by or on behalf of the applicant. The GMO Panel assessed these data for possible new hazards, modified exposure or new scientific uncertainties identified during the authorisation period and not previously assessed in the context of the original application.

Under the assumption that the DNA sequence of the event in oilseed rape GT73 considered for renewal is identical to the sequence of the originally assessed event, the GMO Panel concludes that there is no evidence in the renewal application EFSA-GMO-RX-002 for new hazards, modified exposure or scientific uncertainties that would change the conclusions of the original risk assessment on oilseed rape GT73 (EFSA, 2004).

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.

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1. Introduction

1.1. Background

On 20 July 2016, the European Food Safety Authority (EFSA) received from the European Commission (EC) application EFSA-GMO-RX-002 for the renewal of the authorisation of oilseed rape GT73 (Unique Identifier MON-ØØØ73-7) for feed containing or consisting of GM oilseed rape GT73, excluding cultivation within the European Union (EU), and products other than food and feed containing or consisting of it, submitted by Monsanto Company (hereafter referred to as 'the applicant') under Regulation (EC) No 1829/2003. Before sending the application to EFSA, the European Commission confirmed whether the data submitted in the context of this renewal application were in line with the legal requirements laid down in Articles 11 and 23 of Regulation (EC) No 1829/2003.

Following receipt of application EFSA-GMO-RX-002, EFSA informed the Member States (MS) and made the summary of the application available to the public on the EFSA website.[2]

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003. On 15 December 2016, EFSA declared the application valid in accordance with Articles 6(1) and 18 (1) of Regulation (EC) No 1829/2003, and made the valid application available to the MS and the European Commission. In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of the MS, including national Competent Authorities within the meaning of Directive 2001/18/EC.[3] The MS had three months to make their opinion known on application EFSA-GMO-RX-002 as of the date of validity.

Following the publication of the GMO Panel scientific opinion (EFSA, 2004) on notification C/NL/98/11, the placing on the market of oilseed rape GT73 for feed containing or consisting of GM oilseed rape GT73, excluding cultivation in the EU, and products other than food and feed containing or consisting of it, was authorised by Commission Decision 2005/635/EC.[4] A copy of this authorisation was provided by the applicant.[5]

From the validity date, EFSA and its scientific Panel on Genetically Modified Organisms (hereafter referred to as 'the GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application EFSA-GMO-RX-002. Such time limit was extended whenever EFSA and/or its GMO Panel requested supplementary information to the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and European Commission (for further details, see the section 'Documentation', below).

1.2. Terms of Reference as provided by the requestor

According to Articles 6 and 18 of Regulation (EC) No 1829/2003, EFSA and its GMO Panel were requested to carry out a scientific risk assessment of oilseed rape GT73 for the continued placing on the market of feed containing or consisting of GM oilseed rape GT73 and products other than food and feed containing or consisting of it.

According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation including the opinions of the nominated risk assessment bodies of the MS.[6]

In addition to the present scientific opinion on oilseed rape GT73, EFSA and its GMO Panel were also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No 1829/2003. The relevant information is made available in the EFSA Register of Questions,[7] including the information required under Annex II to the Cartagena Protocol, a labelling proposal, a Post-Market Environmental Monitoring (PMEM) plan as provided by the applicant; the method(s), validated by the

[2] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2016-00478
[3] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.
[4] 2005/635/EC: Commission Decision of 31 August 2005 concerning the placing on the market, in accordance with Directive 2001/18/EC of the European Parliament and of the Council, of an oilseed rape product (Brassica napus L., GT73 line) genetically modified for tolerance to the herbicide glyphosate (notified under document number C(2005) 3110). Official Journal of the European Union L 228/11, 3.09.2005.
[5] Dossier: Oilseed rape GT73 – Annex 1.
[6] Opinions of the nominated risk assessment bodies of EU Member States can be found at the EFSA Register of Questions, http://registerofquestions.efsa.europa.eu/roqFrontend/login, querying the assigned Question Number.
[7] http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2016-00478

Community reference laboratory, for detection, including sampling, identification of the transformation event in the food-feed and/or foods-feeds produced from it and the appropriate reference materials.

2. Data and methodologies

2.1. Data

The data for application EFSA-GMO-RX-002 provided by the applicant at the time of submission, or in reply to requests for additional information, are specified below.

In the context of this renewal application, no new sequencing study was submitted among the additional documents or studies performed by or on behalf of the applicant. In accordance with the GMO Panel guidelines for renewal of applications of GM food and feed authorised under Regulation (EC) No 1829/2003 (EFSA GMO Panel, 2015), the GMO Panel evaluated the data provided in the context of this oilseed rape GT73 renewal application under the assumption that the GT73 event sequence is identical to the sequence of the originally assessed event (EFSA, 2004).

2.1.1. Post-market monitoring reports[8]

Based on the outcome of the initial risk assessment, a post-market monitoring plan for monitoring of GM feed was not required by the authorisation decision. The implementation of a PMEM plan, consisting of a general surveillance plan to check for any adverse effects on the environment arising from oilseed rape GT73, was a condition for the authorisation. As no potential adverse environmental effects were identified in the environmental risk assessment of oilseed rape GT73 (EFSA, 2004), case-specific monitoring was not considered necessary by the GMO Panel.

The applicant provided 11 annual PMEM reports covering a reporting period from July 2007 till July 2018. The annual PMEM plans submitted by the applicant included (1) commodity crop (GM and non-GM) imports into the EU by country of origin and destination; (2) the description of a centralized system established by EuropaBio for the collection of information recorded by various operators [federations involved in oilseed rape seeds import and processing] on any observed adverse effect(s) on human health and the environment arising from handling of oilseed rape possibly containing oilseed rape GT73; (3) the reports of the surveillance activities conducted by such operators; and (4) the review of relevant scientific peer-reviewed studies retrieved from literature searches.

The applicant provided an overall assessment of the annual PMEM reports in the renewal application.

2.1.2. Systematic search and evaluation of literature[9]

In addition to the 11 separate searches provided as part of the annual PMEM reports, the applicant performed several systematic literature searches covering the period from January 2007 till March 2020, in accordance with the recommendations on literature search outlined in EFSA (2010, 2017a).

Searches in electronic bibliographic databases and in websites of relevant organisations were performed to identify relevant publications. Altogether, 204 publications were retrieved (after removal of duplicates). After applying the eligibility/inclusion criteria defined a priori by the applicant, two publications were identified as relevant for food and feed safety assessment or environmental safety assessment. The list of relevant publications is provided in Appendix A.

2.1.3. Updated bioinformatic data[10]

At the time of submission of the renewal dossier, the applicant provided a complete bioinformatic dataset for oilseed rape GT73 event including an analysis of the insert and flanking sequences, an analysis of the potential similarity to allergens and toxins of the newly expressed proteins and of all possible open reading frames (ORFs) within the insert and spanning the junction sites, and an analysis of possible horizontal gene transfer (EFSA, 2017b). Additionally, upon EFSA request during the risk assessment, the applicant provided a new bioinformatic analysis using up-to-date databases. The outcome of the updated bioinformatic analyses is presented in Section 3.3.

[8] Dossier: Oilseed rape GT73 – CD2 (CI) – PMEM; additional information: 10/12/2018; spontaneous information 5/10/2017.
[9] Dossier: Oilseed rape GT73 – Annex 3.1; additional information: 1/8/17, 3/10/17, 30/10/17, 5/12/18, 25/3/19, 16/3/20, 2/4/20.
[10] Dossier: Oilseed rape GT73 – Annex 3; additional information: 12/12/2018 and 20/12/2018.

2.1.4. Additional documents or studies provided by the applicant[11]

In line with the renewal guidance requirements (EFSA GMO Panel, 2015), the applicant provided an overview on the worldwide approvals of oilseed rape GT73 and the full reports of all studies performed by or on behalf of the applicant over the course of the authorisation period and not previously submitted to the EU (Appendix B).

The relevance of the listed studies for molecular characterisation, human and animal safety and the environment was assessed by the applicant.

Upon EFSA request, the applicant provided a 28-day repeated dose toxicity study in mice on the GOXv247 protein, to complement the assessment of oilseed rape GT73 products other than those with trace levels of GOX protein (e.g. oil, toasted meal) (Appendix C).

2.1.5. Overall assessment as provided by the applicant[12]

The applicant provided an overall assessment concluding that information provided in the application EFSA-GMO-RX-002 for renewal of authorisation of oilseed rape GT73 does not change the outcome of the original risk assessment (EFSA, 2004).

2.1.6. Monitoring plan and proposal for improving the conditions of the original authorization[13]

The applicant indicated in the dossier that the environmental monitoring plan is appropriate and does not need any changes.

2.2. Methodologies

The GMO Panel assessed the application EFSA-GMO-RX-002 for renewal of the authorisation of oilseed rape GT73 in accordance with Articles 11 and 23 of Regulation (EC) No 1829/2003. The GMO Panel took into account the requirements described in its guideline for the risk assessment of renewal applications of GM food and feed authorised under Regulation (EC) No 1829/2003 (EFSA GMO Panel, 2015). The comments raised by the nominated risk assessment bodies of the EU Member States were taken into consideration during the scientific risk assessment.

3. Assessment

3.1. Evaluation of the post-market monitoring reports

During the general surveillance activities covering the authorisation period of oilseed rape GT73, no adverse effects were reported by the applicant.

3.2. Evaluation of the systematic search and evaluation of literature

The GMO Panel assessed the applicant's literature searches on oilseed rape GT73. Although the overall quality of the performed literature searches is acceptable, the GMO Panel considers that future searches could be fine-tuned. The GMO Panel therefore recommends the applicant for future searches to:

- ensure that enough search term variation is used (covering possible synonyms, related terms, acronyms, spelling variants, old and new terminology, brand and generic names, lay and scientific terminology, common typos, translation issues);
- ensure that enough truncation is used and used consistently;
- adapt the search to the size of the retrieved publications (and thus not combine search sets when one of the search sets already yields only a small number of publications).

The GMO Panel acknowledges that no publications raising a safety concern for human and animal health and the environment which would change the original risk assessment conclusions on oilseed rape GT73 (EFSA, 2004) have been identified by the applicant.

[11] Dossier: Oilseed rape GT73 – Annex 3; additional information: 08/10/2018.
[12] Dossier: Oilseed rape GT73.
[13] Additional information: 5/10/2017.

3.3. Evaluation of the updated bioinformatic data

The results of the updated bioinformatic analyses of oilseed rape GT73 confirm previous analyses indicating that no known endogenous genes were disrupted by the insert (EFSA, 2004; EFSA GMO Panel, 2009, 2013). Analyses of the amino acid sequence of the newly expressed CP4 EPSPS and GOXv247 proteins show no relevant similarities to toxins or allergens. In addition, bioinformatic analyses of the newly created ORFs within the insert or spanning the junctions with genomic DNA confirm previous conclusions indicating no similarities to toxins or allergens for the event GT73 (EFSA, 2004; EFSA GMO Panel, 2009, 2013).

The updated bioinformatic analysis for oilseed rape event GT73 does not reveal any DNA sequence that could provide sufficient length and identity which could facilitate double homologous recombination (HR), confirming the previous conclusions (EFSA, 2004; EFSA GMO Panel, 2013). Given the results of this analysis and the potential of the recombinant DNA in oilseed rape GT73 to confer selective advantages or increased fitness to microorganisms, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT.

3.4. Evaluation of the additional documents or studies provided by the applicant

The GMO Panel evaluated the full study reports of the additional studies provided (Appendix B) and the 28-day toxicity study on GOXv247 protein provided upon EFSA request (Appendix C). This new information does not raise any concern for human and animal health and the environment, which would change the original risk assessment conclusions on oilseed rape GT73.

3.5. Evaluation of the overall assessment as provided by the applicant

The GMO Panel evaluated the overall assessment provided by the applicant and confirms that there is no evidence in renewal application EFSA–GMO–RX–002 indicating new hazards, relevant changes in exposure or scientific uncertainties that would change previous conclusions on oilseed rape GT73.

3.6. Evaluation of the monitoring plan and proposal for improving the conditions of the original authorisation

The PMEM plan covers general surveillance of imported GM plant material, including oilseed rape GT73. This general surveillance is coordinated by EuropaBio and implemented by selected operators (federations involved in oilseed rape import and processing). In addition, the applicant reviews relevant scientific publications retrieved from literature searches on an annual basis. The GMO Panel is of the opinion that the scope of the plan provided by the applicant is consistent with the scope of application EFSA-GMO-RX-002, but reminds that monitoring is related to risk management, and thus the final adoption and implementation of the PMEM plan falls outside the mandate of EFSA.

4. Conclusions

Under the assumption that the DNA sequence of the event in oilseed rape GT73 considered for renewal is identical to the sequence of the originally assessed event, the GMO Panel concludes that there is no evidence in renewal application EFSA–GMO–RX–002 for new hazards, modified exposure or scientific uncertainties that would change the conclusions of the original risk assessment on oilseed rape GT73 (EFSA, 2004).

Documentation as provided to EFSA

1) Letter from the European Commission to EFSA received on 20 July 2016 concerning a request for the continued marketing of genetically modified oilseed rape GT73 for feed containing or consisting of genetically modified GT73 oilseed rape and products other than food and feed containing or consisting of genetically modified oilseed rape GT73, submitted under Regulation (EC) No 1829/2003 by Monsanto (EFSA-GMO-RX-002).
2) Application EFSA-GMO-RX-002 validated by EFSA, 15 December 2016.
3) Request for supplementary information to the applicant, 23 February 2017.
4) Receipt of supplementary information from the applicant, 19 May 2017.

5) Request for supplementary information to the applicant, 31 May 2017.
6) Request for supplementary information to the applicant, 06 July 2017.
7) Receipt of supplementary information from the applicant, 01 August 2017.
8) Request for supplementary information to the applicant, 02 August 2017.
9) Request for supplementary information to the applicant, 01 September 2017.
10) Receipt of supplementary information from the applicant, 07 September 2017.
11) Receipt of supplementary information from the applicant, 3 October 2017.
12) Request for supplementary information to the applicant, 18 October 2017.
13) Receipt of supplementary information from the applicant, 30 October 2017.
14) Receipt of supplementary information from the applicant, 20 December 2017.
15) Request for supplementary information to the applicant, 31 January 2018.
16) Request for supplementary information to the applicant, 02 October 2018.
17) Receipt of supplementary information from the applicant, 08 October 2018.
18) Request for supplementary information to the applicant, 08 November 2018.
19) Request for supplementary information to the applicant, 03 December 2018.
20) Receipt of supplementary information from the applicant, 05 December 2018.
21) Receipt of supplementary information from the applicant, 10 December 2018.
22) Receipt of supplementary information from the applicant, 12 December 2018.
23) Request for supplementary information to the applicant, 13 December 2018.
24) Receipt of supplementary information from the applicant, 20 December 2018.
25) Request for supplementary information to the applicant, 21 December 2018.
26) Request for supplementary information to the applicant, 24 January 2019.
27) Receipt of supplementary information from the applicant, 25 March 2019.
28) Receipt of supplementary information from the applicant, 01 August 2019.
29) Request for supplementary information to the applicant, 28 October 2019.
30) Receipt of supplementary information from the applicant, 19 December 2019.
31) Request for supplementary information to the applicant, 11 February 2020.
32) Receipt of supplementary information from the applicant, 11 April 2020.

References

EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Notification (Reference C/NL/98/11) for the placing on the market of glyphosate-tolerant oilseed rape event GT73, for import and processing, under Part C of Directive 2001/18/EC from Monsanto. EFSA Journal 2004;2(3):29, 19 pp. https://doi.org/10.2903/j.efsa.2004.29

EFSA (European Food Safety Authority), 2010. Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA Journal 2010;8(6):1637, 90 pp. https://doi.org/10.2903/j.efsa.2010.1637

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2009. Scientific Opinion on applications (EFSA-GMO-RX-GT73[8.1.a] and EFSA-GMO-RX-GT73[8.1.b/20.1.b]) for renewal of the authorisation for continued marketing of existing (1) food and food ingredients produced from oilseed rape GT73; and of (2) feed materials, feed additives and food additives produced from oilseed rape GT73, all under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2009;7(12):1417, 12 pp. https://doi.org/doi:10.2903/j.efsa.2009.1417

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2013. Scientific Opinion on application (EFSA-GMO-NL-2010-87) for the placing on the market of genetically modified herbicide tolerant oilseed rape GT73 for food containing or consisting of, and food produced from or containing ingredients produced from oilseed rape GT73 (with the exception of refined oil and food additives) under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2013;11(2):3079, 26 pp. https://doi.org/10.2903/j.efsa.2013.3079

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2015. Guidance for renewal applications of genetically modified food and feed authorised under Regulation (EC) No 1829/2003. EFSA Journal 2015a;13(6):4129, 8 pp. https://doi.org/10.2903/j.efsa.2015.4129

EFSA (European Food Safety Authority), Devos Y, Guajardo IM, Glanville J and Waigmann E, 2017a. Explanatory note on literature searching conducted in the context of GMO applications for (renewed) market authorization and annual post-market environmental monitoring reports on GMOs authorised in the EU market. EFSA supporting publications 2017:EN-1207, 48 pp. https://doi.org/10.2903/sp.efsa.2017.en-1207

EFSA (European Food Safety Authority), Gennaro A, Gomes A, Herman L, Nogue F, Papadopoulou N and Tebbe C, 2017b. Technical report on the explanatory note on DNA sequence similarity searches in the context of the assessment of horizontal gene transfer from plants to microorganisms. EFSA supporting publication 2017: EN-1273. 11 pp. https://doi.org/10.2903/sp.efsa.2017.en-1273

Abbreviations

ANOVA	analysis of variance
BSA	bovine serum albumin
bw	body weight
ERA	environmental risk assessment
GM	genetically modified
GMO	genetically modified organisms
GMO	Panel EFSA Panel on Genetically Modified Organisms
ORFs	open reading frames
PMEM	post-market environmental monitoring
WBC	white blood cell

Appendix A – List of relevant publications identified by the applicant through the systematic literature search (January 2007 – March 2020)

Reference
Caine WR, Aalhus JL, Dugan ME, Lien KA, Larsen IL, Costello F, McAllister TA, Stanford K, Sharma R. 2007. Growth performance, carcass characteristics and pork quality of pigs fed diets containing meal from conventional or glyphosate-tolerant canola. Meat Research Agriculture and Agri-Food Canada, Research Centre of Alberta, Department of Agricultural, Food and Nutritional Sciences of Alberta, 87, 517–526
Reuter T, Alexander TW, Martínez TF, McAllister TA. 2007. The effect of glyphosate on digestion horizontal gene transfer during in vitro ruminal fermentation of genetically modified canola. Journal of the Science of Food and Agriculture, 87, 2837–2843

Appendix B – List of additional studies performed by or on behalf of the applicant over the course of the authorisation period and not previously submitted to the EU with regard to the evaluation of the safety of the food and feed for humans, animal or the environment from oilseed rape GT73

Study identification	Title
MSL0024742	Amended Report for MSL0013287: Molecular Characterization of Roundup®-tolerant Canola
MSL0024814	Amended Report for MSL0018532: PCR and DNA Sequence Analysis of the Insert in Roundup Ready® Canola Event RT73

Appendix C – Outcome of the assessment of a 28-day oral repeated dose toxicity study in mice on GOXv247

To complement the toxicological assessment of the glyphosate oxidoreductase (GOXv247) protein expressed in oilseed rape GT73, the applicant provided a 28-day toxicity study in mice on GOXv247 conducted in accordance with OECD TG 407 (2008)[14] 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 1000, 100 or 10 mg/kg body weight (bw) per day (GOXv247 protein groups); or 1000 mg/kg bw/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,[15] 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)[15]; 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 (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).[16] Therefore, the GMO Panel concludes that this deviation from OECD TG407 (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 1000, 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 cell (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

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

[15] Additional information 1/8/2019.

[16] EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Notification (Reference C/NL/98/11) for the placing on the market of glyphosate-tolerant oilseed rape event GT73, for import and processing, under Part C of Directive 2001/18/EC from Monsanto, The EFSA Journal (2004) 29, 1-19. https://doi.org/10.2903/j.efsa.2004.29

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[15]. The urea-based purification 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/mg protein and similarly specific vs 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 GOXv247 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 this renewal application. No significant similarities to known toxins were described.

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 GT73 will not cause any adverse effects in animals or humans consuming food and feed containing, consisting and produced from this crop.