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Assessment of genetically modified oilseed rape MS8, RF3 and MS8 × RF3 for renewal authorisation under Regulation (EC) No 1829/2003 (application EFSA-GMO-RX-024)

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Abstract

Following the submission of application EFSA-GMO-RX-024 under Regulation (EC) No 1829/2003 from BASF Agricultural Solutions Seed US LLC, the Panel on Genetically Modified Organisms of EFSA 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 MS8, RF3 and MS8 × RF3, for food and feed uses, excluding cultivation within the European Union. 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 sequences of the events in oilseed rape MS8, RF3 and MS8 × RF3 considered for renewal are identical to the sequences of the originally assessed events, the GMO Panel concludes that there is no evidence in renewal application EFSA-GMO-RX-024 for new hazards, modified exposure or scientific uncertainties that would change the conclusions of the original risk assessment on oilseed rape MS8, RF3 and MS8 × RF3.

Keywords: oilseed rape, MS8, RF3, MS8 × RF3, renewal, Articles 11 and 23, Regulation (EC) No 1829/2003

Requestor: European Commission (DG SANTE)

Question number: EFSA-Q-2021-00121

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Summary

Following the submission of application EFSA-GMO-RX-024 under Regulation (EC) No 1829/2003 from BASF Agricultural Solutions Seed US LLC, the Panel on Genetically Modified Organisms of EFSA (GMO Panel) 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 MS8, RF3 and MS8 × RF3. The scope of the renewal application EFSA-GMO-RX-024 is for the renewal of the placing on the market of products containing, consisting of, or produced from oilseed rape MS8, RF3 and MS8 × RF3, excluding cultivation within the European Union (EU).

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-RX-024, 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-024 contained: post-market environmental monitoring reports, an evaluation of the literature retrieved by a scoping review, additional studies performed by or on behalf of the applicant and updated bioinformatics analyses. 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 events in oilseed rape MS8, RF3 and MS8 × RF3 considered for renewal is identical to the sequence of the originally assessed events, the GMO Panel concludes that there is no evidence in the renewal application EFSA-GMO-RX-024 for new hazards, modified exposure or scientific uncertainties that would change the conclusions of the original risk assessment on oilseed rape MS8, RF3 and MS8 × RF3 (EFSA GMO Panel, 2012, 2017a).

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1. Introduction

1.1. Background

On 8 March 2021, the European Food Safety Authority (EFSA) received from the European Commission (EC) application EFSA-GMO-RX-024 for the renewal of the authorisation of oilseed rape MS8, RF3 and MS8 × RF3 (Unique Identifier ACS-BNØØ5-8, ACS-BNØØ3-6 and ACS-BNØØ5-8 × ACS-BNØØ3-6, respectively), submitted by BASF Agricultural Solutions Seed US LLC (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003[1].

Following receipt of application EFSA-GMO-RX-024, EFSA informed the Member States (MS) and made the summary of the application available to the public on the Open EFSA portal.[2]

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003 and Regulation (EU) No 503/2013[3] and, when needed, asked the applicant to supplement the initial application. On 9 November 2021, EFSA declared the application valid and made the valid application available to the MS and the EC.

Following the submission of applications EFSA-GMO-BE-2010-81 and EFSA-GMO-RX-004 and the publication of the EFSA scientific opinions (EFSA GMO Panel, 2012, 2017a), the placing on the market of oilseed rape MS8, RF3 and MS8 × RF3 for products containing, consisting of, or produced from this GM oilseed rape, excluding cultivation in the EU, was authorised by Commission Implementing Decision 2013/327/EU and (EU) 2019/1301[4]. A copy of these authorisations were 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-024. This 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 MS and EC (for further details, see the section 'Documentation', below).

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[6]. The MS had 3 months to make their opinion known on application EFSA-GMO-RX-024 as of date of validity.

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 MS8, RF3 and MS8 × RF3 for the renewal of authorization for placing on the market of products containing, consisting of, or produced from GM oilseed rape MS8, RF3 and MS8 × RF3 in the context of its scope as defined in application EFSA-GMO-RX-024.

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.[7]

[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.

[2] Available online: https://open.efsa.europa.eu/questions/EFSA-Q-2021-00121

[3] Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L157, 8.6.2013, p. 1–48.

[4] Commission Implementing Decision of 25 June 2013 authorising the placing on the market of food containing or consisting of genetically modified oilseed rape Ms8, Rf3 and Ms8 × Rf3, or food and feed produced from those genetically modified organisms pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council.
Commission Implementing Decision (EU) 2019/1301 of 26 July 2019 amending Implementing Decision 2013/327/EU as regards the renewal of the authorisation to place on the market feed containing or consisting of genetically modified oilseed rape Ms8, Rf3 and Ms8 × Rf3 pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council.

[5] Dossier: Oilseed rape MS8, RF3 and MS8 × RF3 – Annex I.

[6] 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.

[7] Opinions of the nominated risk assessment bodies of EU Member States can be found at the Open EFSA Portal https://open.efsa.europa.eu/questions, querying the assigned Question Number.

In addition to the present scientific opinion on oilseed rape MS8, RF3 and MS8 × RF3, 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 OpenEFSA portal,[8] 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 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-024 submitted according to EFSA requirements (EFSA, 2019a; EFSA GMO Panel, 2015) and provided by the applicant at the time of submission, or in reply to requests for additional information, are specified below.

In the frame of the contracts OC/EFSA/GMO/2018/04, EOI/EFSA/SCIENCE/2020/01 – CT 02 GMO and OC/EFSA/GMO/2014/01, the contractor performed preparatory work and delivered reports on the methods applied by the applicant in performing literature search, and statistical analysis and study design of the 90-day oral repeated dose toxicity study included in this opinion.

2.1.1. Post-market monitoring reports[9]

Based on the outcome of the initial food and feed risk assessment, a post-market monitoring plan for monitoring of GM food and 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 MS8, RF3 and MS8 × RF3, was a condition for the authorisation. As no potential adverse environmental effects were identified in the environmental risk assessment of oilseed rape MS8, RF3 and MS8 × RF3 (EFSA GMO Panel, 2012, 2017a), case-specific monitoring was not considered necessary by the GMO Panel.

The applicant provided 10 annual PMEM reports covering a reporting period from July 2011 till June 2021. The annual PMEM reports 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 centralised system established by EuropaBio[10] for the collection of information recorded by various operators (federations involved in oilseed rape 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 MS8, RF3 and MS8 × RF3; (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.

2.1.2. Systematic search and evaluation of literature[11]

In addition to the separate searches provided as part of the annual PMEM reports, the applicant performed scoping reviews covering the period from June 2013 untill November 2022, in accordance with the recommendations on literature search outlined in EFSA (2010, 2019b).

Searches in electronic bibliographic databases and in websites of relevant organisations were performed to identify relevant publications. Altogether 953 publications (including the updated search) were identified (after removal of duplicates). After applying the eligibility/inclusion criteria defined *a priori* by the applicant, seven publications were identified as relevant for food and feed safety assessment. The relevant publications are listed in Appendix A.

[8] https://open.efsa.europa.eu/questions/EFSA-Q-2021-00121

[9] Dossier: Oilseed rape MS8, RF3 and MS8 × RF3 – Annex II; additional information: 16/5/2022.

[10] The responsibilities of EuropaBio in coordinating activities of technology providers on the post-market environmental monitoring of GM crops were taken over by CropLife Europe as of 1 January 2021.

[11] Dossier: Oilseed rape MS8, RF3 and MS8 × RF3 – Annex III; additional information: 16/5/2022, 10/1/2023.

2.1.3. Updated bioinformatic data[12]

At the time of submission of the renewal dossier, the applicant provided a complete bioinformatic dataset for oilseed rape MS8, RF3 and MS8 × RF3 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, an analysis of possible horizontal gene transfer (EFSA, 2017), and a safety assessment of the newly expressed proteins Barnase, Barstar and PAT regarding their capacity to trigger celiac disease (EFSA GMO Panel, 2017b). The outcome of the updated bioinformatic analyses is presented in Section 3.3.

2.1.4. Additional documents or studies provided by the applicant[13]

In line with the renewal guidance requirements (EFSA, 2019a; EFSA GMO Panel, 2015), the applicant provided an overview on the worldwide approvals of oilseed rape MS8, RF3 and MS8 × RF3 and searched for any available full reports of 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.

2.1.5. Overall assessment as provided by the applicant[14]

The applicant provided an overall assessment concluding that information provided in the application for renewal of authorisation of oilseed rape MS8, RF3 and MS8 × RF3 for food and feed uses in the EU does not change the outcome of the original risk assessment (EFSA GMO Panel, 2012, 2017a).

2.1.6. Monitoring plan and proposal for improving the conditions of the original authorisation[15]

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

2.2. Methodologies

The GMO Panel assessed the application for renewal of the authorisation of oilseed rape MS8, RF3 and MS8 × RF3 for food and feed uses 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 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 MS8, RF3 and MS8 × RF3, 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 MS8, RF3 and MS8 × RF3 and the newly expressed proteins Barnase, Barstar and PAT. The overall quality of the performed literature searches is acceptable.

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 Barnase, Barstar and PAT proteins (EFSA GMO Panel, 2012, 2017a) have been identified by the applicant.

[12] Dossier: Oilseed rape MS8, RF3 and MS8 x RF3 – Annex III; additional information: 17/3/2022.
[13] Dossier: Oilseed rape MS8, RF3 and MS8 x RF3 – Annex III; additional information: 16/5/2022, 21/10/2022.
[14] Dossier: Oilseed rape MS8, RF3 and MS8 x RF3 – Annex III.
[15] Dossier: Oilseed rape MS8, RF3 and MS8 x RF3 – Part I – Request for renewal; additional information 21/10/2022.

3.3. Evaluation of the updated bioinformatic data

The results of the updated bioinformatic analyses to assess the interruption of oilseed rape endogenous genes confirm previous results indicating that RF3 insert may have landed in the 3' UTR of a rotundifolia-like 21 gene while no endogenous genes have been interrupted by the event MS8 (EFSA GMO Panel, 2012, 2017c).

The analyses of the amino acid sequence of the newly expressed PAT, Barnase and Barstar proteins reveal no significant similarities to toxins, allergens or immunogenic gluten-related epitopes. Moreover, the updated bioinformatic analyses of the newly created ORFs within the inserts do not indicate sequence similarities to toxins or allergens in oilseed rape MS8, RF3 and MS8 × RF3. In addition, the updated bioinformatic analysis of the newly created ORFs spanning the junctions with genomic DNA confirms previous results which did not indicate sequence similarities to toxins or allergens in oilseed rape MS8, RF3 and MS8 × RF3 (EFSA GMO Panel, 2012, 2017a,c).

The updated bioinformatic analyses for events MS8 and RF3 reveal three elements of bacterial origin with sufficient length and sequence identity to facilitate homologous recombination with native bacterial genes. However, no pairs of sequences which would facilitate transfer of inserts by double homologous recombination were identified. These results confirm previous conclusions (EFSA GMO Panel, 2012, 2017a,c). Given the results of this analysis and that the recombinant DNA in oilseed rape MS8, RF3 and MS8 × RF3 does not confer selective advantages 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 reports of the additional studies provided (Appendix B) and the 90-day oral repeated dose toxicity study in rat with canola RF3 provided upon EFSA request (Appendix C). Overall, the new additional documents or studies provided by the applicant do not raise any concern for human and animal health and the environment, which would change the original risk assessment conclusions on oilseed rape MS8, RF3 and MS8 × RF3.

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-024 indicating new hazards, relevant changes in exposure or scientific uncertainties that would change previous conclusions on oilseed rape MS8, RF3 and MS8 × RF3.

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 MS8, RF3 and MS8 × RF3. This general surveillance is coordinated by CropLife Europe and implemented by selected operators (federations involved in oilseed rape grains 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-024, 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 events in oilseed rape MS8, RF3 and MS8 × RF3 considered for renewal is identical to the sequence of the originally assessed events, the GMO Panel concludes that there is no evidence in renewal application EFSA-GMO-RX-024 for new hazards, modified exposure or scientific uncertainties that would change the conclusions of the original risk assessment on oilseed rape MS8, RF3 and MS8 × RF3 (EFSA GMO Panel, 2012, 2017a).

5. Documentation as provided to EFSA

- Letter from the European Commission to EFSA received on 8 March 2021 for the continued marketing of genetically modified oilseed rape MS8, RF3 and MS8 x RF3 submitted in accordance with articles 11 and 23 of Regulation (EC) No 1829/2003 by BASF Agricultural Solutions Seed US LLC (EFSA-GMO-RX-024)
- The application was made valid on 9 November 2021
- Additional Information (Clock 1) was requested on 17 December 2021
- Additional Information (Clock 1) was received on 17 March 2022
- Additional Information (Clock 2) was requested on 18 March 2022
- Additional Information (Clock 2) was received on 16 May 2022
- Additional Information (Clock 3) was requested on 3 June 2022
- Additional Information (Clock 3) was received on 21 October 2022
- Additional Information (Clock 4) was requested on 28 November 2022
- Additional Information (Clock 4) was received on 10 January 2023

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EFSA (European Food Safety Authority), Gennaro A, Gomes A, Herman L, Nogué F, Papadopoulou N and Tebbe C, 2017. 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

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OECD (Organisation for Economic Co-operation and Development), 1998. OECD Guideline for the testing of chemicals - Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents. OECD Publishing, Paris.

Abbreviations

GM	genetically modified
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
ORFs	open reading frames
PMEM	post-market environmental monitoring

Appendix A – List of relevant publications identified by the applicant through literature searches (June 2013–November 2022)

Reference
Arpaia S, Birch AN, Edmund CA, du Jardin P, Gathmann A, Gropp J, Herman L, Hoen-Sorteberg, H-G, Jones H, Kiss J, Kleter G, Lagiou P, Lovik M, Messean A, Naegeli H, Nielsen KM, Ovesna J, Perry J, Rostoks N, Tebbe C, Devos Y, Diveki Z and Ehlert C, Fernandez Dumont, 2012. Scientific opinion on application (EFSA-GMO -BE-2010-81) for the placing on the market of genetically modified herbicide -tolerant oilseed rape Ms8, Rf3 and Ms8 × Rf3 for food containing or consisting of and food produced from or containing ingredients produced from, oilseed rape Ms8, Rf3 and Ms8 × Rf3 (with the exception of processed oil) under Regulation (EC) No 1829/2003 from Bayer. EFSA Journal 2012;10(9):2875, 32 pp.
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Siruguri V, Bharatraj DK, Vankudavath RN, Rao Mendu VV, Gupta V and Goodman RE, 2015. Evaluation of Bar, Barnase, and Barstar recombinant proteins expressed in genetically engineered *Brassica juncea* (Indian mustard) for potential risks of food allergy using bioinformatics and literature searches. Food and Chemical Toxicology, 83, 93–102. https://doi.org/10.1016/j.fct.2015.06.003

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 MS8, RF3 and MS8 × RF3

Study identification	Title
18-RSOS0041	Influence of Ionic Strength on PAT/bar Functional Activity
M-479248-01-1	The effect of temperature on microbially produced Barstar assessed by ELISA
M-488334-01-1[(a)]	Comparative assessment of MS8 × RF3 × RT73, MS8 and RF3 *Brassica napus* tolerance to glufosinate herbicide
M-493314-0-1[(a)]	Comparative assessment of MS8 × RF3 × RT73, MS8 and RF3 *B. napus* tolerance to glufosinate and MS8 × RF3 × RT73, MS8 and RF3 to glyphosate herbicide
M-497705-01-1	Comparative assessment of MS8 × RF3 × RT73, MS8 and RF3 *B. napus* tolerance to glufosinate herbicide
M-500088-01-1[(a)]	Confirmation of the absence of vector backbone sequences in *B. napus* MS8
M-500399-01-1	Comparison of the Barstar protein expressed in *B. napus* containing event RF3 and the Barstar protein batch No. 1340 Barstar
M-500404-01-1[(a)]	Quantitative protein expression analysis of Barnase and Barstar proteins in leaf, seed, and whole above-ground plant matrices of MS8 × RF3 × RT73 Canola, MS8 Canola, and RF3 Canola grown in Canada in 2011
M-533563-01-1[(a)]	Structural stability analysis of *B. napus* RF3
M-534293-01-1[(a)]	Confirmation of the absence of vector backbone sequences in *B. napus* RF3
M-537791-01-2[(a)]	MS11xRF3 *B. napus*, MS11 *B. napus* and RF3 *B. napus* – Comparative assessment of tolerance to glufosinate-ammonium herbicide, 2015
M-541224-01-1	Quantitative protein expression analysis of Barstar and PAT/bar proteins in whole plant and raceme matrices over three generations of RF3 (ACS-BNØØ3-6) *B. napus*
M-557508-01-1	The effect of temperature on PAT/bar as assessed by ELISA
M-563611-01-1[(b)]	Channel catfish feeding study with RF3 canola

(a): Additional study previously assessed in application EFSA-GMO-RX-004 (EFSA GMO Panel, 2017a).
(b): The GMO Panel notes that the submitted study report contained limited details about the materials and methods used for the production of the test diets. As the study was not a requirement for the EU, clarification of the limitations was not sought. On evaluation of the available information, no treatment-related adverse effects were identified.

Appendix C – Outcome of the assessment of a 90-day oral repeated dose toxicity study in rat with canola RF3 (study number M_584150-01-1)

In this study, pair-housed Sprague Dawley Crl:CD(SD) rats (16 per sex per group; 2 rats per cage) were allocated to three groups using a randomised complete block design with eight replications per sex. Groups were fed diets containing 15% of incorporation rate of *B. napus* meal either from oilseed rape RF3 treated with the intended herbicides[16] (test material), from the conventional counterpart (control material) or a non-GM reference oilseed rape (Spectrum). The study was adapted from OECD test guideline 408 (OECD, 1998), aligned with EFSA Scientific Committee guidance (EFSA Scientific Committee, 2011) and complied with the principles of good laboratory practice (GLP) with some minor deviations not impacting the study results and interpretation. The stability of the test and control materials was not verified; however, in accordance to product expiration declared by the diet manufacturer, the constituents of the diets are considered stable for the duration of the treatment. The GMO Panel considered this justification acceptable. Diet preparation procedures and regular evaluations of the mixing methods guaranteed the homogeneity and the proper concentration of the test or control substances in them. Event-specific PCR analysis confirmed the presence of the event oilseed rape RF3 in both the GM meals and diets and excluded the presence of the event in the respective controls. Both the GM meals and diets were analysed for nutrients, antinutrients and potential contaminants. Balanced diets were formulated based on the specifications of PMI Nutrition International, LLC (TestDiet®). Feed and water were provided ad libitum. In-life procedures and observations and terminal procedures were conducted in accordance to OECD TG 408 (1998).

An appropriate range of statistical tests were performed on the results of the study. Detailed description of the methodology and of statistically significant findings identified in rats given diets containing meal derived from oilseed rape RF3 is reported in Annex A.

There were no test diet-related incidents of mortality or clinical signs. No test diet-related adverse findings were identified in any of the investigated parameters. A small number of statistically significant findings were noted but these were not considered adverse effects of treatment for one or more of the following reasons:

- were within the normal variation[17] for the parameter in rats of this age;
- were of small magnitude;
- were identified at only a small number of time intervals with no impact on the overall value;
- exhibited no consistent pattern with related parameters or endpoints;
- exhibited no consistency with increasing incorporation levels.

No gross pathology findings related to the administration of the test diet were observed at necropsy, and the microscopic examinations of a wide range of organs and tissues did not identify relevant differences in the incidence or severity of the histopathological findings related to the administration of the test diet compared to the control group.

The GMO Panel concludes that this study is in line with the requirements of Regulation (EU) No 503/2013 and that no treatment related adverse effects were observed in rats after feeding diets containing oilseed rape RF3 meal at 15% of inclusion level for 90 days.

The GMO Panel noted that the incorporation rate of oilseed rape meal in this study is up to 15%, based on nutritional considerations made by the applicant. Although EFSA (2014) proposes the upper limit of 25% for inclusion of rapeeed meal in rodent diets, the GMO Panel considers that further scientific investigations are required to confirm its applicability.

[16] Glufosinate-ammonium herbicide.

[17] Although animal used in a toxicology study are of the same strain, from the same supplier and are closely matched for age and body weight at the start of the study, they exhibit a degree of variability in the parameters investigated during the study. This variability is evident even within control groups. To help reach a conclusion on whether a statistically significant finding in a test group is 'adverse' account is taken of whether the result in the test group is outside the normal range for untreated animals of the same strain and age. To do this, a number of sources of information are considered, including the standardised effect size, the standard deviations and range of values within test and control groups in the study and, if applicable, data from other studies performed in the same test facility within a small timeframe and under almost identical conditions (Historic Control Data).

Annex A – Statistical analysis and statistically significant findings in the 90-day toxicity study in rats on oilseed rape RF3

A.1. Statistical analysis of the 90-day study on oilseed rape RF3 in rats

The following endpoints were statistically analysed: body weights, body weight changes, food consumption, clinical pathology values (as applicable), absolute and relative organ weights, functional observational battery (FOB) data, locomotor activity, and histopathological data. For all continuous endpoints, mean, standard deviation in terms of the standardised effect sizes (SES) of each dose group for each sex, variable and period or time interval were reported.

The main statistical analysis compared the test diet group with the conventional counterpart.

The analysis was performed for sex-separated and pooled data at 5% level of significance. Continuous endpoints were analysed with a linear model (factor: diet group, in addition, sex and interaction 'diet-sex' for pooled analysis, whereas for Locomotor activity data: diet, time and the interaction term 'diet-time' for sex-separated analysis, in addition, sex and the interaction 'diet-sex' term for pooled analysis). Ranges from historical control data were provided to aid the assessment of statistically significant differences between the test and the control diet group. Missing data were considered by the Panel and found not to have an impact on the results (Table A.1).

Table A.1: Statistically significant findings in 90-day study on oilseed rape RF3 in rats

Statistically significant parameter/endpoint	Finding	GMO Panel interpretation
Body weight gain	Reduced (33%)[A] in males at weeks 7–8	Transient result. No impact on final body weight. Within normal variation. Not an adverse effect of treatment.
Ambulatory counts	Reduced at 51–60 min in both sexes combined (50%) and in males (90%)	Transient, with no impact on overall ambulatory count. Within normal variation and consistent with pre-dosing values. Not an adverse effect of treatment.
Motor activity total counts	Reduced in males (70%) at 51–60 min	Transient, with no impact on overall motor activity total counts. Within normal variation. Not an adverse effect of treatment.
Urobilinogen	Increased (150%) in females	Within normal variation, all values were within the concurrent control range (0.2–1.0). Not an adverse effect of treatment.
Seminal vesicle/prostate weight (absolute and relative to brain weight)	Reduced (10%)	Small magnitude. Within normal variation. No associated histopathology findings. Not an adverse effect of treatment.
Thyroid/parathyroid weight (absolute and relative to body weight and brain weight)	Reduced (10–20%) in both sexes combined	Small magnitude. Within normal variation. No associated histopathology findings. Not an adverse effect of treatment.

A: Where changes are given as percentages (e.g. reduced (30%)) this indicates the magnitude of the change relative to the control value (e.g. 30% means a value of 7 in test group animals versus 10 in controls).